THE *IN VITRO* INVESTIGATION OF THE TOXICITY OF COMMERCIALY AVAILABLE HERBAL MIXTURES USED AS AFRICAN TRADITIONAL MEDICINE IN PRETORIA, GAUTENG, SOUTH AFRICA.

A thesis submitted by

Ms ME Mothibe

In partial fulfilment of the requirements for the degree

Doctor of Philosophy

of the

Sefako Makgatho Health Sciences University

Department of Pharmacology and Therapeutics

School of Medicine

Faculty of Health Sciences
DECLARATION

I, Mmamosheledi Elsie Mothibe, hereby declare that the work on which this dissertation is based is original (except where acknowledgements indicate otherwise); and that neither the whole work nor any part of it has been submitted before for any degree or examination at this or any other university.

Signed .............................................. Date........................................
ACKNOWLEDGEMENTS

I would like to thank God, the Almighty, whose presence in my life guaranteed this document come to being.

I would like to express my sincere gratitude to the following people:

❖ My supervisors; Prof CP Kahler-Venter and Prof E Osuch, for their guidance and trust in me.

❖ The staff of the Department of Pharmacology and Therapeutics, who made this work possible. Particularly Mrs Corrie Van Staden for all the assistance and support, and Most specially Sister Selina Mabitsela, for her willingness to help, her availability and professionalism throughout.

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❖ All the people on campus, who engaged with me, shared their stories, wisdom and curiosity about this study. I am richer in wisdom for having encountered each one of them.

❖ My entire family- my children, my sister and her children and my brothers for their unwavering love, support and assistance.

God bless you all for being with me in this expedition.
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Consent form (English)
Consent form (Setswana)
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<th>Description</th>
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<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
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<td>AGEs</td>
<td>Advanced glycation end-products</td>
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<td>ATM</td>
<td>African traditional medicines</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<td>ARC</td>
<td>Agricultural Research Council</td>
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<tr>
<td>ARDS</td>
<td>Acute Respiratory Distress Syndrome</td>
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<td>ARSO</td>
<td>African Standardisation Organisation</td>
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<td>ARSO THC13</td>
<td>ARSO Technical Harmonisation Committee on Traditional Medicine No 13</td>
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<td>AU</td>
<td>African Union</td>
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<td>BCL</td>
<td>Biochemiluminescence</td>
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<td>BHL</td>
<td>Body healing liquid</td>
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<td>CAD</td>
<td>Coronary artery disease</td>
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<td>CAM</td>
<td>Complementary alternative medicines</td>
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<td>CGD</td>
<td>Chronic granulomatous disease</td>
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<td>CHMs</td>
<td>Commercial herbal mixtures</td>
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<td>CHP</td>
<td>Conventional Health Practitioner</td>
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<td>Chemiluminescence</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>COX-1</td>
<td>Cyclooxygenase-1 enzyme</td>
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<td>CSIR</td>
<td>Council for Scientific and Industrial Research</td>
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<td>CVS</td>
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<td>Cyt b</td>
<td>Cytochrome b</td>
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<td>D-U</td>
<td>Drug-positive Urine</td>
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<td>DAG</td>
<td>Diacylglycerol</td>
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<td>Diastolic blood pressure</td>
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<td>DFU</td>
<td>Drug-free urine</td>
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<td>DGMH</td>
<td>Dr George Mukhari Hospital</td>
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<td>DM</td>
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<td>Description</td>
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<tr>
<td>DMSO</td>
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<td>DTM</td>
<td>Directorate: Traditional Medicine</td>
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<td>EDTA</td>
<td>Ethylene-diamine-tetra-acetic acid</td>
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<td>fMLP</td>
<td>N-formyl-methionyl-leucyl-phenylalanine</td>
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<td>FMN</td>
<td>Flavin mononucleotide</td>
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<td>Flavin mononucleotide dihydrogen</td>
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<td>GACP</td>
<td>Good Agricultural Collection Practices</td>
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<td>Good Clinical Practice</td>
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<td>Guanine triphosphate</td>
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<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
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<td>HBSS</td>
<td>Hanks' balanced salt solution</td>
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<td>HIV/AIDS</td>
<td>Human immunodeficiency virus and Acquired Immune deficiency syndrome</td>
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<td>HM</td>
<td>Herbal medicine/mixture</td>
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<td>HMP</td>
<td>Hexose monophosphate</td>
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<td>HOCl</td>
<td>Hypochlorous acid</td>
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<td>HT</td>
<td>Hypertension</td>
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<td>IC</td>
<td>Initial luminescence of the control standard</td>
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<td>IHN</td>
<td>Isolated human neutrophil</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>INF</td>
<td>Interferon</td>
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<td>INH</td>
<td>Inhibition</td>
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<td>%INH</td>
<td>Percentage inhibition</td>
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<td>IP3</td>
<td>Inositol 1,4,5-triphosphate</td>
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<td>IT</td>
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<td>iTHPCSA</td>
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<td>LECL</td>
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<tr>
<td>MCC</td>
<td>Medicines Control Council</td>
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<td>MIC</td>
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<td>NADP</td>
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<td>O$_2^-$</td>
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<td>OH$^-$</td>
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<td>Oral rehydration solution</td>
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<td>Platelet-activating factor</td>
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<td>PAH</td>
<td>Polycyclic aromatic hydrocarbons</td>
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<td>PMNs</td>
<td>Polymorphonuclear leucocytes</td>
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<td>PMP</td>
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<td>Rheumatoid arthritis</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>rpm</td>
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<td>RPMI</td>
<td>Roswell Park Memorial Institute Medium</td>
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<td>SA</td>
<td>South Africa</td>
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<td>SADC</td>
<td>Southern African Development Community</td>
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<td>SAHPRA</td>
<td>South African Health Products Regulatory Authority</td>
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<td>SBP</td>
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<td>SOA</td>
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<td>Solid phase extraction</td>
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<td>T&amp;CM</td>
<td>Traditional and Complementary medicine</td>
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<td>TEAC</td>
<td>Trolox Equivalent Antioxidant Capacity</td>
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<td>Traditional and Complementary medicine</td>
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<td>TNF</td>
<td>Tumour Necrosis Factor</td>
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SUMMARY

African traditional medicine (ATM) is the oldest and most diverse of all medicine systems, even though the medicine systems are poorly recorded (Gurib-Fakim, 2006). African traditional healing is interwoven with cultural practices and religious beliefs, and is therefore regarded as being holistic, involving both the body and the mind (Helwig, 2005; Gurib-Fakim, 2006; Truter, 2007). ATM was generally associated with herbs, remedies and advice from diviners or healers with strong spiritual and cultural components (Truter, 2007). Usually, ATM would be obtained from traditional healers or by self-collection on advice from a healer or someone knowledgeable about the medicine. Commercialisation and marketing has popularised herbal mixtures such that they are available ready for use and packaged from special muthi shops, pharmacies, grocery stores, health shops, streetside vendors and other outlets. The use of commercial herbal medicines with modern packaging and marketing practices was reported to be a booming trade in South Africa. The many herbal mixtures sold in many outlets and over-the-counter (OTC) in pharmacies have not been tested for efficacy and safety the way conventional medicines are tested before use, despite their modern packaging and presentations (Ndhlala, Stafford, Finnie, Van Staden, 2011; Ndhlala & Van Staden) due to lack of control, regulation or monitoring.

The widespread use of the herbal mixtures being marketed as immune boosters, tonics, or medicines that strengthen the immune system may pose a threat to both the healthy and non-healthy persons who use them. There is a belief that they are natural hence they are safe, which predisposes their users to possible unknown adverse and toxic effects. They may also impact negatively on the outcomes of conventional treatment of chronic conditions like asthma, hypertension and diabetes mellitus. It is therefore imperative that these herbal mixtures be investigated for all possible effects on physiological systems, including toxicity.

Hence the aim of the study was to investigate, in vitro, the toxicity of commercially available herbal mixtures used as African traditional medicine in Pretoria, Gauteng, to strengthen the immune system or the body.
The objectives of the study were specifically to:

Use biochemiluminescence assay to investigate the *in vitro* effects of the selected herbal mixtures on isolated human neutrophils (IHNs), platelets and whole blood of healthy volunteers and patients on chronic treatment for asthma, hypertension and for diabetes mellitus;

Investigate whether the selected herbal mixtures have cross-reactivity with commonly tested substances of abuse in urine, using a qualitative rapid test and an automated quantitative assay.

The selected commercial herbal mixtures were found to be common in retail outlets as immune boosters or intended to strengthen the immune system in Pretoria. They are *Maphilisa™* Herbal medicine, *Intlamba Zifo™, Matia™* African medicine for all diseases, *Ngoma™* Herbal Tonic Immune Booster, *Stametta™* Body Healing Liquid, *Vuka Uphile™* Immune Booster.

Blood samples were collected from consenting healthy volunteers and asthma, diabetes mellitus and hypertension patients who were on treatment and met the requirements. Drug-free urine was pooled from samples donated by healthy volunteers. Urine that had tested positive for tetrahydrocannabinol was obtained from the Pharmacology and Therapeutics Department Routine Laboratory. The biochemiluminescence assay was performed on the Orion L Microplate Luminometer supplied by Berthold Detection Systems and the rapid urinalysis screening test was performed with the *Instant view® Multi-Drug of Abuse Test* kit form Lab-stix Diagnostics (Pty) Ltd. The effects of the herbal mixtures were tested through two pathways of stimulation; via a direct mechanism by stimulating protein kinase C and via an indirect G-protein-linked mechanism. Both mechanisms cause the activation of NADPH oxidase system, resulting in the formation of reactive oxygen species within cells.

The six herbal mixtures affected the cells and platelets by generally causing inhibition of their activity at high concentrations and stimulation at low concentrations. The inhibitory effect was mainly a weak inhibition (< 50%) while the stimulatory effect varied from weak (< 50%), moderate (50% - 79%) to potent (≥ 80%) stimulation. The effects of the herbal mixtures were
at times similar on healthy cells and platelets, and on those from asthmatic, diabetic and hypertensive patients. The herbal mixtures in general affected the two pathways of stimulation comparably. However in several cases, the effects were either opposite, or were inhibition or stimulation only. The IHNs of healthy volunteers were inhibited by all the herbal mixtures via both mechanisms at all concentrations. The herbal mixture *Intlamba Zifo™* caused stimulation of whole blood phagocytes at high concentrations and inhibition at low concentration.

The variable effects of the herbal mixtures may have various implications. Inhibition and hence reduced reactive oxygen species in healthy neutrophils and platelets may result in a compromised immune system, which could enhance the susceptibility to infections, with reduced antimicrobial capacity. It would also increase the risk of bleeding which may be a serious complication if such individuals would need conventional intervention such as surgery or lead to serious interactions with conventional drug therapy. Stimulation of the healthy cells and platelets, with increased production of reactive oxygen species, would in turn enhance the reactivity of these cells. The consequence would be exaggerated or uncontrolled inflammatory or immune reaction to stimulus; which could trigger unexpected or unwanted inflammatory conditions. It could also enhance the thrombotic state, leading to unexplained thromboembolic and cardiovascular conditions.

Stimulation of neutrophils and platelets with increased formation of reactive oxygen species would aggravate both hypertension and asthma, and enhance the development and progress of associated complications. The inhibition of these cells may delay or reduce the severity of the two conditions by lowering the oxidative stress; however it may result in a compromised immune system, and the increased risk of bleeding. Stimulation of neutrophils in diabetes mellitus may enhance the functioning of the immune system, and lower the increased risk of infections in the disease while their inhibition would do the opposite and impair the immune system. The stimulation of the platelets which are already hyperactive in diabetes mellitus would greatly increase the risk of development and progress of atherothrombotic and cardiovascular complications.

The stimulatory and inhibitory effects occurred at different concentrations, highlighting the importance of appropriate dosing. The ingredients of the herbal mixtures are quite diverse
while others are not stipulated. The possibility of interactions with drug treatment exists, whether prescription or non-prescription drugs. Of the six herbal mixtures tested, five of them had no influence on the quantitative rapid urinalysis assay. The one herbal mixture which showed different results was *Ngoma™* Herbal Tonic and Immune Booster. This herbal mixture reported false-negative results for tetra-hydrocannabinol (THC) in the urine samples that were positive for THC, making it a potential agent for adulteration of urine samples.

In view of the observed effects on cells and platelets from healthy, diabetic, hypertensive and asthmatic volunteers, the possible drug interactions, and in recognition of the fact that users of African traditional medicines tend not to disclose their use of the medicines; it is therefore advisable to probe patients about the use of African traditional medicines and commercial herbal medicines. It would also be advisable for conventional health care providers to make patients aware that disclosure of traditional medicine use would benefit their conventional treatment as all possible risks and interactions would be considered.
CHAPTER 1

1. Introduction

Traditional medicines have been used by humankind for the treatment of various
diseases since long before the advent of orthodox medicine, and to this day, serve
the health care needs of the majority of the world population. According to the World
Health Organisation (WHO), trends in the use of traditional medicine (TM) and
complementary medicines have been increasing (WHO 2013).

Traditional medicine is an ancient system of healing used in many parts of the world,
involving the extensive use of indigenous plants. WHO defines TM as “the sum total
of knowledge, skills and practices based on the theories, beliefs and experiences
indigenous to different cultures, whether explicable or not, that are used to maintain
health, as well as to prevent, diagnose, improve or treat physical and mental
illnesses” (WHO, 2008). Traditional medicines (TMs), also called herbal medicines
(HMs) include herbs, herbal materials, herbal preparations and finished herbal
products (WHO, 2004). The WHO has been at the forefront of TM matters, collating
information about TM practices worldwide and providing guidelines in advancing the
recognition, acceptance and integration of TM into the health systems of member
countries.

The Draft Policy on African traditional medicine (ATM) for South Africa (SA) defines
ATM as a body of knowledge that is associated with the examination, diagnosis,
therapy, treatment, prevention of, or promotion and rehabilitation of the physical,
mental, spiritual or social wellbeing of humans and animals (Department of Health,
2008). In the Traditional Health Practitioners Act (Act 22 of 2007), TM is defined as
“an object or substance used in traditional health practice for the diagnosis,
treatment or prevention of physical or mental illness or any curative or therapeutic
purpose, including the maintenance or restoration of physical or mental health or
well-being in human beings” (Truter, 2007; Department of Health, 2008).
1.1. The role of WHO in the Traditional medicine sector

The role of the WHO in the recognition, promotion and acceptance of TM dates as far back as 1976. In that year, the World Health Assembly (WHA), which is the governing body of WHO drew attention to the reserve constituted by those practising traditional medicine. The organisation urged its member states to utilise their traditional systems of medicine. In 1978 at the international conference on Primary Health Care at Alma Ata, the WHA recommended that governments prioritise the incorporation of traditional health practitioners (THPs) and birth attendants into the health care team and proven traditional remedies into national drug policies and regulation (WHO, 2000).

In 2000 the WHO Regional Committee for Africa adopted a strategy for the African region titled ‘Promoting the Role of Traditional Medicine in Health Systems: A Strategy for African Countries’. The aim of the strategy was to contribute to the achievement of health for all in the African Region, by optimising the use of TM. The strategy urged member states to develop national policies and legislation on traditional medicine, to improve regional and sub-regional collaboration. It also encouraged the member states to take steps to promote and protect traditional medicine nationally. Consequently, the WHO Regional Office for Africa in collaboration with the Department of Essential Drugs and Medicines Policy organised a series of workshops on the regulation of traditional medicines to assist member states to establish mechanisms for evaluating traditional medicines for registration purposes (WHO Regional Office for Africa, 2011).

The WHO African Regional Strategy on TM was adopted in 2001 by the Organization of African Unity (OAU), now called the African Union. The period 2001-2010 was declared as the Decade for African Traditional Medicine. An annual African Traditional Medicine Day was also declared and it is celebrated on the 31st of August every year. All stakeholders were requested to prepare a Plan of Action for implementation to meet the objectives of the strategy (WHO, 2002).

The WHO Traditional Medicines Strategy 2002-2005 provided a framework for action to promote the use of TM and Complementary Alternative medicine (TM/CAM) in reducing mortality and morbidity impoverished nations. The strategy had four objectives which were:
• “To integrate TM/CAM into national health care systems, where appropriate, by developing and implementing national TM/CAM policies and programmes.
• To promote the safety, efficacy and quality of TM/CAM by expanding the knowledge base of these remedies and by providing guidance on regulatory and quality assurance standards.
• To increase the availability and affordability of TM/CAM where appropriate, focusing on poorer populations.
• To promote therapeutically sound use of appropriate TM/CAM by providers and consumers” (WHO, 2002).

In 2003, the first WHO regional workshop was held in SA in 2003 where a set of guidelines for the registration of traditional medicines was developed. The minimum regulatory requirements for the registration of TMs were determined (WHO Regional Office for Africa, 2004). In the same year, resolution WHA56.31 on TM was adopted at the 56th World Health Assembly (WHA). It urged Member States to establish or expand and reinforce existing national drug safety monitoring systems to monitor herbal medicines and other traditional practices.

In 2004, guidelines were issued – “Guidelines for registration of traditional medicines in the WHO Africa region”. The overall objective of the guidelines was to facilitate the registration, marketing and distribution of traditional medicines of consistent quality in the African Region (WHO Regional Office for Africa, 2004).

The Guidelines on the safety monitoring of herbal medicines in pharmacovigilance system were issued, with the objectives which included promoting the safe and proper use of herbal medicines (WHO, 2004).

By 2004 it had become important for WHO to obtain detailed qualitative and quantitative information about the prevalence of TM/CAM utilisation. A set of indicators had been proposed by a special consultative meeting of experts in 2001 to measure these aspects. The core indicators were expected to provide answers for who uses TM/CAM, what is used, why it is used and what it costs. The indicators were classified into three main groups. Background indicators included amongst others, aspects such as the total number of prescribers and the total number of
TM/CAM providers within and outside of the conventional medicine system. Structural indicators addressed issues such as whether there was official national policy and legislation on TM/CAM. Process indicators provided answers for estimated prevalence of national TM/CAM and estimated prevalence of the five most popular therapies used (WHO, 2001).

The first WHO Congress on Traditional medicine was held in Beijing, China in 2008, to further assess the role of TM/CAM, to review the progress of member states as well as to help these countries integrate TM into their health systems. Part of the Beijing Declaration adopted by Congress stated, among others, that

- “Governments had a responsibility for the health of their people and should formulate national policies, regulations and standards to ensure safe, appropriate and effective use of TM.
- TM should be further developed based on research and innovation”.

It also encouraged improved education, clinical inquiry into traditional medicine and improved communication between health care providers (WHO, 2008).

The 62nd WHA held in 2009 endorsed the resolutions of the Beijing declaration and urged member states to adopt and implement the declaration in accordance with national capacities, priorities, relevant legislation and circumstances (WHO, 2009).

The WHO Traditional Medicine Strategy for 2014-2023, was released in 2012. It was intended to support member states in, among others,

- “Harnessing the potential contribution of TM to health, wellness and people-centred care.
- Promoting the safe and effective use of TM by regulating, researching and integrating TM products, practitioners and practice into health systems where appropriate.
- Education and training of TM and CAM practitioners”.

This strategy also provided a review of the progress made since the strategy 2002-2005 and it sought to build upon that strategy (WHO, 2012).
1.1.1. Progress on TM emanating from WHO strategies

The progress report on the Decade of African traditional medicine (ATM) indicated that many countries popularised TM. They established and strengthened their institutional capacity and developed national policies and regulatory frameworks for TM. Progress was also made in establishing national programmes and setting up expert committees for the development of TM in their health ministries.

By 2010, 22 countries including SA were using WHO guidelines in conducting research on TM for diabetes, hypertension, HIV/AIDs, malaria and sickle cell anaemia. A total of five countries had included TM in their National Essential Drugs list, which was an increase from only one in 1999/2000 to four in 2010. SA and 11 other countries issued market authorisations for TM, ranging from one in Cameroon to more than 1000 in Ghana and Nigeria. Six countries including SA, had tools for the protection of Intellectual Property Rights and the TM knowledge (Kasilo, Trapsida, Mwikisa, Lusamba-Dikassa, 2011).

The African Standardisation Organisation (ARSO) set up the ARSO Technical Harmonisation Committee (THC) on Traditional Medicine No 13 (ARSO THC 13). This committee is aimed to harmonise terminologies and technical terms so as to create a common platform for understanding concepts, theories and technical aspects for stakeholders involved in ATM. The ARSO THC 13 aims to support the quality, safety and effectiveness of TM products, support good manufacturing practices, good agricultural collection practices (GACP) and to assist in the trade and commerce of TM. This initiative will improve the visibility, viability, applicability and acceptability of ATM among professionals in African populations. The committee launched in December 2014 and is currently developing various relevant standards including, among others, the glossary, GMP for herbal medicines and the minimum requirements of registration of herbal medicines (www.arso-oran.org).

The WHO report of 2013 acknowledged that there was growing interest in the world about the use of traditional and complementary medicine (T&CM). The report stated that there had been significant progress in the implementing, regulating and managing T&CM in most regions. Member states that had established or developed national policies for T&CM had increased from 39 in 2003 to 69 in 2012 and those who were regulating herbal medicines had increased from 82 in 2003 to 119.
Member states had developed regulations on the quality, quantity, accreditation and education structures for T&CM practitioners and conventional medicine practitioners who used T&CM. Member states that provided education on T&CM up to university doctoral degrees had increased from none to 39. Some institutions in the African region, including SA had included TM in the curricula of health professions students. Some countries across the world had set up national research institutes in the field of T&CM, and those had increased from 56 in 2003 to 73 in 2012 (WHO, 2012).

1.2. Utilisation and prevalence of traditional medicine in South Africa

TM remains widespread in developing countries, while use of CAM is increasing rapidly in developed countries (Ernst, 2000; Harris & Rees, 2000; WHO, 2008). The use of TM in developing countries has increased since the 1990s, as evidenced by the 70-80% of the population in India and Ethiopia who depend on TM and TM practitioners for primary health care (WHO, 2008).

South Africa is classified as a mixture of both a developing and a developed country (Rautenbach, 2011). Like other African countries, SA has a pluralistic system of healthcare, in which modern medicine practice coexists with other non-conventional health systems. These include a variety of indigenous systems based on traditional practices and beliefs (Meissner, 2009).

It has been estimated that 80% of Africans use TM, compared to 60% of the world’s population in general (WHO, 2008; Helwig, 2005). The use of ATM by the general public has been reported since years back (Kgoatla, 1994; Peltzer, 2009), and with more and more newer studies being published, it indicates that the practice is not abating (Malangu, 2007; Gqaleni, Moodley, Kruger, Ntuli & McLeod, 2007; Otang, Grierson, Ndip Ndip, 2011).

It was reported that in seeking health, people consult traditional healers first before a conventional health provider (CHP) (Meissner, 2009; De Villiers & Ledwaba 2003; Moshabela, 2008; Dambisya, 2003). These people may choose to withhold this information when consulting a CHP and not tell that they use ATM (De Villiers & Ledwaba, 2003; Malangu, 2007; Gqaleni, 2007, Peltzer, Phaswana-Mafuya & Treger, 2009; Otang et al., 2011). Mander et al. (2007) asserted that a large proportion of the Black population makes use of the dual health care system, in
which both the conventional and traditional medicines are demanded depending on
the ailment. It was estimated that 72% of the Black African population use ATM, and
the average frequency of use per consumer was 4.8 times per year.

Most recent cases that demonstrated the dualism/pluralism in health seeking
patterns are those involving the therapy of HIV infection. The majority of people living
with HIV and AIDS consulted THPs first before visiting conventional medicine
practitioners (Peltzer & Mqundaniso, 2008; Morris, 2001). Many individuals on
antiretroviral treatment also reported the use of TM concomitantly (Richter, 2003;
UNAIDS, 2006; Babb, Pemba, Seatlanyane, Charalambous, Churchyard, Grant,

The users of ATMs come from all facets of societal classes, including age
categories, education levels, religion and occupation (Cocks & Moller, 2002; Mander,
et al., 2007, Otang et al., 2011; Hughes, Aboyade, Clark, Puoane, 2013). The use of
TM is a common practice across most sectors of the Black African population and is
not merely confined to the poor, rural or uneducated users (Mander et al., 2007).

ATM is said to be the oldest and most diverse of all medicine systems, even though
the medicine systems are poorly recorded (Gurib-Fakim, 2006). African traditional
healing is interwoven with cultural practices and religious beliefs, and is therefore
regarded as being holistic, involving both the body and the mind (Helwig, 2005;
Gurib-Fakim, 2006; Truter, 2007). There are many reasons cited for the use of TM.
Cultural practice is the main reason for use of ATM, and is commonly mentioned in
many studies (Otang, et al., 2011; Truter, 2007). Other reasons why people consult
traditional healers, and hence use ATM include affordability, availability, accessibility,
spiritual and emotional reasons, emphasis on cure rather than prevention in
conventional treatments, limitations of conventional treatments in dealing with
chronic health problems and a general desire for wellness (Kgoatla, 1994; Truter,
2002, Truter, 2007). Affordability means the monetary cost associated with the
utilisation of TM treatment, consultation or products thereof. Availability refers to the
extent to which TM treatment, provider or products are geographically available to
the user (WHO, 2001).
One reason which may be as major as culture is that consumers of HMs believe that these medicines are natural, hence they are safe to use, which is a misconception (WHO, 2004). In Mander et al. (2007) consumers reported that they chose TM as they felt it was more holistic than western medicine, and that the combined mind and body approach addressed both the cause and the symptoms of disease. They were often prepared to pay an exceptional price for the products and services even if the cost exceeded that of western treatments.

The WHO Traditional Medicine Strategy for 2014-2023, stated that people use TM because of its presence on the ground and being readily affordable. It is also due to cultural and historical influences. It was stated that studies have shown that individuals choose TM due to a general demand for all health services, a desire for more information leading to more awareness of available options, increased dissatisfaction with existing healthcare services and a rekindled interest in treatment of the person as a whole (WHO, 2013).

Latif (2010), in her study to investigate the extent of TM use by local communities in the province of Limpopo found that medications not being readily available at district clinics, and hospital waiting times were some of the reasons that forced people to seek TM help. However, it was reported by Mander et al. (2007) that 97% of traditional healer’s patients in one study, indicated that their use of TM was by choice and not as a result of access and cost issues associated with western medicine.

Nevertheless, financially and legally, the delay in legitimising ATM disadvantages its users. Although THPs have catered for the majority of the population over years, ATM has been marginalised as a result of the legacy of apartheid and colonialism and has been suppressed and disempowered as a discipline (Department of Health, 2008). From a financial point of view, the right to choose a health system is biased towards conventional health care, particularly for the employed citizens who have medical aid (Morris, 2001; Mbatha, Street, Ngcobo & Gcaleni, 2012).

ATMs are used for the treatment of a wide variety of conditions, illnesses and disease states and for general health and wellbeing (Hughes et al., 2013). For purposes of this study, the focus will be the use of ATM for diabetes mellitus, hypertension and asthma. These are dealt with in detail in the chapters 4, 5 and 6.
1.3. Commercialisation of traditional medicine

ATM was generally associated with herbs, remedies and advice from diviners or healers with strong spiritual and cultural components (Truter, 2007). Usually, ATM would be obtained from traditional healers or by self-collection on advice from a healer or someone knowledgeable about the medicine.

Presently, due to commercialisation, some ATMs are available ready for use from various retail outlets. These include grocery stores, muthi markets, health shops, street side vendors, supermarkets and over the counter (OTC) in pharmacies. The formal producers of commercially available TM are the retail muthi shops, health shops that specialise in herbal medicines, pharmaceutical manufacturers and Laissez faire manufacturers (Cocks & Moller, 2002; Mander et al., 2007).

Commercialisation is defined as the process of introducing a new product into the commerce, i.e. making the product available on the market (Wikipedia). Commercialisation of medicinal plants means that the plants are available as processed materials in modern packaging and in various dosage forms such as capsules, ointments, tablets, teas or tinctures (Van Wyk, 2008). Some of them may be in the form of coloured solids, brightly coloured and scented liquids, capsules and incense sticks (Ndhlala & Van Staden, 2012).

Muthi (also spelt muti) is a Zulu word meaning African traditional medicine and is derived from the Zulu word umuthi which means tree. However muthi is generally, traditional medicine issued by a traditional healer, and may be of plant, animal or mineral origin (Wikipedia; thefreedictionary.com). The first muthi shop can be traced as far back as 1934. An African inyanga/ herbalist Malavuke Ngcobo who had a small herbal practice, had turned it by 1934, into five muthi shops in and around Durban, SA (Flint, 2008). There are no clear statistics providing an indication of how many muthi shops are available currently, but these shops form part of the commercial landscapes of many metropolitan centres. The traditional medicines trade is huge and growing, generating billions of rands per annum in South Africa in various sectors, including the pharmaceutical sector (Mander et al., 2007).

Dold and Cocks (2002) interviewed street hawkers (street vendors), owners of muthi shops (amayeza shops in iSiXhosa), THPs and TM users and came to the assertion
that the use and trade of plants for medicinal use is no longer confined to traditional healers but had entered both the formal and informal entrepreneurial sectors of the SA economy.

With marketing some HMs are popularised by extensively running advertisements and promotions in newspapers and on radio stations, especially indigenous language stations. The media therefore also serves a role in increasing awareness, and helps that the ATMs become known even to people who had never used these medicines. *Mafavuke Ngcobo* had also advertised his services and *muthi* shops with pamphlets (Flint, 2008), as in figure 1.1. It was noted that social networks, the internet, radio, television and newspapers have provided a new platform for marketing of ATM by THPs (Bonora, 2001).

This OTC market will grow as it is, since it has become convenient, in that it bypasses the services of the THP. One other reason for the growth may be that people now generally want to take the responsibility of their health and wellbeing. Hence the prevailing conditions in terms of commercialisation and marketing permit and enhance the use of TMs through self-medication. Self-medication, according to WHO (2001) simply means the use of TM products or therapy without the advice or consultation of a traditional healer and it includes the use of home remedies.

![Image](image.png)

**Figure 1.1.** An example of pamphlets advertising the services of a traditional health practitioner typical nowadays.
Figures 1.2, 1.3 and 1.4, show plant material collected for sale as traditional medicines, in various stages of processing/handling.

Figure 1.2. Freshly collected plant material being sold at a traditional medicine market

Figure 1.3. Dried collected plant material being sold at a traditional medicine market
1.4. African Traditional medicine in South Africa: Legislation and regulation

South Africa is a member state of the WHO, the AU and the Southern African Development Community (SADC). All three bodies have accepted resolutions which urge member states to develop and implement national policies on ATM. The SA government has therefore taken steps towards the official recognition, acceptance and institutionalisation of ATM. Institutionalisation means formalisation and official incorporation of TM into the national health system (WHO, 2003).

The National Drug Policy (NDP) for South Africa of 1996 is amongst one of the first documents to recognise the potential role and benefits of traditional medicine for the national health system. It was aimed at investigating 'the use of effective and safe traditional medicines at primary level' and specified the following, with regard to traditional medicine:

- Traditional medicine will be investigated for its 'efficacy, safety and quality with a view to incorporate their use in the health care system'.
- Marketed traditional medicine will be registered and controlled.
- A National Reference Centre for African Traditional Medicine for African Traditional Medicines (the NRCATM) will be established (Department of Health, 2004).
The NRCATM, a virtual reference centre, was established in 2003 by the Medicines Control Council (MCC) of the National Department of Health in collaboration with the Council for Science and Industrial Research (CSIR) and the Medical Research Council (MRC). The purpose of the centre was to gather, harness and synthesise information to promote, regulate and register ATMs derived from plants. Some of its functions included the development of a national database of indigenous plants that have been screened for efficacy and toxicity and to test the effectiveness and safety of traditional medicines, so as to protect the public from unproven claims within the traditional medicines sector (Department of Health, 2004).

A Presidential task team was appointed in 2006 to advice on national policy and appropriate regulatory and legal framework regarding TM institutionalisation. This task team drafted the national policy of ATM in SA after consultation with various stakeholders. The Draft National Policy on ATM in SA was gazetted in 2008 (Department of Health, 2008).

A new directorate, the Directorate: Traditional medicine, (DTM) was established to manage the work related to TM within the NDoH. The vision of the DTM is to advance the contribution of TM to the health and well-being of the population. The mission thereof is to facilitate the institutionalisation of TM in the National Healthcare System (NHS) through validation and production of safe and effective TMs and the promotion of TM practice based on a sound legislative and policy framework. Currently the DTM is headed by Mr FB Mbedzi and he is the coordinator of all activities relating to ATM in liaison with units of TM at provincial level and other government departments and statutory research councils such as CSIR, MRC, ARC (Department of Health, 2008; Mbedzi, 2015).

The Traditional Health Practitioners Act (Act 22 of 2007) has now been enacted. This act was purposed to establish an interim Traditional Health Practitioners Council of SA (iTHPSCSA). It was also aimed to provide for the registration, training and practices of THPs and to serve and protect the interests of the public who use the services of the THPs. The iTHPSCSA was formed and appointed in 2013. The council is tasked to provide

- a regulatory framework to ensure the efficacy, safety and quality of traditional health care services,
- the management and control over the registration, training and conduct of practitioners, students and specified categories of the THPs.

The Traditional Health Practitioners Act stipulates four categories of THPs - diviners, traditional birth attendants, traditional surgeons and herbalists. Prophets are no longer part of this group, since their practice is church-based; hence their regulation falls within the auspices of the Council of Churches. The DTM is in the advanced stages of establishing the office of the Registrar of the iTHPCSA (Mbedzi, 2015).

In keeping with the AU plan of Action for the 2001-2010 Decade of TM, the African traditional medicine day (31 August) as well as the traditional medicine week are commemorated annually.

The Medicines and Related substances act 101 of 1965 which governs legal matters for the registration of all medicines did not cover for the registration, regulation and control of ATM and CAM adequately. Hence ATMs were separated from the category of CAM in the act. The motivation for the removal was that the government would be able to give ATM special priority and identity, which would enable speedy development and effective regulation as a category on their own (Department of Health, 2008).

The act was amended in 2013 to accommodate CAM and was published in the Government Gazette on 15 November 2013. The amendment provided the definition and classification of CAM as well as the specific requirements for labelling, package inserts and patient information leaflets. Complementary medicines are classified as Category D, which are products with medicinal claims linked to an allied healthcare profession.

South Africa currently regulates all CAM practices under the Allied Health Professions Act of 1982. These include the professions - chiropractors, homeopaths, osteopaths, naturopaths, ayurvedic practitioners, Chinese traditional medicine practitioners and aroma-therapists.

Act 101 of 1965 is in the process of being amended again, to allow regulatory oversight for traditional medicines (Department of Health, 2014; Mbedzi, 2015). The intention of Bill 6 of 2014 is to amend the Medicines and Related Substances Act,
1965 when read together with Act 72 of 2008. It therefore intends to establish a new Medicines Regulatory Authority to be called the SA Health Products Regulatory Authority (SAHPRA).

Hence, currently the ATM practice and the use of ATMs are progressing as an unregulated, unmonitored domain. The ATMs used have not been tested for safety and efficacy.

Notwithstanding the progress made by the DTM, there are some challenges they still have to overcome. The slow pace of government processes, the uncoordinated implementation of government strategies around ATM and funding are some of the hurdles that they have to surmount. The NRCATM should be revived, as it was dormant since inception. One of the priorities of the DTM is to facilitate the speedy finalisation of the Draft policy of ATM to make it into policy (Mbedzi, 2015) which can then be enacted. It is very crucial since one of the proposals in the policy is to set up centres that will test ATMs for their pharmacology, toxicology and clinical profiles. The national pharmacovigilance centres, established by the NDoH, one of them being located in the Sefako Makgatho University (previously University of Limpopo (UL), Medunsa campus) could be one of the sites for assisting in compiling some of the profiles of ATMs. The aim of the pharmacovigilance centre in SMU was to detect, assess and prevent adverse reactions to antiretroviral drugs (ARVs) and traditional and complementary medicines, when used by people living with human immunodeficiency virus (HIV) infection (Department of Health, 2004). While they have made strides in terms of adverse events associated with ART, not much has been done regarding ATMs and CAM.

Despite these measures, there is still no regulation for commercially packaged and labeled herbal formulations used as ATMs. However, the use of TMs has been increasing and it generates considerable amounts of revenue (Gqaleni, Moodley, Kruger, Ntuli & McLeod, 2007). The use of commercial herbal medicines (CHMs) with modern packaging and marketing practices was reported to be a booming trade in South Africa. The many herbal mixtures sold in many outlets and OTC in pharmacies have not been tested for efficacy and safety the way conventional medicines are tested before use, despite their modern packaging and presentations (Ndhlala, Stafford, Finnie, Van Staden, 2011; Ndhlala & Van Staden, 2012).
Although they are readily available and used, their efficacy remain unproven and their safety and toxicity profiles remain unknown. This therefore calls for research by the scientific community, which would support the rational basis for the use of the herbal medicines. As noted by Ndhlala et al. (2011), the acceptance and modernisation of the herbal mixtures would rely largely on scientific research output to stipulate the rationale for their usage and safety. Hence this investigation may serve as contribution to the scientific research output, to show the rational basis as well as the safety or toxicity of the CHMs being used.

1.5. Terminology and definitions

It is important to look at the definitions and descriptions of traditional medicine and related words for comprehension and context.

**Traditional medicine (TM)** is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses (WHO, 2008).

TM, according to the Traditional Health Practitioners Act is defined as “an object or substance used in traditional health practice for the diagnosis, treatment or prevention of physical or mental illness or any curative or therapeutic purpose, including the maintenance or restoration of physical or mental health or well-being in human beings, but does not include a dependence-producing or dangerous substance or drug”.

**Complementary medicine** or **alternative medicine (CAM)** refer to a broad set of health care practices that are not part of a country’s own tradition or conventional medicine, and are not fully integrated into the dominant health care system of that country. The terms are used interchangeably with TM in some countries (WHO, 2013). Other terms sometimes used to describe these health care practices include “natural medicine”, “non-conventional medicine” and “holistic medicine” (http://www.who.int/medicines/areas/traditional/definitions /en/).
In countries where the dominant health care system is based on allopathic medicine or where TM has not been incorporated into the national health care system, TM is often termed ‘complementary’, ‘alternative’ or ‘non-conventional’ medicine. For example, traditional Chinese medicine and acupuncture would be termed ‘Traditional Medicine” when used in China, but ‘Complementary and Alternative Medicine’ when used in Europe, North America or South Africa (WHO, 2012).

**Traditional medicines (TM)**, also called herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products (WHO, 2004).

**African traditional medicine (ATM)** is described in the Draft Policy on African Traditional Medicine for SA as a body of knowledge that has been developed and accumulated over tens of thousands of years, which is associated with the examination, diagnosis, therapy, treatment, prevention of, or promotion and rehabilitation of the physical, mental, spiritual or social wellbeing of humans and animals (Department of Health, 2008).
CHAPTER 2: STUDY PROBLEM

2.1 Rationale for the study

Traditionally, a herbal medicine may be prepared in many different ways, and may also be applied or administered in numerous ways depending on the ailment being treated. Steenkamp (2003) noted that the formulation or dosage form, the preparation method and the mode of administration are important, and they differ depending on the indication. Truter (2007) stated that traditional medicines are closely associated with plant material, which may include leaf, bark or root infusions for drinking, bathing, purging, steaming and using as enemas. Some preparations include powders, poultices, ointments, pastes and decoctions (concentrated extracts). Infusions are prepared like tea, by steeping the herbs in boiling water. Decoctions are prepared by adding cold water to the amount of plant material required and allowing it to boil. A poultice is a paste from the crushed fresh plant material, usually mixed in water and then applied on the required part of the body (Gurib-Fakim, 2006).

Normally a THP once having diagnosed the ailment or problem would then provide the HM in a suitable preparation and provide instructions for application. The THP may prepare the medicine and dispense it, or may provide the ‘raw’ material and provide instructions for preparation at home. The prepared medicines are generally sold in recycled plastic containers which may be of poor quality, or are wrapped in newspaper or plastic packets (Ndhlala & Van Staden, 2012). In many instances, the prepared herbal medicine or herbal mixture is usually dispensed in used plastic cooldrink bottles.

Herbal mixtures (HMs) are defined as concoctions of two or more plant species with the same or different uses (Cano and Volpato, 2004). Herbal concoctions are commonly used as traditional medicine, either obtained from THPs, or self-prepared as home remedies. Mixtures are also described as products with medicinal properties and which contain two or more plants or herbs which can act individually, additively or synergistically to restore or maintain health (Gurib-Fakim, 2006). In the “Guidelines for herbal medicines”, WHO defines herbal preparations made from one or more herbs as “finished herbal preparations” (FHPs). These can also be called...
“mixture herbal products” (MHPs). Both FHPs and MHPs may contain excipients in addition to the active compound. The products are not considered to be herbal if they have a chemically defined active substance added (WHO, 2000).

Commercialisation and marketing has popularised herbal mixtures such that they are available ready for use and packaged from special muthi shops, pharmacies, grocery stores, health shops, streetside vendors and other outlets. As a result of lack of control, regulation or monitoring, these commercial herbal mixtures (CHMs) have not been taken through clinical trials where safety studies would be done as is with conventional medicine. Lack of standardisation procedures means that there is no information about the quality of the CHMs in terms of collection, preparation, packaging as well as storage. The lack of standardisation of the raw material, the methods of production and quality control of the product were reported to be some of the hindrances towards integration of TM with conventional medicine (Taylor, Rabe, McGaw, Jager and Van Staden, 2001).

The information supplied with the commercial herbal mixtures is generally limited. Most have information about what they are used for and how to use them; some may also have a few precautions to users and contraindications. There are no information pamphlets/ inserts available. It has been stated that manufacturers of CHMs used the labels for branding and colourful images for marketing purposes instead of being informative. There have been inconsistencies and deceptive tendencies regarding the labels and the actual contents of CHMs, hence it was concluded that labels cannot be trusted about the contents of the containers (Ndhlala & Van Staden, 2012).

Nevertheless, the lack of information about these medicines does not deter their use by the public or their sale by the various available retailers/ vendors, including pharmacies. It was noted that the market for CHMs may be driven by the desire for urbanised populations to use TM but having neither the time nor the resources to produce the medicines (Ndhlala & Van Staden, 2012). Studies done on the knowledge and awareness of pharmacists about ATM or herbal medicines have highlighted the need for information to be made available. Although pharmacists had limited knowledge to be able to provide counselling on ATMs, about the ATM, they still believed that it was appropriate that they continue to sell the medicines to those
who needed them (Mothibe, 2015). So the use of CHMs is solely based on the manufacturer claims and marketing, or on the inherent knowledge about the constituents of the HMs. It may be that it is convenient in that both time and money are saved in bypassing the need for consultation of a THP. There might also be the indirect connotation that since the HMs are sold in pharmacies, then they are guaranteed for safety and efficacy. It was postulated that urban people prefer the CHMs out of the perception that as natural products they signify purity, simplicity and safety, and for the convenience of OTC availability from any herbal or food store without a prescription (Ndhlala et al., 2011).

With the prevalence of human immunodeficiency virus (HIV) infection, there has been an explosion of CHMs that claim to strengthen the immune system. Generally, it is not stipulated as to how the immune system will be strengthened. The immune system is an intricate system whose role is to provide the body with protection against foreign agents (Sherwood, 2001). A dysfunctional immune system may give rise to disease, including the development and progression of chronic diseases (Haddad, Azar, Groom, Boivin, 2005). Most of the CHMs that claim to strengthen the immune system are herbal mixtures fortified with vitamins and minerals and are marketed as immune boosters, tonics, or are said to be beneficial in one or more existing disease conditions. Their widespread use may pose a threat to both the healthy and non-healthy persons who use them. The belief that they are natural hence they are safe may predispose their users to possible unknown adverse and toxic effects. As with other herbal medicines, they are used in a wide variety of conditions, illnesses and disease states and for general health and wellbeing (Hughes et al., 2013). Some of the conditions are chronic conditions in which patients would be on long-term conventional drug treatment like diabetes mellitus, hypertension and asthma and the use of TM in these three conditions has been documented.

It is known that there is dualism/pluralism in health seeking patterns of individuals, in which both conventional and traditional medicines are sought; and that these people may choose to not disclose their use of ATM when consulting a CHP. The use of CAM in managing asthma worldwide has been reported, with herbal preparations being one of the highest used CAM modalities (Huntley & Ernst, 2000; Barnes, Powell-Griner, McFann, Nahin, 2004). Patients on treatment for diabetes mellitus
were reported to also be using herbal medicines (Peltzer, Khoza, Lekhuleni, Madu, Cherian, Cherian, 2001b) and the use of ATM for hypertension has been testified (Peltzer, Khoza, Lekhuleni, Madu, Cherian, Cherian, 2001a; Lotika, 2006; Hughes et al., 2013). Many ethnobotanical surveys revealed a variety of medicinal plants that were used for the treatment of asthma (Van Wyk, Van Oudtshoorn and Gericke, 1997; Thring & Weitz, 2006; Ekpo & Pretorius, 2007; Sonibare & Gbile, 2008; Van Wyk, 2008; Van Wyk, De Wet, Van Heerden, 2008; Fouache, Van Rooyen, Faleschini, 2013; Street & Prinsloo, 2013); diabetes mellitus (Ziyyat, Legssyer, Mekhfi, Dassouli, Serhrouchni, Benjelloun, 1997; Edouks, Maghrani, Ouahidi, Jouad, 2002; Amel, 2013; Mootoosamy & Mahomoodally, 2014; Tsabang, Yedjou, Tsambang, Tchinda, Donfagsiteli, Agbor, Tchounwou, Nkongmeneck, 2015; Yaseen, Ahmad, Zafar, Sultana, Kayani, Cetto, Shaheen, 2015); and hypertension (Ziyyat et al, 1997; Thring & Weitz, 2006; Edouks et al., 2002; Van Wyk et al., 2008; Mensah, Okoli, Turay, Ogie-Odia, 2009; Amel, 2013; Mootoosamy & Mahomoodally, 2014; Tsabang, et al, 2015).

Some of the herbal medicines have been incorporated into various types of formulations and are available over the counter for consumers. As with all medicinal agents, the use of herbal medicines brings forth the possibility of unwanted reactions, adverse effects, unexpected interactions as well as toxic effects. With the concommittant use of the CHMs and conventional medicines, there may be negative impact on the outcomes of conventional treatment of the chronic conditions, by lowering the desired pharmacological effects of prescribed drugs (Hussin, 2001; Spiteri-Staines, 2011). The CHMs available to the public have not been taken through clinical trials; hence their safety and toxicity profiles are unknown. It is therefore imperative that these herbal mixtures be investigated for all possible effects on physiological systems, including side effects, efficacy, safety and toxicity. As stated by Ifeoma and Oluwakanyinsola (2013), toxicity screening of herbal medicines may reveal some risks that may occur with the use of the herbs, which may help in avoiding the harmful effects which may occur when the herbal medicine is used.

Although the CHMs are already being used, the starting point for investigations for their toxicity is by performing laboratory tests. *In vitro* laboratory tests are the alternative to whole animal or human studies for toxicity studies as they are less expensive, more rapid and specific (Ndhlala et al., 2010). Although *in vitro* effects
are not automatically reproduced *in vivo*, the findings of *in vitro* tests play an important role in guiding research. The WHO general guidelines for methodologies on research and evaluation of TM state that the safety data obtained from *in vitro* tests might not be absolute markers of safety, but should be seen as indicators of potential toxicity (WHO, 2000).

The CHMs are solutions containing many compounds and molecules that would distribute into all compatible body tissue and fluids. Since the medicines have not been tested for safety, efficacy and interactions, and have not been validated, the effect of their presence on laboratory diagnostic tests is unknown. The presence of these compounds or their metabolites in the various bodily fluids and their impact on laboratory and diagnostic tests is rarely thought of. No report could be found in literature about their effects. There are also no laboratory tests or diagnostic tests that test for the presence of herbal medicines specifically ATMs, except for some qualitative tests that assess for the urine presence of tetra-hydrocannabinol, which is a metabolite of the herb, marijuana.

However the need for such tests is developing, as the use of CHMs is becoming more prevalent. There was a concern raised about the possible unwarranted benefit that may be there in sports people who use ATM, as there are no specific laboratory tests to determine their presence (Stafford, 2010; Harris, 2010). In contrast to that, some concern could be that the herbal medicines may have properties similar to some banned substances, hence when tested, an athlete could test positive for banned substance unknowingly; or the ATM may be used to alter the test results for other substances due to their interactions. One investigation performed on common household products found that some of the chemicals influenced the urine test for drugs of abuse, giving false-negative results as well as invalid results (Uebel & Wium, 2002); while another study revealed six chemicals that caused false-negative results for cannabinoids (Schwarzhoff & Cody, 1993). There are no reports in literature, showing such tests for herbal medicines in general or for ATMs.
2.2 The aim of the study

The aim of this study was

To investigate, *in vitro*, the toxicity of commercially available herbal mixtures used as African traditional medicine in Pretoria, Gauteng, South Africa, to strengthen the immune system or the body.

2.3 The objectives of the study

The objectives of the study were specifically to:

- Use biochemiluminescence assay to investigate the *in vitro* effects of the selected herbal mixtures on isolated human neutrophils, platelets and whole blood of healthy volunteers
- Use biochemiluminescence assay to investigate the *in vitro* effects of the selected herbal mixtures on isolated human neutrophils, platelets and whole blood of patients on chronic treatment for asthma
- Use biochemiluminescence assay to investigate the *in vitro* effects of the selected herbal mixtures on isolated human neutrophils, platelets and whole blood of patients on chronic treatment for hypertension
- Use biochemiluminescence assay to investigate the *in vitro* effects of the selected herbal mixtures on isolated human neutrophils, platelets and whole blood of patients on chronic treatment for diabetes mellitus
- Investigate whether the selected herbal mixtures have cross-reactivity with commonly tested substances of abuse in urine, using a qualitative rapid test and an automated quantitative assay
CHAPTER 3 LITERATURE STUDY: HERBAL MEDICINES AND MIXTURES

3.1 Background

As explained by Cano and Volpato (2004), herbal mixtures are simply concoctions of two or three species with the same popular medicinal use. They are prepared jointly to enhance the known beneficial effects of each species. The main aim of the mixtures seems to be to potentiate the known therapeutic effects of the single-plant preparations; hence species with common uses are often used in combination. Some mixtures may include species claimed to have different therapeutical actions, so their combination is meant to achieve specific goals.

Plants that are used as ATM are assumed to be safe, based on the knowledge accumulated over their long history of usage (Fennel, Lindsey, McGaw, Sparg, Stafford, Elgorashi, Grace, Van Staden, 2004). In view of the fact that the herbal mixtures are used without having been tested in clinical trials, *in vitro* laboratory tests are the best option for screening possible adverse and toxic effects. There are several sites in SA where toxicology of plant extracts is studied. Some of the tests performed include those for anti-inflammatory, antioxidant, antimicrobial, cytotoxic, mutagenic and genotoxic effects (Taylor et al., 2001). These tests were generally performed after the extraction and concentration of what the researchers believed to be the active compounds within the herbal medicines. Most studies have investigated the toxicity of individual herbs or plant extracts but not mixtures. These are in contrast to how the herbal medicines would normally be used or handled in the traditional medicine practice.

It was highlighted that the challenge with analysis of plant material is that there are usually many compounds present and only one or a few might have pharmacologic or toxicological activity. The concentration of the compounds may differ depending on location, soil and season (Stewart, Steenkamp, Zuckerman, 1998; Ncube, Finnie, Van Staden, 2012). As an example, more than 100 volatile and more than 40 non-volatile metabolites have been isolated from *A. afra*. Only a few of the compounds had antimicrobial, antiplasmodial, antioxidant and other activities. The plant samples collected in four different areas were found to have qualitative and quantitative properties (Liu, Van Der Kooy, Verpoorte, 2009). Ncube, Finnie and Van Staden
(2011) found variations in the types and quantities of plant compounds produced in different seasons. They also found that some plant extracts that had moderate to high inhibition on cyclooxygenase were significantly variable in that activity across seasons (Ncube et al., 2012).

In a review of the safety of ATM, it was revealed that several plants used in SA as TM can cause damage to genetic material (Fennell et al., 2004). Steenkamp (2003) listed 25 plants that are potentially toxic and are used for gynaecological disorders and complaints. Callilepis laureola, a plant commonly used as a TM in KwaZulu-Natal was reported to be cytotoxic, which concurred with the observed hepatotoxicity in clinical cases of poisoning with the plant. It was suggested that the toxicity may be due to the presence of compounds related to potassium atractylate, that the plant contains (Larrey, 1997; Popat, Shear, Malkiewicz, Stewart, Steenkamp, Thompson, Neuman, 2001). An HM used during pregnancy, isihlambezo is said to have beneficial effects on both the mother and the foetus, yet several ingredients of this mixture are plants known to have toxic properties (Varga & Veale, 1997). Evaluation of 13 herbal mixtures commonly sold in KwaZulu-Natal for toxicity, revealed four with mutagenic effects and one with high cytotoxic effects (Ndhlala, Anthonissen, Stafford, Finnie, Verschaeve, Van Staden, 2010).

Therefore the HMs need to be investigated for their toxicity, especially since it was understood that some people do not consult either the conventional health care provider or THPs for their physical illnesses, but do self-treatment by using OTC CHMs (Ndhlala & Van Staden, 2012). The WHO guidelines for methodologies on research and evaluation of TM further stipulated that the methodologies should guarantee the safety and efficacy of herbal medicines and therapies that are traditional procedure-based and should not become obstacles to the application and development of TM (WHO, 2000).
3.2 Herbal medicines as immune boosters

The immune system is a complex physiological system that serves to defend the body against foreign and abnormal cells. It involves both non-specific innate response and an acquired or adaptive immune system (Sherwood, 2001). The two systems work together to protect the body against infections. The innate system responds directly to the presence of microorganisms and involves primary cells that are natural killer cells in the identification and spontaneous lysis of invading targets. The adaptive system is acquired by interaction with the environment, and involves the use of cytotoxic and helper T lymphocytes, bone marrow derived lymphocytes, macrophages and dendritic cells. The two systems are integrated and interdependent, and communicate internally and externally by means of cytokines (Haddad et al., 2005). Cytokines are a large group of small signalling molecules that are extracellular proteins or glycoproteins and serve as intercellular regulators and mobilisers. They are responsible in the selection, initiation and modulation of an appropriate immune response (Haddad et al., 2005; Spelman, Burns, Nichols, Winters, Ottersberg, Tenborg, 2006).

A weakened or an overactivated immune system may give rise to various disorders. A weak system increases the risk of opportunistic infectious diseases, development of cancer and tumour escape. An overactivated one is associated with chronic inflammation and autoimmunity, and diseases such as diabetes mellitus type 1, heart disease, cancer, allergies, asthma, joints and tissue destruction. The immune system is implicated in the development and progression of chronic diseases, which are mainly treated with conventional medicine (Haddad et al., 2005). It is thus understandable why there is a need to maintain a viable, efficient and balanced immune system. It explains the rise and popularity of the use of herbal medicines to boost the immune system.

Herbs are said to be immunostimulants that increase the immune system activity without being specific to a particular disease or antigen (McCaleb, 1997). They are also immunomodulators, which are defined as medicinal plants that alter the activity of the immune system by regulating cytokines, hormones, neurotransmitters and other peptides (Spelman et al., 2006). Some ATMs prescribed by THPs were found to be able to modulate the production of cytokines at low doses. Isolated human peripheral blood mononuclear cells treated with the ATMs showed increased
secretion of various pro-inflammatory cytokines including interferon (IFN), interleukins (IL) and tumour necrosis factor (TNF), that are mediators of the cellular immune system with different biological activities (Gqaleni, Ngcobo, Parboosing, Naidoo, 2012). In the body, cytokines are found in concentrations of nanomolar to picomolar ranges, which implies that dilute herbal mixtures with biologically active compounds may have therapeutic benefit (Spelman et al., 2006).

A herbal mixture called PHELA, which is composed of four ATMs generally used by THPs for wasting conditions, was confirmed in animal studies to be an immunostimulant particularly in a compromised immune system (Lekhooa, Walubo, Du Plessis, Matsabisa, 2012). Spelman et al. (2006) listed many herbal immunomodulators that either increase or decrease the secretion or expression of multiple cytokines. They therefore motivate that the pharmacological effects and therapeutic efficacies of medicinal plants are derived from several compounds acting in synergy, rather than from a single compound.

Following claims of immune boosting action, one CHM sold in retailers and pharmacies in KwaZulu-Natal, Gauteng, Mpumalanga and the Western Cape, uMakhonya®, was investigated for \textit{in vitro} immunomodulatory activity. It was found to have cytotoxic activity at high doses and induced chemokine secretion significantly, but did not have any chemoattractant activity at lower non-cytotoxic doses. At the lowest doses, the CHM was able to maintain or increase the secretion of chemokines by monocytes activated by lipopolysaccharide (LPS). Remarkably, other doses of the CHM induced the secretion of chemokines comparable to LPS, which suggested an identical mechanism of action between uMakhonya® and LPS (Ngcobo, Gcaleni, Ndlovu, Serumula, Sibiya, 2016). It has been stated that \textit{in vitro} tests should be cognizant of clinically relevant concentrations of agents being tested, if they were to serve as a guide towards positive clinical trials that will lead to improved therapies (Smith & Houghton, 2013). This validates the need for testing of the HMs at various concentrations, ranging from millimolar to nanomolar concentrations.

Generally, the actions and effects of CHMs, whether wanted, unwanted or toxic, depend entirely on the active chemicals they contain, the interaction of the components of the mixture and the interaction with endogenous chemicals.
3.3 Selection of the commercial herbal mixtures

There is widespread use and awareness of commercial herbal mixtures (CHMs) being marketed as immune boosters, tonics, or medicines that strengthen the immune system. The CHMs selected for this study were found to be common in retail outlets as immune boosters or intended to strengthen the immune system in Pretoria, Gauteng, SA. They were available over the counter in pharmacies, muthi shops, muthi markets, and some were found in grocery stores.

They are Intlamba Zifo™, Maphilisa™ Herbal medicine, Matla™ African medicine for all diseases, Ngoma™ Herbal Tonic Immune Booster, Stametta™ Body Healing Liquid, Vuka Uphile™ Immune Booster and are shown in Table 3.1. They are denoted HM1 to HM6 respectively, which stands for Herbal Mixture number 1 (HM1) up until Herbal Mixture number 6 (HM6). Currently there are no guidelines available, specifically for information required on the label and package of these herbal mixtures. So the information presented about the mixtures is at the discretion of the producer/ manufacturer and therefore is not consistent. None of the herbal mixtures had a package insert. The information available was written on the package or on the container of the medicine. All the information available on the package and/or the container of each of the CHMs is presented in Table 3.2. It includes the following details where available: the indications, ingredients or contents, the instructions for use (dosage and dosing), the contraindications, storage instructions, warnings, precautions and disclaimers.
Table 3.1 The selected commercial herbal mixtures for investigation

<table>
<thead>
<tr>
<th></th>
<th>Product Name</th>
<th>Description</th>
<th>Code</th>
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<tbody>
<tr>
<td>1</td>
<td><em>Intlamba zifo™</em> (HM1)</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td><em>Maphilisa™</em> Herbal Medicine (HM2)</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td><em>Matla™</em> African medicine for all diseases (HM3)</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td><em>Ngoma™</em> Herbal Tonic Immune Booster (HM4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Stametta™</em> Body healing liquid (HM5)</td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td><em>Vuka Uphile™</em> Herbal remedy (HM6)</td>
<td></td>
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</tr>
</tbody>
</table>
3.4 Literature review of the selected commercial herbal mixtures and their constituents

There are no reports in literature about the CHMs listed in this study, except for *Stametta™ Body healing liquid (BHL)*. There is also not much information provided on the packages of these HMs. However some of the components of the CHMs are well known herbs or plants with known medicinal properties.

*Stametta™ BHL* contains aloe, ascorbic acid, aniseed oil and magnesium sulphate. It was found to have high levels of total phenolic compounds and lower levels of flavonoids. It was the least toxic among 14 HMs tested for toxicity on human liver cells, using the neutral red uptake inhibition bioassay. It also had indirect mutagenic activity when tested using the Ames test (Ndhlala et al., 2010). *Stametta™ BHL* was listed as one of the herbal remedies used by HIV patients in Kwazulu-Natal, before initiation of antiretroviral therapy (ART) but the use was drastically reduced after six months of ART. However it was still being used by some patients after 12 months and had stopped completely at 20 months of treatment (Peltzer et al., 2011). This CHM was reported to be widely used in both rural and urban areas, which might be as a result of advertising as well as its bright and colourful label (Ndhlala & Van Staden, 2012). *Stametta™ BHL* was one of the top ten commonly sold HMs in Tshwane township community pharmacies, and more than 50% of community pharmacists indicated that they knew the ingredients, the indications, dose intervals, storage conditions and the route of administration of this CHM (Mothibe, 2015).

*Ngoma™ herbal tonic* is listed as one of the common HMs sold in Piertermaritzburg, Kwazulu-Natal, SA (Ndhlala, 2010). There is no information about the CHM but its components are well-known herbs with various activities. It is a concoction of echinacea, dandelion, alfalfa-lusern, *Aloe ferox* and *Harpagophytom* (devil’s claw). It is worth noting that there has been reports about labelling discrepancies in products that contained echinacea, with some products not containing the correct species and the actual quantity of the herb (Ndhlala & Van Staden, 2012).

*Maphilisa™ herbal medicine* contains African potato, *Aloe ferox*, African ginger, citric acid, sutherlandia and wild olive. It was also one of the top ten commonly sold HMs in Tshwane township community pharmacies. However, other than the route of
administration, the majority of community pharmacists did not know the ingredients, the indications, dose intervals, storage conditions and other pharmacological properties of this CHM (Mothibe, 2015).

**Intlamba zifo™** contains aloe, water, assorted herbs, H2O2, sunset yellow No 6-CI 15985. It was also one of the top ten commonly sold HMs in Tshwane township community pharmacies. Similarly to **Maphilisa™** herbal medicine, the majority of community pharmacists did not know the ingredients, the indications, dose intervals, storage conditions and other pharmacological properties of this CHM except the route of administration (Mothibe, 2015).

**Vuka Uphile™** is stated to be containing natural herbs.

**Matla™** *African medicine for all diseases* has no information about the contents.

It is important to note that aloe (*Aloe ferox, Aloe vera*) is common in the four CHMs that have ingredients recorded. *Aloe ferox, Aloe vera, African potato and devil’s claw* are listed as some of the famous African medicinal plants (Gurib-Fakim, 2006). *Aloe ferox, devil’s claw, African potato, Sutherlandia frutescens* (Van Wyk, 2008) and wild (African) ginger (Street & Prinsloo, 2013) are included as commercially important southern African medicinal plants. *Aloe ferox* and devil’s claw are included in the list of the ten potent African medicinal plants (Mahomoodally, 2013). African potato and *Sutherlandia* were in 2002, recommended by the Ministries of Health of SA and other SADC countries for use in the treatment of HIV and the management of associated symptoms, despite the lack of evidence of effectiveness at the time (Mills, Cooper, Seely, Kanfer, 2005).

### 3.5 The ethnobotanical uses of the components of the commercial herbal mixtures

Medicinal plants as stated earlier may be used as different plant parts, prepared in various forms and be applied in many ways. They contain mixtures of different chemical compounds, which may act individually, additively or in synergy to improve health. As an example, one plant may contain substances that stimulate digestion, phenolic compounds that have antioxidant activity, antibacterial tannins that serve as
natural antibiotics and diuretic substances that help to eliminate waste products 
(Gurib-Fakim, 2006). This explains why a medicinal plant may have many uses as a 
traditional medicine.

*Aloe ferox* and *Aloe vera* (*kgophane* in Setswana, *impinda* in isiZulu) are the most 
commonly used and commercially available species of *Aloe* (Liliaceae). These 
perennial succulent plants have large, fleshy lance-shaped leaves that are stemless. 
*Aloe* is available in various dosage forms, including tablets, capsules, creams, 
powders and aqueous solutions, and the most common in South Africa is the 
aqueous infusion (Steenkamp & Stewart, 2007). It has been described as one of the 
medicinal plants used for many diverse conditions affecting various systems, 
including the nervous, endocrine, musculoskeletal, respiratory, genitourinary, 
circulatory and digestive systems. It helps in the treatment of pain, infections and 
infestations, skin complaints and in pregnancy (Grace, Simmonds, Smith, Van Wyk, 
2008; Amoo, Aremu, Van Staden, 2014). Leaf and stem decoctions are used for 
emesis. Leaves and roots are boiled in water and used for treatment of stress and 
hypertension. Other preparations are used to treat stomach pains, toothache, 
arthritis, conjunctivitis and sinusitis, while the plant is generally used as a laxative 
(Van Wyk, 2008; Street & Prinsloo, 2013). Pharmacological screening has confirmed 
the antimicrobial, antihelminthic, anticancer, antioxidant, anti-inflammatory, 
immunostimulatory, anticholinesterase, laxative, wound healing and skin repair 
properties of *Aloe ferox* (Vasquez, Avilla, Segura, Escalante, 1996; Pugh, Ross, El 
Sohly, Pasco, 2001; Fawole, Amoo, Ndhlala, Light, Van Staden, 2010; 
Mahomoodally, 2013; Street & Prinsloo, 2013) and *Aloe vera* (Rodriguez-Fragoso, 
Reyes-Esparza, Burchiel, 2008).

Figure 3.1. *Aloe ferox*
**African ginger** (*Siphonochilus aethiopicus*) (*iSiphepheto in IsiZulu*) is a deciduous rare southern African plant. It has an extensive history of use in ATM and the root or rhizome is the plant part used. It is used to treat headache, asthma, coughs, colds, influenza, sinusitis, throat infections, thrush, candida, malaria, premenstrual syndrome and menstrual cramps, diabetes, hypertension, stroke, constipation, catarahh, rheumatism, toothache and gingivitis. It has expectorant effects in the lungs hence relieves cough and chest infections. It is well-known for its antiemetic effects and was found to be highly effective for motion sickness (O’Hara, Kiefer, Farell, Kemper, 1998; Ali, Blunden, Tanira & Nemmar, 2007; Van Wyk, 2008; Zadeh & Kor, 2014; herbalafrica.co.za). A review of the phytochemical, pharmacological and toxicological properties of ginger revealed more than 100 compounds isolated, and confirmed the anti-inflammatory, anti-apoptotic, antioxidant, immunomodulatory, anti-tumorigenic, anti-hyperglycaemic and anti-lipidaemic actions of the plant (McGaw, Jager, Van Staden, 1997, Ali et al., 2007).

![Figure 3.2. African ginger (*Siphonochilus aethiopicus*)](image)

**African potato** (*Hypoxis hemerocallidea*) (*monamaledu in Setswana; inkome, ilabatheka in isiZulu*) is a tuberous perennial herb, with star-shaped, yellow flowers and strap-shaped leaves commonly distributed in southern Africa. In traditional medicine it is used as an immune booster and for the treatment of many diverse conditions such as arthritis, painful joints, dyspepsia, loss of appetite, urinary diseases, tuberculosis, prostate hypertrophy, cancer, diabetes and as a laxative (Ojewole, 2006; Nair, Foster, Arnason, Mills, Kanfer, 2007; Nair, Dairam, Agbonon, Arnason, Foster, Kanfer, 2007; Van Wyk, 2008; Street & Prinsloo, 2013).
African potato, similarly to Stametta™, was used by HIV patients in KZN, before initiation of antiretroviral therapy (ART), after six months of ART and had stopped completely after 12 months of ART (Peltzer et al., 2011). In the majority of patients who used ATMs concurrently with ART, African potato and Aloe vera were the most commonly used (Babb et al., 2007). African potato had been recommended by the Ministries of Health of SA and other SADC countries for use in the treatment of HIV and the management of associated symptoms (Mills et al., 2005). In vivo animal studies have shown that African potato has antidiabetic, antinociceptive and anti-inflammatory activities (Ojewole, 2006). A review of the pharmacological activity of the plant listed various activities including the antimicrobial, antiviral antioxidant, anti-inflammatory, antidiabetic, anticancer, anticonvulsant and cardiovascular activities. The main compounds isolated is hypoxoside and its biologically active metabolite, rooperol (Ncube, Ndhlala, Okem, Van Staden, 2013).

![African potato](image.png)

**Figure 3.3.** African potato (*Hypoxis hemerocallidea*)

**Devil's claw (Harpagophytum procumbens)** (*sengaparile* in Setswana) is a perennial herb native to the southern African countries surrounding the Kalahari. It is a weed with yellow and purple tubular flowers and the fruit is covered with several hooks. Commercial products of the plant are registered in Europe as a herbal medicine or food supplement (Kemper, 1999; Van Wyk, 2008; Street & Prinsloo, 2013; Mahomoodally, 2013). It is available as capsules, tablets and injection solution (Al-Harbi, Al-Ashban, Shah, 2013). Traditionally the dried root was the part used as decoctions or infusions for headache, backache, menstrual pain, rheumatism, arthritis, painful joints, dyspepsia, loss of appetite, heartburn; for gall, liver, kidney
and bladder problems; as body tonic, detoxifying agent; and externally to treat skin ulcers, boils, sores, and allergies (Al-Harbi et al., 2013; Street & Prinsloo, 2013). Pharmacological studies have confirmed the analgesic, anti-inflammatory, antioxidant, antiviral, antidiabetic, antiepileptic, antimalarial activities of the herb; as a result, it is used in Western herbalism for treatment of rheumatoid arthritis, osteoarthritis, low back pain, joint and knee pain (Mncwangi, Chen, Vermaak, Viljoen, Gericke, 2012; Al-Harbi et al., 2013; Street & Prinsloo, 2013). The ethanol root extract of *H. procumbens* was confirmed by *in vitro* studies as an immunomodulator, by modulating the secretion of IL-1B, IL-6 and TNF (Spelman et al., 2006).

![Figure 3.4. Devil’s claw (*Harpagophytum procumbens*)](image)

**Sutherlandia frutescens**, (*phetola* in Setswana; *unwele* in isiZulu) commonly known as cancer bush, is a soft-wooded shrub with reedy stems and is well distributed in southern Africa. It is currently available as tablets, gel and powder, manufactured by Phyto Nova (Pty) (Street & Prinsloo, 2013) and the product is one of the extensively marketed herbal medicines on SA radio as an immune booster. *Sutherlandia* is the only plant claimed to be traditionally used for almost any disease. The conditions include gastrointestinal disorders (indigestion, poor appetite, gastritis, oesophagitis, peptic ulcer, dysentery), respiratory disorders (colds, cough, influenza, asthma, bronchitis), urinary tract infections, kidney and liver disorders, rheumatism, heart failure, diabetes, stress, anxiety backache and fever (Street & Prinsloo, 2013; Van Wyk, 2008; Mills et al., 2005). Pharmacological screening reported anti-cancer, anti-mutagenic effects, anti-HIV activity and stress-relieving properties (Van Wyk, 2008).
A safety study performed in 2002 and a Phase I clinical trial in 2007 found no signs of toxicity of *Sutherlandia* (Van Wyk, 2008; Street & Prinsloo, 2013). These effects may be the motivation why *Sutherlandia* was recommended by the Ministries of Health of SA and other SADC countries for use in the treatment of HIV and the management of associated symptoms (Mills et al., 2005).

![Figure 3.5 Sutherlandia frutescens (cancer bush)](image)

**Wild olive** (*Olea europaea* L. subs *Africana*) (*motlhware* in Setswana; *umhlwati* in isiZulu) is an evergreen tree with a dense spreading crown, is widespread in the European Mediterranean islands, the Arabian Peninsula, the Indian subcontinent and Asia, and Africa, with four species in South Africa (Van Wyk, Van Oudtshoorn, Gericke, 1997; Khan, Panchal, Vyas, Butani, Kumar, 2007; Hashmi, Khan, Hanif, Farooq, Perveen, 2015). It is used traditionally as decoction of dried fruit and dried leaves, hot water extract, infusion of fresh leaves, tincture of leaves and oil from the seeds. Virgin oil is the edible oil extracted and mass produced from the seeds. The olives are not used naturally but are consumed as table olives because of their bitter taste (Somova, Shode, Ramnanan, Nadar, 2003; Khan et al., 2007; Hashmi et al., 2015). *O. europaea* is used as TM to lower blood pressure, cholesterol, uric acid and blood sugar, and to treat diarrhoea, diabetes, hypertension, respiratory and urinary tract infections, stomach and intestinal diseases, asthma, haemorrhoids, rheumatism, sore throat, eye conditions, to improve kidney function, and as a tonic and a laxative (Van Wyk, Van Oudtshoorn, Gericke, 1997; Joffe, 2002; Somova et al., 2003; Khan et al, 2007; Hashmi et al., 2015). Many compounds have been extracted and isolated from the bark, leaves, fruits, seeds, wood and the oil. Most of these compounds were pharmacologically tested and confirmed for various activities.
including the anti-inflammatory, antihypertensive, antioxidant, antidiabetic, analgesic (antinociceptive), anticancer, antimicrobial, antiviral, antiatherosclerotic, gastroprotective, neuroprotective, immunomodulatory as well as wound healing activities (Somova et al., 2003; Khan et al, 2007; Chebbi Mahjoub, Khemiss, Dhidah, Dellai, Bouraoui, Khemiss, 2011; Hashmi, et al., 2015).

![Figure 3.6. Wild olive (Olea europaea L.subs Africana)](image)

**Echinacea (Echinacea purpurea)**, also called the purple coneflower is a native American herb well-known for its immune boosting effects. Although the root was the part used in traditional medicine in the past, all plant parts are used nowadays. Preparations of Echinacea are approved drugs in Europe, and the herb is available commercially as capsules and tablets, creams, gels, powder, liquid extract and as dried root or herb. It is used for treatment of colds, coughs and influenza, for infections such as upper respiratory infections, urinary tract infections, sore throat, herpes and candida infections. Topical preparations are used for treatment of wounds, skin infections, psoriasis, eczema and other inflammatory conditions (O’Hara et al., 1998; Leigh, 2001). Pharmacological tests of the herb have revealed various effects including immunostimulant, anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, antimitagenic, anticancer and anxiolytic activities (McCaleb, 1997; Wojdylo, Oszmianski, Czemerys, 2007; Manayi, Vaziran, Saeidnia, 2015). Various doses of the flower and herb extracts of *E. purpurea* were confirmed by *in vitro* studies as immunomodulators, by modulating the secretion of several ILs and TNF (Spelman et al., 2006). Through animal studies it was confirmed that components of echinacea increase the number of circulating white cells, enhance
phagocytosis, trigger the complement pathway and stimulate the formation of cytokines (O'Hara et al., 1998).

Figure 3.7. Echinacea (*Echinacea purpurea*)

**Alfalfa-luserne** (*Medicago sativa*) is a perennial leguminous herb that originated in Asia but now cultivated and used worldwide. The sprouts are consumed as food (vegetable salad) and the leaves and seeds are commercially packaged as capsules, tablets and powdered herb and are commonly sold as nutritional supplements. Traditionally alfalfa was used as a herbal remedy for many conditions, including arthritis, kidney problems, cough, asthma, bladder disorders, prostate disorders, cancer, scurvy, sore muscles and gastrointestinal disorders. It was also useful for improvement of memory, inflammation, increasing breast milk, appetite stimulation and wound healing (Hong, Chao, Chen, Lin, 2009; Kundan & Anupam, 2011). It contains a variety of compounds including alkaloids, proteins, enzymes, organic acids, vitamins, flavonoids, alkaloids and many other phytochemicals that confer a host of different activities. Pharmacological studies have reported that *M. sativa* reduces hypocholesterolaemic, antiatherosclerotic, oestrogenic, antioxidant, antiulcer, antimicrobial and neuroprotective activities which make it beneficial in the treatment of heart disease, stroke, atherosclerosis, cancer, diabetes and menopausal symptoms in women (Khaleel, Gad, El-Maraghy, Hfnawy, Abdel-Sattar, 2005; Hong, Chao, Chen, Lin, 2009; Rana, Katbamna, Padhya, Dudhrejiya, Jivani, Sheth, 2010; Kundan & Anupam, 2011).
**Figure 3.8. Alfalfa-luserne (Medicago sativa)**

**Dandelion (Taraxacum officinale)** is a perennial weed with yellow florets, largely distributed in the warm temperate areas of the Northern Hemisphere, in fields, roadsides and railway sides (Schutz, Carle, Schieber, 2006; Wright, Van-Buren, Kroner, Koning, 2007). The leaves, roots or the whole plant are used fresh or dried for medicinal purposes and the herb is commercially available in capsules, teas and extracts. The plant parts are also used as food, i.e. leaves are used fresh in salads, or roots are roasted for coffee, or extracts are processed and used as flavour components in various food products (Schultz et al., 2006; Yarnell & Abascall, 2009; Bhat & Moskovitz, 2009; http://nccih.nih.gov/health). Ethnobotanical uses of *T. officinale* include its use as a laxative and for the treatment of diarrhoea, gout, blisters, liver and spleen complaints, kidney disease, dyspepsia, heartburn, diabetes, hepatitis, bronchitis, pneumonia, arthritis and rheumatoid arthritis. The many compounds extracted and isolated from the herb include tannins, saponins, flavonoids, terpenoids, and phenolic compounds. Pharmacological studies of these compounds as well as the extracts of the herb have confirmed the diuretic, anti-inflammatory, choleric, antioxidant, anticancer, anti-allergic, antidiabetic, analgesic, antithrombotic and hepatoprotective activities (Hu & Kitts, 2005; Schutz, Carle, Schieber, 2006; Wright, Van-Buren, Kroner, Koning, 2007; Wojdylo, Oszmianski, Czemerys, 2007; Clare, Conroy, Spelman, 2009; Yarnell & Abascall, 2009; Amin Mir, Sawhey, Jassal, 2013; Ahmad, 2014).
Ascorbic acid (Vitamin C) is well known as an immune modulator, as well as for its antioxidant activities (Mainardi, Kapoor, Bielory, 2009). It is an intracellular and extracellular scavenger of free radicals, hence plays protective role against tissue damage caused by oxygen free radicals (Stamp, James, Cleland, 2005).

3.6 The toxicity effects of the components of the commercial herbal mixtures

While the herbs in the HMs have known or purported effects, the effects of the combination of these herbs are not known. Such combinations may have diverse effects resulting from the additive or synergistic activities of the different compounds that are present. More crucial is the safety of these mixtures, as the combinations may result in toxicity due to additive undesirable effects. Factors that contribute to the potential toxicity of herbal remedies include misidentification of the plant, collection of wrong plant part, variability in the time and location of collection,
contamination during preparation and inconsistency in labelling of the final product (Whiting, Clouston, Kerlin, 2002). Poisoning from HMs may be as a result of contamination during collection and handling of the plant by microbes, heavy metals, pesticides or herbicides, mislabeling of products, improper storage of the HM and inadvertent confusion of toxic species with a non-toxic one (Ndhlala et al., 2011).

The concentration of the active ingredients and other chemicals in the plant differs according to the plant part used, the time of year when harvested, the geography and soil conditions (Woolf, 2003). HMs contain multiple active substances therefore their targets may be various multiple cellular molecules, which should be identified as molecular evidence for toxicity. The induction of toxicity of HMs is as a result of bioactivation of the herbal constituents, causing formation of metabolites, some of which may be reactive and carcinogenic (Zhou, Koh, Gao, Gong, Lee, 2004). Woolf (2003) stated that the safety of herbal remedies is closely linked to the active ingredients they have, their interaction with other herbal products or their inherent/intrinsic toxicity. It is rational to expect that while the intention is to have increased beneficial effect of the herbs, the result may also be increased unwanted or toxic effects. It is pertinent therefore to mention the known or reported unwanted or toxic effects of the ingredients in the selected herbal mixtures.

Aloe vera is known to inhibit prostaglandins and thromboxane A2 (TXA2), which may cause decreased platelet aggregation and prolonged bleeding time (Yagi, Kabash, Okamura, Haraqushi, Moustafa, Khalifa, 2002). This was suggested to be the cause of excessive intraoperative blood loss suffered by a patient given sevoflurane, who had taken aloe tablets before the surgery. Acute toxic hepatitis was reported in three patients who ingested aloe tablets (Yang, Kim, Kim, Sohn, Choi, Choi, 2010). Possible interactions with drugs may result in toxicities which may either be worsened adverse effects of the drugs or exaggerated effect of the drugs. It has been suggested that because aloe gel might lower blood glucose levels, it may increase the risk of hypoglycaemia if taken concurrently with hypoglycaemic drugs. It may also increase the risk of toxicity if used with cardia glycosides, as a result of potassium depletion if aloe is overused (Rodriguez-Fragoso et al., 2008).
**African ginger** caused DNA damage in human white blood cells. This means it may cause damage to genetic material, and hence have long-term effects in people who use the plant (Fennell et al., 2004).

**African potato** extracts were reported to be non-toxic, specifically hypoxoside (Mills et al., 2005). However the bioactive metabolite of hypoxoside, rooperol inhibited the drug metabolising enzymes cytochrome P450, particularly CYP3A4, 3A4 and C19 (Nair et al., 2007). It implies that consumption of the plant extract may result in significant inhibitory effect, which may have impact on drugs taken concomitantly. Rooperol was also reported to be cytotoxic. Chronic infusion of the plant extracts was found to impair kidney function in rats (Ncube et al., 2013).

**Devil’s claw** had no reports of toxicity, whether on acute or chronic use, or when used alone or as an adjunct. Only a few gastrointestinal disturbances were mentioned to occur in patients who were using it for back pain and osteoarthritis (Ibrahim, Al-Ashban, El-Sammani, 2010; Al-Harbi et al., 2013; Street & Prinsloo, 2013). Devil’s claw was found to have low toxicity from acute toxicity studies performed in mice. Cytotoxic effects of extracts and purified substances were reported on murine peritoneal macrophages, and decreased heart rate and arterial blood pressure in rats (Mncwangi et al., 2012).

**Sutherlandia** is apparently a safe herbal medicine as it has a long history of use with no serious side effects reported. A safety study performed with high doses on primates and a phase I clinical trial revealed no indications of toxicity (Street & Prinsloo, 2013; Van Wyk, 2008). Only one preclinical study so far, has reported the possibility of herb-drug interactions which may lead to therapeutic failure if *Sutherlandia* is co-administered with drugs that are CYP3A4 substrates (Minocha, Mandava, Kwatra, Pal, Folk, Earla, Mitra, 2011). However, one compound in *Sutherlandia*, L-canavanine has been associated with systemic lupus erythematos syndrome (Mills et al., 2005).
Wild olive leaf extract was reported to cause alterations in liver and kidney tissue of rats fed higher doses of the extract. It caused necrosis of hepatocytes and some slight haemorrhage. As a result it was suggested that care should be taken if the extract is taken for longer periods at high doses. No symptoms of toxicity were observed when high doses of the compound, maslinic acid, were administered to rats as a single dose or daily dose (Hashmi et al., 2015).

Echinacea: Animal studies on various Echinacea preparations revealed low toxicity of the herb. A few adverse effects were reported in clinical studies; such as rash, angioedema, pruritis, erythema and urticaria (Manayi, Vazirian, Saeidnia, 2015).

Medicago sativa (alfalfa) has been reported to cause a systemic lupus erythematosus-like syndrome in animal studies, and pancytopaenia in humans when ingested as ground seeds. These effects were attributed to the presence of the alkaloid, canavanine and coumistan, coumestrol. A patented preparation free of the two compounds was confirmed to be free of the reported adverse effects (Khaleel, Gad, El-Maraghy, Hifnawy, Abdel-Sattar, 2005; Kundan & Anupam, 2011).

Dandelion was found to have low toxicity due to the absence of alkaloids or other significant toxins. However the herb can cause allergic contact dermatitis due to the presence of sesquiterpenes (Schutz, Carle, Schieber, 2006). It can also cause erythema multiforme or an anaphylaxis reaction in sensitive individuals. The presence of significant amounts of potassium in the herb may cause increased risk of hyperkalaemia if it is used concomitantly with potassium sparring diuretics (Rodriguez-Fragoso et al., 2008).

Ascorbic acid (Vitamin C) is well known as an immune modulator, as well as for its antioxidant activities (Mainardi, Kapoor, Bielory, 2009). The toxicity of Vitamin C may only be evident after ingestion of more that one gram per day. Intake of high doses enhanced formation of kidney stones, water and electrolyte imbalances and red cell lysis (Dollery, 1991).
Table 3.2: The Information found on the packages and/or containers of the selected herbal mixtures.

<table>
<thead>
<tr>
<th>Herbal mixture</th>
<th>Indication/s</th>
<th>Instructions: Dosage and dosing</th>
<th>Ingredients</th>
<th>Storage instructions</th>
<th>Warnings/Contraindications</th>
<th>Warnings/Precautions/Disclaimer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intlamba zifo™ (HM1)</strong></td>
<td>Boosts the immune system, helps combat viruses and removes toxins. May assist in treatment of various ailments such as: acne, allergies, arthritis, bladder infections, candida, chemotherapy side effects, circulation, constipation, colon cleanser, lowers cholesterol, ulcers, diabetes, fluid retention, herpes, hypertension, heartburn, high blood pressure, hangovers, insomnia, kidney infection, mouth ulcers, nausea, nervous stress, rashes, sinus, tiredness. Eases inflammation, fights fungal infections and parasites.</td>
<td>Take three tablespoons (45 ml) of intlamba zifo three times a day, one hour before meals. Drink 8 glasses of clean fresh water per day.</td>
<td>Active: aloe, water, assorted herbs, H2O2, sunset yellow No 6-CI 15985</td>
<td>None.</td>
<td>Not to be taken by children and pregnant or breastfeeding women.</td>
<td>It will support your doctor’s prescription, but is not intended to replace it. Continue your prescribed medication using Intlamba zifo to support and strengthen your immune system, to give safe recovery. No medical claims are made.</td>
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<tr>
<td><strong>Maphilisa™ Herbal Medicine (HM2)</strong></td>
<td>Immune booster To boost energy, prevent sicknesses, and helps with appetite</td>
<td>Take half a glass twice a day after meals. Shake well before use. Do not use if seal is broken.</td>
<td>African potato, aloe ferox, African ginger, citric acid, Sutherlandia and wild olive. Preserved with sodium benzoate</td>
<td>Keep refrigerated once opened. Keep out of reach of children</td>
<td>None.</td>
<td>This product does not substitute normal treatment. Sufferers must seek medical advice.</td>
</tr>
<tr>
<td><strong>Matla™ African medicine for all diseases (HM3)</strong></td>
<td>Written in IsiZulu and</td>
<td>Drink ¼ cup before sleeping and after meals</td>
<td>None.</td>
<td>None.</td>
<td>Do not consume during pregnancy. Not to be used by children under the age of 14.</td>
<td>This product does not use any pharmaceutical ingredients.</td>
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<tr>
<td><strong>Ngoma™ Herbal Tonic Immune Booster (HM4)</strong></td>
<td>An immune booster; antidiabetic agent; a relief of stomach ailments; A relief for symptoms of cold, influenza, piles, inflammation, backache, arthritis, hypertension and stress; A general health booster, anti-blood pressure agent</td>
<td>Shake well before use One tablespoon (10ml) in the morning after meals and one tablespoon in the evening after meals.</td>
<td>Sutherlandia (cancer bush) Echinacea, dandelion, alfalfa-lusern, aloe ferox, harpagophyt om (devil’s claw), alcohol (13.5%)</td>
<td>Keep out of reach of children. Keep under 25°C</td>
<td>Not to be taken by children under six years of age, pregnant and breastfeeding women The use of this medication may lead to drowsiness and impaired concentration that may be aggravated by the simultaneous intake of alcohol or other medication</td>
<td></td>
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<tr>
<td><strong>Stametta™ Body Healing Liquid (HM5)</strong></td>
<td>Regular use will improve general well-being and strengthen the immune system. This product may be used to assist in the treatment of or relief from the following conditions: painful and irregular menstruation, lower back pain, joint pain, fatigue, gum disorders, sexual performance, flatulence, digestive problems, constipation, strengthening of bones and muscle tissue. 50 – 100 ml three or four times a week. Drink water after taking liquid.</td>
<td>Each 500 ml contains: aloe 1.667g, ascorbic acid1.667 g, aniseed oil 0.1817g, magnesium sulphate 71.667g. Preservative: nipastat 0.02% m/v.</td>
<td>Store in a cool, dry place below 25°C. Keep out of reach of children.</td>
<td>If any of the conditions persist for more than three weeks, consult your healthcare practitioner Not to be taken by pregnant women or children under the age of 14 years. Not to be used if suffering from low blood pressure and diarrhoea.</td>
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<tr>
<td><strong>Vuka Uphile™ Herbal Remedy (HM6)</strong></td>
<td>Diabetes, TB, kidneys, Arthritis, Back pain, Boost erection, Flu, joints and Drink a ¼ of a glass after a</td>
<td>Natural herbs</td>
<td>None</td>
<td>Bodicare products are only intended to assist towards the achievement of better health. Always consult your doctor for professional advice.</td>
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**Ngoma** is a powerful immune booster, made of a mixture of selected herbs traditionally used as a remedy against viruses, bacteria and fungus infections, also used to boost the immune system, for weight gain, appetite stimulant, to improve general health and as an anti-blood pressure agent.

**Stametta™** Body Healing Liquid (HM5) is a herbal medicine. No medical claims are made. **Ngoma** is medical aid listed.
| (written in IsiZulu also) | inflammation, Period pains, High blood, Ulcers, Digestion, Dizziness, Purifies blood, Sore bones, Eyes, Ears, Kills worms, Gives appetite, Rheumatic, Diarrhoea treatment, Sweating | meal. One in the morning and one at night. | Safe to use after three months of pregnancy |
CHAPTER 4 LITERATURE STUDY: CHEMILUMINESCENCE

4.1 Background of chemiluminescence

Luminescence is the emission of light by a molecule when it returns to its ground state from an excited state. It is also described as emission of light through a non-thermal process. Chemiluminescence (CL) is when the light emitted is due to a chemical reaction and bioluminescence describes the process when it occurs in living organisms such as fireflies, jellyfish, fungi, and marine bacteria (Campbell, Hallet, Weeks, 1985; Dodeigne, Thunus, Lejeune, 2000). Biochemiluminescence (BCL) therefore combines the emission of light by living organisms and that of a chemical reaction. It is a process in which luminescent chemicals are used to enhance the natural bioluminescence of living organisms, which allows quantification of the light energy emitted.

CL has been used in various fields including environmental chemistry, biotechnology and molecular biology to study the generation of reactive oxygen species (ROS) in different biological systems. It has been used quite notably in laboratories as a diagnostic tool, and has now become an essential tool in medical research and in routine analysis (Dodeigne et al., 2000; Roda, Pasini, Guardigli, Musian, Mirasoli, 2000). CL detection has been used in many fields and analytical systems as it is a highly sensitive system when compared with other colorimetric detection systems. It is also a more sensitive and faster technique than radioactivity-based detection systems. CL detection is based on the principle that when experimental conditions are appropriate and optimum for an analyte, the amount or intensity of light emitted is proportional to the concentration of the analyte, or is related to the activity of the analyte. This allows precise and sensitive quantitative analysis (Roda et al., 2000).

There are advantages of CL detection, which include the low consumption of expensive reagents, the high stability of reagents, the fast emission of light especially when generated in a single flash and the large linear response obtained when CL is used (Dodeigne et al., 2000). CL was reported to be a simple and fast detection method and was widely used for the detection and measurement of ROS because of its sensitivity, reliability and low cost (Caldefie-Chezet, Walrand, Moinard, Tridon, Chassagne, Vasson, 2002). BCL is suitable for direct detection of molecules present in complex biological matrix because of its high detectability when compared to other detection methods. This important property of BCL detection is due to low background which is not affected by the
light-scattering, which is a concern in absorption and fluorescence methods (Roda et al., 2000). The benefits of using CL detection include high specificity, the use of stable and non-toxic reagents, rapid measurements and the possibility to measure analytes in turbid solutions (BioOrbit, Application Note 100).

BCL has been used in immunoassays and non-immunoassays. Immunoassay applications included the determination of amongst others, total cholesterol, glucose, choline, acetylcholine and other biological parameters. It has been used for the prediction of the course of infectious diseases, rheumatoid arthritis, chronic granulomatous disease, tumour development and other diseases related to oxygen metabolites (Dodeigne et al., 2000; Roda et al., 2000; BioOrbit, Application Note 100).

Many cells in the body naturally emit light when activated, although the light intensity may be low. The light emission is associated with the so-called respiratory burst that occurs as a result of increased oxygen consumption when cells are stimulated. The light emitted can be enhanced chemically, and this enhanced CL has been used to study the activities of biological systems, such as isolated cells, subcellular components, tissue homogenates and whole organ systems. The ROS involved in CL reaction mechanisms allows the determination of antioxidant capacity of biologically active compounds in aqueous solutions or in biological fluids (Roda et al., 2000). BCL is the technique used in this investigation to study the effects of CHMs on the activity of whole blood phagocytes, neutrophils and platelets. The CL activity observed is a measure of the amount of light emitted by the cells when activated, which is a direct indication of the amount of ROS generated, which may be interpreted to the viability of the cells under various simulated conditions. Therefore the higher the CL activity, the more active the cells.

4.1.1 Selection of luminescence enhancer for the study: Luminol

Luminol was selected as the CL enhancer in this study. Luminescent substrates are chemical substances that are used in CL tests to amplify the light emitted by stimulated cells being tested. Amplification of the light allows/ improves detection. The most common substrates are luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), isoluminol (6-amino-2,3-dihydro-1,4-phthalazinedione) and lucigenin (bis-N-methylacridinium nitrate) (Nauseef, 2014). Other probes include ferricytochrome, nitroblue tetrazolium, p-
hydroxyphenylacetic acid and dichlorofluorescein (Freitas, Porto, Lima, Fernandes, 2008). Of these, luminol was found to be able to improve luminescence detection when the level of neutrophils was as low as $2 \times 10^4$ cells (Nauseef, 2014).

Luminol in water, water solvent mixtures and lower alcohols, is oxidised by oxygen derivatives like peroxides, molecular oxygen, and superoxide anion to produce light. The reaction is catalysed by enzymes such as peroxidases and other substances like metal complexes (Roda et al., 2000; Dodeigne et al., 2000).

Luminol-enhanced CL has facilitated the widespread use of CL as an indicator of ROS and lipid peroxidation in cells and whole organs (Roda et al., 2000; Dodeigne et al., 2000). It has been shown that luminol directly induces neutrophil CL (Allred, Margetts, Hill, 1980). Luminol was reported to be cell-permeable, hence it could be used for detection of oxidant production both intracellularly and extracellularly (Dahlgren, Karlson, Bylund, 2007). It also provides measurement of overall ROS production as opposed to lucigenin which measures mainly SOA production (Paula, Kabeya, Kanashiro, de Figueiredo, Azzolini, Uyemura, Lucisano-Valim, 2009). It was asserted that luminol-enhanced CL provided a simple and valuable tool for studying kinetic ROS metabolic activity in blood cells (Caldefie-Chezet et al., 2002).

4.1.2 Selection of cell activators: N-formyl-methionyl-leucyl-phenylalanine and phorbol myristate acetate

Chemotactic agonists, phorbol myristate acetate (PMA) and N-formyl-methionyl-leucyl-phenylalanine (fMLP) are activators of choice in this study. They have been used widely in chemiluminescence studies involving neutrophils, platelets and whole blood phagocytes. They stimulate the respiratory burst of blood cells, through different mechanisms, which both result in the activation of the NADPH oxidase system.

NADPH oxidase (NOX) is a complex enzyme system found in phagocytes, which serves an antimicrobial function. It is a transmembrane electron transport chain which reduces
molecular oxygen to microbicidal ROS (Bei et al., 1998; Bylund, Brown, Movitz, Dahlgren, Karlsson, 2010). The enzyme system is also present in non-phagocyte cells, in both humans and animals. It is distributed in all cell types and tissue, with many of the cells expressing more than one NOX isoform. It was reported in vascular system cells including fibroblasts, smooth muscle cells, endothelial cells (Roos, Van Bruggen, Meischl, 2003; Paravicini & Touyz, 2008; Nauseef, 2014) and platelets (Krotz, Sohn, Pohl, 2004).

The ROS include superoxide anion (O$_2^-$) (SOA), hydrogen peroxide (H$_2$O$_2$), singlet oxygen ($^1$O$_2$), hypochlorous acid (HOCl) and hydroxyl radicals (OH$^-$). The NOX-derived oxidants are involved in many biological processes and serve as important elements of signalling pathways and effector systems (Nauseef, 2014). NOX is an enzyme complex with two of its subunits forming a membrane bound heterodimer called cytochrome b (Cyt b) and others being cytosolic polypeptide units (Alba et al., 2004; Roos, et al., 2003; Bylund et al., 2010; El-Benna, My-Chan Dang, Perianin, 2010). In resting cells NOX is dormant and the cytosolic components are not associated with Cyt b. the components are distributed between the cytosol and the plasma membrane and the membranes of cellular granules. On cell stimulation the cytosolic components translocate to the membrane and associate with Cyt $b$ in the process of polymerisation of NOX, and form functional NOX (Caldefie-Chezet et al., 2002; Bylund et al., 2010; El-Benna et al., 2010).

Once cell activation occurs by phosphorylation, NOX transfers two electrons from NADPH to molecular oxygen, resulting in the formation of the oxygen radical, the SOA, O$_2^-$ as outlined in Figure 4.1. This oxygen radical further dismutates to hydrogen peroxide, H$_2$O$_2$. Both O$_2^-$ and H$_2$O$_2$ lead to the formation of highly reactive hydroxyl radicals, OH or HOCl. These can further generate the singlet oxygen. The increased oxygen consumption connected with the whole reaction is termed the respiratory or metabolic burst. It is coupled by emission of light, termed native luminescence, and this is the light which can be enhanced by addition of CL substrates such as luminol, isoluminol or lucigenin, and be detected and measured by a CL detector called a luminometer (Parij et al., 1998, Alba et al., 2004, Campbell et al., 1985; Bylund et al., 2010).
Activation of NOX in phagocytes can be induced by particulate and soluble agents such as latex particles, opsonized bacteria, opsonized zymosan, formylated peptides such as fMLP, and by pharmacological compounds that include calcium ionophores and PKC activators such as phorbol esters like PMA (Roos et al., 2003; El-Benna et al., 2010). The two activators fMLP and PMA have been used extensively in *in vitro* studies, for their mechanism of stimulation of cells. They act on the same pathway of signalling, the phospholipase pathway by different mechanisms.

The agonist, fMLP binds to specific formyl peptide receptors on the cell membrane, which are G-protein-linked. The G-protein is Gi2 protein and it is a guanine triphosphate (GTP) - binding protein. The binding then initiates a cascade of reactions that begin with the activation of phospholipase C (PLC), a phosphatidylinositol specific membrane bound enzyme. PLC then splits phosphatidylinositol biphosphate (PIP2) into inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) (Bianca, et al., 1986, Christiansen, 1988, Hu, et al., 1999). DAG confined to the membrane activates the enzyme protein kinase C (PKC). PKC is responsible for the phosphorylation and activation of NOX, leading to the production of ROS and the respiratory burst of cells. IP3 stimulates the release of calcium ions (Ca^{2+}) from intracellular stores causing a transient increase in cytosolic free Ca^{2+}, which was reported to be important in the regulation and onset of the respiratory burst (Bei et al., 1998; Hu et al., 1999).
PMA, a soluble synthetic analogue of DAG stimulates neutrophils via a mechanism different to fMLP and other chemotactic agonists. It lacks specific cell surface receptors on neutrophils. It directly activates PKC resulting in activation of NADPH oxidase (Bei et al., 1998; Hu et al., 1999; Geiszt et al., 1999; Campbell et al., 1985). It was reported that the activation of PKC was the first step in governing the onset of the respiratory burst (Hu et al., 1999).

PMA and fMLP have been used extensively in both in vitro and in vivo animal studies. The two are used together in studies to contrast the receptor/G-protein-mediated and non-receptor/G-protein-mediated signalling (Bei et al., 1998, Hu et al., 1999, Geiszt et al., 1999, Campbell et al., 1985, Kahler, 2000; Mothibe, 2007, Paula et al., 2009). It has been stated that fMLP is a powerful activator of both neutrophils and macrophages (Corteling, Wyss, Trifilieff, 2002). The use of fMLP and PMA in this study will allow differentiation of the mechanism of the effects of various CHMs on cells, whether direct or indirect, as well as show if the onset of the effects differs.

4.2 The role of neutrophils in immunity and bioluminescence

Neutrophils are key components of the innate immune system and the inflammatory response. They form the largest components of leucocytes and are part of the first line of defense against invading microorganisms. They generally circulate freely and passively in blood and are recruited into inflamed tissue to assist in the removal of pathogens and other inflammatory agents (Bei et al., 1998; Freitas et al., 2008; Cowburn, Condliffe, Farahi, Sumers, Chilvers, 2008). They display features that are crucial in immunity including the ability to adhere to endothelial cells, to migrate to sites of inflammation through vessel walls, to recognize and phagocytose opsonized molecules and to produce and release degradative proteins (Faurschou & Borregaard, 2003; Panasiuk, Wysocka, Maciorkowska, Panasiuk, Prokopowicz, Zak, Radomsji, 2005).

The role of neutrophils as effector cells is achieved by phagocytosing pathogens and destroying them using ROS generated by NOX and hydrolytic granule proteins (Dahlgren
and Karlsson, 1999; Faurschou and Borregaard, 2003; Roos et al., 2003; Bylund et al., 2010, Porter & Kaplan, 2011). When neutrophils are stimulated, either by physiological stimulants or in vitro by chemoattractants such as fMLP or PMA, there is increased oxygen consumption through the activity of NOX. The phospholipase pathway plays a critical role in regulating human neutrophil responses such as chemotaxis, adhesion, phagocytosis, secretion and SOP by the NADPH oxidase complex (Burelout et al., 2004). The action of NOX generates ROS including SOA, H₂O₂, and the more reactive and strongly antimicrobial singlet oxygen, HOCl and OH⁻. The increased oxygen consumption is termed the respiratory burst and it plays a vital role in microbial killing (Dahlgren & Karlsson, 1999; Bjorkman, Dahlgren, Karlsson, Brown, Bylund, 2008; Freitas et al., 2008; Bylund et al, 2010; Jancinova, Perecko, Nosal, Harmatha, Drabikova, 2012).

4.2.1 Significance of NADPH oxidase and the respiratory burst

The effectiveness of neutrophils as phagocytes is determined by a functional NOX system which would generate the potent antimicrobial ROS. In addition to the important role of defense against pathogens, ROS have been said to be a threat to bystander host cells and tissues in the vicinity of the inflammatory reaction, and were implicated in inflammatory tissue damage. The oxygen metabolites may cause damage by destroying surrounding tissue and inducing apoptosis in other immune reactive cells (Dahlgren & Karlsson, 1999; Droge, 2002; Bjorkman et al., 2008; Tintinger, Steel, Theron, Anderson, 2008). Parij et al. (1998) reported that the ROS and granule enzymes contribute to the pathogenesis of immune and non-immune chronic inflammatory conditions, by causing damage to molecules, cells and tissues that are important in inflammation. Conditions that are accompanied by tissue damage caused by neutrophil-derived products include asthma, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis (RA), gout, autoimmune vasculitis and types of glomerulonephritis (Roos et al., 2003; Ciz, Denev, Kratchanova, Vasiczek, Ambrozova, Lojek, 2012).

The involvement of NOX in calcium availability intra and extracellularly has led to findings that NOX mediated the neutrophil membrane depolarisation as well as modulation of the rate of repolarisation. The enzyme also serves to protect the cell from cytosol Ca²⁺ flooding. This important role was seen in patients who do not have a functional NOX and do not produce or produce subnormal levels of ROS as in chronic granulomatous disease.
(CGD). The patients are predisposed to proinflammatory activity which includes the increased release of proteolytic enzymes and cytokines, leading to increased frequency of inflammatory disorders (Tintinger et al., 2008; Bylund et al., 2010). Therefore activation of NOX may also reveal unforeseen anti-inflammatory properties (Tintinger et al., 2008). Normal NOX-derived ROS production appears to be important in keeping inflammatory diseases under control (Bjorkman et al., 2008; Bylund et al., 2010).

Neutrophils (PMNs) have been studied extensively, using BCL. Studies on leucocytes have allowed a better understanding of inflammatory processes and diseases related to oxygen metabolites; immune based diseases; the influence of drugs on neutrophil metabolism, the process of phagocytosis, pathological conditions such as rheumatoid arthritis, infectious diseases, CGD and tumour development (Carulli, Minucci, Angiolini, Azzara, Ambrogi, 1995; Roda et al., 2000; Dodeigne et al., 2000). It has been shown that CL measurement and analysis of phagocyte function may be useful in the diagnosis and staging of infection. The circulating phagocyte function correlated with clinical condition, the severity and the outcome of acute infections (Stevens, Bryant, Huffman, Thompson, Allen, 1994). It was shown by studies of CGD phagocytes, that phagocytes that lack a functional NOX are hyperinflammatory (Bylund et al., 2010). It was also noted that inappropriate activation of neutrophils contributed to the development and progress of many acute and chronic inflammatory disorders of infective and non-infective origin. The airways and the cardiovascular system (CVS) were reported to be particularly vulnerable and some of the conditions include asthma, RA, atherosclerosis and COPD (Tintinger et al., 2008). Other conditions in which the extensive release of intracellular oxidising agents by phagocytes was implicated were stroke, myocardial infarction and respiratory disease syndrome (Caldefie-Chezet et al., 2002).

4.2.2 Studies of effects of herbal medicines on neutrophils and phagocytes

Several studies have been done to investigate the effects of HMs on neutrophils and phagocytes using luminol-enhanced CL (LECL). Extracts of plants traditionally used in Malaysia for treatment of microbial infections, fever, allergies and other inflammatory conditions were tested for their effects on ROS production of PMNs. A few of the extracts had high inhibitory activity (Jantam, Harun, Septama, Murad, Mesaik, 2011). The extract of
the tamarind fruit pulp, traditionally used in some Asian and American countries was found to have a higher inhibitory effect on neutrophil NOX activated by PMA than that activated with fMLP (Paula et al., 2009). Various extracts of *Tinospora cordifolia*, a plant used in Indian herbal preparations for different ailments, significantly increased the phagocytic function of human neutrophils. The activity of the extracts was confirmed by observing increased ROS generation of the cells after incubation with the extracts, via a LECL assay (Sharma, Bala, Kumar, Singh, Munshi, Bhalerao, 2012).

Selected Sudanese medicinal plants commonly used against infectious diseases were said to have in vitro immunomodulatory activity after being tested on PMNs. The extracts had variable inhibitory activity on the PMA-induced oxidative burst of the PMNs, from potent, moderate to weak activity (Koko, Mesaik, Yousaf, Galal, Choudhary, 2008). Some medicinal plants used as TM in Mauritius were also found to have significant inhibitory activity on the ROS generation of human neutrophils when tested using a LECL assay on PMA-activated PMNs (Mahomoodally, Mesaik, Choudhary, Subratty, Gurib-Fakim, 2012).

### 4.3 The role of platelets in immunity

Platelets are anucleate cells derived from megakaryocytes and are well known for their important role in haemostasis. They are involved in initiation of the coagulation process by thrombus formation and regulate and maintain vessel wall integrity. They generally circulate in inactive state and are activated when a blood vessel is damaged (Krotz et al., 2004; Ghoshal & Bhattacharyya, 2014; Trzeciak-Rycek, Torkaz-Deptula, Deptula, 2013). Their involvement in hemostasis includes activation, adhesion to subendothelium, aggregation, further platelet recruitment and the resultant thrombus formation (Drabikova, Jancinova, Nosal, Danihelova, 2000; Krotz, et al., 2004; Thijs, Nuyttens, Deckmyn, Broos, 2010). The platelet plasma membrane is the site where many different surface receptors are expressed, and they aid in cell signaling and intracellular trafficking. They also contain granules that store biologically active molecules that function in coagulation and inflammation.

On activation, the surface receptors stimulate the release of α granules that play various roles in addition to haemostasis, including atherosclerosis, angiogenesis, wound repair, antimicrobial host defence and inflammation (Ghoshal & Bhattacharyya, 2014). The many
surface receptors allow interaction with various ligands that are important in haemostasis. These include collagen receptors – integrin α2β1 and glycoprotein VI (GP VI); TP receptor and G-protein coupled receptors which include the serotonin receptor, the platelet activating factor (PAF) receptor and the V1 receptor for vasopressin. Activation of platelets and thrombin generation through the clotting cascade are the key elements of haemostasis and lead to fibrin formation and coagulation (Thisj et al., 2010; Ghoshal & Bhattacharyya, 2014). The chemical molecules secreted from granules for haemostasis include adenosine diphosphate (ADP), ATP, calcium, serotonin and catecholamines (Ghoshal & Bhattacharyya, 2014).

Platelets play a role in the inflammatory response and the immune system. This role is enabled by the range of surface receptors that allow platelets to be effector cells in the inflammatory response, and important elements of the immunological response, both directly and indirectly. Platelets release cytokines whose role in immunological response is to mobilise and direct leucocytes to the inflammation site, and affect other immune cells outside of the circulatory system (Li, Hu, Lindqvist, Wikström-Jonsson, Goodall, Hjemdahl, 2000; Peters, Heyderman, Hatch, Klein, 1997; Ghoshal & Bhattacharyya, 2014; Trzeciak-Rycek et al., 2013). Upon activation, platelets secrete a range of chemical mediators which promote the recruitment of neutrophils to inflammatory tissue (Zarbock, Polanowska-Grabowska, Ley, 2007; Semple & Freedman, 2010).

The platelet effect on immune cells during the inflammation leads to formation of aggregates with neutrophils, monocytes and lymphocytes. Platelets also increase the activity of triggering receptors expressed on neutrophils and monocytes, and contribute to the acceleration of chemokine synthesis in monocytes. They secrete substances that are chemoattractants which activate lymphocytes such as T helper cell, cytotoxic T cells, B cells and natural killer (NK) cells. These activities involve the surface receptors such as CD40, P-selectin and many others to which various ligands bind (Li, et al., 2000; Ghoshal & Bhattacharyya, 2014; Trzeciak-Rycek et al., 2014). CD40 plays a role, amongst others, in promoting influx of leucocytes to the inflammation site and enhances the cytolytic activity lymphocytes. P-selectin was said to recruit neutrophils (Ghoshal & Bhattacharyya, 2014); and enhance the interaction between platelets and neutrophils and endothelial cells (Li et al., 2000). The most important receptors for the immunological response are the Toll-like receptors (TLRs) which play a role in activation of the immune system cells,
determining the specific and non-specific responses (Semple & Freedman, 2010; Trzeciak-Rycek et al., 2013).

In addition to their indirect role in immunity, by involvement of immune cells, platelets are capable of directly destroying pathogens by secreting substances that destroy and damage bacteria. They can also detect and surround bacteria by adhesion, which helps to reduce the bacteria from circulation and therefore prevents infection spreading. Activated platelets release antibacterial platelet microbicidal proteins (PMP) from α granules, that damage the bacterial membranes by increasing their permeability and depolarisation (Trzeciak-Rycek et al., 2013). TXA2 was reported to be involved in the mechanism that destroys Toxoplasma gondii (Yong, Chi, Fritsche, Henderson, 1991). Some of the substances released by activated platelets include ROS (Krotz et al., 2004). Figure 4.2 shows the many functions of platelets and roles in relevant pathologies.

Figure 4.2. The multifunctional platelet (adapted from Goshal & Battacharyya, 2014).
4.3.1 The role of reactive oxygen species and NADPH oxidase in platelets and chemiluminescence

It has been reported that platelets generate ROS via several enzymatic sources. The sources include platelet NOX, xanthine oxidase, COX-1 and during mitochondrial respiration. The reported ROS include $O_2^-$, $H_2O_2$, and $OH^-$ (Krotz et al., 2004; Nauseef, 2014). Other pathways that generate ROS include during nitric oxide synthesis, the glutathione cycle, initiating metabolism of arachidonic acid and phosphoinositol (Miedzobrodzki, Panz, Plonka, Zajac, Dracz, Pytel, Mateuszuk, Chlopicki, 2008). The platelet NOX was reported to be similar to the phagocyte NOX, and was found to generate $O_2^-$ on stimulation, and could be stimulated by agonists that induce platelet activation. Direct activation of PKC on platelets resulted in increased SOA release, whereas the inhibition caused reduced production of SOA. Platelet-derived ROS release was inhibited by hyperpolarisation of the platelet membrane (Krotz et al., 2004).

Different types of in vitro tests may be done to measure platelet activity and the influence of various mediators on the activity. Some of the tests include platelet aggregometry, radio-labelled fibrinogen binding assays, platelet density estimations and the measurement of soluble release products such as TXA2 and flow cytometry (Peters et al., 1997). CL studies of platelets have been performed to show the activity of platelets within the immune system and the inflammatory response and the role of platelets in various CVS disorders. The studies were also performed to show the interaction of platelets with other immune cells, especially neutrophils. Platelets were found to emit a burst of CL on stimulation by several mediators including arachidonic acid, linoleic acid and a few polyunsaturated fatty acids (Mills, Gerrard, Filipovich, White, Quie, 1978). It was stated that the NOX-dependent production of $O_2^-$ increased the recruitment of neutrophils to a growing thrombus (Krotz et al., 2004).

It was shown that platelets in the proximity of activated neutrophils reduce the neutrophil CL (Drabikova et al., 2000; Jancinova, Drabikova, Nosal, Petrikova, Ciz, Lojek, Danihelova, 2003; Jancinova, Drabikova, Petrikova, Nosal, 2004). This effect was postulated to be a protective mechanism by platelets, which may be active only in cases of emergency and at sites that are exposed to toxic effects of ROS (Jancinova et al., 2004). It was proven, in vitro, that platelets enhanced the CL response of neutrophils that were activated by opsonized and non-opsonised bacteria, specifically Escherichia coli and...
Staphylococcus aureus (Miedzobrodski et al., 2008). This effect concurred with the report that the interaction of platelets and neutrophils in the presence of bacterial lipopolysaccharides was such that neutrophil activation was increased, resulting in increased production of ROS by neutrophils (Trzeciak-Rycek et al., 2013). It also corroborates the view that platelet-neutrophil complexes perform phagocytosis, cytotoxicity and cytolysis more effectively than neutrophils on their own (Semple, Italiano, Freedman, 2011). Platelet-neutrophil (P-N) interactions have been suggested to have a role in diseases such as acute respiratory distress syndrome, myocardial ischaemia and atherosclerosis (Ruf, Schlenk, Maras, Morgenstern, Petscheke, 1992). An increase in P-N conjugates was suggested to be one of the factors causing more severe cardiovascular disease in diabetic patients (Tuttle, Davis-Gorman, Goldman, Copeland, McDonagh, 2003).

Although many studies on Western herbal medicines have been reported on their antiplatelet effects, studies of ATM effects on platelets could not be found. Herbal medicines such as garlic, ginger, ginkgo biloba, ginseng and feverfew have been reported to have antiplatelet effects, which may cause prolonged bleeding time and interactions with anticoagulant therapy (Owen, 2005; Samuels, 2005). However there are several studies done which show plant compounds or TM to have anti-inflammatory activity by inhibition of cyclooxygenase (Fennel et al., 2004). These indicate that there could be possible effects on platelets or platelet function.

4.4 Chemiluminescence of whole blood

Whole blood CL is the technique in which the whole blood sample is tested, as opposed to isolation of the various cellular components. It measures the CL activity of phagocytes in their natural matrix, plasma as well as in the presence of red blood cells. Generally, neutrophils form the largest fraction of all white blood cells. Although all the phagocytes are present in the sample, the CL signal obtained in whole blood is a function of the quantity and activity of neutrophils (Ristola & Repo, 1989). The activity of other cells-lymphocytes, monocytes and platelets was found to be non-significant and their contribution towards the CL signal obtained was considered to be negligible (DeSole, 1989). It was suggested that the CL responses of whole blood were not just a function of the total number of neutrophils, but was also indicative of their metabolic activity...
Various whole blood CL studies were carried out, to investigate the effects of drugs as well as plant compounds (Nosal, Drabikova, Jancinova, Perecko, Ambrozova, Ciz, Lojek, Pekarova, Smidrkal, Harmatha, 2014; Nosal, Drabikova, Jancinova, Macickova, Pecivova, Perecko, Harmatha, Smidrkal, 2015).
CHAPTER 5 LITERATURE STUDY: TESTING FOR CROSS-REACTIVITY OF HERBAL MEDICINES WITH COMMONLY TESTED SUBSTANCES OF ABUSE IN URINE

5.1 Background

The use of herbal medicines or TM is common, and the reasons for this usage are diverse. As stated in Chapter 1, an estimated 80% of Africans use ATM (Helwig, 2005) and the use of ATM by the general public has been reported (Kgoatla, 1994; Peltzer, 2009). It was also documented most people consult a traditional health practitioner before a primary health practitioner (Truter, 2007; Peltzer, 2009); and that they do not disclose this fact during consultation with a healthcare provider (De Villiers and Ledwaba, 2003; Ernst, 2000). The use of CHMs with modern packaging and marketing practices was reported to be widespread in South Africa (Ndhlala & Van Staden, 2012).

Since the medicines have not been tested for safety, efficacy and interactions, and have not been validated, the effect of their presence on laboratory diagnostic tests is unknown. There are no reports in literature, showing such tests for herbal medicines or TM. There has been a concern about the possible unwarranted benefit that may be there, in sports people who use ATM, as there are no specific tests to determine their presence. During the 2010 FIFA world cup hosted in SA, such a concern was raised, that the use of ATM extracted from local plants may grant unfair advantage to soccer players who used them as they may contain untraceable/ undetectable amounts of stimulants (Stafford, 2010; Harris 2010). The officials were concerned that some herbal materials used may produce compounds that may have been undetectable by the laboratory tests available at the time while other products of the medicinal plants used may have not been included in the list of banned substances of the World Anti-Doping Agency (WADA). The only plants or plant-related compounds present on the official list of WADA are cannabis, cocaine and ephedra and they are listed as stimulants (Stafford, 2010; http://list.wada-ama.org). There are no listed compounds related to ATM.

However, there could be more issues of concern regarding the use of herbal medicines by sports people. The herbal medicines may have properties similar to some banned substances, hence when tested, a sports person could test positive for banned substances unknowingly. This could have serious repercussions as testing positive for banned substance constitutes an offence which may result in the offender being banned from participation in the sporting activity for some time. There are several common substances...
that are known for causing false-positive results on urine screening tests for drugs of abuse. These include the Vicks® inhaler testing positive for amphetamine, NSAIDS such as ibuprofen and naproxen testing positive for barbiturates and cannabinoids and some fluoroquinolones testing positive for opiates (Baden, Horowitz, Jacoby, Eliopoulos, 2001; Vincent, Zebelman, Goodwin, 2006; Reisfield, Goldberger, Bertholf, 2009). That is why it is recommended and it is standard practice, that a positive urine drug screen should be confirmed by a more specific method such as gas chromatography and mass spectrometry (GC-MS) (Eskridge, Guthrie, 1997; Vincent et al., 2006).

Another aspect could be the intentional adulteration of samples to influence test results. Adulteration is a process of deliberate interference with the process of specimen collection, transport or analysis, with the intention to avoid legitimate test results. In vivo adulteration is the intake of interfering substances such as drugs, or lots of water before sample collection. In vitro adulteration is when substances are added into the urine sample after collection, to alter the results either to cause a false negative. Some of the substances include drug-free human, animal or synthetic urine or assay interferants (Reisfield, Salazar, Bertholf, 2007; Laurens, www.be.up.ac.za/forensicanalysis).

5.2 Introduction to urinalysis

Generally, urine is tested routinely for substances of abuse in health facilities to aid the diagnosis of potential abusers and some psychiatry patients on admission. Qualitative screening tests are used initially to test for the presence of the drugs of abuse (DoA). The substances tested for include commonly amphetamine, cocaine, morphine, methamphetamine, tetrahydrocannabinol (THC) and methylenedioxyamphetamine (MDMA).

The other important testing is for banned substances in special populations such as those whose work environment requires that they be drug-free; and for people involved in professional sport. With such testing, there is always concern about possible use of substances to adulterate the samples being submitted for testing. Urine samples have been tested for various possible chemicals that may be used for adulteration. Among the common household chemicals tested by Uebel and Wium (2002), it was found that gluteraldehyde (G-cide) and Perle hand soap may cause false-negative results for
methaqualone, and chloroxylenol (Dettol®) may cause false-negative results for the cannabis test. Addition of other chemicals like ethanol caused invalid results.

Rapid urinalysis assay which is based on an immunoassay method, is the routine initial screening test commonly used for substances of abuse. It is based on the principle of antigen-antibody reaction in which the drug or drug metabolite present in urine will interact with a labelled polyclonal or monoclonal antibody provided (Kapur, 2001; Moeller, Lee, Kissack, 2008). The results of this assay are generally used to indicate whether further testing is required. Testing urine for DoA generally follows the protocol indicated in the flow diagram in Figure 5.1.

![Flow diagram for urine testing for substances of abuse](image)

Figure 5.1. Flow diagram for urine testing for substances of abuse

Once a sample tests positive after screening, a confirmation test is performed, involving more sensitive, specific and accurate techniques. These would detect the presence of the tested substance as well as provide the quantity in the sample. GC-MS is regarded as the gold standard for confirmation testing as the method is specific, can detect many types of drugs and is highly sensitive, i.e. it can detect very low levels of drugs present (Kapur, 2001; Moeller et al., 2008, Reisfield et al., 2009). However, there are many substances
that may interfere with the screening test, and result in false-negative or false-positive results.

False-negative is when the method of testing tells that the drug is not present, when it is actually present. Such results may occur when the drug is present in the sample but the detection limit of the method is too high, or the actual concentration of the drug in the sample is very low (Kapur, 2001). False-negative results may also be caused by cross-reactivity of the antibody in the assay, the time lapse between drug ingestion and specimen collection and the cutoff concentration for positive results (Saitman, Park, Fitzgerald, 2014). Although false-negative results are rare; there are substances whose presence in the sample has caused this negative interference on urine immunoassays. Tolmetin registered false-negative results for opiates, amphetamines and THC, while salicylates caused false-negative result for cocaine, on the enzyme-multiplied immunoassay technique (Reisfield et al, 2009).

False-negative results may also occur as a result of adulteration of the sample. A study by Uebel and Wium (2002) investigated the effect of common household products on urine tested for drugs of abuse. Some of the chemicals influenced the positive test, giving false-negative results while others gave invalid results. Another study investigating 16 chemicals commonly used as adulterants revealed that the cannabinoid test was the most adulterated test, with six chemicals causing false-negative results for cannabinoids (Schwarzhoff & Cody, 1993).

False-positive is when the drug is absent in the sample but the method reports otherwise. The results occur when a substance present in the urine sample interferes with the tested substance and cross-reacts with the test reagents (Kapur, 2001). Several substances known for their interference with immunoassays which result in false-positive results are presented in table 5.1.
### Table 5.1. A list of substances that cause false-positive results on immunoassays

<table>
<thead>
<tr>
<th>Drug tested</th>
<th>Interfering substance causing false-positive results on immunoassays</th>
</tr>
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<tbody>
<tr>
<td>Opiates</td>
<td>Rifampicin, quinolones, ofloxacin, levofloxacin, pentazocine, tramadol, poppy seeds</td>
</tr>
<tr>
<td>THC</td>
<td>Ibuprofen, naproxen, pantoprazole, efavirenz,</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>Ephedrine, phentermine, tyramine, trazodone, bupropion, ciprofloxacin, mefenamic acid, metronidazole, tolmetin, chlorpromazine, promethazine</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Indomethacin, fenoprofen, ketoprofen, tolmetin, sertraline, oxaprozin,</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Coca tea</td>
</tr>
</tbody>
</table>

(Adapted from Baden et al., 2001; Vincent et al., 2006; Moeller et al., 2008; Reisfield et al., 2007; Reisfield et al., 2009, Saitman et al., 2014).

Cross-reactivity is defined as a phenomenon that is associated with immunoassay-based screening tests, wherein an antibody for a drug has variable degrees of reactivity towards the drug and/or its metabolite with similar or unrelated chemical structures (Reisfield et al., 2009). Cross-reactivity occurs with immunoassay based screening tests because they are generally designed to detect a class of drugs as opposed to a specific agent. Hence their specificity for individual drugs is poor (Kapur, 2001). However the immunoassay based urine screening tests are commonly used as they are easy to use and automate, offer rapid results and are relatively largely inexpensive (Reisfield et al., 2007; Saitman et al., 2014). Urine is usually the preferred sample for analysis amongst biological samples, because of ease of collection which is non-invasive; also because drugs and metabolite concentrations are higher than in plasma, which allows a reasonably longer detection time after ingestion (Eskridge & Guthrie, 1997; Moeller et al., 2008).

### 5.3 Cross-reactivity of herbal medicines with immunoassay screening tests

The use of CAM, similarly with conventional medicines, may affect laboratory test results. It has been reported that herbal medicines may cause altered results due to contamination of the herbal product with orthodox medicines, and or interfere directly with laboratory assays. Commonly, direct intereference with immunoassays occurs as a result of cross-reactivity of the components of the herbal medicine with the test reagents (Dasgupta & Bernard, 2006). A few herbal products, mainly Chinese herbal medicines are known to interfere with the immunoassays that are used for monitoring the levels of therapeutic
drugs. The medicines, *Chan Su* and *Dan Shen* caused altered serum digoxin concentration when the fluorescence polarization immunoassay (FPIA) and the microparticle enzyme immunoassay (MEIA) were used. They caused elevated and lowered serum digoxin levels on FPIA and MEIA respectively. These effects were due to the structural similarities that the components of the herbal medicines have with digoxin. Siberian and Asian ginseng also had moderate but similar interference with serum digoxin concentration measurements using FPIA and MEIA (Dasgupta, 2003; Dasgupta & Bernard, 2006).

A toxicology screening test for a patient who had taken *Ma huang*, a Chinese herbal medicine tested positive for amphetamines. This was confirmed to be a cross-reaction of the ephedrine contained in the product, causing false-positive results (Haller & Benowitz, 2000). Moderate consumption of poppy seeds has been reported to cause false-positive results for opiates and a positive confirmatory test for codeine and morphine, while coca leaf tea reported false positive results for both the screening and the confirmatory test for cocaine (Reisfield et al., 2007).

A literature search provided no results for cross reactivity of ATMs, or the influence of ATMs on laboratory tests. Nonetheless, the use of ATMs is well documented including the non-disclosure behaviour of the users thereof. Therefore this study sought to establish if any of the commonly used CHMs may affect the outcomes of a qualitative test for substances of abuse. It was intended to show whether the presence of the CHMs would alter the results of a rapid urinalysis assay. Dasgupta (2003) acknowledged that abnormal laboratory results may be found in otherwise healthy people, as a result of the use of herbal medicines, and that these abnormal results are the evidence of the toxicity of the herbs. Hence the outcomes of this investigation may help in initiating research in that direction, specifically for African traditional medicines.
CHAPTER 6 LITERATURE STUDY: AFRICAN TRADITIONAL MEDICINES IN ASTHMA, HYPERTENSION AND DIABETES MELLITUS TYPE II

This chapter details the use of ATM in the three conditions, asthma, diabetes mellitus and hypertension; including the roles of neutrophils and platelets in these conditions.

6.1 The use of traditional medicine in asthma

Asthma is a respiratory system disease, characterised by diffuse airway inflammation. It can be caused by various triggering stimuli, resulting in complete or partial reversible bronchoconstriction. The symptoms that mainly denote the narrowed airways include wheezing, cough, chest tightness and dyspnoea (shortness of breath). The pathophysiology of asthma includes bronchoconstriction, airway oedema, inflammation, hyperreactivity and remodelling (Dipiro, Talbert, Yee, Matzke, Wells, Posey, 2008; Porter & Kaplan, 2011). The various agents and events that may trigger asthma include respiratory infections such as influenza; allergens such as fungal spores and dust mites; environmental conditions such as cold air and smoke; emotions like anxiety and laughter; exercise; drugs such as aspirin and other NSAIDS; and occupational stimuli such as flour for bakers and hay mould for farmers (Dipiro et al., 2008).

Asthma is a chronic condition of the modern world, affecting millions of adults and children worldwide. WHO estimated that about 235 million people worldwide suffer from asthma (WHO, 2011). Its prevalence was increasing (Porter & Kaplan, 2011; Li & Brown, 2009) and there was evidence that the incidence and severity of the condition was also increasing (Huntley & Ernst, 2000; Porter & Kaplan, 2011). However the mortality was reported to be decreasing and prognosis was good if there was access and adherence to treatment (Porter & Kaplan, 2011). The prevalence of asthma in Africa was found to be increasing in the decades 1990-2010, and it was also suggested that the findings could have been an underestimate (Adeloye, Chan, Rudan, Campbell, 2013). Its prevalence in SA was estimated to be 6-10% in adults and was rising, and SA was ranked to be the fourth or fifth highest country in the world on asthma death rate (Zar & Laloo, 2013).

The management of asthma includes classes of drugs for treatment of chronic asthma and its exacerbations, and for maintenance to prevent attacks. The classes which are either controllers or relievers include bronchodilators, corticosteroids, mast cell stabilisers,
leukotriene modifiers and methylxanthines. The mechanisms of action of β2 agonists as bronchodilators are to relax bronchial smooth, inhibit microvascular leakage into the airways, increase mucociliary clearance and decrease mast cell degranulation and histamine release. Anticholinergics and methylxanthines relax bronchial smooth muscle. Corticosteroids inhibit airway inflammation and cytokine production and the activation of adhesion proteins. Mast cell stabilisers reduce the release of histamine from mast cells and they lessen the hyperreactivity of the airway. Leukotriene modifiers exert anti-inflammatory and bronchodilator effects by blocking the effects of leukotrienes in the airways (Dipiro et al., 2008; Porter & Kaplan, 2011, Brenner & Stevens, 2012; Rossiter, 2012).

There is no complete cure for asthma. Chronic asthma is clinically described as a disease of exacerbation and remission in which the patient may have no signs or symptoms at the time of examination, but may complain of episodes of dyspnoea, chest tightness, coughing and wheezing (Dipiro et al., 2008). The fact that sufferers have to take drugs for life is driving the search for alternative therapies for management of the condition. Furthermore, some of the pharmacological agents used are associated with adverse effects (Li & Brown, 2009). There is more evidence of the use of CAM in managing asthma worldwide. CAM was reported to be used by 38% of adult asthmatics in the USA (Barnes et al., 2004), by 60 to 70% of asthmatics in Australia (McLenna, Wilson, Taylor, 1996), 61% of adult asthmatics in primary health care clinics in Malaysia (Alshagga, Al-Dubai, Faiq, Yusuf, 2011), asthmatic patients attending a speciality care facility in Trinidad (Clement, Williams, Arenda, Chase, Watson, Mohammed, Stubbs, Williamson, 2005) and by 50,5% of adult Nigerians visiting a specialist asthma clinic (Adeyeye, Onadeko, Ogunleye, Bamisile, Olubusi, 2011).

Herbal preparations were reported to be one of the highest used CAM modalities. Traditional herbal medicine (Chinese, Japanese, Kampo, Ayurveda) have been used for asthma with some of them having shown some clinical effect (Huntley & Ernst, 2000; Ziment & Tashkin, 2000; Clement et al., 2005; Passalacqua, Bousquet, Carlsen, Kemp, Lockey, Niggemann, Pawankar, Price, Bousquet, 2006; Li & Brown, 2009; Alshagga et al., 2011). The use of ATM for asthma includes several plant species, either singly or in combination. Local herbs were the most used CAM by adult Nigerian asthmatics in an urban tertiary health centre (Adeyeye et al., 2011) and *Garnicia kola* (bitter kola) is one of the plants used in Nigeria as ATM for treatment of asthma, among its other medicinal uses.

It is therefore reasonable to expect that asthmatics would also use CHMs, in the same way that other sufferers of chronic conditions do.

### 6.1.1 The role of neutrophils and other phagocytes in asthma

It has been reported that all cells of the airways are involved and become activated in airways inflammation in asthma. These include eosinophils, mast cells, macrophages, T-cells and other cells (Dipiro et al., 2008). However, eosinophil infiltration of the airways is an important feature of patients with asthma. They are recruited into lung tissue by activated platelets in the late phase of asthma. The eosinophils release inflammatory mediators that cause lung tissue inflammation resulting in the known symptoms of asthma—coughing and wheezing. Although eosinophils are important, neutrophils are reported to be the first cells that enter the airways in response to the presence of an allergen and they release chemical mediators which may prolong asthma symptoms (Vickers, 2014; Macdowell & Peters, 2007; Monteseirin, 2009).

Neutrophils have been shown to contribute to the pulmonary allergic inflammatory process and exacerbation of asthma (MacDowell, Peters, 2007; Tintinger, Steel, Theron, Anderson, 2008). They are present in the airways of asthma patients, and in asthma exacerbations without infections (Cowburn et al., 2008; Monteseirin, 2009). They were found in high numbers in the airways of patients who had severe disease and in those who died due to sudden-onset fatal asthma (Dipiro et al., 2008). Activated neutrophils release inflammatory mediators and proteases that contribute towards the airway inflammation, airway remodelling and the bronchial hyperresponsiveness that occur in asthma. They are
thus capable of causing tissue damage by secretion of metalloproteinases, elastases, lactoferrin, myeloperoxidases and ROS. The oxidants may interact with the proteases to increase the tissue damage in asthma. Oxygen radicals were found in higher amounts in asthma patients with worsening symptoms (Cowburn et al., 2008; Dipiro et al., 2008; Monteseirin, 2009).

It has been shown by CL technique, that isolated neutrophils of asthma patients have increased capacity to produce ROS. It was also shown that there was correlation between the neutrophil production of ROS and bronchial hyperactivity in patients with asthma (Meltzer, Goldberg, Lad, Easton, 1989; Kato, Nakano, Morikawa, Kimura, Shigeta, Kuroume, 1991). There was also correlation found between the magnitude of the neutrophil ROS production and the severity of the asthma symptoms (Kato et al., 1991). Nordman et al. (1994) reported an increased PMA-induced whole blood chemiluminescence which they attributed to the activation of neutrophils. The findings of the study suggested that the whole blood CL assay may be a useful systemic inflammatory marker for patients with bronchial hyperactivity.

6.1.2 The role of platelets in asthma

Platelets have been suggested to be role-players in the pathophysiology of asthma since they produce and release a large variety of biologically-active chemical mediators that are capable of inducing or increasing inflammatory responses (Moritani, Ishioka, Haruta, Kambe, Yamakido, 1998; Kornerup & Page, 2007). They are said to act as fully functional inflammatory cells by expressing adhesion molecules on their surface, undergoing chemotaxis and being able to be activated by chemical mediators they release and those released by other inflammatory cells. It was found that platelets in asthma are easily activated when stimulated, resulting in the enhanced release of chemokines. These chemokines then serve to attract eosinophils into lung tissue (Moritani et al., 1998).

Platelets have been shown to be involved in bronchoconstriction by their release of bronchoconstricting mediators such as PAF, serotonin, arachidonic acid metabolites and histamine. Their role in airways inflammation was through the recruitment of leucocytes and the formation of platelet-leucocyte aggregates which are mediated by the expression of P-selectin on platelets surface. They contribute to airway remodeling by direct and
indirect stimulation of the airway smooth muscle and epithelial proliferation (Kornerup & Page, 2007; De Boer, Majoor, Van't Veer, Bel, Van der Poll, 2012).

Clinically, it has been shown that there was increased activation of platelets in atopic asthma patients, and that there were increased circulating platelet-leucocytes aggregates in patients with asthma attacks. It was also found that asthmatics have prolonged bleeding time, which is due to reduced aggregation as a result of platelet desensitization. The desensitization was as a result of chronic activation which was theorized to be ‘exhausted platelets’ (Kornerup & Page, 2007; Semple & Freedman, 2010; De Boer et al., 2012).

However, the observed activities of platelets in asthma were also found to respond to various pharmacological interventions. The increased release of chemokine RANTES may be inhibited by theophylline (Moritani et al., 1998), and the prolonged bleeding time and altered sensitivity may be normalized by treatment with glucocorticoids (Kornerup & Page, 2007). It is therefore worth investigating, how the herbal medicines may affect the activity of platelets in asthma.

6.2 The use of traditional medicine in diabetes mellitus

Diabetes Mellitus (DM) is a heterogeneous, multifactorial, polygenic disease characterised by impaired insulin action and secretion. DM Type 1 is characterised by the absence of insulin production, as a result of the autoimmune destruction of pancreatic B-cells. Type 2 DM, also called non-insulin dependent DM (NIDDM) occurs as a result of inadequate insulin secretion or peripheral insulin resistance, leading to persistent hyperglycaemia (Porter & Kaplan, 2011). Type 2 DM is regarded as a disorder of lifestyle, which is a major global threat as it affects millions of people worldwide. It is a risk factor for many CVS disorders, including CAD, coronary heart disease and ischaemic stroke (Ferreiro, Gomez-Hospital, Angiolillo, 2010; Porter & Kaplan, 2011).

Complications of DM include microvascular conditions such as nephropathy, retinopathy and neuropathy, which are associated with the oxidative stress in DM (Aydin, Orhan, Sayal, Ozata, Sahin, Isimer, 2001). DM is a chronic condition, managed by lifestyle modifications such as appropriate diet and exercise, insulin for type 1 DM, oral antihyperglycaemic drugs with or without insulin, and adjunct drugs to prevent complications such as aspirin or statins (Dipiro et al., 2008; Porter & Kaplan, 2011).
The principal goals of therapy are to reduce the microvascular and macrovascular complications, to improve the symptoms, lower the mortality rate and to improve the quality of life of the patients (Dipiro et al., 2008).

As in other chronic conditions, alternative therapies are always sought, to improve health and wellbeing. The use of ATM for diabetes has been reported. Patients who were diagnosed and were on treatment for DM also consulted THPs and used herbal medicines (Peltzer, Khoza, Lekhuleni, Madu, Cherian, Cherian, 2001b). Ethnobotanical surveys have revealed many plants and plant species used for treatment of DM. The surveys were done by questionnaires and interviews of traditional healers, patients or community members. A total of 111 plant species, some incorporated into 30 herbal formulations were confirmed for ethnobotanical use against DM and diabetes related complications in Mauritius (Mootoosamy & Mahomoodally, 2014), 120 plant species were reported in Pakistan (Yaseen, Ahmad, Zafar, Sultana, Kayani, Cetto, Shaheen, 2015), 38 plants in the north-east region (Ziyyat, Legssyer, Mekhfi, Dassouli, Serhrrouchni, Benjelloun, 1997) and 37 in the south-east region (Edouks, Maghrani, Lemhardi, Ouahidi, Jouad, 2002) of Morocco, 28 plants in Algeria (Amel, 2013) while 33 plants in Cameroon were identified to be used for hypertension and/or diabetes (Tsabang, Yedjou, Tsambang, Tchinda, Donfagsiteli, Agbor, Tchounwou, Nkongmeneck, 2015). In his study on the utilization and practice of TM/CAM in SA, Peltzer (2009) listed DM and its complications as one of the prevalent conditions treated at different TM/CAM outpatient settings. It indicated that there were people who sought TM for the treatment of diabetes.

### 6.2.1 The role of neutrophils in diabetes mellitus

DM is characterised by insulin deficiency and persistent hyperglycaemia. Immune dysfunction is one of the major complications which occur as a direct consequence of the hyperglycaemia on cellular immunity (Porter & Kaplan, 2011). Numerous changes in the functional capacity of neutrophils as first-line of defence in the body were reported. Some of the changes include decreased bactericidal activity, impaired phagocytosis and migration, diminished release of lysosomal enzymes and reduced production of ROS (Hatanaka, Monteagudo, Marrocos, Campa, 2006; Alba-Loureiro, Munhoz, Martins, Cerchiaro, Scavone, Curi, Sannomiya, 2007).
It was shown that the sustained hyperglycaemia causes a reduction of response to agonist stimulation in neutrophils. There have also been reports that the neutrophils in DM have enhanced functional responsiveness (McManus, Bloodworth, Prihoda, Blodgett, Pinckard, 2001). However, the treatment of diabetes has been reported to improve the functional capacity of the neutrophils (Alba-Loureiro et al., 2007). Nonetheless, it remains to be seen how the intake of CHMs for immune boosting purposes may affect diabetic cells and platelets that are on treatment.

6.2.2 The role of platelets in diabetes mellitus

DM is characterised by a high risk of atherothrombotic events. Factors that contribute to this prothrombotic state are increased coagulation, impaired fibrinolysis, endothelial dysfunction and platelet hyperreactivity (or hyperactivity) (Ferreiro, Sibbing, Angiolillo, 2010). Platelet hyperactivity and hyperaggregation play a critical role in the development of the angiopathies in DM (El Haouari & Rosado, 2008), hence are regarded as important inflammatory markers that are relevant in CVS diseases associated with DM type 2 (Ghoshal & Bhattacharyya, 2014).

The abnormalities of the platelets include morphological changes such as shape, size, volume, membrane fluidity. Functional changes include increased adhesion, adhesion molecule expression, thromboxane production, aggregation in response to agonists, calcium mobilisation, platelet specific protein release; and a reduced sensitivity to anti-aggregating mediators (Trovati & Anfossi, 2002; Arjomand, Roukoz, Surabhi, Cohen, 2003; Yngen, 2005; El Haouari & Rosado, 2008; Kakouros, Rade, Kourliouros, Resar, 2011; Ghoshal & Bhattacharyya, 2014).

The platelet abnormalities are influenced by the conditions in DM - hyperglycaemia, and the insulin resistance, as well as associated metabolic disorders and other cellular abnormalities. Insulin resistance effects on the platelets include the increased resistance to anti-aggregating agents, impairment of platelet and collagen interactions, reduction of nitric oxide production and increased expression of adhesive proteins including glycoprotein receptors, CD40 ligands, von Willebrand factor, P-selectin and others. Hyperglycaemia causes translocation of PKC to the membrane, increased intracellular calcium by modulating the function of the calcium exchanger, decreased NO availability as
well as increased platelet-leucocyte aggregates (Vinik, Erbas, Park, Nolan, Pittenger, 2001; Trovati & Anfossi, 2002; Ferreiro, Gomez-Hospital et al., 2010; Kakouros et al., 2011; Ghoshal & Bhattacharyya, 2014).

The hyperactivity or hyperreactivity behaviour of platelets in DM is associated with the increased oxidative stress in DM, and the reduced antioxidant capacity in platelets. It is also linked to the increased intracellular calcium. On stimulation by agonists, diabetic platelets react by producing excessive ROS, increased intracellular free calcium and tyrosine phosphatase activity. These actions result in increased synthesis of TXA2, which enhances the hyperaggregation of platelets (Trovati & Anfossi, 2002; El Haouari & Rosado, 2008; Kakouros et al., 2011). These observed changes in platelet function in DM have provided the motivation for improved strategies in the management of the condition that include reducing the risk of the CVS complications. Although some antidiabetic drugs have added beneficial effects on platelets, antiplatelet therapy is recommended for DM patients and low-dose aspirin therapy is the preferred drug. It was reported that it reduced the risk of MI and stroke in intermediate-to-high risk patients who had established vascular disease. However, there is not sufficient data that warrants antiplatelet therapy in DM patients with stable vasculopathy (Arjomand et al., 2003; Yngen, 2005; Kakouros et al., 2011; Ghoshal & Bhattacharyya, 2014; Santilli, Simeone, Liani, Davi, 2015). Therefore, it would be interesting to see how the diabetic platelet on antidiabetic treatment responds to herbal medicines.

6.3 The use of traditional medicine in hypertension

Hypertension (HT) is a chronic condition in which the blood pressure is increased above normal, and is considered clinical when the systolic pressure (SBP) is above 140 mmHg and the diastolic (DBP) is above 90 mmHg. It is then classified as mild (SBP ≥ 140-159 mmHg, DBP ≥ 90-99 mmHg), moderate (SBP ≥ 160-179 mmHg, DBP ≥ 100-109 mmHg) or severe (SBP ≥ 180 mmHg, DBP ≥ 110 mmHg). Primary or essential HT has no known cause and secondary HT may be linked to a specific cause, which in most instances is renal dysfunction. The WHO reported the prevalence of HT worldwide in 2008 as 40% of adults aged above age 25 with a total of one billion people living with the condition. The prevalence of HT was highest in the Africa region with 46% of adults aged 25 and above having been diagnosed with the condition (WHO, 2013).
The prevalence in SA in 2008 was reported to be high among older adults in SA, with the rates of awareness, treatment and control being very low (Peltzer & Phaswana-Mafuya, 2013). HT is regarded as a disease of lifestyle, and is a risk factor for many other conditions including coronary artery disease, stroke, myocardial infarction and renal failure. The goal of HT treatment is to decrease the morbidity and mortality associated with the condition, and are related to target-organ damage (Dipiro et al., 2008; Porter & Kaplan, 2011). HT may be well-controlled by lifestyle modifications and drug management which is initiated when the lifestyle changes fail, or when the BP is too high on diagnosis. Lifestyle modification can lower BP in patients with known HT and reduce the progression to HT in patients with with prehypertensive BP values. The drug management includes various classes of antihypertensive drugs. There are sympatholytics (β- Blockers), vasodilators and diuretics, and these may be used singly or in combination for life (Dipiro et al., 2008; Porter & Kaplan, 2011, Brenner & Stevens, 2012; Rossiter, 2012). The limitations of conventional treatments in dealing with chronic health problems was one of the reasons why patients sought alternative therapies of treatment such as consulting traditional healers and using TM. The use of ATM for hypertension has been reported (Peltzer, Khoza, Lekhuleni, Madu, Cherian, Cherian, 2001a; Lotika, 2006; Hughes et al., 2013).

Tabassum and Ahmad (2011) provided a review of natural herbs with hypotensive and antihypertensive effects. Some of these plants had been validated for these effects via scientific studies. Ginger is one of the listed medicinal plants, with extracts of this herb having been tested and confirmed for reduction of BP in hypertensive rats. Twenty plant species were identified for use as traditional medicine against hypertension in Mauritius (Mootoosamy & Mahomoodally, 2014), 73 plants in the south-east region (Edouks, Maghrani, Lemhardi, Ouahidi, Jouad, 2002) and 18 plants in the north-east region of Morocco (Ziyyat et al., 1997), 14 plant species in Edo State, Nigeria (Mensah, Okoli, Turay, Ogie-Odia, 2009), 15 plants in Algeria (Amel, 2013) with 33 plants in Cameroon identified for hypertension and/or diabetes (Tsabang et al., 2015). Surveys done in SA also revealed medicinal plant species used for the treatment of hypertension (Van Wyk et al., 2008; Thring & Weitz, 2006).

6.3.1 The role of neutrophils and phagocytes in hypertension

Hypertension is characterised by increased oxidative stress (Lassegue & Giendling, 2004; Hirata & Satonaka, 2001; Paravicini &Touyz, 2008). The oxidative stress which was said to
precede HT occurs as a result of increased ROS formation and it contributes towards the pathophysiology of the condition by affecting multiple tissues. Some of the effects include vascular damage, hypertrophic remodelling in the myocardium and enhance reabsorption of salt in the kidneys (Lassegue & Griendling, 2004; Zuo, Rose, Roberts, He, Barnes-Bercelli, 2014). High levels of the SOA inactivate the vasodilator nitric oxide, and cause endothelial dysfunction and vasoconstriction, which is associated with HT (Paravicini & Touyz, 2008).

It was observed that the ROS participated and maintained HT. Hypertensive patients have reduced antioxidant capacity, while there is increased PMN and platelet production of ROS (Paravicini & Touyz, 2008). Hypertensive neutrophils were proven to produce more oxygen radicals than normal neutrophils (Salamino, Sparatore, De Tullio, Mengotti, Melloni, Pontremoli, 1991) and that on stimulation with PMA, they produced more SOA than normotensive neutrophils (Ramasamy, Maqbool, Mohamed, Noah, 2010). Neutrophil-derived ROS was reported to cause significant endothelial cell injury with H2O2 and the OH− being the oxidants responsible for the injury (Tsukimori, Fukushima, Tsushima, Nakano, 2005). Antihypertensive drugs have the capacity to correct the oxidative stress in HT, in addition to their hypotensive effects (Hirata & Satonaka, 2001). Hence it would be interesting to see how the neutrophils in HT on treatment are affected by HMs.

6.3.2 The role of platelets in hypertension

As it is well-known, HT is a risk factor for thrombotic conditions wherein platelets play a crucial role. It is reported that platelets in hypertension are hyperactive and therefore are implicated in the development of CVS complications such as myocardial ischaemia and infarction (El Haouari & Rosado, 2009; Gkaliagkousi, Passacquale, Douma, Zamboulis, Ferro, 2010; Du & Kiriazis, 2013). The increased platelet activation is caused by the endothelial dysfunction that prevails in HT. There are both morphological and functional abnormalities of platelets in HT. Morphological changes include lower mean platelet granularity, higher mean platelet mass and mean platelet volume and increased platelet size. Functional abnormalities include changes in intracellular chemical signalling resulting in spontaneous aggregation and enhanced sensitivity to agonists. Platelets of hypertensive patients were reported to have increased intracellular calcium under resting conditions and
after agonist stimulation as compared to normal persons (Hiraga, Oshira, Yoshimura, Matsuura, Kajiyama, 1998; El Haouari & Rosado, 2009).

An important alteration in platelets in HT is the increased production of ROS and the general oxidative stress. Platelets from hypertensive patients have reduced antioxidant capacity which causes the presence of higher amounts of ROS, particularly SOA. It has been reported that agonist stimulated SOA production is increased in hypertensive patients than normotensive patients. The increased ROS is associated with platelet hyperactivity and may be implicated in the development of the thrombotic disorders linked to HT (El Haouari & Rosado, 2009).
CHAPTER 7 METHODS AND MATERIALS

7.1 BIOCHEMILUMINESCENCE

The biochemiluminescence assay was performed on the Orion L Microplate Luminometer supplied by Berthold Detection Systems. The luminometer is specially designed to detect chemiluminescence and biochemiluminescence. It has an integrated injector system that allows up to two injections per measurement.

The components of the luminometer include the following:

- Light detector: a highly sensitive photomultiplier tube that measures light in the visible spectral range between 300 and 600nm and is housed within the detection assembly.
- Two injectors with the pumps and reagent holders inside the instrument chamber, and the tips of the injectors located within detection assembly.
- The microplate holder is an automatic plate on to which microplates are loaded and unloaded, one at a time.
- The measurement chamber is a chamber into which the microplate is drawn and exposed to the photomultiplier tube, for measurements to be taken.
- The luminometer makes use of standard 96-well microplates, and white microplates were used in this study as they reflect light from the samples and increase light sensitivity of the luminometer.

7.1.1 Reagents and their preparation

The reagents used included:

- Hanks’ balanced salt solution (HBSS) supplied by Sigma. It was supplied as a 10 times (10X) concentrate. Every 100 ml contains sodium chloride (80 g/l), potassium chloride (4.0 g/l), glucose (10 g/l), potassium phosphate, monobasic (600 mg/l), sodium phosphate, dibasic (475 mg/l) and phenol red (170 mg/ml). This solution does not contain calcium, magnesium or bicarbonate.

- Lysis buffer. The buffer was prepared by dissolving 8.3 g of ammonium chloride (NH₄Cl) and 1.0 g of potassium hydrogen carbonate (KHCO₃) in 1000 ml of distilled water.
Percoll solution as supplied by Sigma-Aldrich. This is a density gradient medium for cell separation and suspension. It is a colloidal suspension of polyvinyl pyrrolidine (PVP) coated silica particles and has a density of 1.130; pH 8.9 and viscosity of 10 cps at 20°C. A volume of 15 ml of Percoll working solution (PWS) was prepared by adding 9.5 ml of Percoll, 1.5 ml Hanks medium (10X concentrate) and four millilitres (4 ml) distilled water. The solution turned dark pink in colour. This Percoll solution had density 1,088 g/ml and is of appropriate osmolality and viscosity for cell viability.

Dimethyl sulfoxide (DMSO) supplied by Sigma, \(\text{C}_2\text{H}_6\text{SO}\), formula weight 78.13g.

PMA from Sigma; \(\text{C}_{36}\text{H}_{56}\text{O}_8\), formula weight 616.8 g.
A mass of 10 mg of PMA was dissolved in 1 ml of DMSO in a 10 ml glass tube and made up to a volume of 10 ml with Roswell Park Memorial Institute (RPMI) Medium.

fMLP from Sigma, \(\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5\text{S}\), formula weight 437.6 g. A mass of 0.00437 g (4.37 mg) fMLP was dissolved in 1 ml DMSO and made up to 10 ml with RPMI medium.

Luminol (5-amino-2,3 dihydro-1,4- phthalazinedione) from Sigma, \(\text{C}_8\text{H}_6\text{N}_3\text{O}_2\text{Na}\), formula weight 199.1 g. A mass of 0.00199 g (1.99 mg) luminol was dissolved in 1 ml DMSO and stirred vigorously. The volume was made up to 10 ml with RPMI medium, to yield a 0.199 mg/ml (1 mM) solution. From this stock solution, a working solution was made up by taking 1 ml of the 1 mM solution and making it up to 10 ml with HBSS.

Tyrode solution. This solution was prepared by adding 4.00 g NaCl, 0.1 g KCl, 0.1 g MgCl\(_2\), 0.035 g NaH\(_2\)PO\(_4\) and 0.5 g glucose in 500 ml distilled water. The pH of the solution was adjusted to 7.3 with NaHCO\(_3\).

Tyrode with ethylene-diamine-tetra-acetic acid (EDTA). The tyrode solution was prepared as above and 1.00 g EDTA was added. The pH of the solution was also adjusted to 7.3.
7.1.2 The commercial herbal mixtures

The six CHMs selected for the study, denoted HM1 till HM6 were as follows:

HM1- *Intlamba Zifo™*
HM2- *Maphilisa™* Herbal medicine
HM3- *Matla™* African medicine for all diseases
HM4- *Ngoma™* Herbal Tonic Immune Booster
HM5- *Stametta™* Body Healing Liquid
HM6- *Vuka Uphile™* Immune Booster

The CHMs were kept in the refrigerator at 4°C before use.

7.1.2.1 Preparation of the herbal mixture test samples

Four serial dilutions of each of the six herbal mixtures were prepared, from 10x, 100x, 1000x to 10,000x with the HBSS.

The neat herbal mixture (the undiluted CHM) and its diluted solutions were tested each in triplicate in the luminometer. So for each CHM, there were five samples for testing.

For statistical purposes, each assay was performed a minimum eight times (Means of $n = 8$ indicated on graph), on a different test variable (whole blood, isolated human neutrophils, platelets) each time.

7.1.3 Collection of blood samples

The blood samples were collected from healthy volunteers and from consenting confirmed asthma, diabetes mellitus and hypertension patients who were on treatment. The healthy volunteers included males and females aged between 20 and 31 years, and were non-smokers, not on any medication and had not taken any self-medication such as painkillers, vitamin supplements or any herbs or herbal mixtures in the two weeks preceding the day of collection of sample. The patients’ ages ranged from 23 to 54, and were on their regular treatment; and had not taken any self-medication such as painkillers, vitamin supplements or any herbs or herbal mixtures in the 14 days preceding the day of collection. For DM there were four males and four females; for asthma and hypertension there were five females and three males each. For DM patients, all patients who were taking antiplatelet treatment were excluded.
Each participant was informed by the researcher about the study and the requirements. They each signed a consent form after being informed about voluntary participation and guaranteed confidentiality of the results. The blood samples were collected and handled confidentially by a qualified professional nurse in the clinic at the Department of Pharmacology and Therapeutics. Each collected blood sample was handled and analysed on the same day of collection.

In compliance with ethical requirements, clearance (MREC/M/09/2011:PG) for the study was obtained from the UL Medunsa Campus Research Committee (MREC) and permission was obtained from the Superintendent of Dr George Mukhari Hospital (DGMH). The letter of clearance, letter of permission, the consent form and the information leaflet to participants are appended to this document.

### 7.1.3.1 Isolation of neutrophils.

Neutrophils were isolated from whole blood by using the Percoll sedimentation method outlined below, which was developed and adapted in the Department of Pharmacology laboratory of Sefako Makgatho health Sciences University (SMU). This method was developed and modified from comparison of several documented methods of isolation of white blood cells (Kahler, 2000). It was found to be reliable, sensitive and cost-effective in isolation of neutrophils.

Approximately 30 ml of heparinised blood was collected from each suitable donor. It had been reported that the viability of isolated neutrophils was independent of anticoagulant used during collection, but their activation differed with various anticoagulants used. Cells collected on heparin showed higher activation than those collected on EDTA and citrate (Freitas et al., 2008).

Equal volumes of the blood sample were distributed into two graduated 50 ml conical centrifuge tubes. The volume in each tube was approximately 15 ml. Each of the samples was topped up with RPMI to a volume of 30 ml.
In another two graduated 50 ml conical centrifuge tubes, 15 ml each of the PWS was added. Each blood sample was carefully layered onto the PWS, such that the blood floated on top of the Percoll and was not submerged.

The samples were centrifuged at 2000 rpm (800 g) for 30 minutes. The samples were carefully removed from the centrifuge, without being shaken. The supernatant (the top clear/amber layer including the buffy layer) was aspirated to leave the dark red layer at the bottom.

Clean absorbent gauze was used to wipe the inside of each tube. This served to remove the remaining monocytes clinging to the sides which could interfere with the results.

In each of the two tubes, a small volume of lysis buffer was added to resuspend the cells. A pipette was used to mix the solution so that the pellet was completely dislodged and dissolved. Each tube was filled up to 50 ml with the lysis buffer to lyse the red blood cells. Then they were capped tightly and gently inverted twice to mix the samples well.

Each tube was immersed in wet ice for five minutes and then centrifuged at 1000 rpm (250 g) for five minutes. The tubes were removed carefully from the centrifuge. A white pellet of cells was visible when the tubes were viewed from the bottom. All the supernatant was decanted by inverting each tube once.

The cells were resuspended in the lysis buffer by adding a small volume of the buffer first and using a pipette to dislodge the pellet of cells. The volume of the lysis buffer was then made up to 50 ml. The samples were kept on wet ice for one minute and centrifuged at 1000 rpm (250 g) for five minutes.

The tubes were carefully removed from the centrifuge and all the supernatant was decanted. Approximately 2 ml of HBSS was added in each tube to suspend the cells. The two samples were added into one conical 15 ml graduated tube. The volume was made up to seven millilitres (7 ml) with HBSS. The tube was closed tightly and kept on wet ice. A white cell count was performed at the NHLS, Haematology section, to obtain the total amount of neutrophils isolated.
7.1.3.2 Isolation of platelets

Approximately 10 ml of blood was collected in tubes with citrate preservative. The blood was centrifuged at 1000 rpm (250 g) for 10 minutes. Then the platelet-rich plasma (PRP) was collected, approximating to 4 ml of plasma. A volume of 40 µl of citric acid was added to the PRP, mixed well and then centrifuged at 2200 rpm (1000 g) for 10 minutes. The supernatant was discarded, and then 4 ml of a tyrode plus EDTA solution was added to the pellet and mixed. The solution was allowed to stand for 10 minutes at room temperature. Then it was centrifuged at 2200 rpm (1000 g) for six minutes. The supernatant was discarded and a tyrode solution was added to the pellet, to a final volume of 6 ml. A platelet count was performed at the NHLS, Haematology section, to obtain the total amount of platelets isolated. A volume of 25 µl of the sample was used in each well for CL test on the luminometer. The platelet counts were corrected to $1 \times 10^3$ cells/ul.

7.1.3.3 Preparation of whole blood samples for luminescence

A whole blood sample was collected in a heparinised tube, and then a full blood count test with differential count was performed on the sample. Then a 50 times dilution of the sample was made, for use in the luminescence determination- 50 µl of the sample was added to 2450 µl of HBSS to make a final volume of 2.5 ml of diluted sample, which was then kept in ice-water until used. The 50 times dilution factor was taken into consideration when presenting the luminescence results. Total cells used for luminescence = total white cell count x 50/2500 x 25 µl.
7.1.4 Procedure for luminescence measurement

In a 96 well microplate, the following were added

- 25 µl HBSS in each control well.
- 25 µl test solution in each test well (neat herbal mixture, followed by the 10x, 100x, 1000x and 10000x diluted solutions.
- 25 µl of the cell suspension/platelet suspension/diluted whole blood sample.

The plate was covered tightly with parafilm wrap and incubated in a waterbath at 37°C for 30 minutes, but not submerged in the water.

Then the luminometer injectors were used to add

- 25 µl working luminol,
- 25 µl of either fMLP or PMA,
- and 100 µl of HBSS, to make the final volume up to 200 µl in each well.

This was followed immediately by reading the luminescence of each well (time = 0 minutes), measured in Relative Luminescence Units (RLU), with total integral values set with repeated scans at 30 s intervals and 1 second points measuring time.

Then each plate was read further for luminescence for a total of 60 minutes, at five minute intervals till 30 minutes, then at 15 minute interval until 60 minutes. So the results are the recorded luminescence readings at time 0, 5, 10, 15, 20, 25, 30, 45 and 60 minutes.

Luminescence values are a reflection of the amount of light emitted by the cells once stimulated, which is related proportionally to the activity of the cells after incubation with the various HMs, which reflects the amount of ROS formed. Therefore the higher the RLU value, the higher the activity of the cells, the more the ROS produced. The lower the values, the lower the activity of the cells.

7.1.5 Flow diagram outlining the approach to testing each herbal mixture

Each HM (of the six) and its four diluted solutions were tested on three components-platelets, neutrophils and whole blood phagocytes. Each of the components was tested in two different conditions, using either fMLP or PMA, to differentiate the mechanisms of the effects of the HMs on the components. The same protocol as outlined in figure 7.1 was followed for components of blood collected from healthy volunteers, and people with the diseases -hypertension, asthma and diabetes mellitus.
7.1.6 Data collection, handling and analysis

The luminometer used in this study was coupled to a data collection device. The raw data from the luminometer were entered into a Microsoft Excel™ program to obtain descriptive statistics. The cell count for the luminescence activity measured were each corrected for $1 \times 10^3$ cells/ul. The average luminol-enhanced luminescence (LEL) activity of each HM and controls were calculated per time interval. Graphs were constructed to show luminescence activity (RLU) over time (minutes). The percentage inhibition (% inh) for each HM were calculated compared to the controls, using the formula:

$$\% \text{ INH} = \left( \frac{\text{RLU}_{\text{ctrl}} - \text{RLU}_{\text{test}}}{\text{RLU}_{\text{ctrl}}} \right) \times 100$$

The values were displayed on graphs of % inhibition versus time. The % inhibitions were graded as potent ($\geq 80\%$), moderate (50-79%) and low (weak) (< 50%) which was in line with other studies. Then average (mean) LEL activities for the 60 minutes and the corresponding % inhibitions were also calculated and displayed in tables.
7.1.7 Statistical analysis

The luminescence activity of each HM was compared with the controls using the Microsoft Excel™ data analysis tool, t-Test: Two sample Assuming Unequal Variance. Statistical significance was declared where the differences had the p-value equal or less than 0.05. (p ≤ 0.05). The results are displayed as means in graphs and mean ± standard deviation (SD) in the tables.

7.2 Cross-reactivity of herbal medicines with substances of abuse: Method and materials

7.2.1 The rapid urinalysis assay

The test kit used was the Instant view® Multi-Drug of Abuse Test kit form Lab-stix Diagnostics (Pty) Ltd. It is composed of test cassettes that have six panels for testing for six substances concurrently. The test device panels are specific for amphetamine (AMP), cocaine (COC), methamphetamine (MET), morphine (MOR), tetra-hydrocannabinol (THC) and methylenedioxymethamphetamine (MDMA) which is known by its street name as ecstasy.

7.2.1.1 Principle of the rapid urinalysis test

This test is a one-step lateral flow chromatographic immunoassay. Each panel contains drug-protein conjugate immobilised on a porous membrane support. The test is based on the principle of competition for limited binding sites on the antibody between the drug or drug metabolite present in the urine sample and the immobilized drug-protein conjugate. When a urine sample is poured on the test panel, it moves by capillary action to the test area, where it also mobilises the drug-protein conjugate. If the drug is absent in the urine, or is present in below cut-off levels, the coloured conjugate will interact with the drug antigen immobilised in the test line and form a coloured line. If the drug or drug metabolite is present, it will bind to and saturate all the binding sites on the antibody conjugate, preventing any binding by the drug antigen at the test area. Hence no colour line will form in the test area.

- The results are negative if two lines appear. The intensity of the line does not matter; hence even a faint line forming is regarded as negative.
The results are positive if only one colour line appears in the control site. No colour line should form on the test site.

The results are invalid if no colour line appears on the test as well as the control site.

7.2.2 The dipstick test

A 10 test dipstick kit was bought from Labstix Diagnostics (Pty). Each dipstick tests qualitatively for pH and specific gravity, and for the presence of leukocytes, nitrites, urobilinogen, protein, blood, ketones, bilirubin and glucose. The stick is immersed in a urine sample and then observed within five minutes for results. The results are the observed colour changes as compared to the standard provided.

7.3 Test Procedure

7.3.1 Test 1: Testing drug-free urine

Ten urine samples were collected from healthy volunteers who each confirmed that they had not taken any drugs, herbal medicines or any over-the-counter (OTC) medicines within the preceding seven days. Each sample was tested using a dipstick for pH and specific gravity, and for the presence of leukocytes, nitrites, urobilinogen, protein, blood, ketones, bilirubin and glucose. The urine, designated drug-free urine (DFU) was pooled and six 50 ml aliquots were then taken from the pool. The remaining DFU was stored in the refrigerator at 4°C. From each of the six CHMs, three serial dilutions were made, i.e 10, 100 and 1000 times dilution with distilled water. Four aliquots of 9 ml each of DFU were made for each of the six CHMs. A volume of 1 ml each of the neat HM and the three dilutions were added to the aliquots.

\[ 9 \text{ ml DFU} + 1 \text{ ml neat CHM} = 10 \text{ ml spiked DFU} \]

\[ 9 \text{ ml DFU} + 1 \text{ ml dilute CHM} = 10 \text{ ml spiked DFU} \]

A dipstick test was done on each spiked DFU sample and the results recorded, specifically for pH, specific gravity. The neat CHMs and each of these aliquots was then tested using the Instant view® Multi-Drug of Abuse Test cassette, following the instructions on the test
kit manual test. The CHM5 and CHM6 were too dark in appearance, hence a two times dilution of each of them was made with distilled water and they were also tested. Each of the spiked DFU was then tested for drugs of abuse using the test cassettes and the results were recorded. The test was repeated in all the samples at the time intervals of 8, 24, 48, 72, 96 and 120 hours. The results were recorded for day 1 at 0 hour and 8 hours; and day 2 till day 5. The samples were stored in the refrigerator at 4°C daily.

Six aliquots of each DFU sample were each spiked with one of the HMs at 40% v/v (i.e 2400 µl D-U + 1600 µl CHM). The control for each sample was composed of a volume of 2400 µl of the D-U plus 1600 µl of DFU to make up for the dilution. These samples were tested following the same procedure and the results were recorded.

7.3.2 Test 2: Testing urine that is positive for drugs of abuse

This test was performed as a follow-up to test 1. Urine samples that had been sent to the Pharmacology Laboratory for routine testing, and had tested positive for any of the tested substances were collected. The samples (D-U) were labelled starting from FNT1 to FNT8. Each sample was centrifuged for 5 minutes at 1000 rpm (250 g). The supernatant was collected and stored in the refrigerator at 4°C until needed.

Six aliquots of each D-U sample were each spiked with one of the HMs at 40% v/v (2400 µl D-U + 1600 µl CHM). The control for each sample was composed of a volume of 2400 µl of the D-U plus 1600 µl of DFU to make up for the dilution. These samples were tested following the same procedure as in Test 1 and the results were recorded.

7.3.3 Assay procedure

The test pouch and the urine samples for testing were removed from the fridge and were allowed to equilibrate to room temperature before testing.

The test cassette/device was labelled accordingly with the sample number. Then the dropper provided with the kit was used to collect the urine sample, up to the mark, and then the urine was transferred on to the sample well on the cassette.

The results were then read after five minutes. The test recommends that the results be read between four and seven minutes.
CHAPTER 8 RESULTS: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM HEALTHY VOLUNTEERS

This chapter details the effect of the six CHMs on the luminescent activities of the isolated human neutrophils, platelets and whole blood phagocytes obtained from healthy volunteers. The results for all the three components are first displayed in graphs of luminescence (in RLUs) versus time (in minutes), which indicated the kinetics observed over 60 minutes. Then the percentage inhibitions were calculated, to give an overall of the effect relative to the control for each of the components, also over 60 minutes.

8.1. Luminescence activity of whole blood phagocytes from healthy volunteers

The graphs below depict luminol-enhanced luminescence (LEL) activity of whole blood phagocytes stimulated with (A) fMLP and (B) PMA for each neat HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibitions of the HMs on the cells at the various time intervals.
Figure 8.1. Luminol-enhanced luminescence (LEL) activity of healthy whole blood phagocytes after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph). CTRL = control

On stimulation with fMLP (A), the control (CTRL) cells started showing an increasing LEL activity. The activity peaked at 10 minutes, started declining and then remained constant above the baseline (point of stimulation, t = 0) until 60 minutes. The cells incubated with
neat HM1 and the 10x HM1 diluted standard had high activity on stimulation, which increased further and peaked at 10 minutes. The activity gradually decreased and was the same as that of the CTRL cells at 45 and 60 minutes of testing, respectively. Cells in the other three diluted HM1 standards showed activity much closer to that of the CTRL cells, but the 1000x HM1 diluted standard had activity lower than the CTRL cells.

The PMA-induced CTRL cells (B) had activity that declined slightly after stimulation, but was back to the baseline level (6 RLU) at 20 minutes. The activity then remained at the baseline level until 60 minutes. The cells incubated with neat HM1 and the diluted standards, except the 1000x standard had variable increased activity on stimulation, with the neat HM1 cells having the highest activity. The activity of these cells declined gradually. The activity of the cells in the 10000x diluted standard mimicked the CTRL cells much more closely. The cells incubated with the 1000x diluted HM1 showed LEL activity below that of the CTRL cells, from start, which then increased slowly to the level of the CTRL cells at 20 minutes. The activity of the cells in the 10x diluted standard stabilised after 25 minutes, but remained above that of the CTRL cells for the remaining time. The LEL activities of all the other PMA-induced cells stabilised from 25 minutes and returned to the baseline level at 60 minutes.

In comparison, the baseline (t = 0) LEL activity of the PMA-induced cells was higher than the fMLP cells, including the CTRL cells. The activity of the fMLP-induced cells increased after stimulation whereas the activity of the PMA-induced cells declined after stimulation.
Figure 8.2. The % inhibition of healthy whole blood phagocytes after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The calculated % inhibitions were congruent with the observed LEL activities. The 1000x diluted HM1 standard was the only one with weak inhibitory effect on both the PMA and fMLP-induced cells, the effect being slightly higher in the PMA-induced cells. The neat HM1 had the moderate stimulatory effect at the beginning, which tapered with time, followed by the 10x diluted standard with a lower stimulatory effect. The 100x diluted standard had a low stimulatory effect, much more evident on the PMA- than the fMLP-induced cells.
Figure 8.3. Luminol-enhanced luminescence (LEL) activity of healthy whole blood phagocytes after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The cells incubated with the 10x diluted HM2 had notable LEL activity for both the PMA and the fMLP-induced experiments but the PMA-induced cells had much higher RLU values than the fMLP-induced cells on stimulation. The fMLP-induced cells’ activity peaked
at 10 minutes, to the level almost equal to the baseline level of the PMA-induced cells. The activity of the fMLP induced cells in the 10x diluted HM1 standard stabilised at 30 minutes, but remained increased above the CTRL cells’ activity after 60 minutes. The cells incubated with the other test standards as well as the neat HM2 had comparable activities, closer to the controls, above and below the CRTL cells. The activities ranged from 4-8 RLU for fMLP-induced cells and 4-6 RLU for the PMA-induced cells.

Figure 8.4. The % inhibition of healthy whole blood phagocytes after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The 10x diluted HM1 standard had the highest stimulatory effect of both the PMA-induced and the fMLP-induced cells, from potent and to moderate for 25 minutes and low for the remaining time. All the other standards had weak stimulatory effect throughout, except the neat HM2 which had weak inhibitory effect throughout.

**HM3- Matla™ African medicine for all diseases**

Figure 8.5. Luminol-enhanced luminescence (LEL) activity of healthy whole blood phagocytes stimulated after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of \( n = 8 \) indicated on graph).
The cells incubated with the neat HM3 had LEL activity less than that of the CTRL cells, whether PMA- or fMLP induced. The cells in the other standards had activity more than that of the CTRL cells that stabilised over time but remained above the CTRL after 60 minutes. The activity of the cells, on stimulation, was higher in the PMA-induced cells than the fMLP cells. The fMLP-induced cells (A) in the diluted standards peaked at different time intervals, while the PMA-induced cells had their highest activity at the beginning. On stimulation, the activities of the PMA-induced cells were higher than that of the fMLP-induced cells.

Figure 8.6. The % inhibition of healthy whole blood phagocytes after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM3 had a weak inhibitory effect on both the PMA- and the fMLP-induced cells, however the effect was more pronounced in the fMLP-induced cells. The other test standards had low stimulatory effect on the cells, with the 2 lower dilution solutions (10x and 1000x) exerting moderate inhibitory effect on the PMA-induced cells at t = 0.

**HM4- Ngoma™ Herbal Tonic Immune Booster**

![Diagram of LEL activity of whole blood phagocytes](image)

Figure 8.7. Luminol-enhanced luminescence (LEL) activity of healthy whole blood phagocytes stimulated after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The cells in the diluted HM4 standards had higher activity than the CTRL on stimulation, and the activities mimicked that of the CTRL throughout. The cells in the neat HM4 had activity lower than that of the CTRL cells for the whole 60 minutes. The cells in the diluted HM4 standards had activity above that of the CTRL, which peaked at different time intervals, and stabilised and tapered to levels slightly above their baselines.

The PMA-induced (B) CTRL cells had activity that decreased from baseline, which recovered to peak at 25 minutes and then stabilised to a level almost equal to the baseline at 60 minutes. The cells in the neat HM4 had activity that followed the same pattern but at levels below that of the CTRL cells. Cells in the diluted standards had activity higher than the CTRL cells, which on average remained the same for each of the standards with time. That is with the exception of the cells in the 10x diluted standard, which decreased gradually from its baseline, to the level of the CTRL cells at 60 minutes.
Figure 8.8. The % inhibition of healthy whole blood phagocytes after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM4 had a weak inhibitory effect on both induced cells, with the effect being much lower on the PMA-induced cells. The diluted HM4 standards generally had low stimulation effect on the cells but the 10x and the 100x diluted standard had a moderate stimulatory effect for the first ten minutes on the PMA-induced cells.
Figure 8.9. Luminol-enhanced luminescence (LEL) activity of healthy whole blood phagocytes stimulated after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The activities of the fMLP-induced cells in the neat HM5 and the 10x diluted standard showed LEL activities much close to that of the CRTL cells, at points being above or
below. The PMA-induced cells in the 10x diluted standard were in turn more active than the CTRL cells. Both PMA and fMLP-induced cells in the 100x and 1000x diluted standards had a much higher activity, which decreased gradually and were lowest at 60 minutes, although the behaviour of the PMA-induced cells in the 1000x diluted standard differed between five and 30 minutes. The cells in the 10000x diluted standard had activity above that of the CTRL cells, which remained constant for the entire testing time.

Figure 8.10. The % inhibition of healthy whole blood phagocytes after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat and the 10x diluted HM5 standard had weak inhibitory effect, while the other diluted standards had stimulatory effect. The 100x diluted HM5 standard had a very high \((t = 0)\) stimulatory effect which lowered to moderate effect within the 30 minutes of testing, whereas the 1000x diluted standard had a high to moderate effect for the fMLP-induced cells, and a weak stimulatory effect in the PMA-induced cells.

**HM6- Vuka Uphile™ Immune Booster**

Figure 8.11. Luminol-enhanced luminescence (LEL) activity of healthy whole blood phagocytes stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of \(n = 8\) indicated on graph).
The fMLP-induced cells in the neat HM6 had reduced activity, below the control, whereas the PMA-induced cells had activity much close to that of the CTRL cells either above or below. The cells incubated with the 10x diluted HM6 standards showed comparable LEL activities on stimulation, but the activity increased in the fMLP-induced cells and peaked at 10 minutes. The activity declined in the PMA-induced cells and was lowest for both cells at 60 minutes, close to but above the control which remained consistent for the 60 minutes of test time. The PMA-induced cells in the 10000x diluted HM6 standard showed the highest LEL activity at 5 minutes after stimulation, which decreased gradually and became constant from 30 minutes onwards.

Figure 8.12. The % inhibition of healthy whole blood phagocytes after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM6 had a weak inhibitory effect on both the PMA- and the fMLP- induced cells. The 10x diluted HM6 standard had a high to moderate stimulation effect on the cells which became weak from 15 minutes onwards. All the other standards had weak stimulatory effect throughout.

Table 8.1. The average LEL activity of healthy whole blood phagocytes incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% inhibition</td>
<td>LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control (CTRL)</td>
<td>6.07 ± 0.59</td>
<td>-19.0 ± 19.4</td>
<td>7.29 ± 1.04*</td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>7.20 ± 0.85*</td>
<td>-11.8 ± 10.4</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>6.86 ± 1.25</td>
<td>-0.3 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>6.11 ± 0.62</td>
<td>4.0 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>5.84 ± 0.62</td>
<td>2.8 ± 8.7</td>
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<td></td>
<td>10000x dilution</td>
<td>5.92 ± 0.65</td>
<td>-11.8 ± 10.4</td>
</tr>
<tr>
<td>HM2</td>
<td>Neat</td>
<td>4.76 ± 0.374*</td>
<td>-6.5 ± 9.3</td>
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<td></td>
<td>10x dilution</td>
<td>10.48 ± 2.47*</td>
<td>-5.0 ± 8.6</td>
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<td></td>
<td>100x dilution</td>
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<td>-4.2 ± 6.3</td>
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<td></td>
<td>1000x dilution</td>
<td>6.45 ± 1.11</td>
<td>-4.2 ± 6.3</td>
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<td></td>
<td>10000x dilution</td>
<td>6.36 ± 0.81</td>
<td>-4.2 ± 6.3</td>
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<td>-6.5 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>7.66 ± 0.61*</td>
<td>-22.4± 11.5</td>
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<td></td>
<td>100x dilution</td>
<td>7.44 ± 0.72*</td>
<td>6.44 ± 0.44</td>
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<td></td>
<td>1000x dilution</td>
<td>7.56 ± 1.02*</td>
<td>6.36 ± 0.65*</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>7.26 ± 1.11*</td>
<td>-23.5 ± 6.3</td>
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<td>-23.5 ± 6.3</td>
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<tr>
<td></td>
<td>100x dilution</td>
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<td>100x dilution</td>
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<td>-7.91 ± 38.2</td>
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<td>-23.5 ± 6.3</td>
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<tr>
<td></td>
<td>10000x dilution</td>
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<td>Neat</td>
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<td>-18.1 ± 6.9</td>
</tr>
<tr>
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<td>10x dilution</td>
<td>8.96 ± 1.81*</td>
<td>-21.7 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>7.42 ± 0.94*</td>
<td>-21.7 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>6.92 ± 0.51*</td>
<td>-14.1 ± 11.9</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>6.79 ± 0.66*</td>
<td>-11.3 ± 3.4</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The table presents the overall average LEL activities of the test standards in 60 minutes with the corresponding average % inhibitions, also calculated over 60 minutes. They are
presented as mean ± SD for each standard. Positive values signify overall inhibitory effect, while negative values denote stimulatory effect. The values presented in the table allow comparison of LEL activity and % inhibition at various concentrations for each standard. It shows where the stimulatory or inhibitory effect of the CHM is weak, moderate or potent. Hence where possible, it is clear whether the stimulatory or inhibitory effect is directly or inversely proportional to the concentration or it does not correlate, whether it differs for each mechanism of stimulation of the cells (fMLP or PMA) and it shows the statistically significant effects (p ≤ 0.05) The specific details of the table are addressed in the discussion chapter (chapter 9).
8.2 Luminescence activity of isolated human neutrophils from healthy volunteers

The graphs below depict luminol-enhanced luminescence (LEL) activity of isolated human neutrophils (IHNs) stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the cells at the various time intervals.

HM1- *Intlamba Zifo™*

Figure 8.13. Luminol-enhanced luminescence (LEL) activity of healthy isolated human neutrophils after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The LEL activities of the cells were higher in the fMLP-induced cells than the PMA-induced cells. All the cells had the highest activity on stimulation, which reduced rapidly within five minutes. Thereafter the CRTL cells’ activities fluctuated but on average were stable around the same value. The PMA-induced cells remained constant till 60 minutes, whereas the fMLP induced cells’ activity declined further after 25 minutes and only stayed constant thereafter.

The cells in the neat HM1 had activity below that of the CTRL cells which was consistent throughout the 60 minutes of observation. The fMLP-induced (A) cells in the 10x diluted HM1 standard also had activity below the CTRL cells for 60 minutes. All the other cells had activities close to that of the CTRL cells, which fluctuated either above or below the CTRL activity.
Figure 8.14. The % inhibition of healthy isolated human neutrophils after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM1 and its diluted solutions had weak to moderate inhibitory effect on both fMLP- and PMA-induced IHNs. This effect varied only where the 100x, the 1000x and the 10000x diluted standards had weak stimulatory effect on the cells.
Figure 8.15. Luminol-enhanced luminescence (LEL) activity of healthy isolated human neutrophils after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

All the cells incubated with the HM2 had activity lower than the CTRL cells. The cells in the neat HM1 had activity lowest to that of the CTRL cells, which then increased gradually to peak at 15 minutes for fMLP-induced cells and at 25 minutes in the PMA-induced cells. The activity stabilised but remained above the baseline level at 60 minutes of observation. All the other cells had activities that closely mimicked that of the CTRL cells, but remained below the CTRL activity.
Figure 8.16. The % inhibition of healthy isolated human neutrophils after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM2 and its diluted standards had weak to moderate inhibitory effect on both the PMA and the fMLP-induced IHNs, which was sustained variably for the 60 minutes of testing.
Figure 8.17. Luminol-enhanced luminescence (LEL) activity of healthy isolated human neutrophils after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The cells incubated with the HM3, with few exceptions, had activity lower than the CTRL cells. The cells in the neat HM1 had activity lowest to that of the CTRL cells. The fMLP-induced cells in the 10000x diluted HM3 standard were closest to the CTRL values. The exceptional activity was that of the PMA-induced cells in the 100x diluted HM3 standard, which was above that of the CTRL cells between 30 and 45 minutes, but decreased to be below at 60 minutes.
Figure 8.18. The % inhibition of healthy isolated human neutrophils after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM3 and its diluted standards had a weak to moderate inhibitory effect on both the PMA and the fMLP-induced IHNs, which was sustained variably for the 60 minutes of testing, except for the 100x diluted standard.
Figure 8.19. Luminol-enhanced luminescence (LEL) activity of healthy isolated human neutrophils stimulated after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The activity of the cells incubated with the HM4 and its diluted standards was generally below that of the CTRL cells. The fMLP-induced cells in the neat HM4 had activity lowest to that of the CTRL cells. While the activities of the cells in the diluted standards oscillated, the activity remained below the CTRL cells’ activity. The deviant behaviour was from the
fMLP-induced cells in the 10000x diluted standard which was close to but above that of the CTRL cells between 10 and 30 minutes.

Figure 8.20. The % inhibition of healthy isolated human neutrophils after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM4 and its diluted standards had a weak to moderate inhibitory effect on both the PMA and the fMLP-induced IHNs, which was sustained variably for the 60 minutes of testing, except for the 10000x diluted standard which had weak stimulatory effect on fMLP-induced cells from 15 to 30 minutes, and on PMA-induced cells at 45 minutes.
HM5- Stametta™ Body Healing Liquid

Figure 8.21. Luminol-enhanced luminescence (LEL) activity of healthy isolated human neutrophils stimulated after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The activity of the cells incubated with the HM5 and its diluted standards was generally below that of the CTRL cells. The fMLP-induced cells in the neat HM5 had activity lowest to that of the CTRL cells. The activities of the cells in the diluted standards oscillated, but it remained below the CTRL cells’ activity. The deviant behaviour from that was from the fMLP-induced cells in the 1000x (t =10 minutes) and 10000x (t = 25 minutes) diluted standard and in the PMA-induced cells (t = 5, 10 and 20 minutes) which were above that of the CTRL cells.
Figure 8.22. The % inhibition of healthy isolated human neutrophils after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM5 and its diluted standards had a weak to moderate inhibitory effect on both the PMA- and the fMLP-induced IHNs, which was sustained variably for the 60 minutes of testing, with a few minor exceptions that match the increased activity described in figure 8.21 above.
Figure 8.23. Luminol-enhanced luminescence (LEL) activity of healthy isolated human neutrophils stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The activity of the cells incubated with the HM6 and its diluted standards was generally below that of the CTRL cells. The fMLP-induced cells in the neat HM6 had activity lowest to that of the CTRL cells. The activities of the cells in the diluted standards oscillated, but it remained below the CTRL cells’ activity. The deviant behaviour was from the fMLP-induced cells in the 10000x (t = 0, 15 minutes) diluted standard which were above that of the CTRL cells.
Figure 8.24. The % inhibition of healthy isolated human neutrophils after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

All the diluted HM6 standards had a weak inhibitory effect on both the PMA and the fMLP-induced IHNs, which was sustained variably for the 60 minutes. The neat HM6 had moderate inhibitory effect at a few time intervals (t = 0 both cells and t = 15, 30 minutes of fMLP induced cells). The 1000x and the 10000x diluted standards had weak stimulatory effect on fMLP-induced cells at t = 0, 15, 25 and 30 minutes.
Table 8.2. The average LEL activity of healthy isolated human neutrophils incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% inhibition</td>
<td>LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control</td>
<td>16.17 ± 3.57</td>
<td>14.21 ± 2.64</td>
<td></td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>9.29 ± 1.06</td>
<td>40.7 ± 12.1</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>11.17 ± 4.41</td>
<td>22.4 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>13.67 ± 3.38</td>
<td>15.4 ± 8.5</td>
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<tr>
<td></td>
<td>1000x dilution</td>
<td>15.38 ± 2.38</td>
<td>3.3 ± 12.6</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>15.58 ± 3.50</td>
<td>3.6 ± 7.9</td>
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<tr>
<td>HM2</td>
<td>Neat</td>
<td>9.41 ± 1.70</td>
<td>39.4 ± 16.1</td>
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<tr>
<td></td>
<td>10x dilution</td>
<td>10.41 ± 1.37</td>
<td>33.9 ± 12.2</td>
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<tr>
<td></td>
<td>1000x</td>
<td>12.69 ± 2.80</td>
<td>21.3 ± 8.9</td>
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<td>10000x</td>
<td>13.52 ± 2.39</td>
<td>15.5 ± 10.2</td>
</tr>
<tr>
<td>HM3</td>
<td>Neat</td>
<td>8.81 ± 1.64</td>
<td>44.3 ± 12.2</td>
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<tr>
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<td>10x dilution</td>
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<td>34.5 ± 11.2</td>
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<td>100x</td>
<td>13.86 ± 2.03</td>
<td>11.7 ± 14.4</td>
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<tr>
<td></td>
<td>1000x</td>
<td>14.10 ± 2.37</td>
<td>11.4 ± 13.3</td>
</tr>
<tr>
<td></td>
<td>10000x</td>
<td>14.21 ± 2.27</td>
<td>10.4 ± 15.0</td>
</tr>
<tr>
<td>HM4</td>
<td>Neat</td>
<td>8.95 ± 1.35</td>
<td>42.9 ± 12.7</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>10.70 ± 1.81</td>
<td>31.3 ± 16.4</td>
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<td></td>
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<td>1000x</td>
<td>13.37 ± 1.75</td>
<td>15.4 ± 13.6</td>
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<tr>
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<td>10000x</td>
<td>14.97 ± 2.31</td>
<td>5.6 ± 14.6</td>
</tr>
<tr>
<td>HM5</td>
<td>Neat</td>
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<td>37.7 ± 16.0</td>
</tr>
<tr>
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<td>10x dilution</td>
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<td>27.2 ± 14.7</td>
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<td>12.62 ± 1.41</td>
<td>20.1 ± 12.1</td>
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<tr>
<td></td>
<td>1000x</td>
<td>12.96 ± 2.24</td>
<td>17.5 ± 16.0</td>
</tr>
<tr>
<td></td>
<td>10000x</td>
<td>14.39 ± 2.28</td>
<td>9.8 ± 11.9</td>
</tr>
<tr>
<td>HM6</td>
<td>Neat</td>
<td>9.34 ± 1.79</td>
<td>39.8 ± 16.7</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>11.04 ± 1.30</td>
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<td>100x</td>
<td>12.44 ± 4.22</td>
<td>23.9 ± 13.2</td>
</tr>
<tr>
<td></td>
<td>1000x</td>
<td>14.27 ± 5.35</td>
<td>12.9 ± 16.5</td>
</tr>
<tr>
<td></td>
<td>10000x</td>
<td>16.28 ± 4.64</td>
<td>0.1 ± 7.3</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 8.2 allow comparison of LEL activity and % inhibition at various concentrations for each standard. It shows that the CHMs had overall weak inhibitory effects on the isolated human neutrophils, both fMLP and PMA induced. The inhibitory effect was directly or inversely proportional to the concentration or it did not correlate. Some of the effects were statistically significant (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 9).
8.3 Luminescence activity of healthy platelets

The graphs below depict luminol-enhanced luminescence (LEL) activity of platelets stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the platelets at the various time intervals.

**HM1- *Intlamba Zifo™***

![Graph A](image)

![Graph B](image)

Figure 8.25. Luminol-enhanced luminescence (LEL) activity of healthy platelets after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The LEL activity of the fMLP-induced (A) CTRL cells increased rapidly from baseline after stimulation, peaked at 5 minutes, decreased gradually and returned to baseline level at 60 minutes. The activities of the cells in the diluted HM1 standards had activities very close to that of the CTRL platelets, and also mimicked the behaviour of the CTRL platelets. The activity of the PMA-induced CTRL platelets was highest on stimulation, and gradually declined until 60 minutes. The activities of the PMA-induced platelets in the diluted HM1 standards had activities very close to that of the CTRL platelets, above and below and also mimicked the behaviour of the CTRL platelets. Both the PMA- and fMLP-induced platelets in the neat HM1 had activity below the CTRL platelets, which generally did not change throughout the 60 minutes of testing.

Figure 8.26. The % inhibition of healthy platelets after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM1 had weak inhibitory effect on the platelets, which was sustained for the whole 60 minutes. The diluted standards had weak inhibitory effect, which was generally much lower than that of the neat HM1. There were few times where the diluted standards variably had weak stimulatory effect.

**HM2- Maphilisa™ Herbal medicine**

![Graph A](image)

![Graph B](image)

Figure 8.27. Luminol-enhanced luminescence (LEL) activity of healthy platelets after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the PMA- and fMLP-induced platelets in the neat HM2 had activity below the CTRL platelets, which stayed the same throughout the 60 minutes. The fMLP-induced platelets in the lowest three diluted standards had activity higher than the CTRL at stimulation which decreased until five minutes. Thereafter the activity increased, peaked at 10 minutes and gradually reduced until 60 minutes. The activity of the platelets in the 10x diluted HM2 started decreasing after stimulation until 60 minutes. The activities of the PMA-induced platelets in the diluted HM2 standards had activities below that of the CTRL platelets on stimulation. The activities remained very close to that of the CTRL platelets, above and below and also mimicked the behaviour of the CTRL platelets until 60 minutes of testing.

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Figure 8.28. The % inhibition of healthy platelets after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM2 had weak to moderate inhibitory effect on the fMLP-induced platelets, and a weak one on the PMA-induced platelets, which were sustained for the whole 60 minutes. The diluted standards had weak inhibitory effect, which was generally much lower than that of the neat HM2 except at $t = 0$ for fMLP-induced platelets where the effect was a weak stimulatory effect. There were few exceptions where the diluted standards variably had weak stimulatory effect.

**HM3- Matla™ African medicine for all diseases**

![Graph A](image1)

![Graph B](image2)

**Figure 8.29.** Luminol-enhanced luminescence (LEL) activity of healthy platelets after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of $n = 8$ indicated on graph).
The activities of both induced platelets in the diluted HM3 standards had activities very close to that of the CTRL platelets, and fluctuated with time either above or below that of the CTRL platelets. Both the induced platelets in the neat HM3 had activity below the CTRL platelets, which generally did not change throughout the 60 minutes of testing.

Figure 8.30. The % inhibition of healthy platelets after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of \( n = 8 \) are indicated).

The neat HM3 had weak to moderate inhibitory effect on the fMLP-induced platelets, and a weak one on the PMA-induced platelets, which were sustained for the whole 60 minutes. The diluted standards had weak inhibitory effect, much lower than that of the neat HM3 except at \( t = 0 \) for fMLP-induced platelets where the effect was a weak stimulatory effect.
There were few exceptions where the diluted standards variably had weak stimulatory effect, particularly the 10000x diluted HM3 standard.

**HM4- Ngoma™ Herbal Tonic Immune Booster**

![LEL activity of platelets](image)

Figure 8.31. Luminol-enhanced luminescence (LEL) activity of healthy platelets after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both the PMA- and fMLP-induced induced platelets in the neat HM4 and the 10x diluted standard had activities below the CTRL platelets, which generally remained constant until
60 minutes. The activities of both induced platelets in the other diluted HM4 standards had activities close to that of the CTRL platelets, and fluctuated with time either above or below that of the CTRL platelets.

Figure 8.32. The % inhibition of healthy platelets after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM4 and the 10x diluted HM4 standard had weak to moderate inhibitory effect on the fMLP-induced platelets, and a weak one on the PMA-induced platelets, which were sustained for the whole 60 minutes. These effects though were much more pronounced than the weak inhibitory effect of the other diluted standards. The other diluted standards had generally weak stimulatory effect on the platelets.
Figure 8.33. Luminol-enhanced luminescence (LEL) activity of healthy platelets after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both the PMA- and fMLP-induced induced platelets in the neat HM5 and the 10x diluted standard had activities below the CTRL platelets, which generally remained constant until 60 minutes. The activities of both induced platelets in the other diluted HM5 standards had activities close to that of the CTRL platelets, and fluctuated with time either above or below that of the CTRL platelets.
The neat HM5 had weak to moderate inhibitory effect on the fMLP-induced platelets, and a weak one on the PMA-induced platelets. The 10x diluted HM5 standard had a weak inhibitory effect on both induced platelets. The effects of the neat and the 10x diluted HM5 were persistent for the full 60 minutes. These effects though were much more pronounced than the weak inhibitory effect of the other diluted standards. The other diluted standards also had generally weak stimulatory effect on the platelets at few various time intervals.
Figure 8.35. Luminol-enhanced luminescence (LEL) activity of healthy platelets stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both the PMA- and fMLP-induced induced platelets in the neat HM6 and the 10x diluted standard had activities below the CTRL platelets, which remained constant until 60 minutes. The fMLP-induced platelets in the 100x diluted HM6 standard had the highest initial activity, which reduced rapidly until 5 minutes after which the activity remained stable until 20 minutes, followed by a slight decline until 60 minutes. The platelets in the 10000x diluted HM6 had activity which increased from baseline level which was above the CTRL platelets, and peaked at 10 minutes followed by a slight decline, yet remained above that
of the CTRL platelets until 60 minutes. The activity of the platelets in the 1000x diluted standard was below that of the CTRL platelets.

The PMA-induced platelets in the other three diluted HM6 standards had activities close to that of the CTRL platelets, and fluctuated with time either above or below that of the CTRL platelets, though the cells in the 100x diluted standard had the initial activity above the CTRL platelets.

Figure 8.36. The % inhibition of healthy platelets after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM6 had weak to moderate inhibitory effect on the fMLP-induced platelets, and a weak one on the PMA-induced platelets. The 10x diluted HM6 also had weak inhibitory effect on the platelets. These inhibitory effects were sustained for the whole 60 minutes were much more pronounced than the weak inhibitory effect of the other diluted standards. The other diluted standards had generally weak stimulatory effect on the platelets, while the 100x diluted standard had a moderate stimulatory effect on fMLP-induced platelets after stimulation.

Table 8.3. The average LEL activity of healthy platelets incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced platelets</th>
<th>PMA-induced platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>Control</td>
<td>Neat</td>
<td>0.00352 ± 0.00020*</td>
<td>40.5 ± 11.5</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.00569 ± 0.00077</td>
<td>5.7 ± 7.0</td>
</tr>
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<td></td>
<td>100x dilution</td>
<td>0.00594 ± 0.00096</td>
<td>1.9 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.00580 ± 0.00072</td>
<td>3.7 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.00572 ± 0.00075</td>
<td>7.8 ± 7.7</td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>0.00316 ± 0.00020*</td>
<td>46.2 ± 12.0</td>
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<tr>
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<td>10x dilution</td>
<td>0.00490 ± 0.00063*</td>
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<td>100x</td>
<td>0.00560 ± 0.00061</td>
<td>6.0 ± 15.2</td>
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<tr>
<td></td>
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<td>8.1 ± 11.4</td>
</tr>
<tr>
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<td>10000x</td>
<td>0.00529 ± 0.00043</td>
<td>11.2 ± 12.6</td>
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<tr>
<td>HM2</td>
<td>Neat</td>
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<td>48.2 ± 10.3</td>
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<td>10x dilution</td>
<td>0.00502 ± 0.00057*</td>
<td>15.7 ± 14.4</td>
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<td>0.00580 ± 0.00058</td>
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<td>0.00625 ± 0.00077</td>
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<td></td>
<td>10x dilution</td>
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<td>42.6 ± 10.7</td>
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<td>0.00627 ± 0.00081</td>
<td>-4.6 ± 13.8</td>
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<td>0.00602 ± 0.00079</td>
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<td>-2.7 ± 10.5</td>
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<td></td>
<td>10000x</td>
<td>0.00552 ± 0.00066</td>
<td>8.0 ± 11.0</td>
</tr>
<tr>
<td>HM5</td>
<td>Neat</td>
<td>0.00340 ± 0.00023*</td>
<td>42.6 ± 10.9</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.00467 ± 0.00050*</td>
<td>22.1 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>100x</td>
<td>0.00586 ± 0.00114</td>
<td>1.5 ± 25.4</td>
</tr>
<tr>
<td></td>
<td>1000x</td>
<td>0.00555 ± 0.00072</td>
<td>7.3 ± 14.6</td>
</tr>
<tr>
<td></td>
<td>10000x</td>
<td>0.00657 ± 0.00066</td>
<td>-9.5 ± 10.4</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 8.3 allow comparison of LEL activity and % inhibition at various concentrations for each standard. The values indicate the stimulatory and
inhibitory effects of the CHMs ranging from weak to moderate. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they do not correlate. The effects are generally comparable between the two mechanisms of stimulation of the platelets (fMLP or PMA), and some are statistically significant effects (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 9).
CHAPTER 9 DISCUSSION: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM HEALTHY VOLUNTEERS

The determination of chemiluminescence activity is a direct measurement of the amount of light emitted by cells when stimulated. The light emitted is derived from the respiratory burst of cells, which is the increased oxygen consumption that occurs, resulting in the formation of ROS. Therefore the light emitted is directly proportional to the amounts of ROS formed, and is also a measure of whether the cells are stimulated and how much are they stimulated. Luminol was the luminescence enhancer used to amplify the light signal detected; hence the study was investigating the luminol-enhanced luminescence (LEL) activity of cells. The two chemotactic agonists/activators –PMA and fMLP were used to stimulate the cells. These two agonists act via different mechanisms on the cells to stimulate production of ROS by the cells hence their use was of importance in the differentiation of response of the cells when stimulated by different mechanisms. PMA directly activates protein kinase C (PKC) resulting in activation of NADPH oxidase (NOX). The agonist, fMLP binds to specific G-protein-linked formyl peptide receptors on the cell membrane, initiating a cascade of reactions that begin with the activation of phospholipase C (PLC) leading to activation of PKC, which in turn activates NOX.

The graphs shown before in chapter 8 indicated the time-based changes that occurred in the LEL activity of the cells and platelets in 60 minutes. It was however important to show average activities for that time and the corresponding % inhibitions so as to be able to make comparisons and draw conclusions. The average LEL activities and % inhibitions are displayed in the tables 8.1 (page 104), 8.2 (page 119) and 8.3 (132). The average effects of each HM and its dilutions on the whole blood phagocytes (table 8.1), the neutrophils (table 8.2) and the platelets (table 8.3) are discussed below.

HM1- Intlamba Zifo™

- The highest concentration of HM1 (neat) and its two lower dilutions (10x, 100x) caused stimulation of whole blood phagocytes, indicated by LEL activity which is higher than the CTRL, and negative % inhibitions, which fall in the range of weak stimulation
(<50%). The values reflected in table 8.1 showed that the weak stimulation was directly concentration dependent, meaning that the higher the concentration of HM1, the higher the stimulation effect. The two higher dilutions of HM1 (1000x, 10000x) had weak inhibitory effect on the phagocytes, which was also directly concentration dependent. Therefore, the higher the concentration of HM1, the higher the inhibition (the lower the activity) of whole blood phagocytes.

The behaviour of the fMLP-induced cells and PMA-induced cells was similar. The time-based response to stimulation differed (figure 8.1, page 90), with the highest LEL activities occurring on stimulation in PMA-induced cells, and at 10 minutes after stimulation in fMLP-induced cells. On average the LEL activity and % inhibitions were comparable. There was no statistical significance to the difference in activity between fMLP-induced cells and those induced with PMA. Therefore, the effect of HM1 on whole blood phagocytes was similar, whether via a G-protein receptor binding mechanism (fMLP) or a direct PKC activating mechanism (PMA).

- The neat HM1 and its dilutions had weak inhibitory effects on fMLP- and PMA-induced isolated human neutrophils (IHNs). The effects were directly concentration dependent among the neat HM1 and the three lower diluted standards (10x, 100x, 1000x) in fMLP-induced cells. The effects were directly concentration dependent among the neat and the two lower diluted standards (10x, 100x) in PMA-induced cells. The behaviour of the neutrophils was identical, in that both the PMA and the fMLP-induced cells had their highest LEL activity on stimulation. The difference was that the fMLP-induced cells had higher activity than the PMA-induced cells. It implies that the IHNs incubated with HM1 were more responsive to stimulation by fMLP than PMA. Since fMLP is a receptor based mechanism, this could mean that the HM1 sensitises the receptor in some way, or has some permissive effect on the formyl peptide receptors on the cell membrane. However, the difference in the initial LEL activity of the IHNs was statistically not significant.

- The neat HM1 and its dilutions had a weak inhibitory effect on fMLP-induced platelets, which was directly concentration dependent among the neat and the two lower diluted standards (10x, 100x). The effect was however, inversely concentration dependent among the three higher diluted standards (100x, 1000x, 10000x). The higher the concentration of HM1, the higher the inhibition effect and the lower the LEL activity. Yet the lower the concentration, the lower the inhibition and the higher the activity, however the activity did not rise above the control. The neat HM1 and its dilutions had weak inhibitory effects on PMA-induced platelets, which were directly concentration dependent
among the neat and the diluted standards, excluding the 1000x diluted standard which had a weak stimulatory effect on the platelets. The differences in LEL activities of both induced platelets in the neat HM1 were statistically significant when compared to the controls.

**HM2- Maphilisa™ Herbal medicine**

- The neat HM2 had a weak inhibitory effect on both fMLP- and PMA-induced whole blood phagocytes, with statistically significant activity in the fMLP-induced cells. The diluted HM2 standards had weak to moderate stimulatory effect which was directly concentration dependent. The 10x diluted HM2 had moderate stimulatory effects on both fMLP and PMA-induced cells. The cells had the same highest LEL activity (on stimulation in PMA-induced cells, and after 10 minutes of stimulation in fMLP-induced cells), and the differences as compared to the control were both statistically significant.

- HM2 had a weak inhibitory effect on IHNs, which was directly concentration dependent. The LEL activity of the neutrophils decreased as the strength of the solutions increased. The lowest activity was observed in the IHNs in the neat HM2. The LEL activities were statistically significant in the neat HM2 and the three lower dilutions in fMLP induced cells, and in the neat HM2 and the 10x diluted HM2 in PMA-induced cells. All the IHNs except those in the neat HM2 had their highest activity on stimulation (Figure 8.15, page 109).

- The HM2 and dilutions had weak inhibitory effects on both PMA and fMLP-induced platelets. The effects were directly concentration dependent among the neat and the two lower diluted standards (10x, 100x). The effects were however, inversely concentration dependent among the three higher diluted standards (100x, 1000x, 10000x) in fMLP-induced platelets, and in the two higher diluted standards (1000x, 10000x). The higher the concentration of HM2, the higher the inhibition effect and the lower the LEL activity. Yet the lower the concentration, the lower the inhibition and the higher the activity, however the activity did not rise above the control. Statistically significant differences in the LEL activities were noted between the controls and the neat HM2 and the 10x diluted HM2 standard in both induced platelets, and in the 100x diluted HM2 standard in PMA-induced platelets.
HM3- *Matla™* African medicine for all diseases

- The neat HM3 caused reduced LEL activity in whole blood phagocytes exerting a weak inhibitory effect; and its dilutions caused increased activity, exerting a weak stimulatory effect. All the LEL activities were statistically significant. The stimulatory effects were non-systematic, but were directly concentration dependent among the three higher diluted standards (100x, 1000x, 10000x).
- The HM3 had weak inhibitory effects on the fMLP- and PMA-induced IHNs. The effects were directly concentration dependent in the fMLP-induced cells and among the neat and the two lower diluted standards (10x, 100x) in PMA-induced neutrophils. The effects were inversely concentration dependent among the three higher diluted standards (100x, 1000x, 10000x). Statistically significant differences in the LEL activities were noted between the controls and the neat HM3 and the 10x HM3 diluted standards in both the induced cells.
- With the exclusion of the 10000x HM3 diluted standard, the HM3 had a weak inhibitory effect on the fMLP- and PMA-induced platelets. The effects were directly concentration dependent in the fMLP-induced platelets and among the neat and the two lower diluted standards (10x, 100x) in PMA-induced platelets. The 10000x HM3 diluted standard had a weak stimulatory effect on both induced platelets. Statistically significant differences in the LEL activities were noted between the controls and the neat HM3 and the 10x HM3 diluted standards in both the induced platelets. Therefore HM3 had weak, concentration dependent inhibitory effect at higher concentrations, and a weak stimulatory effect at very low concentrations on platelets.

HM4- *Ngoma™* Herbal Tonic Immune Booster

- The neat HM4 exerted a weak inhibitory effect in both induced whole blood phagocytes, causing reduced LEL activity; while its dilutions exerted weak stimulatory effects, causing increased activity. The stimulatory effects were directly concentration dependent among the three higher diluted standards (100x, 1000x, 10000x). It meant that the higher the concentration, the higher the stimulation. The differences in the LEL activities were all statistically significant when compared to the controls. The HM4 therefore, affected the fMLP- and PMA-induced cells comparably.
• The HM4 had a direct concentration dependent and weak inhibitory effect on the IHNs. The higher the concentration, the lower the LEL activity, and the higher the inhibitory effect. Statistically significant differences in the LEL activities were noted between the controls and the neat HM4 and the two lower HM4 dilutions in both the fMLP- and PMA-induced neutrophils. The HM4 and its dilutions affected the fMLP- and the PMA-induced cells similarly.

• The neat HM4 and the two diluted standards (10x, 10000x) had weak, direct concentration dependent inhibitory effect on both the PMA- and fMLP-induced platelets. The other 100x and 1000x diluted standards had weak, inversely concentration dependent stimulatory effects on the platelets. The LEL activities of the platelets in the neat HM4 and the 10x diluted standard had statistically significant differences when compared to the controls.

**HM5- Stametta™ Body Healing Liquid**

• The neat HM5 and the 10x HM5 diluted standard exerted weak inhibitory effects and caused reduced LEL activity in both fMLP- and PMA-induced whole blood phagocytes. The three higher HM5 diluted standards had weak (by the 10000x diluted HM5) and moderate (by the 100x and the 1000x diluted HM5) directly concentration dependent stimulatory effects on the cells. The differences in LEL activities when compared to the controls were statistically significant except those of the fMLP-induced cells in the neat HM5 and the PMA-induced cells in the 10x diluted HM5 standard. Therefore HM5 had a weak concentration dependent inhibitory effect at high concentrations, and weak to moderate concentration dependent stimulatory effect at low concentrations, similarly on the fMLP and PMA-induced whole blood phagocytes.

• The HM5 and diluted standards had a directly concentration dependent and weak inhibitory effect on the fMLP-induced IHNs. The higher the concentration, the higher the inhibitory effect and the lower the LEL activity. The HM5 and diluted standards had weak inhibitory effects on the PMA-induced neutrophils, which were directly concentration dependent if the 100x diluted standard was excluded. The differences in LEL activities when compared to the controls were statistically significant in all the cells except those in the 1000x diluted HM5 (fMLP-induced) and the 100x and 10000x diluted HM5 standards (PMA-induced).
With the exclusion of the 1000x diluted HM5, the neat HM5 and the other diluted standards exerted weak inhibitory effects on the fMLP- and the PMA-induced platelets. The effects were directly concentration dependent in the PMA-induced platelets and among the neat and the two lower diluted standards (10x, 100x) in fMLP-induced platelets. The 10000x HM5 diluted standard had a weak stimulatory effect on fMLP-induced platelets, while it had a weak inhibitory effect on PMA-induced platelets which did not correlate with the effects of the other platelets. The differences in LEL activities when compared to the controls were statistically significant in the neat and the 10x HM3 diluted standard.

**HM6- Vuka Uphile™ Immune Booster**

- The neat HM6 exerted a weak inhibitory effect in whole blood phagocytes, causing reduced LEL activity; while its dilutions exerted weak stimulatory effects, causing increased activity. The stimulatory effects in the fMLP-induced cells were directly concentration dependent. The stimulatory effects on the PMA-induced cells were directly concentration dependent in the three lower diluted standards, and were non-systematic when all the four diluted standards were compared. The differences in LEL activities when compared to the controls were statistically significant, except that of the PMA-induced cells in the 10000x diluted standard.

- The HM6 and diluted standards had weak inhibitory effects on the fMLP- and PMA-induced IHNs. The effects in the fMLP-induced cells were directly concentration dependent. The effects in the PMA-induced neutrophils were different with each standard, but non-systematic. The differences in LEL activities when compared to the controls were statistically significant in the neat HM6, the 10x and the 1000x diluted HM6 standards in both the fMLP- and PMA-induced neutrophils.

- The HM6 and its three lower dilutions had weak, direct concentration dependent inhibitory effect on fMLP-induced platelets, while the 10000x diluted HM6 had a weak stimulatory effect. HM6 had a weak inhibitory effect on PMA-induced platelets which was directly concentration dependent in the neat HM6 and the two lower diluted standards (10x, 100x). The differences in LEL activities when compared to the controls were statistically significant in the neat and the 10x diluted HM6 of both induced platelets.
Table 9.1. Summary of the overall effects of the HMs on healthy cells and platelets, from the highest to the lowest concentration

<table>
<thead>
<tr>
<th></th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>HM1</td>
<td>+↓</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>HM2</td>
<td>↓+†</td>
<td>↓+†</td>
<td>↓</td>
</tr>
<tr>
<td>HM3</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
<tr>
<td>HM4</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
<tr>
<td>HM5</td>
<td>↓+†</td>
<td>↓+†</td>
<td>↓</td>
</tr>
<tr>
<td>HM6</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
</tbody>
</table>

↓ weak inhibition; ↓↓ moderate inhibition; ↓↓↓ potent inhibition
+ weak stimulation; † moderate stimulation; ‡ potent stimulation

9.1 Implications of the effects of the HMs on the healthy cells and platelets

It was stated in the WHO general guidelines for methodologies on research and evaluation of TM that the safety data obtained from in vitro tests might not be absolute markers of safety, but should be seen as indicators of potential toxicity (WHO, 2000). It is imperative to describe what the results obtained in the study infer. In this study, the increase in luminescence activity when compared to the control is translated as stimulation of the cells, and reduction in activity as inhibition. PMA and fMLP are chemotactic agonists that result in the activation of the NADPH oxidase system, which causes formation of ROS within cells. Neutrophils were tested while in whole blood and when isolated. Whole blood CL is the technique that provides information about the quantity and the metabolic activity of neutrophils in plasma and in the presence of other cellular elements including other phagocytes and the erythrocytes (Ristola & Repo, 1989; Nordman, Nyberg, Linko, 1994).

There was overwhelming inhibition of the isolated neutrophils by all the six HMs, while in whole blood there was variable inhibition as well as degrees of stimulation at different concentrations of the HMs. In other studies, extracts of medicinal plants were tested and found to have inhibitory effects on neutrophils and other phagocytes. It was concluded that extracts that inhibited the generation of ROS and hence were able to modulate the innate immune response may serve as new and natural immunomodulatory agents (Jantam et al., 2011; Mahomoodally et al., 2012). In concordance with those studies, the HMs in this
study inhibited the LEL activity of neutrophils when isolated as well as in whole blood. Inhibition of the production of ROS in the cells confers *in vitro*, immunomodulatory potential to these HMs. It therefore provides the rationale for the use of these HMs as immune boosters. The results tend to validate the use of these HMs though they have not been tested *in vivo*.

The cells were stimulated in the presence of the various HMs, to different levels. Activation of the cells means there was increased activity of the NOX, which resulted in the formation of ROS. The ROS include SOA, H$_2$O$_2$, singlet oxygen, HOCl and hydroxyl radicals. The weak stimulation by the HMs was generally sustained for the 60 minutes of testing. This implied that the formation of the ROS could be maintained at those levels within the cells for at least an hour. Stimulation via the fMLP route signifies that in the presence of the HMs, the formyl peptide receptor was more responsive, meaning that the HM may have sensitized the receptor. It could also be that the HM acted on other sites, which then have permissive effects on the formyl peptide receptor activity, resulting in the increased activity of NOX. Stimulation via the PMA route means that in the presence of the HM, some elements of PKC signalling route are sensitised, such that when PKC is activated, the response by the cell is amplified. It could also be that the enzyme itself, PKC is sensitised. The HMs therefore served as "priming agents" which means they did not induce a strong activation directly, but potentiated significant respiratory burst in the cells in response to other activators. They induced a weak production of ROS, which would enhance a stronger production of ROS after exposure to another activator (Ciz et al., 2012). Stimulation of neutrophils and other phagocytes may be beneficial, as part of immune-boosting effects. The rationale is that activated neutrophils would respond rapidly and highly, and be more efficient as elements of the immune system. Hence it may assist in the body resisting infections and diseases.

The presence of ROS may have both negative and positive attributes in a system. As it was stated earlier the effectiveness of neutrophils as phagocytes is determined by a functional NOX system which generates ROS. ROS serve as potent antimicrobial agents that play an important role of defence against pathogens. They were also said to be a threat to bystander host cells and tissues in the vicinity of an inflammatory reaction. ROS have been implicated in inflammatory tissue damage, that they may cause damage by destroying surrounding tissue and inducing apoptosis in other immune reactive cells (Dahlgren & Karlsson, 1999; Bjorkman et al., 2008; Tintinger, Steel, Theron, Anderson,
It was reported that inappropriate activation of neutrophils and extensive release of intracellular oxidising agents contributed to the development and progress of many acute and chronic inflammatory disorders of infective and non-infective origin. The airways and the cardiovascular system were noted to be particularly vulnerable and some of the conditions included respiratory disease syndrome, asthma, RA, atherosclerosis, COPD, stroke, and myocardial infarction (Caldefie-Chezet et al., 2002; Tintinger et al., 2008). Droge (2002) stated that an excessive and/or sustained increase in ROS production was associated with the pathogenesis of many diseases, and as such the functions of neutrophils must be regulated appropriately, to avoid tissue damage and to achieve proper host defense. Paula et al. (2009) also stated that increased neutrophil activation was implicated in the pathogenesis of inflammatory and autoimmune disorders such as systemic lupus erythematosus, RA, emphysema, atherosclerosis and Chron’s disease. It therefore needs to be noted that while it is suggested that the stimulation of neutrophils and other phagocytes may be beneficial for healthy individuals, it may also lead to the development of acute and chronic inflammatory disorders and immune diseases mentioned. Healthy individuals are simply persons undiagnosed with any specific condition; however these people may be at risk of, or have a genetic predisposition to undiagnosed conditions. The intake of the HMs may therefore accelerate progression towards or trigger the onset of any of the mentioned conditions.

The inhibition of LEL activity means that in the presence of the HMs, the cells could not respond when prompted. It implied that the HMs inhibited the pathways of stimulation. This could have occurred by blocking the receptor, specifically the formyl peptide receptor, such that the agonist, fMLP could not bind to the receptor. It could also have occurred by components of the HM binding to and inhibiting some elements of the NOX, which could then prevent the assembly of the enzyme if the receptor was stimulated. The inhibition of the PMA pathway could have occurred by binding to the enzyme PKC and inhibiting its activity, or also by inhibiting some components of the NOX which would have prevented its assembly. As postulated by Mahomoodally et al. (2012), inhibition of both fMLP and PMA mechanisms suggests that the HMs do not affect a specific pathway, instead they may directly inhibit a common biochemical target site such as NOX or scavenge the ROS. Also, herbal medicines that inhibit ROS production may be useful in averting tissue destruction and therefore delaying the onset of numerous diseases. Perez-Garcia et al. (2001) also noted that optimal function of a host defence system depends upon an ample supply of
antioxidants that can act as preventative or therapeutic agents inhibiting ROS production. They reported that the inhibitory effects of plant extracts suggested that the extracts act as scavenging agents, reacting directly with the free radicals. Therefore, the inhibitory effects of the HMs in this study may be indicative of the role of the HMs as antioxidants or scavengers of ROS, which may benefit the users as prophylaxis against the onset and progress of the various conditions mentioned above.

Inhibition of neutrophils could have serious implications in the health and wellbeing of individuals. The role of neutrophils as effector cells of the immune system is fulfilled by their ability to respond to stimuli, whereby they would then migrate to sites of inflammation through vessel walls, recognize and phagocytose opsonized molecules and pathogens, and destroy them using ROS generated by NOX and hydrolytic granule proteins (Dahlgren & Karlsson, 1999; Faurschou & Borregaard, 2003; Roos et al., 2003; Panasiuk et al., 2005; Bylund et al., 2010; Porter & Kaplan, 2011). The inhibition of neutrophils in healthy individuals means that the immune system would be compromised. Although there was generally a weak inhibitory effect, it still suggests that the neutrophils would always be operating at a non-optimum level. This may predispose individuals to infections and increase the risk of acquiring diseases. It has been stated that neutrophils (polymorphonuclear leukocytes) play a crucial role in the resistance of infections, and the reduced function thereof is linked to susceptibility to bacterial infections (McManus et al., 2001). This may be the first point of possible negative impact of the use of HMs on the conventional treatment of infectious diseases. The success of bacteriostatic antimicrobials as treatment of infections depends on a viable immune system. Therefore, in the presence of HMs, there may be failure of these antimicrobials, or a need for prolonged duration of administration.

The results in the study indicated the inhibitory effects of the HMs to be directly or inversely related to the concentration of the HM. The stimulation was also directly or inversely related to the concentration. In other instances, the effects were random, hence they could not be extrapolated whether they would increase or decrease from one concentration to the next. The implication is that the effect may be dose-related either incrementally or decrementally, or unforeseeable. Since the doses of most of the HMs are generally unspecified or unknown, it is critical to note that the effect may therefore be unpredictable.
Platelets are a key element of the process of haemostasis and also form a crucial part of the inflammatory response and the immune system. Chemiluminescence studies of platelets are done to show their activity within the immune system and the inflammatory response. These studies have also been done to show the role of platelets in various cardiovascular disorders. Platelets normally circulate in inactive state and become activated when a blood vessel is damaged (Krotz et al., 2004; Ghoshal & Bhattacharyya, 2014; Trzeciak-Rycek, Torkaz-Deptula, Deptula, 2013). They may also be activated by the presence of chemical mediators released by other cells involved in inflammation and the immune response. The platelets in the study were inhibited or stimulated depending on the HM and the concentration thereof.

The weak stimulation of platelets by the HMs implies that platelets may be circulating in sensitised form. An immune-boosting role would be that activated platelets might respond rapidly and favourably, and be more beneficial as components of the immune system. Generally, activated platelets secrete chemical mediators which enhance the interaction of platelets with other cells of the immune system. These include the recruitment of neutrophils to inflammatory tissue (Zarbock, Polanowska-Grabowska, Ley, 2007; Semple & Freedman, 2010) and the formation of aggregates with neutrophils, monocytes and lymphocytes (Ghoshal & Bhattacharyya, 2014). They also secrete substances that directly destroy pathogens. Some of the substances include antibacterial platelet microbicidal proteins (PMP) from α granules and ROS generated by platelet NOX. The reported ROS include O$_2^-$, H$_2$O$_2$, and OH$^-$ (Krotz et al., 2004; Nauseef, 2014). The platelet-neutrophil complexes perform immune functions such as phagocytosis, cytotoxicity and cytolysis more effectively than neutrophils on their own (Semple, Italiano, Freedman, 2011).

The weak stimulation of platelets by the HMs also implies that a stimulus may cause a response which may seem to be exaggerated. Such hyperreactive/ hyperresponsive platelets may increase the risk of thromboembolic disorders. The presence of platelet-derived ROS, predominantly NOX-dependent O$_2^-$ increased the recruitment of neutrophils to a growing thrombus (Krotz et al., 2004). The hyperreactive/ hyperresponsive platelets may also result in uncontrolled and /or exaggerated inflammatory response and immune reaction, which is one of the bases of development of allergic reactions. Platelet-neutrophil interactions were implicated in the development and progression of diseases such as ARDS, myocardial ischaemia and atherosclerosis (Ruf et al., 1992).
The weak inhibitory effects on platelets imply that platelets may be circulating in hypoactive form, as in the principle of routine platelet inhibition therapy (antiplatelet therapy) such as intake of low dose aspirin. In healthy individuals with normal functioning platelets, suppression of platelets may be more harmful than beneficial. The risk of bleeding or excessive bleeding is a known complication of antiplatelet therapy as well as any drug with antiplatelet side effects. Similarly, the weak inhibition of platelets by the HMs may increase the risk of bleeding in the healthy individuals who take the HMs. Although in vivo tests are the most valid to verify pharmacokinetics of the HMs, the study suggests that the inhibition on platelets may last as long as 60 minutes after administration, especially in high doses. Therefore the risk of bleeding may be as long as the inhibition lasts. It was noted that patients who are on a similar antiplatelet regimen may have a highly variable antiplatelet response. Some patients may have little or no response, while others may have high inhibitory effects, which oblige for platelet function testing in such patients (Ferreiro, Sibbing et al., 2010). Since the inhibitory effects on platelets in the study were directly or inversely concentration dependent, or random, it suggests the possibility of variable responses in users of the HMs. It was also stated that high and low levels of inhibition of platelets are closely linked to the risk of bleeding events as well as ischaemic events, respectively (Ferreiro, Sibbing et al., 2010). Therefore the platelet inhibition due to the presence of the HMs may lead to increased risk of either bleeding or ischaemic events.

The inhibition of platelets may also have negative impact on the clinical outcomes, since patients or users of ATMs tend not to disclose their use. Prolonged and/or excessive bleeding may be a complication of among others anaesthesia, trauma and dermatologic surgery. Because of their effect, it is recommended that herbal medicines be stopped before any procedure, with time periods ranging from one day to about two weeks before (Lawrence & Whitaker, 2001; Owen, 2005; Wong & Townley, 2011; Dippenaar, 2015). Acute postoperative wound haemorrhage had occurred in a patient who had continued using some herbal medicines until the day of surgery. The HM that the patient had taken included ginkgo, which has one compound, ginkgolide, a known inhibitor of platelet activating factor (Jayasekera, Moghal, Kashif, 2005). Postoperative bleeding in dermatologic surgery was said to cause necrosis, increased risk of infections, wound dehiscence, scar formation and pain (Lawrence & Whitaker, 2001). Hence it is needed that patients going for surgery be probed about the use of TMs and CHMs as some contain herbs such as ginger that are known to have antiplatelet activity.
CHAPTER 10 RESULTS: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM ASTHMATIC PATIENTS

The effects of the six CHMs on the LEL activities of the IHNs, PLTs and whole blood phagocytes obtained from asthma patients are shown below. The results for all the three components are first displayed in graphs of luminescence (in RLUs) versus time (in minutes) and the % inhibitions which indicated the kinetics observed over 60 minutes. Then the average LEL activities and the % inhibitions over 60 minutes were calculated and are displayed in the tables below.

10.1 Luminescence activity of whole blood phagocytes from asthmatic patients

The graphs below depict LEL activity of whole blood phagocytes stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibitions of the HMs on the cells at the various time intervals.
The activity of the fMLP-induced (A) CTRL cells increased after stimulation peaked at 10 minutes, decreased, and then remained constant until 60 minutes. The cells in the neat HM1 had activity higher than the CTRL cells on stimulation, which then decreased gradually, stabilised around 25 minutes and stayed constant till 60 minutes. The LEL activities of the cells in the diluted HM1 standards increased variably after stimulation, all peaking at 10 minutes and gradually decreased and stabilised until 60 minutes. Their activity mimicked that of the CTRL cells.
The PMA-induced (B) CTRL cells’ activity remained the same from point of stimulation, which was the same as that in the fMLP-induced cells, until 60 minutes. This signifies that the cells did not respond to PMA stimulation. The cells in the 10x diluted HM1 standard had high activity on stimulation, which initially reduced rapidly until 5 minutes, then gradually and then remained stable until 60 minutes. The cells in the other diluted standards had activities above the CTRL cells on stimulation, but the activities reduced and stabilised till 60 minutes. It is worth noting that the activities seemed to be slightly increased around 45 minutes.

Figure 10.2. The % inhibition of asthmatic whole blood phagocytes after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The HM1 and dilutions generally had variable weak stimulatory effect on whole blood phagocytes, except in the fMLP-induced cells where the neat HM1 had a weak inhibitory effect.

**HM2- Maphilisa™ Herbal medicine**

Figure 10.3. The LEL activity of asthmatic whole blood phagocytes after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of $n = 8$ indicated on graph).
The cells incubated with the 10x diluted HM2 had notable LEL activity in both the PMA and the fMLP pathways, but the fMLP -induced cells had much higher RLU values than the PMA -induced cells, and they were all significant.

The cells incubated with the other test standards as well as the neat HM2 had comparable activities, closer to the controls. The cells exposed to the neat HM2 showed LEL activity less than the controls in both the fMLP- and PMA-induced cells.

Figure 10.4. The % inhibition of asthmatic whole blood phagocytes after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
There was variable weak inhibitory effect in fMLP-induced cells (A) by the neat HM2. This effect was weaker in the PMA-induced cells. Other than that exception, there was general stimulatory effect on cells, as evidenced by the negative values of % inhibition. The highest stimulation effect was caused by the 10x diluted HM2 in both the fMLP and PMA-induced cells, though the effect was greatly pronounced in the PMA-induced cells. The stimulation effect in the cells varied from weak, moderate and high.

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**Figure 10.5.** The LEL activity of asthmatic whole blood phagocytes after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the fMLP-induced and the PMA-induced cells incubated with neat HM3 had the lowest LEL activities, lower than the CTRL cells activities. The fMLP-induced cells in the 10000x diluted HM3 had the highest activity of all the cells. All the cells in the other standards had activities closer to those for the CTRL cells. The activities decreased between 0 and 5 minutes, increased thereafter, peaking at 10 minutes for fMLP-induced cells and at various times for the PMA-induced cells (10, 15 or 20 minutes), then declined and stayed constant until 60 minutes.

Figure 10.6. The % inhibition of asthmatic whole blood phagocytes after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM3 had a weak inhibitory effect on both the fMLP- and PMA-induced cells. The 10x diluted HM3 standard had opposite effects on the cells, - a weak inhibitory effect on fMLP-induced cells (except at t = 0) and a weak stimulatory effect on the PMA-induced cells. The other test standards had stimulatory effect on the cells, with the lowest concentration solution (10000x dilution) exerting the highest effect on average.

HM4- *Ngoma™* Herbal Tonic Immune Booster

Figure 10.7. The LEL activity of asthmatic whole blood phagocytes after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The cells in the 100x diluted HM4 standard had the highest LEL activity on stimulation which reduced rapidly in 5 minutes. This activity was equally high in both PMA- and fMLP-induced cells and statistically significant. The activity stabilised and reduced until 60 minutes in the PMA-induced cells. The fMLP-induced cells’ activity rose and then started declining after 10 minutes until 60 minutes. The cells in the other diluted HM4 standards had activity close to the CTRL cells, either above or below the activity of the CTRL cells. Cells in the neat HM4 displayed the lowest activities, below the CTRL cells.

Figure 10.8. The % inhibition of asthmatic whole blood phagocytes after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA (Means of n = 8 are indicated).
The neat HM4 and the 10x diluted standard had weak inhibitory effects on both induced cells, but the effect was more pronounced in the fMLP-induced cell. The other diluted HM4 standards had weak stimulatory effect on the cells. The 100x diluted HM4 had a stimulatory effect which was high on both cells on stimulation, was generally weak on the fMLP-induced cells and moderate on the PMA-induced cells.

**HM5- Stametta™ Body Healing Liquid**

Figure 10.9. The LEL activity of asthmatic whole blood phagocytes after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the fMLP- and the PMA-induced cells in the neat HM5 showed the lowest LEL activities, below the CTRL cells. The activities of the cells in the diluted standards closely mimicked that of the CTRL cells, with the exception of fMLP-induced cells in the 10x diluted standard. These cells showed their highest activity on stimulation. PMA-induced cells in the 100x diluted HM5 standard had high LEL activity, which declined rapidly until 5 minutes, then stabilised until 60 minutes. The cells in the other diluted HM5 had activities that closely mimicked that of the CTRL cells, with the activities almost the same on stimulation and at 60 minutes.

Figure 10.10. The % inhibition of asthmatic whole blood phagocytes after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM5 had weak inhibitory effects on both induced cells, but the effect was more
pronounced in the fMLP-induced cell. The other diluted HM5 standards had weak
stimulatory effect on the cells. The 100x diluted HM5 had a stimulatory effect which was
high on both cells on stimulation, was thereafter generally weak on the fMLP-induced cells
and moderate on the PMA-induced cells. The 10x diluted standard had opposite effects on
the cells, - a weak inhibitory effect on fMLP-induced cells (except at t = 0) and a weak
stimulatory effect on the PMA-induced cells.
The cells incubated with the diluted HM6 standards showed comparable LEL activities, close to but above and below the control, and the activities remained consistent for the 60 minutes of test time. The fMLP-induced cells in the neat HM6 had reduced activity, below the control, whereas the PMA-induced cells had activity much close to that of the CTRL cells.
Figure 10.12. The % inhibition of asthmatic whole blood phagocytes after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM6 had a weak inhibitory effect on the fMLP-induced cells. The 10x diluted M6 standard had the lowest stimulation effect on the fMLP-induced cells, yet it had a weak stimulatory effect (t= 0 min) on these cells. The other dilute standards had weak inhibitory effect on the fMLP-induced cells, except the 10000x diluted standard which also had weak stimulatory effect. The neat HM6 and its diluted standards had variably weak stimulatory and weak inhibitory effects on PMA-induced cells.
### Table 10.1. The average LEL activities of asthmatic whole blood phagocytes incubated with the HMs and the % inhibitions over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>Control</td>
<td>Neat</td>
<td>7.07 ± 0.70</td>
<td>5.70 ± 0.18</td>
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<tr>
<td>HM1</td>
<td>Neat</td>
<td>5.83 ± 0.94*</td>
<td>16.29 ± 19.66</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>9.20 ± 1.52*</td>
<td>-29.82 ± 13.48</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>7.41 ± 1.35</td>
<td>-4.13 ± 11.70</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>7.93 ± 1.75</td>
<td>-11.60 ± 17.14</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>7.13 ± 0.76</td>
<td>-1.14 ± 10.93</td>
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<td>HM2</td>
<td>Neat</td>
<td>4.74 ± 0.49*</td>
<td>32.30 ± 10.84</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>9.57 ± 1.07*</td>
<td>-36.31 ± 20.43</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>7.31 ± 0.77</td>
<td>-3.46 ± 5.77</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>7.81 ± 1.21</td>
<td>-10.04 ± 7.85</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>6.74 ± 0.60</td>
<td>4.46 ± 6.08</td>
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<tr>
<td>HM3</td>
<td>Neat</td>
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<td>32.06 ± 4.89</td>
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<td>10x dilution</td>
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<td>10.10 ± 8.97</td>
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<td></td>
<td>100x dilution</td>
<td>7.19± 0.89</td>
<td>-1.71 ± 7.69</td>
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<tr>
<td></td>
<td>1000x dilution</td>
<td>7.41± 0.79</td>
<td>-5.48 ± 15.08</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>8.18± 1.31*</td>
<td>-15.44 ± 12.09</td>
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<tr>
<td>HM4</td>
<td>Neat</td>
<td>4.36± 0.45*</td>
<td>37.83 ± 8.48</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>5.91± 0.89*</td>
<td>16.24 ± 11.23</td>
</tr>
<tr>
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<td>100x dilution</td>
<td>10.50± 2.66*</td>
<td>-49.89 ± 45.01</td>
</tr>
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<td></td>
<td>1000x dilution</td>
<td>8.68± 0.94*</td>
<td>-22.83 ± 8.72</td>
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<td></td>
<td>10000x dilution</td>
<td>8.49± 1.07*</td>
<td>-20.13 ± 10.44</td>
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<td>26.66 ± 8.12</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>6.74± 1.20</td>
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<td>1000x dilution</td>
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<td>10000x dilution</td>
<td>8.12± 1.35</td>
<td>-14.79 ± 14.62</td>
</tr>
<tr>
<td>HM6</td>
<td>Neat</td>
<td>4.82± 0.39*</td>
<td>31.62± 5.71</td>
</tr>
<tr>
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<td>10x dilution</td>
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<td>14.69± 13.29</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>6.80± 0.62</td>
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<tr>
<td></td>
<td>1000x dilution</td>
<td>6.71± 0.49</td>
<td>4.82± 6.72</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>7.70± 1.13</td>
<td>-9.30± 15.75</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 10.1 allow comparison of LEL activity and % inhibition at various concentrations for each standard. The values indicate the weak stimulatory and inhibitory effects of the CHMs. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they do not correlate. The effects are generally comparable between the two mechanisms of stimulation of the platelets (fMLP or PMA), and some are statistically significant effects (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 11).
10.2 Luminescence activity of isolated human neutrophils from asthmatic patients

The graphs below depict LEL activity of isolated human neutrophils (IHNs) stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the cells at the various time intervals.

HM1- *Intlamba Zifo™*

Figure 10.13. The LEL activity of asthmatic isolated human neutrophils after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the fMLP- and the PMA-induced neutrophils in the neat HM1 showed the lowest LEL activities, below the CTRL cells which were comparable. The cells incubated with the diluted HM6 standards showed comparable LEL activities, close to but above and below the control, and the activities were highest on stimulation but decreased and remained consistent for the 60 minutes of test time.

Figure 10.14. The % inhibition of asthmatic isolated human neutrophils after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM1 and the 10x diluted standard had weak inhibitory effects on both induced cells, but the effect was moderate at the beginning. The other diluted HM1 standards had variable weak stimulatory and weak inhibitory effect on the cells, with the 10000x standard having a high stimulatory effect at t = 0.
Figure 10.15. The LEL activity of asthmatic isolated human neutrophils after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The fMLP-induced cells in the neat HM2, the 10x and the 100x diluted standards had LEL activities lower than the CTRL cells that changed very little from stimulation till 60 minutes. The cells in the two higher dilutions of HM4 had activities that were close to the CTRL cells.
that also had little change after 10 minutes of stimulation. The PMA-induced cells in the neat and the 10x diluted HM2 standard had activities below the CTRL cells, while the cells in the other diluted standards had activities close to the CTRL cells which wavered above or below at different time intervals. All the cells had high activity on stimulation which reduced rapidly until five minutes.

Figure 10.16. The % inhibition of asthmatic isolated human neutrophils after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM2 and its dilutions had weak inhibitory effects on the fMLP-induced cells, which was constant for the 60 minutes of test time. However there was very weak stimulatory
activity for the higher diluted standards. The neat HM2, the 10x and the 100x diluted standards had consistent weak inhibitory effect on the PMA-induced cells, while the other two diluted standards had variable weak to moderate stimulatory effects.

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![Figure 10.17](image)

Figure 10.17. The LEL activity of asthmatic isolated human neutrophils after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the fMLP- and the PMA-induced cells in the neat HM3 showed the lowest LEL activities, below the CTRL cells. The fMLP-induced neutrophils in the 1000x diluted standard had activity lower than CTRL cells while the activity of the PMA-induced ones was above. The activities of the cells in the 10000x diluted standard were close to and above the CTRL cells. The activity of the neutrophils in the 10x and 100x diluted standards were below the CTRL cells.

Figure 10.18. The % inhibition of asthmatic isolated human neutrophils after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM2 and the two lower diluted standards had weak inhibitory effect on both the fMLP- and the PMA–induced IHNs. The 1000x diluted standards had weak to moderate stimulatory effects on PMA-induced cells and a weak inhibitory effect to fMLP-induced neutrophils. The 10000x diluted standards had variable weak stimulatory and inhibitory effects to both induced cells.

**HM4- Ngoma™ Herbal Tonic Immune Booster**

![Graph A](image1)

**Figure 10.19.** The LEL activity of asthmatic isolated human neutrophils after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The LEL activities of the fMLP-induced neutrophils in the neat HM4 and the two lower diluted standards were below that of the CTRL cells. The cells in the 1000x diluted HM4 standard had activity that increased above that of the CTRL cells between 25 and 30 minutes. The cells in the 10000x diluted HM4 standard had activity close to the CTRL cells, but was above or below the CTRL cells at different time intervals. The LEL activities of the PMA-induced neutrophils in the neat HM4 and the two lower diluted standards were below that of the CTRL cells. The cells in the higher diluted HM4 standards had activities that were above or below the CTRL cells at different time intervals.

Figure 10.20. The % inhibition of asthmatic isolated human neutrophils after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM4 and the two lower diluted standards had weak inhibitory effect on both the fMLP- and the PMA–induced neutrophils. The two higher diluted standards had variable weak stimulatory and inhibitory effects to both induced cells.

**HM5- *Stametta™* Body Healing Liquid**

![Diagram](image)

Figure 10.21. The LEL activity of asthmatic isolated human neutrophils stimulated after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The LEL activities of the fMLP-induced neutrophils in the neat HM5 and the three lower diluted standards were below that of the CTRL cells. The cells in the 10000x diluted HM5 standard had activity that increased above that of the CTRL cells between 10 and 20 minutes. The LEL activities of the PMA-induced neutrophils in the neat HM5 and the 10x diluted standard were below that of the CTRL cells. The cells in the 100x diluted HM5 standard had activity close to the CTRL cells that were above or below the CTRL cells at different time intervals. The cells in the higher diluted HM5 standards had activities that were below the CTRL cells on stimulation, but were above the CTRL cells thereafter until 60 minutes.

Figure 10.22. The % inhibition of asthmatic isolated human neutrophils after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM5 and the diluted standards, except the 10000x diluted standard, had a weak inhibitory effect on the fMLP-induced cells. The neat HM5 and the 10x diluted standard had weak inhibitory effect on both the PMA-induced cells. The other diluted standards had weak inhibitory effects at the beginning and variable and consistent weak stimulatory effects thereafter.

**HM6- Vuka Uphile™ Immune Booster**

Figure 10.23. The LEL activity of asthmatic isolated human neutrophils stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The fMLP-induced cells in the neat HM6 and its dilutions had reduced activity, below the control, except the 10000x diluted standard in which the activity was close to but above from 20 to 30 minutes. The activity of the PMA-induced cells in the 10000x diluted standard were above that of the CTRL cells while those in the 1000x were much close to but above and below that of the CTRL cells. The cells incubated with the neat and the 10x diluted HM6 standards showed comparable LEL activities below the CTRL cells, and the activities remained consistent for the 60 minutes of test time.

Figure 10.24. The % inhibition of asthmatic isolated human neutrophils after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM6 and its diluted standards, except the 10000x diluted standard (between 20 and 30 minutes) had a weak inhibitory effect on the fMLP-induced cells. The neat HM6, the 10x and the 100x diluted standards had weak inhibitory effect on the PMA-induced neutrophils. The other diluted standards had variable weak stimulatory effects on the neutrophils.

Table 10.2. The average LEL activities of asthmatic isolated human neutrophils incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>19.36 ± 3.04</td>
<td>18.14 ± 4.71</td>
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<tr>
<td>HM1</td>
<td>Neat</td>
<td>11.84 ± 1.09*</td>
<td>38.0 ± 8.4</td>
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<td></td>
<td>10x dilution</td>
<td>17.03 ± 2.38</td>
<td>11.5 ± 9.8</td>
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<td>100x dilution</td>
<td>20.79 ± 2.94</td>
<td>-7.8 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>20.42 ± 2.54</td>
<td>6.3 ± 11.6</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>19.43 ± 2.71</td>
<td>-1.2 ± 13.4</td>
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<td>HM2</td>
<td>Neat</td>
<td>12.26 ± 1.25*</td>
<td>35.7 ± 9.7</td>
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<td>10x dilution</td>
<td>11.98 ± 1.23*</td>
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<td>100x dilution</td>
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<td>10000x dilution</td>
<td>18.91 ± 2.11</td>
<td>1.5 ± 10.1</td>
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<td>Neat</td>
<td>12.54 ± 1.55*</td>
<td>34.4 ± 10.0</td>
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<td>10x dilution</td>
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<td>100x dilution</td>
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<td>24.7 ± 7.6</td>
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<td>12.88 ± 0.84*</td>
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<td>10000x dilution</td>
<td>22.94 ± 6.32</td>
<td>5.5 ± 9.0</td>
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</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 10.2 allow comparison of LEL activity and % inhibition at various concentrations for each standard. The values show weak stimulatory and inhibitory...
effects of the CHMs. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they do not correlate. The effects are generally comparable between the two mechanisms of stimulation of the platelets (fMLP or PMA), and some are statistically significant effects ($p \leq 0.05$). The specific details of the table are addressed in the discussion chapter (chapter 11).
10.3 Luminescence activity of platelets from asthmatic patients

The graphs below depict LEL activities of platelets stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the platelets at the various time intervals.

HM1 - *Intlamba Zifo™*

![Graph A](image1)

![Graph B](image2)

Figure 10.25. The LEL activity of asthmatic platelets stimulated after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the PMA- and fMLP-induced platelets in the neat HM1 had activity below the CTRL platelets, which stayed the same throughout the 60 minutes. The fMLP-induced platelets in the diluted HM1 standards had activity higher than the CTRL on stimulation which decreased steadily until 10 minutes. Thereafter the activity remained close to the CTRL platelets, but were above or below at different time intervals. The activity of the PMA-induced CTRL platelets was highest on stimulation, and gradually declined until 60 minutes. The activities of the PMA-induced platelets in the diluted HM1 standards had activities higher than the CTRL platelets on stimulation which decreased and were close to that of the CTRL platelets, above and below until 60 minutes.

Figure 10.26. The % inhibition of asthmatic platelets after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM1 had weak inhibitory effects on both the fMLP- and the PMA-induced platelets. The diluted HM1 standards had variable weak inhibitory and stimulatory effect on the platelets.

**HM2- *Maphilisa™* Herbal medicine**

![Graph A](image)

![Graph B](image)

Figure 10.27. The LEL activity of asthmatic platelets stimulated after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the PMA- and fMLP-induced platelets in the neat HM2 had activity below the CTRL platelets, which was almost the same throughout the 60 minutes. The platelets in the diluted HM2 standards had activities that were close to the CTRL platelets, but above or below at different time intervals.

Figure 10.28. The % inhibition of asthmatic platelets after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM2 and the 10x diluted HM2 standards had weak inhibitory effects on both the fMLP- and the PMA-induced platelets (except for the 10x diluted standard at t=0 and 20 minutes). The other diluted HM2 standards had variable weak inhibitory and stimulatory effect on the platelets at different time intervals.

**HM3- Matla™ African medicine for all diseases**

![Graph A](image1.png)

**Figure 10.29.** The LEL activity of asthmatic platelets stimulated after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

![Graph B](image2.png)
Both the PMA- and fMLP-induced platelets in the neat HM3 had activity below the CTRL platelets, which was almost the same throughout the 60 minutes. The platelets in the diluted HM3 standards had activities that were close to the CTRL platelets, but above or below at different time intervals.

Figure 10.30. The % inhibition of asthmatic platelets after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM3 and the 10x diluted HM3 standards had weak inhibitory effects on both the fMLP- and the PMA-induced platelets (except for the 10x diluted standard at $t = 0$ and 5 minutes). However, the effect was more pronounced in the fMLP-induced platelets. The other diluted HM3 standards had variable weak inhibitory and stimulatory effect on the platelets at different time intervals.

**HM4- Ngoma™ Herbal Tonic Immune Booster**

Figure 10.31. The LEL activity of asthmatic platelets stimulated after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of $n = 8$ indicated on graph).
The FMLP-induced platelets in the neat HM4 had LEL activity above the CTRL cells, whereas the PMA-induced cells’ activity was below. The fMLP-induced platelets in the 10x diluted HM3 standard had activity below the CTRL platelets which was also the lowest activity amongst all the platelets. Both induced platelets in the other diluted HM4 standards had activities close to but mainly above the CTRL platelets.

![Graph A](image1)

![Graph B](image2)

Figure 10.32. The % inhibition of asthmatic platelets after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat and the 10x diluted HM4 standards had weak inhibitory effects on both the fMLP- and the PMA-induced platelets (except for the 10x diluted standard at t = 5, 10 and 30
minutes). The other diluted HM4 standards had variable weak inhibitory and weak to moderate stimulatory effect on the platelets at different time intervals.

**HM5- Stametta™ Body Healing Liquid**

![Graph](image)

**Figure 10.33.** The LEL activity of asthmatic platelets after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the PMA- and fMLP-induced platelets in the neat HM5 had activity below the CTRL platelets, which was almost the same throughout the 60 minutes. The platelets in the diluted HM5 standards had activities that were close to the CTRL platelets, but above or below at different time intervals.

Figure 10.34. The % inhibition of asthmatic platelets after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA.
(Means of n = 8 are indicated).

The neat HM5 had weak inhibitory effects on both the fMLP- and the PMA-induced platelets. The other diluted HM5 standards generally had variable weak stimulatory effects on the platelets at different time intervals.
Figure 10.35. The LEL activity of asthmatic platelets after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA.
(Means of n = 8 indicated on graph).

The fMLP-induced platelets in the neat HM6, the 10x and the 100x diluted HM6 standards had activity below the CTRL platelets. The platelets in the other diluted HM6 standards had activities that were close to and above the CTRL platelets.
The PMA-induced platelets had activities that were much closer to the CTRL platelets. The platelets in the neat HM5 had activity below the CTRL, those in the 10000x were above and those in the other two diluted standards were above or below at different time intervals.

Figure 10.36. The % inhibition of asthmatic platelets after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM6, the 10x and the 100x diluted HM6 standards had weak inhibitory effects on both the fMLP- and the PMA-induced platelets. However the effects were more pronounced in the fMLP-induced platelets. The other diluted HM6 standards had variable weak stimulatory effect on the platelets at different time intervals.
Table 10.3. The average LEL activities of asthmatic platelets incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
<td>Average LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control</td>
<td>0.0048 ± 0.0003</td>
<td>30.9 ± 4.2*</td>
<td>0.0036 ± 0.0003</td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>0.0048 ± 0.0006</td>
<td>0.7 ± 13.8</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>-2.7 ± 8.1</td>
<td>6.8 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>-1.8 ± 14.7</td>
<td>0.0050 ± 0.0010</td>
</tr>
<tr>
<td>HM2</td>
<td>Neat</td>
<td>0.0037 ± 0.0005*</td>
<td>31.4 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>2.5 ± 15.7</td>
<td>0.0040 ± 0.0007</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>1.5 ± 10.5</td>
<td>0.0045 ± 0.0004</td>
</tr>
<tr>
<td>HM3</td>
<td>Neat</td>
<td>0.0034 ± 0.0002*</td>
<td>28.7 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>-2.0 ± 23.1</td>
<td>0.0042 ± 0.0007</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>-6.9 ± 13.1</td>
<td>0.0041 ± 0.0005</td>
</tr>
<tr>
<td>HM4</td>
<td>Neat</td>
<td>0.0059 ± 0.0010*</td>
<td>20.5 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>20.5 ± 5.0</td>
<td>0.0040 ± 0.0003</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>-25.8 ± 15.3</td>
<td>0.0044 ± 0.0004</td>
</tr>
<tr>
<td>HM5</td>
<td>Neat</td>
<td>0.0034 ± 0.0003*</td>
<td>28.2 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>-16.3 ± 21.3</td>
<td>0.0048 ± 0.0007*</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>-9.7 ± 11.4</td>
<td>0.0047 ± 0.0005*</td>
</tr>
<tr>
<td>HM6</td>
<td>Neat</td>
<td>0.0035 ± 0.0004*</td>
<td>27.2 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>17.0 ± 9.2</td>
<td>0.0039 ± 0.0005</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>15.5 ± 12.1</td>
<td>0.0041 ± 0.0005</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 10.3 allow comparison of LEL activity and % inhibition at various concentrations for each standard. The values show weak stimulatory and inhibitory effects of the CHMs on platelets from asthmatic patients. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they do not correlate. The effects are generally higher in the fMLP based mechanism than the PMA mechanisms of stimulation. There are statistically significant effects (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 11).
CHAPTER 11 DISCUSSION: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM ASTHMATIC PATIENTS

A total of six herbal mixtures and four serial dilutions of each one were tested for their effects on the LEL activity of cells stimulated by fMLP and PMA. The cells were all obtained from individuals suffering from asthma, and were on their regular anti-asthma medicine. Their daily regimen included one or combination of the following medicines—salbutamol, beclometasone, salmeterol, fluticasone and montelukast. The average effects of each CHM and its dilutions on the whole blood phagocytes (table 10.1, page 160), the neutrophils (table 10.2, page 173) and the platelets (table 10.3, page 187) are discussed below.

HM1- Intlamba Zifo™

- The highest concentration of HM1 (neat) caused opposite effects in whole blood phagocytes, inhibition in the fMLP–induced and stimulation in the PMA-induced ones. The reduction in LEL activity (inhibition) was statistically significant when compared to the controls. The diluted standards had stimulatory effects that were concentration dependent in PMA-induced cells. The effects were significant in the higher concentration standards (lower dilutions- 10x and 100x dilutions). The stimulatory effects in the fMLP-induced cells were random with the different concentrations of HM1, and statistically significant difference was noted in the 10x diluted HM1 standard when compared to the control.
- The neat HM1 and the 10x dilution had weak inhibitory effects on fMLP-induced isolated human neutrophils (IHNs) and a reduction in LEL activity that were both concentration dependent. There was statistically significant difference between the control and the neat HM1. At low concentration (higher dilutions), HM1 had a random weak stimulatory effect on fMLP-induced neutrophils. At high concentration, HM1 had a weak concentration dependent inhibition and reduction of LEL activity of PMA-induced IHNs. The higher the concentration, the lower the LEL activity and the higher the inhibitory effect. At low concentration, however, HM1 had weak stimulatory effect and increasing LEL activity which were also concentration dependent.
- The highest concentration of HM1 (neat) had weak inhibitory effect on the platelets activated via fMLP as well as PMA and the difference was statistically significant.
compared to the controls. At low concentration, HM1 caused weak, non-systematic stimulation of the PMA-induced platelets. The low concentration HM1 caused random weak stimulation and inhibition of fMLP-induced platelets.

**HM2- Maphilisa™ Herbal medicine**

- The neat and the highest dilution HM2 had a concentration dependent inhibitory effect on fMLP-induced whole blood phagocytes. The reduction in LEL activity of the two showed statistically significant difference between the neat HM2 and the controls. The other (lower) concentrations between the extreme two had random weak stimulatory effect on the fMLP-induced cells. The neat HM2 had a weak inhibitory effect on the PMA-induced whole blood phagocytes with statistically significant difference compared to the controls. At lower concentrations, the HM2 had concentration dependent stimulatory effect on these cells, also with statistically significant difference compared to the controls. The higher the concentration, the higher the stimulatory effect on the cells, and the higher the LEL activity.

- HM2 had a non-systematic weak inhibitory effect on fMLP-induced IHNs and the differences in LEL activities were statistically significant in the neat and two lower dilution standards when compared to the controls. In the PMA-induced IHNs, there was a concentration dependent inhibitory effect in the neat and the two lower dilution standards, and the differences in LEL activities were also statistically significant in the neat and the 10x diluted standard compared to the controls. The higher dilution standards had a weak concentration dependent stimulatory effect on the cells. The higher the concentration the lower the stimulation.

- The HM2 had a weak concentration dependent inhibitory effect on fMLP-induced platelets, with the exclusion of the 10000x diluted standard. The differences in LEL activities were statistically significant in the neat and the 10x diluted standard against the controls. The 10000x diluted standard had a weak stimulatory effect on the fMLP-induced platelets. At higher concentrations, the HM2 also had a concentration dependent inhibitory effect on PMA-induced platelets, but only the neat HM2 had statistically significant difference of LEL activities when compared to the controls.
HM3- *Matla™* African medicine for all diseases

- The neat HM3 and 10x diluted standard had weak concentration dependent inhibitory effect causing reduced LEL activity in fMLP-induced whole blood phagocytes, and the differences in the LEL activities were statistically significant against the controls. The higher the concentration, the higher the inhibition. The higher dilution standards had weak stimulatory effects causing increased activity, with a statistically significant difference only in the highest dilution standard. The lower the concentration, the higher the stimulatory effect. The neat HM3 had a weak and significant inhibitory effect on PMA-induced cells. The diluted HM3 solutions had weak non-systematic stimulatory effect and the differences in the LEL activities were all statistically significant against the controls.

- The HM3 had a concentration dependent and weak inhibitory effect on the IHNs. The higher the concentration, the lower the LEL activity, and the higher the inhibitory effect. The differences in activity compared to the controls were statistically significant in the neat HM3 and the 10x HM3 dilutions in both the fMLP and PMA-induced neutrophils. The HM3 and dilutions affected the fMLP- and the PMA-induced cells comparably.

- The neat HM3 had a weak inhibitory effect on the fMLP- and PMA-induced platelets, and the differences in the LEL activities were statistically significant against the controls. The diluted standards had a weak concentration dependent stimulatory effects on the fMLP-induced platelets and non-systematic weak stimulatory effects on the PMA-induced platelets.

HM4- *Ngoma™* Herbal Tonic Immune Booster

- The HM4 exerted a weak concentration dependent inhibitory effect in whole blood phagocytes, at higher concentrations. In lower concentrations it had concentration dependent stimulatory effect. The higher the concentration, the lower the stimulatory effect. The differences in the LEL activities were statistically significant against the controls except for PMA-induced cells in the 10x diluted standard. The 100x diluted HM4 had notably high % inhibition values, from weak to moderate. Nevertheless, the effect of HM4 was equivalent in both the fMLP and the PMA-induced cells.

- The HM4 had a concentration dependent and weak inhibitory effect on the IHNs. The higher the concentration, the lower the LEL activity, and the higher the inhibitory effect. The differences in the LEL activities were statistically significant against the controls in the
neat HM4 and the two lower HM4 dilutions in both the fMLP and PMA-induced neutrophils. However, at very low concentrations (1000x, 10000x dilutions) the HM4 had weak concentration dependent stimulatory effect on PMA-induced neutrophils.

- The HM4 exerted a weak concentration dependent inhibitory effect on both the fMLP- and PMA-induced platelets, at higher concentrations. In lower concentrations it had concentration dependent stimulatory effect but affected the platelets in an opposite way. In fMLP-induced platelets, the higher the concentration the higher the stimulatory effect. In the PMA-induced cells, the higher the concentration, the lower the stimulatory effect. The differences in the LEL activities were statistically significant against the controls in the neat HM4 for both induced platelets, and the three lower dilutions in fMLP-induced platelets.

**HM5- *Stametta™* Body Healing Liquid**

- The neat HM5 and the 10x HM5 diluted standard caused reduced LEL activity in whole blood phagocytes exerting a weak concentration dependent inhibitory effect on fMLP-induced cells. Only the neat HM5 had inhibitory effect on the PMA-induced cells. The differences in the LEL activities were statistically significant against the controls in the fMLP-induced cells of the neat HM5 and1000x dilution, and all the standards in the PMA-induced cells except the 10000x dilution. The HM5 diluted standards had non-systematic weak stimulatory effects on both the fMLP and PMA-induced cells.
- The HM5 and diluted standards had a concentration dependent and weak inhibitory effect on the fMLP-induced IHNs. The higher the concentration, the lower the LEL activity, and the higher the inhibitory effect. The differences in activity were statistically significant in the neat HM5 and the lower dilution standards against the controls. The HM5 also had weak concentration dependent effects in the PMA-induced neutrophils, and a weak concentration dependent stimulatory effect in the higher dilution standards.
- The HM5 affected the fMLP-and the PMA-induced platelets in the same manner. The neat HM5 had a weak inhibitory effect on both induced platelets with statistically significant differences against the controls. The diluted standards had random weak stimulatory effects on the platelets, significant in the 10x dilution standard.
HM6- *Vuka Uphile™* Immune Booster

- The HM6 exerted a non-systematic weak inhibitory effect in fMLP-induced whole blood phagocytes, causing reduced LEL activity; while the 10000x dilution exerted weak stimulatory effects, causing increased activity. The differences in LEL activities were statistically significant between the controls and the neat and the 10x dilution standard. The effects in the fMLP-induced cells were non-systematic weak inhibitory and stimulatory effects.
- The HM6 and diluted standards had a concentration dependent and weak inhibitory effect on both the fMLP- and the PMA-induced IHNs. The higher the concentration, the lower the LEL activity, and the higher the inhibitory effect. However the 10000x diluted HM6 had a weak stimulatory effect on PMA-induced IHNs. The differences in the LEL activities were statistically significant against the controls in the neat HM6, the 10x and the 10000x diluted HM6 standards in the fMLP-induced neutrophils.
- The HM6 affected the fMLP- and the PMA-induced platelets in the same manner. The neat HM6 and the two lower dilutions had weak concentration dependent inhibitory effect, with statistically significant differences in LEL activity compared to the controls. In low concentrations, the HM6 had a weak concentration dependent stimulatory effect. The higher the concentration, the lower the stimulatory effect.

Table 11.1. Summary of the overall effects of the HMs on the cells and platelets from asthma patients, from the highest to the lowest concentration

<table>
<thead>
<tr>
<th></th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>HM1</td>
<td>↓ +</td>
<td>+</td>
<td>↓ +</td>
</tr>
<tr>
<td>HM2</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓</td>
</tr>
<tr>
<td>HM3</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓</td>
</tr>
<tr>
<td>HM4</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓</td>
</tr>
<tr>
<td>HM5</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓</td>
</tr>
<tr>
<td>HM6</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓</td>
</tr>
</tbody>
</table>

↓ weak inhibition; ↑↑ moderate inhibition; ↑↑↑ potent inhibition
+ weak stimulation; † moderate stimulation; ‡ potent stimulation
11.1 Implications of the effects of the commercial herbal medicines on cells and platelets from asthmatic patients

As in other studies, the researched *in vitro* functional responsiveness of IHNs is a reflection of the *in vivo* physiological capabilities of these cells, which include chemotaxis, adherence, phagocytosis, ROS production (SOA and other oxygen radicals), degranulation, and microbial killing (McManus et al., 2001). In accordance with WHO guidelines, data obtained from *in vitro* tests should be seen as indicators of potential toxicity (WHO, 2000). Though *in vivo* tests should be performed, it is imperative to describe what the findings of this study indicate.

There was general weak inhibition (at high concentration) and weak stimulation of neutrophils in whole blood, isolated neutrophils and platelets by all the six HMs, through both the PMA and the fMLP pathways. The inhibition of the neutrophils by the six HMs means that their functional capacity would be reduced as evidenced by the reduction of ROS production in the study. It means that in the presence of higher concentrations of the HMs, the cells could not respond when stimulated. It implied that the HMs at high doses, inhibited the pathways of stimulation. The inhibition could have occurred by blocking the formyl peptide receptor, such that the agonist, fMLP could not bind to and stimulate the receptor. The inhibition of the PMA pathway could have occurred by binding to the enzyme PKC and inhibiting its activity. Mahomoodally et al. (2012) postulated that inhibition of both fMLP and PMA mechanisms suggested that the HMs do not affect a specific pathway, instead they may directly inhibit a common biochemical target site such as NOX or scavenge the ROS. The activity of the isolated neutrophils in this study were in agreement with the findings of Kato et al. (1991), that isolated neutrophils of asthma patients have increased capacity to produce ROS. This was observed by the higher LEL activities of asthmatic IHNs as compared to healthy IHNs.

Inhibition of neutrophils with the resultant reduced production of ROS may be beneficial in asthma. It has been reported that neutrophils were the first cells that enter the airways in response to the presence of an allergen (Macdowell & Peters, 2007; Monteseirin, 2009). It was also noted that activated neutrophils released inflammatory mediators including ROS which may interact with proteases to increase the tissue damage in asthma, thereby contribute towards the airway remodelling that occurs in asthma (Cowburn et al., 2008;
Monteseirin, 2009). In the presence of the HMs, the neutrophils were weakly inhibited and produced ROS in amounts lower than the cells without the HMs (CTRL). The inhibition of the neutrophils would be valuable to avert the worsening of asthma symptoms due to ROS, since it had been shown that there was correlation between the neutrophil production of ROS and bronchial hyperactivity in patients with asthma (Meltzer et al., 1989; Kato et al., 1991) and since oxygen radicals were found in higher amounts in asthma patients with worsening symptoms (Cowburn et al., 2008; Monteseirin, 2009).

As noted before, ROS have been implicated in inflammatory tissue damage, and could contribute to the development and progress of many acute and chronic inflammatory disorders (Dahlgren & Karlsson, 1999; Bjorkman et al., 2008; Tintinger et al., 2008). The airways and the cardiovascular system were noted to be particularly vulnerable and in addition to asthma, some of the conditions that could develop included RA, atherosclerosis, COPD, stroke, and myocardial infarction (Caldefie-Chezet et al., 2002; Tintinger et al., 2008). Therefore it appears that the inhibition of the neutrophils may therefore also help in preventing other disorders that may occur as complications in asthma as a result of the presence of ROS.

The weak inhibition of platelets occurred at higher concentrations of the HMs. The role of platelets in the pathophysiology of asthma is through the production and the release of biologically-active chemical mediators that are capable of inducing or increasing inflammatory responses (Moritani et al., 1998; Kornerup & Page, 2007). They were found to be easily activated when stimulated, resulting in the enhanced release of chemokines which attract eosinophils into lung tissue (Moritani et al., 1998), and release bronchoconstricting mediators such as PAF, serotonin, arachidonic acid metabolites and histamine. They are involved in airways inflammation by recruiting leucocytes and forming platelet-leucocyte aggregates. They directly and indirectly stimulate the airway smooth muscle and enhance epithelial proliferation, thereby contributing to airway remodeling in asthma (Kornerup & Page, 2007; De Boer et al., 2012). The inhibition of the platelets by the HMs may be beneficial in reducing the sequelae of platelet activation. The reduction of release of inflammatory mediators of bronchoconstriction and reduced airway inflammation may be particularly useful. It would be beneficial in atopic asthma patients, as there was increased activation of platelets in the condition. Notwithstanding, the inhibition of platelets may cause increased risk of bleeding. Clinically it was found that asthmatics have prolonged bleeding time, which is due to reduced aggregation as a result of platelet
desensitization. The desensitization was as a result of chronic activation in asthma (Kornerup & Page, 2007). Therefore platelet inhibition in the presence of HMs may complicate the prolonged bleeding further.

The weak stimulation of neutrophils occurred in presence of the HMs at low concentrations. It implied that the HMs at low doses enhanced the stimulation of the neutrophils by fMLP and PMA. Activation of the cells means there was increased activity of the NOX, which resulted in the formation of ROS. As already stated, neutrophils in asthma have the capacity for increased formation of ROS (Kato et al., 1991); which are associated with the tissue damage that occurs in asthma, the bronchial hyperactivity and the worsening asthma symptoms (Cowburn et al., 2008; Monteseirin, 2009; Dahlgren & Karlsson, 1999; Bjorkman et al., 2008; Tintinger et al., 2008). Therefore the weak stimulation of the neutrophils by the HMs may worsen the condition by increased tissue damage and worsening the symptoms further. The increase in bronchial hyperactivity may contribute towards increasing the frequency of attacks (Porter & Kaplan, 2011). Therefore, the intake of these HMs at low doses may be harmful to the asthmatic patient.

The weak stimulation of platelets occurred at lower concentrations of the HMs. In noting the role of platelets in the pathophysiology of asthma as well as the fact that they were found to be easily activated, it leads to the notion that the stimulation of these platelets by the HMs may be detrimental to the asthma patient. The added stimulation may cause heightened response, such as increased production and the release of biologically-active chemical mediators; bronchoconstricting mediators, enhanced release of chemokines, increased stimulation of the airway smooth muscle, increased recruitment of leucocytes and formation of platelet-leucocyte aggregates (Moritani et al., 1998; Kornerup & Page, 2007). All these activities may worsen the asthma and the symptoms, and increase the frequency of attacks. Platelet-neutrophil interactions were implicated in the development and progression of diseases such as ARDS, myocardial ischaemia and atherosclerosis (Ruf et al., 1992). Hence the stimulation of platelets and the subsequent formation of platelet-neutrophil aggregates may lead to increased occurrence of other complicating disorders. De Boer et al. (2012) observed that asthma patients show signs of increased activation of coagulation in the airways. Krotz et al. (2004) stated that the presence of platelet-derived ROS, predominantly NOX-dependent $O_2^-$ increased the recruitment of neutrophils to a growing thrombus. Therefore stimulation of platelets may enhance this
status, and result in uncontrolled thrombosis in the airways, causing the worsening of asthma.

Normally, activated platelets secrete chemical mediators which include ROS generated by platelet NOX. The chemical mediators enhance the interaction of platelets with other cells of the immune system (Krotz et al., 2004; Nauseef, 2014). The added stimulation of platelets in asthma by the HMs may result in extensive production and release of ROS. As reported by Parij et al. (1998), the ROS and granule enzymes cause damage to molecules, cells and tissues of inflammation and contribute to the pathogenesis of immune and non-immune chronic inflammatory conditions. Therefore the presence of the HMs in an asthmatic person and their effects on the platelets may cause worsening of the asthma and symptoms, and hasten the initiation and frequency of other relevant complications.

It is worth noting that the blood cells and platelets used in the study were from chronic asthma patients, none of whom were having an active attack. The HMs therefore inhibited and stimulated the cells of asthma patients on treatment. It therefore suggests interaction with the anti-asthma medicines – salbutamol, beclometasone, salmeterol, fluticasone and montelukast. It may be important to caution asthma patients about concomitant intake of HMs. It is also important for CHPs to be aware of the effects and the potential for interactions which may impact negatively on conventional treatment outcomes.
CHAPTER 12 RESULTS: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM DIABETIC MELLITUS PATIENTS

The effects of the six CHMs on the LEL activities of the IHNs, PLTs and whole blood phagocytes obtained from type 2 DM patients are shown below. The results for all the three components are first displayed in graphs of luminescence (in RLUs) versus time (in minutes) and the % inhibitions which indicated the kinetics observed over 60 minutes. Then the average LEL activities and the % inhibitions over 60 minutes were calculated and are displayed in the tables below.

12.1 Luminescence activity of whole blood phagocytes from type 2 diabetic mellitus patients

The graphs below depict LEL activity of whole blood phagocytes stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of LEL activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibitions of the HMs on the cells at the various time intervals.
Figure 12.1. The LEL activity of diabetic whole blood phagocytes after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The LEL activity of the fMLP-induced CTRL cells started increasing after stimulation and peaked at 10 minutes, declined gradually and then remained constant above the baseline (point of stimulation, t= 0) until 60 minutes. The LEL activity of the cells incubated in HM1 and its dilutions were close to that of the CTRL cells, above and below at different time intervals. The PMA-induced CTRL cells (B) had activity that declined slightly after stimulation, and then stabilised until 60 minutes. The cells incubated with neat HM1 had activity below that of the CTRL cells, while those in the 10x HM1 diluted standard had
activity above the CTRL cells. Cells in the other three diluted HM1 standards showed activity much closer to that of the CTRL cells, but were above and below at different time intervals.

Figure 12.2. The % inhibition of diabetic whole blood phagocytes after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM1 had weak inhibitory and weak stimulatory effect on fMLP-induced cells, which were not significant. The neat HM1 had moderate stimulatory effect fMLP-induced cells on stimulation and had weak inhibitory effect on PMA-induced cells. The 10x diluted HM1 standard had weak stimulatory effects, while the other diluted standards had variable weak inhibitory and stimulatory effects.
Figure 12.3. The LEL activity of diabetic whole blood phagocytes stimulated after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The cells in the neat HM2 had activity below the CTRL cells, while those in the 10x diluted standard had activity above that of the CTRL cells, for both the fMLP- and the PMA-induced cells. Cells in the other three diluted HM2 standards showed activity much closer
to that of the CTRL cells, mimicking the CTRL cells, but were above and below at different time intervals.

Figure 12.4. The % inhibition of diabetic whole blood phagocytes after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of $n = 8$ are indicated).

The neat HM2 had weak inhibitory effect on fMLP-induced cells, while the 10x diluted HM2 standard had weak stimulatory effects, except on stimulation in fMLP-induced cells where it had potent stimulatory effect. The other diluted standards had variable weak inhibitory and stimulatory effects.
Figure 12.5. The LEL activity of diabetic whole blood phagocytes stimulated after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

In both induced cells, the neat HM3 had activity below that of the CTRL cells, while the other cells had variable activity, close to the CTRL cells and oscillated above and below at different time intervals, but were all above on stimulation.
Figure 12.6. The % inhibition of diabetic whole blood phagocytes after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM3 had weak inhibitory effect on both the fMLP- and PMA-induced cells, while the 100x and the 1000x diluted HM3 standard had weak stimulatory effects on fMLP-induced cells. The other diluted standards had variable weak inhibitory and stimulatory effects, except on stimulation where the 10x diluted standard had moderate stimulatory effects. The neat HM3 and the 10000x diluted HM3 standard had weak inhibitory effects on the PMA-induced cells. The other diluted standards had variable weak inhibitory and stimulatory effects.
Figure 12.7. The LEL activity of diabetic whole blood phagocytes stimulated after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of $n = 8$ indicated on graph).

The cells in the neat HM2 had activity below the CTRL cells, while those in the 10x diluted standard had activity above that of the CTRL cells, for both the fMLP- and the PMA-induced cells. Both fMLP- and PMA-induced cells in the 100x diluted HM4 had remarkably
high LEL activity, far above the CTRL cells and cells in other standards. The PMA-induced cells showed their highest activity on stimulation, while the fMLP-induced increased after stimulation and reached the highest activity at 10 minutes after stimulation. The cells in the other standards had variable activity, close to the CTRL cells and oscillated above and below at different time intervals.

Figure 12.8. The % inhibition of diabetic whole blood phagocytes after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

In identifying with the LEL activity, the 100x diluted HM4 standard had overwhelming moderate to potent stimulatory effects on both fMLP- and PMA-induced cells, though weak
at a few points. The neat HM4 had weak to moderate inhibitory effect to both induced cells. The 10x diluted HM4 had a weak inhibitory effect on PMA-induced cells, and variable weak inhibitory and stimulatory effect on fMLP-induced cells. The other two diluted standards had variable weak inhibitory and stimulatory activity, close to the CTRL cells.

**HM5- *Stametta™* Body Healing Liquid**

Figure 12.9. The LEL activity of diabetic whole blood phagocytes after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The cells in the neat HM5 had activity below the CTRL cells, while those in the 100x and the 1000x diluted standards had activity above that of the CTRL cells, for both the fMLP- and the PMA-induced cells. Cells in the other two diluted HM5 standards showed activity much closer to that of the CTRL cells; however the PMA-induced cells in the 10x diluted had activity below the CTRL cells. All the fMLP-induced cells in the diluted standards had activity higher than the CTRL cells on stimulation.

Figure 12.10. The % inhibition of diabetic whole blood phagocytes after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM5 had weak inhibitory effect on both the fMLP- and PMA-induced cells, while the 100x and 1000x diluted HM5 standards had weak stimulatory effects on those cells.
The 10000x diluted HM5 standard had weak to potent stimulatory effects on the cells. The 10x diluted standards had variable weak inhibitory and stimulatory effect.

**HM6- Vuka Uphile™ Immune Booster**

**Figure 12.11.** The LEL activity of diabetic whole blood phagocytes stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both induced cells in the neat HM6 had activities below that of the CTRL cells, while the other cells had variable activities, close to the CTRL cells and oscillated above and below at different time intervals.

Figure 12.12. The % inhibition of diabetic whole blood phagocytes after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM6 had weak inhibitory effect on both the fMLP- and PMA-induced cells, while the other diluted standards had variable weak inhibitory and stimulatory effects, except on stimulation where the 10x diluted standard had moderate stimulatory effects on the PMA-induced cells.
Table 12.1. The average LEL activities of diabetic whole blood phagocytes incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
<td>LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control</td>
<td>7.52 ± 1.20</td>
<td>7.0 ± 28.1</td>
<td>7.10 ± 0.79</td>
</tr>
<tr>
<td>HM1</td>
<td>6.75 ± 0.96</td>
<td>-9.6 ± 13.0</td>
<td>5.45 ± 0.57*</td>
</tr>
<tr>
<td></td>
<td>8.12 ± 0.72</td>
<td>-8.5 ± 11.6</td>
<td>8.48 ± 1.26*</td>
</tr>
<tr>
<td></td>
<td>8.07 ± 0.93</td>
<td>6.0 ± 10.3</td>
<td>7.52 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>7.01 ± 0.96</td>
<td>-2.3 ± 10.4</td>
<td>6.83 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>7.60 ± 0.85</td>
<td></td>
<td>6.54 ± 0.60</td>
</tr>
<tr>
<td>HM2</td>
<td>4.78 ± 0.39*</td>
<td>35.1 ± 10.9</td>
<td>4.72 ± 0.32*</td>
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<tr>
<td></td>
<td>10.73 ± 2.00*</td>
<td>-47.1 ± 47.4</td>
<td>8.51 ± 1.72*</td>
</tr>
<tr>
<td></td>
<td>8.19 ± 1.35</td>
<td>-9.3 ± 8.4</td>
<td>7.53 ± 0.85</td>
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<tr>
<td></td>
<td>7.71 ± 1.24</td>
<td>-2.9 ± 8.0</td>
<td>6.94 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>7.86 ± 1.45</td>
<td>-4.3 ± 6.2</td>
<td>7.20 ± 0.87</td>
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<tr>
<td>HM3</td>
<td>4.87 ± 0.44*</td>
<td>34.1 ± 9.3</td>
<td>4.66 ± 0.50*</td>
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<td></td>
<td>7.76 ± 0.80</td>
<td>-6.3 ± 27.1</td>
<td>7.16 ± 0.98</td>
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<td></td>
<td>9.26 ± 0.62*</td>
<td>-25.6 ± 19.5</td>
<td>7.54 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>8.55 ± 0.86</td>
<td>-15.0 ± 11.4</td>
<td>7.33 ± 0.81*</td>
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<td></td>
<td>7.88 ± 1.01</td>
<td>-5.6 ± 9.2</td>
<td>6.42 ± 0.31*</td>
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<tr>
<td>HM4</td>
<td>4.69 ± 0.51*</td>
<td>35.9 ± 13.1</td>
<td>4.74 ± 0.46*</td>
</tr>
<tr>
<td></td>
<td>7.58 ± 1.23</td>
<td>-0.9 ± 5.1</td>
<td>5.54 ± 0.57*</td>
</tr>
<tr>
<td></td>
<td>12.42 ± 1.80*</td>
<td>-66.5 ± 17.3</td>
<td>11.25 ± 1.70*</td>
</tr>
<tr>
<td></td>
<td>8.25 ± 0.57</td>
<td>-11.7 ± 15.0</td>
<td>6.79 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>7.92 ± 1.04</td>
<td>-6.1 ± 10.2</td>
<td>6.53 ± 0.71</td>
</tr>
<tr>
<td>HM5</td>
<td>5.28 ± 0.56*</td>
<td>28.4 ± 12.6</td>
<td>4.94 ± 0.44*</td>
</tr>
<tr>
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<td>6.74 ± 0.47</td>
<td>8.6 ± 14.4</td>
<td>6.92 ± 0.55</td>
</tr>
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<td></td>
<td>10.07 ± 0.90*</td>
<td>-37.5 ± 30.5</td>
<td>9.15 ± 1.27*</td>
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<td>10.22 ± 1.35*</td>
<td>-35.1 ± 16.1</td>
<td>9.14 ± 1.31*</td>
</tr>
<tr>
<td></td>
<td>8.33 ± 0.92</td>
<td>-11.9 ± 11.0</td>
<td>7.50 ± 1.08</td>
</tr>
<tr>
<td>HM6</td>
<td>5.36 ± 0.40*</td>
<td>27.4 ± 10.1</td>
<td>4.93 ± 0.54*</td>
</tr>
<tr>
<td></td>
<td>7.03 ± 1.16</td>
<td>3.2 ± 31.4</td>
<td>6.49 ± 1.03</td>
</tr>
<tr>
<td></td>
<td>7.15 ± 0.77</td>
<td>3.4 ± 13.6</td>
<td>6.30 ± 0.61</td>
</tr>
<tr>
<td></td>
<td>7.17 ± 0.77</td>
<td>3.4 ± 11.4</td>
<td>6.62 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>8.37 ± 1.16</td>
<td>-12.7 ± 15.6</td>
<td>7.80 ± 0.90</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 12.1 allow comparison of LEL activity and % inhibition at various concentrations for each standard. The values indicate the weak stimulatory and inhibitory effects of the CHMs on the cells. The stimulatory or inhibitory effects are directly or inversely proportional to the concentration in some cases, and in others they do not correlate. The effects are generally comparable between the two mechanisms of stimulation of the platelets (fMLP or PMA), and some are statistically significant (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 13).
12.2 Luminescence activity of isolated human neutrophils from diabetic patients

The graphs below depict luminol-enhanced luminescence (LEL) activity of isolated human neutrophils (IHNs) stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the cells at the various time intervals.

**HM1- *Intlamba Zifo™***

![Graph A](image)

![Graph B](image)

Figure 12.13. The LEL activity of diabetic isolated human neutrophils stimulated after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The fMLP-induced IHNs in the CTRL had LEL activity that increased after stimulation, peaked at 10 minutes and gradually declined until 60 minutes where the level was almost equal to the levels at stimulation. The PMA-induced cells however had activity that decreased after stimulation, stabilised and slightly increased and at 60 minutes was at the level equal to the one on stimulation. Both induced cells in the neat HM1 had activity below that of the CTRL cells, while IHNs in the diluted HM1 standards had variable activity, close to and above the CTRL cells and their LEL behaviour imitated the CTRL cells throughout the 60 minutes.

Figure 12.14. The % inhibition of diabetic isolated human neutrophils after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM1 had moderate inhibitory effect on both fMLP- and PMA-induced IHNs. All the diluted standards had stimulatory effects on the cells, ranging from weak to potent. The 10x diluted standard had the highest stimulatory effects.

**HM2 - Maphilisa™ Herbal medicine**

Figure 12.15. The LEL activity of diabetic isolated human neutrophils stimulated after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both PMA- and fMLP-induced cells in the neat HM2 and the 10x diluted standard had activity below the CTRL cells. The fMLP-induced cells’ activity in the 10x diluted were
slightly above those in the neat HM2, but the activity of the PMA-induced were equal to that of the CTRL cells. The IHNs in the highest two diluted standards had activity close to but below the CTRL, and mimicked the CTRL cells throughout. The fMLP-induced IHNs in the 100x diluted standard had activity below the CTRL cells while the PMA-induced cells' activity was much closer to the CTRL cells.

![Graph](image1)

Figure 12.16. The % inhibition of diabetic isolated human neutrophils after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM2 and the 10x diluted standard had inhibitory effect on the FMLP- and the PMA-induced cells; moderate to potent in the neat HM2 and weak to moderate in the 10x
diluted standard. The other diluted standards generally had weak inhibitory effects on PMA-induced cells and variable weak inhibitory and stimulatory effects on fMLP-induced cells.

HM3- Matla™ African medicine for all diseases

Figure 12.17. The LEL activity of diabetic isolated human neutrophils stimulated after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both induced IHNs in the neat HM3 and the two lower diluted standards had LEL activities lower than the CTRL cells, while those in the two higher diluted standards had activities close to the CTRL cells, above and below the CTRL cells at different time intervals.

Figure 12.18. The % inhibition of diabetic isolated human neutrophils after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA (Means of n = 8 are indicated).

The neat HM3 and the two lower diluted standards had weak to potent inhibitory effects on both fMLP- and PMA-induced cells, while the two higher diluted standards had variable weak inhibitory and stimulatory effects on the cells.
Figure 12.19. The LEL activity of diabetic isolated human neutrophils stimulated after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both induced IHNs in the neat HM4 and the two lower diluted standards had LEL activities lower than the CTRL cells, while those in the two higher diluted standards had activities close to the CTRL cells, above and below the CTRL cells at different time intervals. However the activity of the PMA-induced cells in the 1000x diluted standard was above that of the CTRL cells.
Figure 12.20. The % inhibition of diabetic isolated human neutrophils after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM4 and the two lower diluted standards had inhibitory effects on both fMLP- and PMA-induced cells, ranging from weak to potent. The two higher diluted standards had variable weak inhibitory and stimulatory effects on the fMLP-induced cells. On the PMA-induced cells, the 10000x diluted standard had weak inhibitory effects, and the 1000x diluted standard was the only one which had stimulatory effects on the cells.
Figure 12.21. The LEL activity of diabetic isolated human neutrophils stimulated after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both induced IHNs in the neat HM5 and the two lower diluted standards had LEL activities lower than the CTRL cells. The PMA-induced IHNs in the two higher diluted standards had activities close to the CTRL cells, above and below the CTRL cells at different time intervals. However the activities of the PMA-induced cells in the two diluted standards were much lower than that of the CTRL cells.
Figure 12.22. The % inhibition of diabetic isolated human neutrophils after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM5 and the two lower diluted standards had inhibitory effects on both fMLP- and PMA-induced cells, ranging from weak to potent. The two higher diluted standards had variable weak inhibitory and stimulatory effects on the fMLP-induced cells and the PMA-induced cells.
Figure 12.23. The LEL activity of diabetic isolated human neutrophils stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both induced IHNs in the neat HM6 and the two lower diluted standards had equivalent LEL activities lower than the CTRL cells. The IHNs in the two higher diluted standards had activities below the CTRL cells but that mimicked the CTRL cells activity throughout the 60 minutes.
Figure 12.24. The % inhibition of diabetic isolated human neutrophils after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM6 and its dilutions had inhibitory effects from weak to potent, on both induced cells for 60 minutes, except for the minor stimulatory effect on stimulation of PMA-induced cells.
Table 12.2. The average LEL activities of diabetic isolated human neutrophils incubated with the HMIs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
<td>LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control</td>
<td>33.08 ± 13.15</td>
<td>25.37 ± 2.54</td>
<td></td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>7.39 ± 0.82*</td>
<td>72.5 ± 15.4</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>40.30 ± 12.31</td>
<td>-27.5 ± 27.0</td>
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<td></td>
<td>100x dilution</td>
<td>40.79 ± 16.09</td>
<td>-24.5 ± 12.8</td>
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<tr>
<td></td>
<td>1000x dilution</td>
<td>43.72 ± 18.17</td>
<td>-31.7 ± 8.8</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>41.23 ± 15.42</td>
<td>-26.7 ± 14.7</td>
</tr>
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<td>HM2</td>
<td>Neat</td>
<td>7.13 ± 0.64*</td>
<td>74.5 ± 12.1</td>
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<td></td>
<td>10x dilution</td>
<td>10.34 ± 1.04*</td>
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<td>100x dilution</td>
<td>27.07 ± 5.67</td>
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<td>1000x dilution</td>
<td>36.02 ± 8.74</td>
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<td>10000x dilution</td>
<td>39.83 ± 11.65</td>
<td>-28.2 ± 16.0</td>
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<td>Neat</td>
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<td>10x dilution</td>
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<td>100x dilution</td>
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<td>1000x dilution</td>
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<td>-14.4 ± 30.5</td>
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<td>10000x dilution</td>
<td>38.18 ± 9.03</td>
<td>-24.4 ± 28.2</td>
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<td>Neat</td>
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<td>39.25 ± 12.16</td>
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<td>9.42 ± 1.03*</td>
<td>66.7 ± 15.3</td>
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<td>10000x dilution</td>
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<td></td>
<td>10000x dilution</td>
<td>26.38 ± 7.98</td>
<td>15.9 ± 17.4</td>
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</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 12.2 allow comparison of LEL activity and % inhibition at various concentrations for each standard. The values show stimulatory and inhibitory effects of the CHMIs ranging from weak to moderate effects. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they are not systematic. The effects are generally comparable between the two mechanisms of stimulation of the platelets (fMLP or PMA), and some are statistically significant effects (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 13).
12.3 Luminescence activity of platelets from diabetic patients

The graphs below depict LEL activities of platelets stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the platelets at the various time intervals.

HM1- Inlamba Zifo™

Figure 12.25. The LEL activity of diabetic platelets stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the PMA- and fMLP-induced platelets in the neat HM1 had activity below the CTRL platelets, which stayed the same throughout the 60 minutes. The diluted HM1 standards had activities higher than the CTRL on stimulation which decreased steadily until five minutes. Thereafter the activities remained close to the CTRL platelets, but were mainly above at different time intervals.

Figure 12.26. The % inhibition of diabetic platelets after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM1 had weak inhibitory effects on both the fMLP- and the PMA-induced platelets. The diluted HM1 standards had variable stimulatory effect on the platelets which was weak to potent on stimulation and weak for the remaining time.
HM2- *Maphilisa™* Herbal medicine

Figure 12.27. The LEL activity of diabetic platelets stimulated after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of *n* = 8 indicated on graph).

Both the PMA- and fMLP-induced platelets in the neat HM2 had activity below the CTRL platelets, which was almost the same throughout the 60 minutes. The fMLP-induced platelets in the 10x diluted HM2 standards had activities above the CTRL platelets while the PMA-induced had activities below. Both induced platelets in the other diluted HM2
standards had activities that were above the CTRL platelets and had their highest activity on stimulation.

Figure 12.28. The % inhibition of diabetic platelets after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated)

The neat HM2 had weak inhibitory effects on both the fMLP- and the PMA-induced platelets. The 10x diluted HM2 standards had weak inhibitory effects on the fMLP-induced
platelets, and moderate to potent stimulatory effects on the PMA-induced platelets. The other diluted HM2 standards had variable weak to potent stimulatory effect at different time intervals on both the fMLP- and PMA-induced platelets.

**HM3- Matla™ African medicine for all diseases**

![LEL activity of platelets](image)

**Figure 12.29.** The LEL activity of diabetic platelets stimulated after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both the PMA- and fMLP-induced platelets in the neat HM3 had activity below the CTRL platelets, which was almost the same throughout the 60 minutes. The platelets in the diluted HM3 standards had activities that were above the CTRL platelets and they all had their highest activity on stimulation.
Figure 12.30. The % inhibition of diabetic platelets after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA.

(Means of n = 8 are indicated).

The neat HM3 had weak inhibitory effects on both the fMLP- and the PMA-induced platelets (except for the 10x diluted standard at 60 minutes). The diluted HM3 standards had variable weak to potent stimulatory effect on the platelets at different time intervals.
Figure 12.31. The LEL activity of diabetic platelets stimulated after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of $n = 8$ indicated on graph).

Both fMLP- and PMA-induced platelets in the neat HM4 and the 10x diluted HM4 standard had activity below the CTRL platelets above the CTRL platelets. Both induced platelets in the 1000x and the 10000x diluted HM4 standards had activities above the CTRL platelets. The fMLP-induced platelets in the 100x diluted HM4 had activities that were above the
CTRL platelets, but the PMA-induced platelets’ activities were below. All the platelets with activities above the CTRL had their highest activity on stimulation.

Figure 12.32. The % inhibition of diabetic platelets after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat and the 10x diluted HM4 standards had weak inhibitory effects on both the fMLP- and the PMA-induced platelets, while the 1000x and the 10000x diluted HM4 standards and weak to potent stimulatory effect on the platelets. The 100x diluted standard had weak to moderate stimulatory effects on fMLP-induced platelets and variable weak inhibitory and stimulatory effects on PMA-induced platelets.
Figure 12.33. The LEL activity of diabetic platelets stimulated after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The activities of the platelets incubated with HM5 were similar to their activities under HM4. Both fMLP- and PMA-induced platelets in the neat HM5 and the 10x diluted HM5 standard had activity below the CTRL platelets above the CTRL platelets. Both induced platelets in the 1000x and the 10000x diluted HM4 standards had activities above the CTRL platelets. The fMLP-induced platelets in the 100x diluted HM5 had activities that
were above the CTRL platelets, but the PMA-induced platelets’ activities were below. All the platelets with activities above the CTRL had their highest activity on stimulation

![Graph A: % Inhibition of HM5](image)

Figure 12.34. The % inhibition of diabetic platelets after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat and the 10x diluted HM5 standards had weak inhibitory effects on both the fMLP- and the PMA-induced platelets, while the 1000x and the 10000x diluted HM4 standards and weak to potent stimulatory effect on the platelets. The 100x diluted standard had weak
to moderate stimulatory effects on PMA-induced platelets and weak inhibitory and stimulatory effects (at $t = 0$ and 60) on fMLP-induced platelets.

**HM6- Vuka Uphile™ Immune Booster**

![Graph A](image1)

![Graph B](image2)

Figure 12.35. The LEL activity of diabetic platelets stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of $n = 8$ indicated on graph).
Both fMLP- and PMA-induced platelets in the neat HM6 had activity below the CTRL platelets. The fMLP-induced platelets in the 10x diluted HM6 had activities that were below the CTRL platelets, but the PMA-induced platelets’ activities were above. Platelets in the neat and the 10x diluted HM6 had the same activities on stimulation and at 60 minutes of testing. Both induced platelets in the 1000x and the 10000x diluted HM6 standards had activities above the CTRL platelets. The fMLP-induced platelets in the 100x diluted HM6 had activity close to that of the CTRL, above and below at various times, but the activity of the PMA-induced platelets was above that of the CTRL platelets.

Figure 12.36. The % inhibition of diabetic platelets after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM6 had weak inhibitory effects on both the fMLP- and the PMA-induced platelets. The 10x diluted HM6 standards had weak inhibitory effects on the fMLP-induced platelets, and weak stimulatory effects on the PMA-induced platelets. The 1000x and the 10000x diluted HM6 standards had weak to potent stimulatory effect on the platelets. The 100x diluted HM6 standards had weak inhibitory and stimulatory effects on fMLP-induced platelets, and weak to potent stimulatory effect on the PMA-induced platelets.
Table 12.3. The average LEL diabetic activities of platelets incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
<td>LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control</td>
<td>0.006 ±0.0004</td>
<td>0.0058 ±0.0003</td>
<td></td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>0.0039 ±0.0004*</td>
<td>42.8 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0069 ± 0.0005*</td>
<td>-38.0 ± 35.5</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0094 ± 0.0025*</td>
<td>-25.5 ± 19.2</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.0084 ± 0.0014*</td>
<td>-23.0 ± 19.7</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0088 ± 0.0013*</td>
<td>-29.2 ± 17.3</td>
</tr>
<tr>
<td>HM2</td>
<td>Neat</td>
<td>0.0044 ±0.0003*</td>
<td>36.5 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0047 ±0.0006*</td>
<td>31.5 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0105 ±0.0020*</td>
<td>-53.0 ± 27.5</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.0085 ±0.0011*</td>
<td>-24.7 ± 14.9</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0091 ±0.0016*</td>
<td>-33.5 ± 22.0</td>
</tr>
<tr>
<td>HM3</td>
<td>Neat</td>
<td>0.0048 ±0.0005*</td>
<td>30.1 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0108 ±0.0040*</td>
<td>-56.3 ± 56.5</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0120 ±0.0027*</td>
<td>-75.1 ± 36.4</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.0105 ±0.0013*</td>
<td>-54.1 ± 17.3</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0101 ±0.0017*</td>
<td>-48.2 ± 24.4</td>
</tr>
<tr>
<td>HM4</td>
<td>Neat</td>
<td>0.0044 ±0.0004*</td>
<td>35.3 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0044 ±0.0003*</td>
<td>36.3 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0094 ±0.0009*</td>
<td>-37.1 ± 15.6</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.0106 ±0.0018*</td>
<td>-40.9 ± 21.2</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0106 ±0.0015*</td>
<td>-55.4 ± 20.7</td>
</tr>
<tr>
<td>HM5</td>
<td>Neat</td>
<td>0.0045 ±0.0004*</td>
<td>35.1 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0050 ±0.0005*</td>
<td>26.9 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0065 ±0.0005</td>
<td>4.6 ± 11.5</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.0082 ±0.0014*</td>
<td>-19.5 ± 20.3</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0107 ±0.0014*</td>
<td>-56.1 ± 18.9</td>
</tr>
<tr>
<td>HM6</td>
<td>Neat</td>
<td>0.0043 ±0.0003*</td>
<td>37.1 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0057 ±0.0003*</td>
<td>16.3 ± 8.8</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0066 ±0.0013</td>
<td>3.7 ± 19.5</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.0081 ±0.0015*</td>
<td>-18.2 ± 21.2</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0099 ±0.0018*</td>
<td>-44.5 ± 24.6</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 12.3 allow comparison of LEL activity and % inhibition at various concentrations for each standard. The values show stimulatory and inhibitory effects of the CHMs, ranging from weak to potent effects. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they do not correlate. The effects are generally comparable between the two mechanisms of stimulation of the platelets (fMLP or PMA), and most are statistically significant (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 13).
CHAPTER 13 DISCUSSION: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM DIABETIC MELLITUS PATIENTS

A total of six herbal mixtures and four serial dilutions of each one were tested for their effects on the LEL activity of cells and platelets stimulated by fMLP and PMA. The cells were all obtained from individuals suffering from type 2 diabetes mellitus, and were on their regular antihyperglycaemic medicine which included one or combination of the following medicines- insulin, metformin, gliclazide and glimepiride. The graphs presented before (chapter 12) showed time based changes that occurred in LEL activity of the cells and platelets. The average effects of each CHM and its dilutions on the whole blood phagocytes (table 12.1, page 210), the isolated human neutrophils (IHNs) (table 12.2, page 223) and the platelets (table 12.3, page 238) are discussed below.

HM1- *Intlamba Zifo™*

- The highest concentration of HM1 (neat) caused inhibition of the fMLP- and the PMA-induced whole blood phagocytes. All the diluted standards had stimulatory effects on the cells, which were non-systematic. The differences in the LEL activities of the cells in the neat and the 10x diluted standard were statistically significant when compared to the controls. Hence, at high concentrations, HM1 inhibited the cells via both mechanisms, while causing weak and random stimulation at low concentrations.

- Similar to the effects on whole blood phagocytes, the neat HM1 caused inhibition of the fMLP- and the PMA-induced IHNs, with statistically significant differences when compared to the controls. All the diluted standards had stimulatory effects on both induced cells, which were non-systematic. The differences in LEL activities of the PMA-induced cells were statistically significant against the controls. Hence at high concentrations, HM1 inhibited the IHNs via both mechanisms, while causing weak and random stimulation at low concentrations.

- The HM1 had similar effects on platelets as on whole blood phagocytes and the IHNs. The neat HM1 caused inhibition of the fMLP- and the PMA-induced platelets, significantly. All the diluted standards had stimulatory effects on the platelets, which were non-systematic. The differences in LEL activities of all the fMLP-induced platelets and the PMA-induced platelets in the 10x and 1000x dilutions were statistically significant against
the controls. Hence at high concentrations, HM1 inhibited the cells via both mechanisms, while causing weak and non-systematic stimulation at low concentrations.

**HM2- Maphilisa™ Herbal medicine**
- The neat HM2 had a weak inhibitory effect on both fMLP- and PMA-induced whole blood phagocytes, with statistically significant differences in LEL activities against the controls. The three lower diluted standards had a weak stimulatory effect on the fMLP-induced cells that was directly concentration dependent. The higher the concentration, the higher the stimulation. The 10000x standard also had a weak stimulatory effect on these cells, but which did not follow the pattern of the other diluted standards. At lower concentrations, the HM2 had weak and random stimulatory effect on PMA-induced cells, and only the 10x diluted HM2 standard had statistically significant difference against the control.
- The neat HM2 and the two lower dilutions had moderate and directly concentration dependent inhibitory effect on fMLP-induced IHNs, while the two higher diluted standards had weak and inversely concentration dependent stimulatory effect on these cells. The neat HM2 and the three lower dilutions had moderate and directly concentration dependent inhibitory effect on PMA-induced IHNs, while the 10000x diluted standards had weak stimulatory effect on the cells. Therefore HM2 had inhibitory effect at high concentrations, and stimulatory effect at low concentrations. The differences in LEL activities of cells in the neat HM2 and the 10x diluted standards were statistically significant when compared to the controls.
- The neat HM2 and the 10x diluted standard had a weak, direct concentration dependent inhibitory effect on fMLP-induced platelets. The other diluted standards had weak non-systematic stimulatory effect on those platelets. The neat HM2 also had a weak inhibitory effect on PMA-induced platelets, while the diluted standards had weak non-systematic stimulatory effects on those platelets. Statistically significant differences in the LEL activities were noted in all the platelets when compared to the controls.

**HM3- Matla™ African medicine for all diseases**
- The neat HM3 had weak inhibitory effect causing reduced LEL activity in fMLP- and PMA-induced whole blood phagocytes, and the differences in LEL activities against the controls were statistically significant. The three higher dilution standards had weak direct
concentration dependent stimulatory effects on the fMLP-induced cells. The 10x diluted standard had a weak stimulatory effect on the cells, which was not in sync with the effect of the other three standards. The three lower diluted HM3 standards had weak non-systematic stimulatory effect on the PMA-induced cells, while the 10000x dilution standard had weak inhibitory effect.

- The neat HM3 and the two lower dilutions had moderate and directly concentration dependent inhibitory effect on fMLP-induced IHNs, while the two higher diluted standards had weak and inversely concentration dependent stimulatory effect on these cells. The neat HM3 and the three lower dilutions had moderate and directly concentration dependent inhibitory effect on PMA-induced IHNs, while the 10000x diluted standards had weak stimulatory effect on the cells. Hence HM2 had inhibitory effect at high concentrations, and stimulatory effect at low concentrations. The differences in the LEL activities of cells in the neat HM2, the 10x and the 100x diluted standard were statistically significant when compared to the controls.

- The neat HM3 had a weak inhibitory effect on the fMLP- and PMA-induced platelets, and the differences in LEL activities against the controls were statistically significant. The three higher diluted standards had weak and directly concentration dependent stimulatory effects on both induced platelets, while the 10000x diluted standard also had weak stimulatory effects on the platelets, which were not correlating with the effects of the other three standards.

**HM4-Ngoma™ Herbal Tonic Immune Booster**

- The neat HM4 had a weak inhibitory effect and the three higher diluted standards had weak and directly concentration dependent stimulatory effects on the fMLP-induced whole blood phagocytes. The 10x diluted standard also had weak stimulatory effects on the cells, which were not correlating with the effects of the other three standards. The neat HM4 and the diluted standards, excluding the 10x standard had weak non-systematic inhibitory effects on PMA-induced IHNs. The 100x diluted HM4 had moderate stimulatory effect on PMA-induced cells.

- The neat HM4 and two lower dilutions had a directly concentration dependent and weak inhibitory effect on the fMLP-induced IHNs, and the differences in LEL activities against the controls were statistically significant. The two higher diluted standards had weak and inversely concentration dependent stimulatory effects on those cells. The 1000x
diluted HM4 standard had a weak stimulatory effect on PMA-induced IHNs, while the neat and the other diluted standards had weak and direct concentration dependent inhibitory effects on those cells.

- The neat HM4 and the 10x diluted standard had a weak, direct concentration dependent inhibitory effect on fMLP-induced platelets. The other diluted standards had weak inversely concentration dependent stimulatory effect on those platelets. The differences in the LEL activities of all those platelets were statistically significant against the controls. The neat HM4 and the two lower dilutions also had a weak, direct concentration dependent inhibitory effect on PMA-induced platelets, while the higher diluted standards had weak directly concentration dependent stimulatory effects on those platelets. The differences in the LEL activities of the platelets in the 10000x diluted standard were statistically significant when compared to the controls.

**HM5- Stametta™ Body Healing Liquid**

- The neat HM5 and the 10x HM5 diluted standard caused reduced LEL activity in whole blood phagocytes exerting a weak and direct concentration dependent inhibitory effect on both induced cells. The three other diluted standards had weak and directly concentration dependent stimulatory effects on the cells. The differences in the LEL activities of cells in the neat HM5, the 100x and the 1000x diluted standard were statistically significant when compared to the controls.
- The HM5 and diluted standards had a concentration dependent and weak inhibitory effect on the fMLP-induced IHNs. The differences in the LEL activities against the controls were statistically significant in all the HM5 standards except the 10000x dilution standard. The neat HM5 and the two lower HM5 diluted standards also had weak and direct concentration dependent effects on the PMA-induced neutrophils. The two higher diluted HM5 standards had weak and direct concentration dependent stimulatory effect.
- The neat HM5 and two lower dilutions had a directly concentration dependent and weak inhibitory effect on the fMLP-induced platelets. The two higher diluted standards had weak and inversely concentration dependent stimulatory effects on those platelets. The differences in the LEL activities of fMLP-induced platelets in the neat HM5, the 10x, the 1000x and the 10000x diluted standard were statistically significant against the controls. The neat HM5 and the 10x diluted standard had directly concentration dependent and weak inhibitory effects on the PMA-induced platelets. The three other diluted standards
had weak and inversely concentration dependent stimulatory effects on those platelets. Statistically significant differences in the LEL activities of PMA-induced platelets were noted between the controls and the neat HM5, the 1000x and the 10000x diluted standard.

**HM6- Vuka Uphile™ Immune Booster**
- The neat HM6 and the three lower diluted standards exerted a non-systematic weak inhibitory effect on both the fMLP- and induced whole blood phagocytes. The 10000x diluted standard exerted weak stimulatory effects on both induced cells. The differences in the LEL activities of cells in the neat HM5 were statistically significant against the controls.
- The neat HM6 and the two lower diluted standards had weak and directly concentration dependent inhibitory effects on the fMLP-induced IHNs, with statistically significant differences in LEL activities against the controls. The two higher diluted HM6 standards had weak and inverse concentration dependent stimulatory effect. The neat HM6 had weak inhibitory effect on the PMA-induced IHNs. The diluted HM6 standards had weak non-systematic stimulatory effect on the PMA-induced cells. The differences in LEL activities were statistically significant in the neat HM6, the 10x, 100x and the 1000x diluted HM6 standards in the PMA-induced neutrophils when compared with the controls.
- The neat HM6 and the two lower diluted standards had weak and directly concentration dependent inhibitory effects on the fMLP-induced platelets, with statistically significant differences in LEL activities against the controls, except in the 100x diluted standard. The two higher diluted HM6 standards had weak and inverse concentration dependent stimulatory effects on those platelets, with statistically significant differences in LEL activities. The neat HM6 had a weak inhibitory effect on the PMA-induced platelets while the diluted standards had weak non-systematic stimulatory effects on those platelets, also with statistically significant LEL activities against the controls.
Table 13.1. Summary of the overall effects of the CHMs on the cells and platelets of diabetes mellitus patients, from the highest to the lowest concentration

<table>
<thead>
<tr>
<th></th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>HM1</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓↓ +</td>
</tr>
<tr>
<td>HM2</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓↓ +</td>
</tr>
<tr>
<td>HM3</td>
<td>↓ +†</td>
<td>↓ +†</td>
<td>↓↓ +</td>
</tr>
<tr>
<td>HM4</td>
<td>↓ +†</td>
<td>↓ +†</td>
<td>↓↓ +</td>
</tr>
<tr>
<td>HM5</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓↓ +</td>
</tr>
<tr>
<td>HM6</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓↓ +</td>
</tr>
</tbody>
</table>

↓ weak inhibition; ↓↓ moderate inhibition; ↓↓↓ potent inhibition
+ weak stimulation; † moderate stimulation; ‡ potent stimulation

13.1 Implications of the effects of the commercial herbal mixtures on cells and platelets from diabetes mellitus patients

As stated in other investigations, the studied in vitro functional responsiveness of IHNs is a reflection of the in vivo physiological capabilities of these cells, which include chemotaxis, adherence, phagocytosis, ROS production (SOA and other oxygen radicals), degranulation, and microbial killing (McManus et al., 2001). As guided by the WHO, data obtained from in vitro tests should be seen as indicators of potential toxicity (WHO, 2000). Though in vivo tests should be performed, it is imperative to describe what the results obtained in this study suggest.

There was overwhelming weak inhibition (at high concentration) of neutrophils in whole blood and weak to moderate inhibition of the isolated neutrophils by all the six HMs. It is known that neutrophils in DM have impaired functional capacity as a result of the insulin deficiency and persistent hyperglycaemia (Hatanaka et al., 2006; Alba-Loureiro et al., 2007). However the treatment of diabetes has been reported to improve their functional capacity (Alba-Loureiro et al., 2007). The inhibition of the cells by the six HMs means that their functional capacity is reduced as evidenced by the reduction of ROS production in the study.
It is well-known that the reduced neutrophil function in DM is linked with increased susceptibility to microbial infections. Reduced production of ROS and decreased bactericidal activity are some of the functional changes that occur in neutrophils in DM (Hatanaka et al., 2006; Alba-Loureiro et al., 2007). ROS are potent antimicrobial agents that play an important role of defence against pathogens and the effectiveness of neutrophils as phagocytes is determined by a functional NOX system which generates ROS. As postulated by Mahomoodally et al. (2012), inhibition of the cells by both fMLP and PMA pathways, suggests that the HMs do not affect a specific pathway, but directly inhibit a common biochemical target site such as NOX or scavenge the ROS. Inhibition via the fMLP pathway could have occurred by antagonistic action on the formyl peptide receptor, or by desensitisation of the receptor. This would be in accordance with the report that stated that there was decreased responsiveness to agonists that initiate stimulus response through G-protein-coupled receptors, which contributes to the PMN dysfunction in DM (McManus et al., 2001). The inhibition of ROS production by the HMs means that the impaired functional capacity is worsened. This may therefore further increase the risk of acquiring infections. DM patients are advised to take proper care of feet, to avoid fungal and bacterial infections which may lead to ulcerations and gangrene (Porter & Kaplan, 2011). It has been mentioned that DM patients have a worse prognosis once infection is established, and that abnormalities in neutrophil function contribute to the increased susceptibility to infections and to the severity of the infection (Alba-Loureiro et al., 2007). Therefore the intake of the HMs may impair the immune system further, and require more vigilant care and avoidance of acquiring infections.

There was weak to moderate stimulation (at low concentration) of neutrophils in whole blood together with weak stimulation of the IHNs by the HMs. Activation of the cells means there was increased activity of the NOX, which resulted in the formation of ROS. In view of the fact that reduced ROS production is implicated in the neutrophils’ dysfunction of DM, the increase in ROS production should therefore be a favourable effect. It should enhance the functional capacity of the neutrophils, and improve on the prognosis and the susceptibility to infections. It is important to note that the stimulation (which is not potent or excessive) of the cells occurred at very low concentrations of the HMs, and were mainly inversely related which meant that the stimulation increased as the concentration decreased. It agrees with the studies which found that neutrophils in DM have increased responsiveness (Hatanaka et al., 2006; Alba-Loureiro et al., 2007). The stimulation at low
concentrations should augur well for the user since low doses of medicines generally do not have many side effects or adverse and toxic effects. The stimulation occurred via both the fMLP and PMA mechanisms. This means the HMs have the capacity to activate or sensitise the formyl peptide receptor, applying the corollary to Mahomoodally et al. (2012), it therefore suggests that the HMs do not affect a specific pathway, but directly activate a common biochemical target site such as NOX.

The platelets in the study were weakly inhibited at high concentrations via both the fMLP and PMA mechanisms. It has been reported that platelets in DM exist in a state of hyperactivity and hyperaggregation as a result of the insulin deficiency and persistent hyperglycaemia (El Haouari & Rosado, 2008). The morphological and functional changes of platelets in DM play a critical role in the development of the angiopathies and cardiovascular diseases associated with DM (Ghoshal & Bhattacharyya, 2014).

Inhibition of the ROS production of platelets means suppression of one of the abnormalities associated with the hyperreactivity nature of the platelets, i.e. the increased oxidative stress amidst reduced antioxidant capacity. Under normal circumstances, on stimulation by agonists, diabetic platelets react by producing excessive ROS, increased intracellular free calcium and tyrosine phosphatase activity. These actions result in increased synthesis of TXA2, which enhances the hyperaggregation of platelets (Trovaï & Anfossi, 2002; El Haouari & Rosado, 2008; Kakouros et al., 2011). Increased ROS in DM platelets, in the presence of hyperglycaemia causes increased production of advanced glycation end-products (AGEs), which participate in the development of atherosclerotic complications (Ferreiro, Gomez-Hospital et al., 2010; Kakouros et al., 2011; Santilli et al., 2015). In the presence of high concentrations of the HMs, the platelets in the study failed to produce ROS, which would be beneficial in the treatment of DM. The inhibition of ROS production by the HMs would lower the risks of the cardiovascular complications due to platelet hyperreactivity in DM.

The inhibition of the platelets occurred via both the fMLP and the PMA pathways. Inhibition via the fMLP pathway could have occurred by antagonistic action on the formyl peptide receptor, or by desensitisation of the receptor. It is well-known that many proaggregants such as TXA2, thrombin and collagen initiate platelet aggregation by binding to G-protein coupled receptors, leading to activation of PKC, which is a known mediator of platelet activation (Vinik et al., 2001). Inhibition of the G-coupled receptors by the HMs would
therefore inhibit the proaggregant and prothrombic mechanisms that are detrimental in DM. The inhibition of the PMA pathway could have occurred by binding to PKC and directly inhibiting its activity. This would similarly, inhibit a mechanism that increases the risks of cardiovascular complications in DM. As proposed by Mahomoodally et al. (2012), inhibition of cells by both fMLP and PMA pathways, indicates that the HMs do not affect a specific pathway, but directly inhibit a common biochemical target site such as NOX or scavenge the ROS. This would reduce the oxidative stress which would reduce the amplified platelet aggregation response that prevails in DM, and also reduce the formation of AGEs, thereby lowering the risk of CVS complications. Therefore, patients taking high doses of the HMs may have added benefit in the same way that conventional antiplatelet therapy provides. It should however be noted that high doses of medicines, in general have more adverse and toxic effects, and increased unwanted interactions, hence these patients might be at risk of bleeding or excessive bleeding which is a known complication of antiplatelet therapy.

Inhibition of platelets may also impact on their role as mediators in the immune system and inflammatory response. Platelets generally interact with other immune cells and enhance the immune response. Platelets are also capable of directly destroying pathogens by secreting substances that destroy and damage bacteria such as ROS. Krotz et al. (2004) stated that the NOX-dependent production of $O_2^-$ increased the recruitment of neutrophils to a growing thrombus, and Semple et al. (2011) observed that platelet-neutrophil complexes perform phagocytosis, cytotoxicity and cytolysis more effectively than neutrophils on their own. However, in DM, there are increased platelet-leukocytes aggregates which contribute to the development of vascular ischaemic disease (Trovatti & Anfossi, 2002), and there is increased formation of ROS (oxidative stress). Hence in the presence of the HMs, the immune system may be further compromised by the inhibition of the platelets and the impaired interaction with other immune cells.

There was general weak stimulation of the whole blood phagocytes and IHNs, with the exception of HM4 which also had moderate inhibition on the cells. The stimulation occurred at low concentration of HMs. Activation of the cells means there was increased activity of the NOX, which resulted in the formation of ROS, and it occurred via both the fMLP and PMA pathways. Stimulation via the fMLP route denoted that in the presence of the HMs, the formyl peptide receptor was more responsive, meaning that the HM may have sensitised the receptor. It could also be that the HMs acted on other sites, which
have permissive effects on the formyl peptide receptor activity, resulting in the increased activity of NOX. Stimulation via the PMA route meant that in the presence of the HMs, the enzyme PKC or some elements of PKC signalling route are sensitised, such that when PKC is activated the response by the cell is amplified. These responses at low concentrations of HMs are in agreement with the report by McManus et al. (2001) and Hatanaka et al. (2006), that neutrophils in DM have enhanced functional responsiveness. The increased responsiveness is also observed by the higher baseline responses (CTRL) of the DM cells when compared with the non-DM cells (healthy volunteers).

Stimulation of neutrophils may be beneficial as part of immune-boosting effects. The rationale is that activated neutrophils would respond rapidly and favourably, and be more efficient as elements of the immune system. As reported before, functional capacity changes that occur in DM neutrophils include decreased bactericidal activity, impaired phagocytosis and migration, diminished release of lysosomal enzymes and reduced production of ROS (Hatanaka et al., 2006; Alba-Loureiro et al., 2007). Stimulation of the neutrophils, by activating NOX and the increased ROS formation may be worthwhile in improving bactericidal activity and other functional impairments. This would reduce the susceptibility to, and the severity of the infections and improve on the prognosis once infection is established in DM all of which were associated with the functional abnormalities in diabetic neutrophils. However, as Roos et al. (2003) mentioned, the activity of NOX should be controlled well, so as to avert unwanted damage to host tissue. Another concern is that activation of NOX may also reveal unforeseen anti-inflammatory reactions (Tintinger et al., 2008). Oxidative stress in DM is closely associated with microvascular and cardiovascular complications (Aydin et al., 2001). Other conditions in which the extensive release of intracellular oxidising agents by phagocytes was implicated were stroke, myocardial infarction and respiratory disease syndrome (Caldefie-Chezet et al., 2002).

There was weak, moderate and potent stimulation of DM platelets by the HMs. As stated before, DM is characterised by a high risk of atherothrombotic events due to factors such as increased coagulation, impaired fibrinolysis, endothelial dysfunction and platelet hyperreactivity (or hyperactivity) (Ferreiro, Gomez-Hospital et al., 2010). Stimulation of the platelets means there was increased activity of the NOX, which resulted in the formation of ROS, and it occurred via both the fMLP and PMA pathways, and at low concentrations of the HMs. The baseline responses of the DM platelets were lower than non-DM platelets,
which did not indicate hyperreactivity behaviour of DM platelets. This may be due to the effect of the antidiabetic therapy. Antidiabetic drugs have a variety of effects on different aspects of platelet dysfunction in DM, with metformin being the only one with direct impact on the oxidative stress. Metformin has been reported to be useful in restoring the antioxidant status of cells in type 2 DM. Amongst other effects it has, metformin was said to decrease ROS generation, stabilise SOA production and lower the risk of CVD (Ghoshal & Bhattacharyya, 2014).

The stimulatory effect by the HMs suggested the hyperactivity or hyperreactivity behaviour of platelets in DM when compared with non-DM platelets. In agreement with other investigations (Trovati & Anfossi, 2002; El Haouari & Rosado, 2008; Kakouros et al., 2011), on stimulation by agonists, the DM platelets reacted by producing excessive ROS. The stimulation effect implies that in the presence of the HMs the platelets may be circulating in sensitised form. It means that the already increased oxidative stress in DM, with reduced antioxidant capacity in platelets will be heightened further in the presence of the HMs. The excessive ROS production was reported as one of the factors that contribute towards the hyperaggregation of DM platelets and the formation of AGEs which lead to CVS complications (Kakouros et al., 2011). The diabetic platelet is generally atherogenic and prothrombotic in nature (Ferreiro, Gomez-Hospital et al., 2010; Kakouros, et al., 2011; Santilli et al., 2015); therefore the intake of the HMs may aggravate the increased risk of cardiovascular disease that occur in DM such as coronary artery disease (CAD) and acute coronary syndrome (ACS).

Activated platelets normally secrete chemical mediators which enhance the interaction of platelets with other cells of the immune system. Kakouros et al. (2011) stated that diabetic platelets were hyperreactive through various pathophysiological mechanisms, and were therefore more sensitive to stimulation by even weak agonists. One of the mechanisms noted was the number and adhesiveness of surface platelet glycoprotein receptors which were significantly increased in DM (Ghoshal & Bhattacharyya, 2014). Therefore in the presence of the HMs, these platelets may have an exaggerated response to stimuli such as bacteria, with excessive ROS production which may also harm host tissue. It may also result in uncontrolled or exaggerated inflammatory response and immune reaction, which are the basis of development of allergic reactions. There may be increased formation of aggregates with neutrophils, monocytes and lymphocytes, with increased release of the chemical mediators by these cells. Parij et al. (1998) reported that the ROS and granule
enzymes cause damage to molecules, cells and tissues of inflammation and contribute to the pathogenesis of immune and non-immune chronic inflammatory conditions. As reported by Ruf et al. (1992), platelet-neutrophil interactions are associated with occurrence and progression of diseases such as acute respiratory distress syndrome (ARDS), myocardial ischaemia and atherosclerosis. Hence, the presence of the HMs may cause increased occurrence of known and unexpected complications of DM.
CHAPTER 14 RESULTS: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM HYPERTENSIVE PATIENTS

The effects of the six CHMs on the LEL activities of the IHNs, PLTs and whole blood phagocytes obtained from hypertensive patients are shown below. The results for all the three components are first displayed in graphs of luminescence (in RLUs) versus time (in minutes) and the % inhibitions which indicated the kinetics observed over 60 minutes. Then the average LEL activities and the % inhibitions over 60 minutes were calculated and are displayed in the tables below.

14.1 Luminescence activity of whole blood phagocytes from hypertensive patients

The graphs below depict luminol-enhanced luminescence (LEL) activity of whole blood phagocytes stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibitions of the HMs on the cells at the various time intervals.
Figure 14.1. The LEL activity of hypertensive whole blood phagocytes after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The fMLP-induced cells (A) in the control (CTRL) had LEL activity which increased after stimulation and peaked at 10 minutes. Then the activity decreased gradually and stabilised until 60 minutes. The PMA-induced (B) CTRL cells had activity that declined slightly after stimulation, but the levels remained the same throughout the 60 minutes of testing.

The fMLP-induced LEL activity of the phagocytes incubated with the neat HM1 was higher than that of the control initially, then it gradually lowered below the control till t = 25
minutes, and then it remained constant, close to that of the control for the rest of the testing time. The cells incubated with the 10x diluted HM1 showed activity above that of the control, from start till 60 minutes. The cells in the other lower diluted standards had activities close to the controls. The PMA-induced cells incubated with the 10x diluted HM1 showed LEL activity above that of the control, from start till 60 minutes, with the activity at $t = 0$ being the highest. The cells in the neat HM1 and the other lower diluted standards had activities close to the controls.

In general, the highest concentration of HM1 and the lower concentrations (100x, 1000x, 10000x dilutions) had similar effects on the activity of the cells, either very little or none. The exception occurred in the time interval between 5 and 25 minutes where the activity was reduced below that of the controls with the neat HM1 in fMLP induced cells. The 10x diluted HM1 however, caused increased activities of the cells, whether PMA- or fMLP induced. Also, the fMLP induced cells had much higher LEL activity than those induced with PMA. With the exception of the 10x diluted HM1, when compared at the same test standard and time intervals, the RLU values or the fMLP-induced cells were higher than PMA-induced cells.
Figure 14.2. The % inhibition of hypertensive whole blood phagocytes after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

There was indication of weak inhibitory effect in fMLP-induced cells (A), between the time intervals of 5 to 25 minutes. Other than that exception, there was general stimulatory effect on cells, as evidenced by the negative values of % inhibition. The highest stimulation effect was caused by the 10x diluted HM1 in both the fMLP and PMA-induced cells, though the effect was greatly pronounced in the PMA-induced cells. The stimulation effect in the fMLP cells was low (< 50%) while that in PMA-induced cells was moderate (> 50%) to potent (> 80%).
**Figure 14.3.** The LEL activity of hypertensive whole blood phagocytes after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The cells incubated with the 10x diluted HM2 had notable LEL activity for both the PMA and the fMLP pathways but the PMA-induced cells had much higher RLU values than the fMLP-induced cells, and they were all significant. The activity of the PMA-induced cells
declined rapidly in the first five minutes, and progressed gradually until 60 minutes similar to the decline in the activity of the fMLP-induced cells. The cells incubated with the other test standards as well as the neat HM2 had comparable activities, closer to the controls. The cells exposed to the neat HM2 showed LEL activity less than the control in both mechanisms.

Figure 14.4. The % inhibition of hypertensive whole blood phagocytes after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

There was variable weak inhibitory effect in fMLP-induced cells (A) by the neat HM2. This effect was weaker in the PMA-induced cells. Other than that exception, there was general
stimulatory effect on cells, as evidenced by the negative values of % inhibition. The highest stimulation effect was caused by the 10x diluted HM2 in both the fMLP and PMA-induced cells, though the effect was greatly pronounced in the PMA-induced cells. The stimulation effect in the fMLP cells was low (< 50%) while that in PMA-induced cells was moderate (> 50%) to potent (> 80%).

HM3- Matla™ African medicine for all diseases

Figure 14.5. The LEL activity of hypertensive whole blood phagocytes after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
All the fMLP-induced cells incubated with the diluted HM3 standards showed LEL activity slightly above that of the control. The cells exposed to the neat HM3 had consistent low activity, below that of the control for the entire 60 minutes of test time. The PMA-induced cells in the neat HM3 had activity equal to that of the control, which meant that the neat HM3 had no effect on the cells. The cells in the lower concentration standards had activities just above that of the control, with the highest activity in the 100x diluted HM3.

Figure 14.6. The % inhibition of hypertensive whole blood phagocytes after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM3 had a weak inhibitory effect on the fMLP-induced cells, except at \( t = 10 \) minutes, where the effect was moderate. The other test standards had stimulatory effect on the cells, with the lowest concentration solution (10000x dilution) exerting the highest effect on average. All the HM3 test standards had a stimulatory effect on the PMA-induced cells, with the 100x diluted standard having effect greater than 50%.

**HM4- Ngoma™ Herbal Tonic Immune Booster**

![Graph A](image1)

Leukocyte extracellular lysis (LEL) activity of whole blood phagocytes after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of \( n = 8 \) indicated on graph).
Both fMLP and PMA-induced cells incubated with the 100x diluted HM4 standard showed LEL activity much higher than those incubated with the other solutions. The cells in the other test standards showed activities close to the control, but above, consistently for 60 minutes. Cells incubated with the neat HM4 had reduced LEL activities, close to but below that of the controls.

![Graph A](image1.png)

![Graph B](image2.png)

Figure 14.8. The % inhibition of hypertensive whole blood phagocytes after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM4 had a weak inhibitory effect on both induced cells, with the effect being much lower on the PMA-induced cells. The diluted HM4 standards had stimulation effect
on the cells, and the 100x diluted standard had the high and most potent inhibitory effect on the cells (> 100%).

**HM5- Stametta™ Body Healing Liquid**

Figure 14.9. The LEL activity of hypertensive whole blood phagocytes after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Cells incubated with the neat HM5 had reduced LEL activities, close to but below that of the controls. The fMLP-induced cells had LEL activity above that of the control, and it was consistent throughout. The PMA-induced cells incubated with the 100x and the 1000x diluted HM5 standards showed comparable LEL activity higher than those incubated with the other two dilute solutions. The cells in the other test standards showed activities close to but above the control consistently for 60 minutes.

Figure 14.10. The % inhibition of hypertensive whole blood phagocytes after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM5 had a weak inhibitory effect on both induced cells, with the effect being much lower on the PMA-induced cells. The 10x diluted M5 standards had the lowest stimulation effect on the cells, followed by the 100x diluted standard. The 1000x and the 10000x diluted standards had comparable high stimulatory effect on the fMLP-induced
cells. However the 100x and 1000x diluted HM5 standards had comparable moderate to high stimulatory effect on PMA-induced cells.

HM6- Vuka Uphile™ Immune Booster

Figure 14.11. The LEL activity of hypertensive whole blood phagocytes stimulated after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The cells incubated with the diluted HM6 standards showed comparable LEL activities, close to but above the control which remained consistent for the 60 minutes of test time.
The fMLP-induced cells in the neat HM6 had reduced activity, below the control, whereas the PMA-induced cells had activity same as that of the control on average.

![Graph A](image)

![Graph B](image)

Figure 14.12. The % inhibition of hypertensive whole blood phagocytes after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated)

The neat HM6 had a weak inhibitory effect on the fMLP-induced cells, with the effect being the lowest at t = 0 minutes. The 10x diluted M6 standard had the lowest stimulation effect on the fMLP-induced cells, yet it had the highest stimulatory effect (t = 0 min) of all the standards on these cells for the entire 60 minutes. The other dilute standards had low to moderate stimulatory effect on the fMLP-induced cells. The HM6 diluted standards had a general comparable low stimulatory effect on PMA-induced cells.
Table 14.1. The average LEL activities of hypertensive whole blood phagocytes incubated with the HMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>IFMLP-induced cells</th>
<th>PMA-induced cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
<td>Average LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5.44 ± 0.75</td>
<td>-</td>
<td>4.77 ± 0.19</td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>5.04 ± 0.45</td>
<td>5.82 ± 17.12</td>
<td>5.08 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>6.99 ± 0.90*</td>
<td>-28.88 ± 9.71</td>
<td>8.19 ± 1.49*</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>5.85 ± 0.72</td>
<td>-7.81 ± 5.67</td>
<td>5.40 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>5.84 ± 0.78</td>
<td>-7.6 ± 9.09</td>
<td>5.11 ± 0.35</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>5.69 ± 0.55</td>
<td>-5.25 ± 7.78</td>
<td>4.97 ± 0.35</td>
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<tr>
<td>HM2</td>
<td>Neat</td>
<td>3.84 ± 0.29*</td>
<td>28.50 ± 10.20</td>
<td>4.46 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>9.07 ± 1.73*</td>
<td>-68.99 ± 41.60</td>
<td>10.31 ±3.34*</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>7.20 ± 0.78*</td>
<td>-33.92 ± 20.55</td>
<td>5.76 ± 0.42*</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>5.74 ± 0.46</td>
<td>-6.60 ± 12.21</td>
<td>5.57 ± 0.37*</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>6.43 ± 0.58*</td>
<td>-19.08 ± 10.64</td>
<td>5.82 ± 0.42*</td>
</tr>
<tr>
<td>HM3</td>
<td>Neat</td>
<td>3.81 ± 0.44*</td>
<td>28.81 ± 12.34</td>
<td>4.73 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>6.16 ± 0.39*</td>
<td>-15.64 ± 21.48</td>
<td>6.65 ± 0.92*</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>7.64 ± 0.68*</td>
<td>-42.87 ± 26.29</td>
<td>8.15 ± 1.31*</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>6.25 ± 0.29*</td>
<td>-45.25 ± 14.00</td>
<td>6.36 ± 0.47*</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>6.79 ± 0.90*</td>
<td>-57.35 ± 20.67</td>
<td>5.79 ± 0.53*</td>
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<tr>
<td>HM4</td>
<td>Neat</td>
<td>3.75 ± 0.18</td>
<td>30.13 ± 8.64</td>
<td>4.19 ± 0.39</td>
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<tr>
<td></td>
<td>10x dilution</td>
<td>7.07 ± 1.29</td>
<td>-29.77 ± 15.83</td>
<td>5.69 ± 0.52*</td>
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<tr>
<td></td>
<td>100x dilution</td>
<td>10.66 ± 1.80</td>
<td>-97.79 ± 37.98</td>
<td>12.15 ± 2.71*</td>
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<td>1000x dilution</td>
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<td>-40.81 ± 16.32</td>
<td>5.67 ± 0.62*</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>6.05 ± 0.79</td>
<td>-39.74 ± 11.98</td>
<td>5.34 ± 0.33*</td>
</tr>
<tr>
<td>HM5</td>
<td>Neat</td>
<td>3.93 ± 0.26</td>
<td>26.38 ± 12.12</td>
<td>4.33 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>6.10 ± 0.59</td>
<td>-14.06 ± 20.90</td>
<td>6.14 ± 0.64*</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>8.42 ± 1.09</td>
<td>-56.20 ± 23.68</td>
<td>9.26 ± 2.27*</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>8.96 ± 0.88</td>
<td>-108.31 ± 28.73</td>
<td>8.74 ± 1.84*</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>8.29 ± 1.11</td>
<td>-91.08 ± 14.69</td>
<td>6.71 ± 0.32*</td>
</tr>
<tr>
<td>HM6</td>
<td>Neat</td>
<td>4.00 ± 0.35*</td>
<td>25.36 ± 11.50</td>
<td>4.48 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>5.85 ± 1.24</td>
<td>-10.27 ±36.04</td>
<td>6.01 ± 1.13*</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>6.57 ± 0.59*</td>
<td>-22.91 ± 22.49</td>
<td>6.40 ± 0.49*</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>6.61 ± 0.59*</td>
<td>-53.23 ± 13.75</td>
<td>5.84 ± 0.58*</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>6.69 ± 0.48*</td>
<td>-55.33 ± 13.10</td>
<td>6.31 ± 0.41*</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 14.1 allow comparison of LEL activity and % inhibition of each standard. The values show weak inhibitory effects and stimulatory effects ranging from weak to remarkably potent effects. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they are non-systematic. The effects are generally variable between the fMLP based mechanism and the PMA mechanisms of stimulation, with some being statistically significant (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 15).
14.2 Luminescence activity of isolated human neutrophils from hypertensive patients

The graphs below depict the LEL activity of isolated human neutrophils (IHNs) stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the cells at the various time intervals.

**HM1- Intlamba Zifo™**

![Graph A](image1)

![Graph B](image2)

Figure 14.13. The LEL activity of hypertensive isolated human neutrophils after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the fMLP- and PMA-induced cells incubated in the neat HM1 had activity below that of the CTRL cells which was consistent throughout the 60 minutes of observation. All the fMLP-induced cells in the diluted HM1 standards had activities close to the CTRL cells, and oscillated above and below the CTRL at various time intervals. The PMA-induced cells in the diluted HM1 standards had activities close to the CTRL, but were mainly above. Both induced cells had their highest LEL activities on stimulation that decreased thereafter.

![A diagram showing % inhibition of HM1 over time](image)

![A diagram showing % inhibition of HM1 over time](image)

Figure 14.14. The % inhibition of hypertensive isolated human neutrophils after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM1 had weak inhibitory effects on both fMLP- and PMA-induced cells. The 10x diluted HM1 standard had weak inhibitory effects on fMLP-induced cells and weak
stimulatory effects on PMA-induced cells. The 100x diluted standard had variable weak inhibitory and stimulatory effects on both induced cells at different time intervals. The 1000x and the 10000x diluted standards had weak to moderate stimulatory effects on both induced cells.

**HM2- Maphilisa™ Herbal medicine**

![Graph A](image)

**Figure 14.** The LEL activity of hypertensive isolated human neutrophils after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both PMA- and fMLP-induced cells in the neat HM2 and the 10x diluted standard had activity below the CTRL cells. The IHNs in the other diluted standards had activities close to the CTRL, above and below and mimicked the CTRL cells throughout. Both induced cells had their highest LEL activities on stimulation that decreased thereafter, except the cells in the 10x diluted standard whose activity peaked at 10 minutes after stimulation.

Figure 14.16. The % inhibition of hypertensive isolated human neutrophils after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The neat HM2 had weak to moderate inhibitory effect on the fMLP- and the PMA-induced cells. The 10x diluted standard had weak inhibitory effects on those cells. The other diluted HM2 standards had variable weak inhibitory and stimulatory effects on both induced cells.

HM3- Matla™ African medicine for all diseases

Figure 14.17. The LEL activity of hypertensive isolated human neutrophils after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The cells incubated with the 10000x diluted HM3 had notable high LEL activity for both the PMA- and the fMLP-induced experiments which were equivalent and significant. The activities decreased rapidly until five minutes and then gradually to be equal at 20 minutes. The cells incubated with the other test standards as well as the neat HM3 had comparable activities, closer to the control. The cells exposed to the neat HM3 showed LEL activity less than the controls in both the fMLP- and PMA-induced cells. Both induced cells had their highest LEL activities on stimulation.

Figure 14.18. The % inhibition of hypertensive isolated human neutrophils after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
In correlation with the LEL activities, the neat HM3 and the 10x diluted standard had weak inhibitory effects on the fMLP- and PMA-induced IHNs. The other diluted HM3 standards had variable weak inhibitory and stimulatory effect on the cells. The 10000x diluted standard had the weak to moderate stimulatory effects and was the standard with the highest stimulatory effects.

**HM4- Ngoma™ Herbal Tonic Immune Booster**

![Graph A](image1)

![Graph B](image2)

**Figure 14.19.** The LEL activity of hypertensive isolated human neutrophils after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
Both the fMLP-induced and the PMA-induced cells incubated with neat HM4 and the 10x
diluted standard had LEL activities lower than the CRTL cells activities. All the cells in the
other standards had activities closer to that of the CTRL cells but the fMLP-induced cells in
the 1000x diluted HM4 had activity much higher than the cells in other diluted standards.
The fMLP-induced IHNs in the 10000x diluted standard had the highest activity on
stimulation, and the lowest activity from 25 minutes onwards.

Figure 14.20. The % inhibition of hypertensive isolated human neutrophils after incubation
with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA.
(Means of n = 8 are indicated).
The neat HM4 and the 10x diluted standard had weak inhibitory effects on the fMLP- and PMA-induced IHNs. The other diluted HM3 standards had variable weak inhibitory and stimulatory effect on the fMLP-induced cells, while they had distinct weak to moderate stimulatory effects on PMA-induced cells.

**HM5- *Stametta™ Body Healing Liquid***

![Graph A](image)

**Figure 14.21.** The LEL activity of hypertensive isolated human neutrophils after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The IHNs incubated with the HM5 and diluted standards had activities that were close to the CTRL cells, but were highly unstable and variable for 25 minutes of testing. The PMA-
induced IHNs in the neat HM5, the 10x and the 10000x diluted standards were the ones with distinct activity, below that of the CTRL and cells (neat, 10x) and above (10000x).

![Graph A](image1)

**Figure 14.22.** The % inhibition of hypertensive isolated human neutrophils after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM5 and the 10xdiluted standard had weak inhibitory effect on both the PMA- and the fMLP-induced cells. In correlation with variable LEL activities, all the other diluted
HM5 standards had highly variable weak inhibitory and stimulatory effects at different times on both the fMLP- and PMA-induced cells.

**HM6- Vuka Uphile™ Immune Booster**

![Graph showing LEL activity of isolated human neutrophils](image)

Figure 14.23. The LEL activity of hypertensive isolated human neutrophils after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The fMLP-induced IHNs in HM6 had LEL activities which were close to that of the CTRL cells, above and below at different times but were all below the CTRL on stimulation. The PMA-induced IHNs in the neat HM6, the 10x and 100x diluted standards had activities below that of the CTRL cells, while the activities of the IHNs in the higher two diluted standards.

Figure 14.24. The % inhibition of hypertensive isolated human neutrophils after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).
The HM6 and its dilutions had variable weak inhibitory and stimulatory effects on fMLP-induced IHNs. The HM however, had clear and distinct effects on PMA-induced cells. The neat HM6, the 10x and 100x diluted standards had weak inhibitory effects on the cells, while the 1000x and 10000x diluted standards had weak stimulatory effects, except for the 10000x standard at 60 minutes.

Table 14.2. The average LEL activities of hypertensive isolated human neutrophils incubated with the HM over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>17.49 ± 3.59</td>
<td>20.03 ± 2.44</td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>13.25 ± 1.55*</td>
<td>22.54 ± 12.99</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>15.60 ± 2.35</td>
<td>9.93 ± 8.00</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>16.63 ± 4.32</td>
<td>5.23 ± 10.55</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>18.15 ± 5.36</td>
<td>-36.47 ± 14.79</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>16.62 ± 4.39</td>
<td>-25.89 ± 17.04</td>
</tr>
<tr>
<td>HM2</td>
<td>Neat</td>
<td>13.52 ± 1.17*</td>
<td>20.19 ± 15.14</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>13.74 ± 1.81*</td>
<td>19.20 ± 17.29</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>16.32 ± 3.10</td>
<td>6.12 ± 10.09</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>17.16 ± 6.03</td>
<td>3.73 ± 12.31</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>18.33 ± 6.06</td>
<td>-3.24 ± 11.41</td>
</tr>
<tr>
<td>HM3</td>
<td>Neat</td>
<td>13.67 ± 0.88*</td>
<td>19.64 ± 13.14</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
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<td>13.52 ± 12.51</td>
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<tr>
<td></td>
<td>100x dilution</td>
<td>15.68 ± 2.56</td>
<td>9.74 ± 7.19</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>16.98 ± 5.07</td>
<td>3.90 ± 10.79</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>18.08 ± 8.70</td>
<td>-0.01 ± 22.64</td>
</tr>
<tr>
<td>HM4</td>
<td>Neat</td>
<td>13.60 ± 0.98*</td>
<td>20.07 ± 13.42</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>14.60 ± 1.77</td>
<td>14.47 ± 15.32</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>17.42 ± 3.42</td>
<td>-0.03 ± 10.96</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>18.22 ± 5.23</td>
<td>-3.18 ± 8.77</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>15.49 ± 7.12</td>
<td>14.22 ± 21.38</td>
</tr>
<tr>
<td>HM5</td>
<td>Neat</td>
<td>15.01 ± 2.13</td>
<td>12.31 ± 16.24</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>16.25 ± 2.01</td>
<td>4.76 ± 17.39</td>
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<td>100x dilution</td>
<td>16.09 ± 1.36</td>
<td>5.58 ± 15.58</td>
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<tr>
<td></td>
<td>1000x dilution</td>
<td>17.71 ± 1.85</td>
<td>-3.44 ± 16.42</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>16.76 ± 1.75</td>
<td>2.23 ± 14.04</td>
</tr>
<tr>
<td>HM6</td>
<td>Neat</td>
<td>14.74 ± 1.72</td>
<td>13.56 ± 16.04</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>14.72 ± 0.92</td>
<td>13.23 ± 15.58</td>
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<td>100x dilution</td>
<td>16.07 ± 1.08</td>
<td>5.85 ± 13.91</td>
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<tr>
<td></td>
<td>1000x dilution</td>
<td>16.40 ± 2.25</td>
<td>5.30 ± 7.72</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>18.20 ± 2.40</td>
<td>-5.34 ± 10.81</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.
The values presented in the table 14.2 allow comparison of LEL activity and % inhibition of each standard. The values show weak inhibitory and stimulatory effects of the CHMs on the neutrophils. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they are non-systematic. The effects are generally comparable between the fMLP based mechanism and the PMA mechanisms of stimulation, with some being statistically significant (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 15).
14.3 Luminescence activity of platelets from hypertensive patients

The graphs below depict the LEL activity of platelets stimulated with (A) fMLP and (B) PMA for each HM and its diluted test solutions. The graphs show kinetics of activity over 60 minutes for each of the test standards. These are followed by graphs indicating the percentage (%) inhibition of the HMs on the platelets at the various time intervals.

HM1- *Intlamba Zifo™*

![Graph A](image1)

![Graph B](image2)

Figure 14.25. The LEL activity of hypertensive platelets after incubation with the neat HM1 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).
The activity of the fMLP-induced CTRL platelets was consistent from stimulation until 60 minutes. The activity of the fMLP-induced platelets in the neat HM1 and the 10x diluted standard had activities below that of the CTRL platelets. The activities of the platelets in the other diluted standards were close to the CTRL, above and below at the different time intervals. The PMA-induced platelets in the neat HM1 had LEL activity below that of the CTRL cells, while those in the diluted standards had activities closer to the CTRL cells, above and below at the different time intervals.

Figure 14.26. The % inhibition of hypertensive platelets after incubation with the HM1 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM1 and its dilutions had weak inhibitory effects generally on fMLP-induced platelets. The neat HM1 had notable weak inhibitory effects on PMA-induced cells, while the diluted HM1 standards had variable weak inhibitory and stimulatory effects.
Figure 14.27. The LEL activity of hypertensive platelets after incubation with the neat HM2 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

The platelets incubated with the HM2 and diluted standards had activities that were close to the CTRL platelets, but were unstable and variable for 25 minutes of testing. The PMA-induced platelets in the neat HM2 and the 10x diluted standards were the ones with distinct activity below that of the CTRL platelets. The platelets in the other three diluted
standards had activities that were close to the CTRL platelets and mimicked the CTRL platelets.

Figure 14.28. The % inhibition of hypertensive platelets after incubation with the HM2 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

HM2 and its dilutions generally had weak inhibitory effects on both the fMLP- and the PMA-induced platelets and the effect was more prominent in the neat HM2.
Figure 14.29. The LEL activity of hypertensive platelets after incubation with the neat HM3 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

As in the HM2, the platelets incubated with the HM3 and diluted standards had activities that were close to the CTRL platelets, but were unstable and variable for 25 minutes of
testing. The PMA-induced platelets in the neat HM3 had activity below that of the CTRL platelets. The platelets in the diluted standards had activities that were close to the CTRL platelets, with the activities of those in the 10x and 10000x diluted above the CTRL.

Figure 14.30. The % inhibition of hypertensive platelets after incubation with the HM3 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

HM3 and its dilutions with the exception of the 10000x diluted standard generally had weak inhibitory effects on both the fMLP- and the PMA-induced platelets and the effect was more prominent in the neat HM3. The 10000x diluted HM3 standard had weak stimulatory and inhibitory effects on the platelets.
Figure 14.31. The LEL activity of hypertensive platelets after incubation with the neat HM4 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both the fMLP- and PMA-induced platelets in the neat HM4 and the 10x diluted standard had activities below that of the CTRL platelets. The activities of the platelets in the other diluted standards were close to the CTRL, above and below at the different time intervals.
Figure 14.32. The % inhibition of hypertensive platelets after incubation with the HM4 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM4 and its dilutions had weak inhibitory effects generally on fMLP-induced platelets. The neat HM4 and the 10x diluted standard had notable weak inhibitory effects on PMA-induced platelets, while the other diluted HM4 standards had variable weak inhibitory and stimulatory effects.
Figure 14.33. The LEL activity of hypertensive platelets after incubation with the neat HM5 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 indicated on graph).

Both the fMLP- and PMA-induced platelets in the neat HM5 had activities below that of the CTRL platelets. The activities of the platelets in the other diluted standards were close to the CTRL, above and below at the different time intervals.
Figure 14.34. The % inhibition of hypertensive platelets after incubation with the HM5 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The HM5 and its dilutions had weak inhibitory effects generally on fMLP- and PMA-induced platelets; which was prominent in the neat HM5 and the 10x diluted standard.
HM6- Vuka Uphile™ Immune Booster

Figure 14.35. The LEL activity of hypertensive platelets after incubation with the neat HM6 and its diluted test solutions and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

Both the fMLP- and PMA-induced platelets in the neat HM6 had activities below that of the CTRL platelets. The activities of the fMLP-induced platelets in the other diluted standards were close to the CTRL, above and below at the different time intervals. The PMA-induced platelets in the neat HM6, the 1000x and 10000x diluted standards had activities above
that of the CTRL cells, while the activities of the platelets in the 100x diluted standards were below.

**Figure 14.36.** The % inhibition of hypertensive platelets after incubation with the HM6 and diluted standards, and stimulation with (A) fMLP and (B) PMA. (Means of n = 8 are indicated).

The neat HM6 had weak inhibitory effects generally on fMLP-induced platelets. The diluted HM6 standards had variable weak inhibitory and stimulatory effects those platelets. The neat HM6 and the 10x diluted standard had weak inhibitory effects on PMA-induced platelets, while the other diluted standards had variable weak inhibitory and stimulatory effects on those platelets.
Table 14.3. The average LEL activities of hypertensive platelets incubated with the CHMs over 60 minutes

<table>
<thead>
<tr>
<th>Extracts tested</th>
<th>Strength of extract</th>
<th>fMLP-induced cells</th>
<th>PMA-induced cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEL activity in 60 minutes (RLU)</td>
<td>% Inhibition</td>
<td>LEL activity in 60 minutes (RLU)</td>
</tr>
<tr>
<td>Control</td>
<td>0.0053 ± 0.0003</td>
<td>0.0057 ± 0.0007</td>
<td>38.0 ± 5.8</td>
</tr>
<tr>
<td>HM1</td>
<td>Neat</td>
<td>0.0040 ± 0.0003*</td>
<td>23.4 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0047 ± 0.0003*</td>
<td>11.3 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0048 ± 0.0002*</td>
<td>9.7 ± 4.4</td>
</tr>
<tr>
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<td>1000x dilution</td>
<td>0.0053 ± 0.0005</td>
<td>-0.3 ± 10.2</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0050 ± 0.0004</td>
<td>5.8 ± 8.8</td>
</tr>
<tr>
<td>HM2</td>
<td>Neat</td>
<td>0.0042 ± 0.0003*</td>
<td>20.0 ± 8.3</td>
</tr>
<tr>
<td></td>
<td>10x dilution</td>
<td>0.0046 ± 0.0004*</td>
<td>13.6 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>100x dilution</td>
<td>0.0047 ± 0.0006*</td>
<td>10.6 ± 11.0</td>
</tr>
<tr>
<td></td>
<td>1000x dilution</td>
<td>0.0050 ± 0.0005</td>
<td>5.2 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0047 ± 0.0005*</td>
<td>8.8 ± 8.3</td>
</tr>
<tr>
<td>HM3</td>
<td>Neat</td>
<td>0.0042 ± 0.0003*</td>
<td>21.1 ± 7.7</td>
</tr>
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<td>10x dilution</td>
<td>0.0045 ± 0.0004*</td>
<td>15.3 ± 9.5</td>
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<td>100x dilution</td>
<td>0.0047 ± 0.0003*</td>
<td>11.3 ± 4.6</td>
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<td>1000x dilution</td>
<td>0.0050 ± 0.0005</td>
<td>5.6 ± 8.6</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0052 ± 0.0005</td>
<td>2.2 ± 7.9</td>
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<td>Neat</td>
<td>0.0040 ± 0.0004*</td>
<td>24.1 ± 6.2</td>
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<tr>
<td></td>
<td>10x dilution</td>
<td>0.0039 ± 0.0003*</td>
<td>25.8 ± 8.3</td>
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<td>100x dilution</td>
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<td>5.0 ± 9.3</td>
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<td>1000x dilution</td>
<td>0.0054 ± 0.0006</td>
<td>-1.3 ± 11.2</td>
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<tr>
<td></td>
<td>10000x dilution</td>
<td>0.0051 ± 0.0004</td>
<td>4.1 ± 6.4</td>
</tr>
<tr>
<td>HM5</td>
<td>Neat</td>
<td>0.0042 ± 0.0004*</td>
<td>20.5 ± 6.7</td>
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<td></td>
<td>10x dilution</td>
<td>0.0046 ± 0.0003*</td>
<td>12.8 ± 7.2</td>
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<td>100x dilution</td>
<td>0.0050 ± 0.0003</td>
<td>5.9 ± 6.1</td>
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<td>10000x dilution</td>
<td>0.0054 ± 0.0005</td>
<td>-1.0 ± 6.5</td>
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<td>HM6</td>
<td>Neat</td>
<td>0.0043 ± 0.0003*</td>
<td>19.3 ± 7.7</td>
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<tr>
<td></td>
<td>10x dilution</td>
<td>0.0047 ± 0.0009*</td>
<td>10.9 ± 16.8</td>
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<td></td>
<td>1000x dilution</td>
<td>0.0049 ± 0.0006</td>
<td>7.4 ± 10.5</td>
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<tr>
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<td>10000x dilution</td>
<td>0.0056 ± 0.0005</td>
<td>-5.8 ± 7.2</td>
</tr>
</tbody>
</table>

Results presented as: mean ± standard deviation; *p ≤ 0.05 as statistical significance.

The values presented in the table 14.3 allow comparison of LEL activity and % inhibition of each standard. The values show weak inhibitory and stimulatory effects of the CHMs on the platelets. In some cases the stimulatory or inhibitory effects are directly or inversely proportional to the concentration and in others they are non-systematic. The effects are generally comparable between the fMLP based mechanism and the PMA mechanisms of stimulation, with some being statistically significant (p ≤ 0.05). The specific details of the table are addressed in the discussion chapter (chapter 15).
CHAPTER 15 DISCUSSION: THE EFFECTS OF COMMERCIAL HERBAL MIXTURES ON ISOLATED HUMAN NEUTROPHILS, PLATELETS AND WHOLE BLOOD PHAGOCYTES FROM HYPERTENSIVE PATIENTS

A total of six herbal mixtures and four serial dilutions of each one were tested for their effects on the LEL activity of cells stimulated by fMLP and PMA. The cells were all obtained from individuals suffering from hypertension, and were on their regular antihypertensive medicine. The graphs presented before (chapter 14) showed time based changes that occurred in LEL activity of the cells and platelets. The average effects of each CHM and its dilutions on the whole blood phagocytes (table 14.1, page 263), the isolated human neutrophils (IHNs) (table 14.2, page 276) and the platelets (table 14.3, page 290) are discussed below.

HM1- *Intlamba Zifo™*

- The highest concentration of HM1 (neat) had a weak inhibitory effect of the fMLP-induced whole blood phagocytes. All the diluted standards had weak stimulatory effects on the cells that were directly concentration dependent. That means the higher the concentration (which means the lower the dilution), the higher the stimulatory effect. The neat HM1 and the diluted standards had weak stimulatory effects on the PMA-induced cells, which were directly concentration dependent among the diluted standards only. The effect of the neat HM1 did not correlate with the other standards. The differences in the LEL activities of the cells in the 10x diluted standard were statistically significant when compared to the controls. Therefore, at high concentrations, HM1 inhibited the whole blood phagocytes but at lower concentrations it stimulated the cells. On the other hand, HM1 stimulated PMA-induced cells at all concentrations.
- The neat HM1 and the two lower diluted standards had a weak inhibitory effect on fMLP-induced IHNs that was directly concentration dependent. The two higher diluted standards had a weak and directly concentration dependent stimulatory effect on the cells. The neat HM1 had a weak inhibitory effect on PMA-induced IHNs. The diluted standards had a weak stimulatory effect that was non-systematic. The differences in the LEL activities were statistically significant between the controls and all the neat HM1 standards.
• The neat HM1 and the diluted standards had weak and directly concentration dependent inhibitory effects on both fMLP- and PMA-induced platelets, except for the 1000x and the 10000x diluted standards. The two diluted standards had weak stimulatory effects on the fMLP- and PMA- induced platelets respectively. The differences in the LEL activities of the fMLP-induced platelets in the neat HM1, the 10x and 100x dilutions, and the PMA-induced platelets in the neat HM1 and the 10x diluted standard were statistically significant when compared with the controls.

HM2- *Maphilisa™* Herbal medicine

• The neat HM2 had a weak inhibitory effect on both fMLP- and PMA-induced whole blood phagocytes. The three lower diluted standards had a weak, moderate (10x diluted HM2 on fMLP-induced cells) and potent (10x diluted HM2 on PMA-induced cells) stimulatory effect on both the induced cells that was directly concentration dependent. The 10000x standard also had a weak stimulatory effect on these cells, which did not correlate with the other diluted standards. The differences in the LEL activities against the controls were statistically significant in all the HM2 standards except for fMLP-induced cells in the 1000x diluted HM2 and PMA-induced cells in the neat HM2.

• The neat HM2 and the three lower dilutions had weak and directly concentration dependent inhibitory effects on fMLP-induced IHNs, while the 10000x diluted standard had weak stimulatory effect on these cells. The neat HM2 and the two lower dilutions had weak and directly concentration dependent inhibitory effect on PMA-induced IHNs, while the two higher diluted standards had weak and inversely concentration dependent stimulatory effect on the cells. Therefore HM2 had inhibitory effect at high concentrations, and stimulatory effect at low concentrations. The differences in the LEL activities of cells in the neat HM2 and the 10x diluted standards were statistically significant against the controls.

• The neat HM2 and the three lower diluted standards had weak, direct concentration dependent inhibitory effects on both fMLP- and PMA-induced platelets. The 10000x diluted standard also had a weak inhibitory effect on those platelets, which did not correlate with the effects of the other standards. The differences in the LEL activities of all the fMLP-induced platelets were statistically significant compared to the controls, except those in the 1000x diluted standard. Only the PMA-induced platelets in the neat HM2 and the 10x diluted standard had statistically significant differences in LEL activities when compared to the controls.
**HM3- Matla™ African medicine for all diseases**

- The neat HM3 had weak inhibitory effect causing reduced LEL activity in fMLP- and PMA-induced whole blood phagocytes. All the diluted standards had weak stimulatory effects on the cells. The stimulatory effect was inversely concentration dependent in the fMLP-induced cells. That means the lower the concentration (the higher the dilution), the higher the stimulation. It was directly concentration dependent in the PMA-induced cells in the three higher dilution standards. The 10x diluted standard had a weak stimulatory effect on the cells, which did not correlate with the effect of the other three standards. The differences in the LEL activities against the controls were statistically significant in all the cells except for the PMA-induced cells in the neat HM3.

- The neat HM3 had weak inhibitory effect on both fMLP- and PMA-induced IHNs, and the diluted standards had weak to potent (PMA-induced cells in the 100x diluted standard) stimulatory effect on these cells. The stimulatory effect was inversely concentration dependent on fMLP-induced IHNs, and non-systematic on PMA-induced cells. The differences in the LEL activities against the controls were statistically significant except for the PMA-induced cells in the neat HM3.

- The neat HM3 and all the diluted standards had weak, direct concentration dependent inhibitory effects on both fMLP-induced platelets, with significant LEL activities in the neat HM3 and the two lower diluted standards. The neat HM3 and the two lower diluted standards had weak, direct concentration dependent inhibitory effects on the PMA-induced platelets. The 1000x diluted standard had a weak inhibitory effect on the PMA-induced platelets that did not link with the inhibitory effect of the other standards; while the 10000x diluted standard had a weak stimulatory effect on the platelets. The differences in the LEL activities against the controls were statistically significant in PMA-induced platelets in the neat HM3 and the 10000x diluted standard.

**HM4- Ngoma™ Herbal Tonic Immune Booster**

- The neat HM4 had a weak inhibitory effect on both the fMLP- and PMA-induced whole blood phagocytes. All the diluted standards had stimulatory effects on the cells, which were weak (10x, 1000x and 10000x diluted standards) and potent (100x diluted standard). The effects in the three higher diluted standards directly concentration dependent, while the 10x diluted standard effects which were not correlating with the
effects of the other three standards. The differences in the LEL activities against the controls were statistically significant in PMA-induced cells in the neat HM4 and the fMLP-induced cells in the 10x and the 100x diluted standard.

- The neat HM4 had weak inhibitory effect on the fMLP- and PMA-induced IHNs. Similar to the effects in whole blood phagocytes, all the diluted standards had stimulatory effects on the cells, which were weak (10x, 1000x and 10000x diluted standards) and potent (100x diluted standard). The effects in the three higher diluted standards directly concentration dependent, while the 10x diluted standard effects did not correlate with the effects of the others. The differences in the LEL activities against the controls were statistically significant except for the PMA-induced cells in the neat HM4 and the fMLP-induced cells in the 1000x and the 10000x diluted standard.

- The neat HM4 and the diluted standards had weak and directly concentration dependent inhibitory effects on fMLP-induced platelets, except for the 1000x diluted standards which had a weak stimulatory effect. The neat HM4, the 10x and the 10000x diluted standards had weak, direct concentration dependent inhibitory effects on PMA-induced platelets. The two other diluted standards had weak stimulatory effects that were directly concentration dependent on the PMA-induced platelets. The differences in the LEL activities against the controls were statistically significant in both induced platelets in the neat HM4 and the 10x diluted standard.

**HM5- Stametta™ Body Healing Liquid**

- The neat HM5 caused reduced LEL activity in whole blood phagocytes exerting a weak inhibitory effect on both fMLP- and PMA-induced cells. All the diluted standards had weak stimulatory effects on the cells. The stimulatory effect of the three lower diluted standards was inversely concentration dependent in the fMLP-induced cells. It was directly concentration dependent in the PMA-induced cells in the three higher dilution standards. The 10x and the 10000x diluted standards had weak stimulatory effect on the fMLP- and PMA-induced cells respectively, which did not correlate with the effect of the other three standards. The differences in the LEL activities against the controls were statistically significant in all the cells except for the PMA-induced cells in the neat HM1 and the 10x diluted standard in the fMLP-induced cells.

- The neat HM5 had weak inhibitory effect on both fMLP- and PMA-induced IHNs, and the diluted standards had weak, moderate and potent stimulatory effect on these cells.
The stimulatory effects of the three lower diluted standards were inversely concentration dependent on the fMLP-induced IHNs, while the 10000x diluted standard effects did not correlate with the other standards. The stimulatory effects of the three higher diluted standards were inversely concentration dependent on PMA-induced cells, while the effect of 10x diluted standard effects did not correlate. The differences in the LEL activities against the controls were statistically significant except for the PMA-induced cells in the neat HM5 and the fMLP-induced cells in the 10x diluted standard.

- The neat HM5 and the three lower diluted standards had weak directly concentration dependent inhibitory effects on both fMLP- and PMA-induced platelets. The 1000x diluted standard had a weak stimulatory effect on fMLP-induced platelets and a weak inhibitory effect on PMA-induced platelets that did not correlate with the inhibitory effects of the other standards. The differences in the LEL activities against the controls were statistically significant in both induced platelets in the neat HM5 and the 10x diluted standard and the PMA-induced platelets in the 1000x diluted standard.

HM6- Vuka Uphile™ Immune Booster

- The neat HM6 had weak inhibitory effect causing reduced LEL activity in fMLP- and PMA-induced whole blood phagocytes. All the diluted standards had weak stimulatory effects on the cells. The stimulatory effect was inversely concentration dependent in the fMLP-induced cells. It was non-systematic in the PMA-induced cells. The differences in the LEL activities against the controls were statistically significant in all the cells except for the PMA-induced cells in the neat HM1 and the 10x diluted standard in the fMLP-induced cells.
- The neat HM6 had weak inhibitory effect on both fMLP- and PMA-induced IHNs, and the diluted standards had weak to moderate (fMLP-induced cells in the 1000x and 10000x diluted standards) stimulatory effect on those cells. The stimulatory effect was inversely concentration dependent on fMLP-induced IHNs, and non-systematic on PMA-induced cells. The differences in the LEL activities against the controls were statistically significant in all the cells except for the PMA-induced cells in the neat HM6 and the fMLP-induced cells in the 10x diluted standard.
- The neat HM6 and the two lower diluted standards had weak and directly concentration dependent inhibitory effects on the fMLP- and PMA-induced platelets. The 1000x diluted standard had a weak inhibitory effect on fMLP-induced platelets that did not
correlate with the inhibitory effects of the other standards. The 10000x diluted standard had a weak stimulatory effect on those platelets. The two higher diluted HM6 standards had weak and inverse concentration dependent inhibitory effects on PMA-induced platelets. The differences in the LEL activities against the controls were statistically significant in the fMLP-induced platelets in the neat HM6 and the 10x diluted standard, and all the PMA-induced platelets except in the 100x diluted standard.

Table 15.1. Summary of the overall effects of the CHMs on the cells and platelets of hypertensive patients, from the highest to the lowest concentration

<table>
<thead>
<tr>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>HM1</td>
<td>↓ +</td>
<td>+†</td>
</tr>
<tr>
<td>HM2</td>
<td>↓ +†</td>
<td>↓ +‡</td>
</tr>
<tr>
<td>HM3</td>
<td>↓ +†</td>
<td>↓ +†</td>
</tr>
<tr>
<td>HM4</td>
<td>↓ +‡</td>
<td>↓ +‡</td>
</tr>
<tr>
<td>HM5</td>
<td>↓ +†‡</td>
<td>↓ +‡</td>
</tr>
<tr>
<td>HM6</td>
<td>↓ +†</td>
<td>↓ +</td>
</tr>
</tbody>
</table>

↓ weak inhibition; † moderate inhibition; ‡ potent inhibition
+ weak stimulation; † moderate stimulation; ‡ potent stimulation

15.1 Implications of the effects of the commercial herbal medicines on cells and platelets of hypertensive patients

As stated in other investigations, the studied in vitro functional responsiveness of IHNs is a reflection of the in vivo physiological capabilities of these cells, which include chemotaxis, adherence, phagocytosis, ROS production (SOA and other oxygen radicals), degranulation, and microbial killing (McManus et al., 2001). As guided by the WHO, data obtained from in vitro tests should be seen as indicators of potential toxicity (WHO, 2000). Though in vivo tests should be performed, it is imperative to describe what the results obtained in this study suggest.
There was weak inhibition (at high concentration) of neutrophils in whole blood, IHNs and platelets by all the six HMs. There was weak, moderate and potent stimulation of neutrophils in whole blood, together with weak stimulation of IHNs and platelets. Hypertensive neutrophils are characterised by increased ROS production, which contributes to the pathology of the condition. The weak inhibition of the neutrophils in the presence of the HMs means that there was reduced ROS production by the neutrophils. This may be beneficial in the condition, since there would be reduced oxidative stress. Since there is lowered antioxidant capacity in HT, a reduction of ROS would be a beneficial effect. However inhibited neutrophils imply a weakened immune system. There may be increased susceptibility to infections with reduced capacity of phagocytes and reduced ability to destroy pathogens.

The weak inhibition of platelets occurred at higher concentrations of the HMs. It has been reported that platelets in hypertension are hyperactive and therefore are implicated in the development of CVS complications such as myocardial ischaemia and infarction. They have enhanced sensitivity to agonists and undergo spontaneous aggregation due to changes in intracellular chemical signalling resulting in HT (El Haouari & Rosado, 2009; Gkaliagkousi et al., 2010; Du & Kiriazis, 2013). Inhibition of the platelets by the HMs may be beneficial, in lowering the hyperactive nature of the platelets, and reduce the risk of the CVS complications. An important alteration in platelets in HT is the increased production of ROS and the general oxidative stress, which are implicated in the development of the thrombotic disorders linked to HT (El Haouari & Rosado, 2009). Hence inhibition of the platelets may suppress the development of thrombotic disorders and other associated disorders. Since the inhibition occurred via both the fMLP and PMA mechanisms, according to Mahomoodally et al. (2012), it suggests that the HMs do not affect a specific pathway, but directly inhibit a common biochemical target site such as NOX or interact directly with the ROS. However the general inhibition of platelets in HT may be harmful by increasing the risk of bleeding or causing excessive bleeding.

Inhibition of platelets may have a negative impact on their role as mediators of the immune system and inflammatory response. Their interaction with other immune cells which includes the recruitment of neutrophils and the formation of platelet-neutrophil complexes that perform phagocytosis, cytotoxicity and cytolysis may be impeded. Due to the reduced
ROS formation, the platelets would be incapable of directly destroying pathogens. Consequently, in the presence of the HMs, the immune system may be weakened which would cause increased susceptibility of the hypertensive patient to infections.

The stimulation of the neutrophils in the presence of the HMs means that there was increased ROS production by the neutrophils. This may worsen the already existing oxidative stress in the condition, resulting in accelerated and heightened tissue damage including endothelial dysfunction, vasoconstriction and vascular damage (Lassegue & Griendling, 2004; Zuo, Rose, Roberts, He, Barnes-Bercelli, 2014). The weak stimulation by the HMs implies the HMs serve as priming agents; therefore any activating stimulus would cause a stronger response, with highly increased ROS production. It was stated that extensive release of oxidising agents contributed to the development and progress of many acute and chronic inflammatory disorders of infective and non-infective origin such as respiratory disease syndrome, asthma, RA, atherosclerosis, COPD, stroke, and myocardial infarction (Caldefie-Chezet et al., 2002; Tintinger et al., 2008). Therefore, the stimulation of the hypertensive neutrophils by the HMs may worsen the condition and the complications associated and may also cause unrelated conditions. This is in agreement with the assertion by Ramasamy et al. (2010) that the respiratory burst of neutrophils is altered by HT; therefore their resultant increased SOA production may be a factor that augments the HT-related complications.

In terms of the immune response, the weak stimulation of neutrophils by HMs may cause an exaggerated or uncontrolled immune response to normal stimuli. This could result in or contribute to the pathogenesis of inflammatory and autoimmune disorders such as systemic lupus erythematosus, RA, emphysema, atherosclerosis and Chron’s disease (Paula et al., 2009).

The weak stimulation of hypertensive platelets by the HMs occurred via both the fMLP and PMA pathways, and at low concentrations of the HMs. As stated before platelets in hypertension are hyperactive and have enhanced sensitivity to agonists (El Haouari & Rosado, 2009; Gkaliagkousi et al., 2010; Du & Kiriazis, 2013). They have increased production of ROS and general oxidative stress amidst reduced antioxidant capacity which causes the presence of higher amounts of ROS. The stimulation of the HT platelets by the HMs therefore increases the ROS formation further, which may increase the oxidative
stress. Since the HT platelets are hyperactive and sensitive to agonists, their weak stimulation by the HMs may similarly to the neutrophils, cause exaggerated or uncontrolled inflammatory response and immune reaction. These responses may lead to the development and progression of diseases such as ARDS, myocardial ischaemia, infarction and atherosclerosis (Ruf et al., 1992). The hyperactive platelets may increase the risk of thromboembolic disorders as the presence of platelet-derived ROS was reported to increase the recruitment of neutrophils to growing thrombi (Krotz et al., 2004). The pro-inflammatory role of platelets has been reported; that activated platelets in HT interact with and activate leukocytes, and may contribute to the cardiac complications in HT such as cardiac myopathy, fibrosis and inflammatory infiltration (Du & Kiriazis, 2013).

Hence the intake of the CHMs which at low concentrations have stimulatory effects on both hypertensive platelets and neutrophils, might be unfavourable to the hypertensive patient on treatment.
CHAPTER 16 RESULTS AND DISCUSSION: CROSS-REACTIVITY OF HERBAL MEDICINES WITH COMMONLY TESTED SUBSTANCES OF ABUSE IN URINE

16.1 Results: Testing drug-free urine

Healthy drug-free urine (DFU) had been spiked with aliquots of the commercial herbal medicines (CHMs), to test for cross-reactivity of the herbal mixtures with substances of abuse. A quantitative lateral flow chromatography immunoassay was used. The urine samples obtained from healthy volunteers appeared normal. In terms of appearance, the colour of the urine were all amber, with various shades, but none of the observed parameters deviated from the normal ranges as indicated by the dipstick test. The pH and SG of the individual urine samples differed, but the values for the pooled sample were pH 6.0 and SG 1.020.

The results of the rapid qualitative test on the neat HMs were all negative for the tested substances of abuse. HM5 and HM6 were too dark and could not show clear results on the test panel. Then a two times dilution of each was made with distilled water. These samples were less intense in colour and showed clear negative results.

Figure 16.1. Rapid test cassette showing negative results for all the tested substances of abuse
The results remained negative throughout testing from day 1 till day 5. The dipstick results for pH and SG also remained the same for each of the samples throughout the 5 days of testing.

The results of the 40% v/v spiked urine samples were also negative, and remained negative throughout the 5 days of testing. The SG for DFU spiked with HM1, HM2, HM4 was 1,010 with pH 5.0, and HM3 and HM5 was 1,025 with pH 6.0 and 5.0 respectively. The values for DFU with HM6 remained the same as the pooled urine, with pH 6.0 and SG 1,020.

16.2 Results: testing urine that is positive for substances of abuse

Eight urine samples positive (D-U) for THC were used in this assay. The pH of seven samples were between 5 and 7.5 and the SG ranged from 1,005 to 1,030. The pH of the controls was between 5 and 6.5 and the SG between 1,010 and 1,030. One sample denoted FNT4 was positive for nitrites, had protein present (3+), had a pH of 8.5 and SG 1,005.

The controls for all the D-U samples remained positive for THC. The results of seven D-U samples spiked with HM1- Intlamba Zifo™, HM2- Maphilisa™ Herbal medicine, HM3- Matla™ African medicine for all diseases, HM5- Stametta™ Body Healing Liquid, and HM6- Vuka Uphile™ Immune Booster remained positive for THC in all concentrations of the HMs tested, from the lowest to the highest (40% v/v).

The D-U samples spiked with HM4- Ngoma™ Herbal Tonic Immune Booster tested negative for THC at the highest concentration (40% v/v). Because this HM is the only one which is alcohol-based, alcohol had to be excluded as a possible cause of the false-negative result. A 13.5% alcohol (methanol) solution was prepared and used to spike some D-U samples, which were then taken through the same procedure. The results of the spiked D-U samples remained positive, which meant that the alcohol did not alter the results.

The D-U sample FNT4 stayed positive for THC.
Figure 16.2. Rapid test cassette results for D-U sample FNT1 showing positive results for THC. Note the results for the HM4 sample.
Figure 16.3. Rapid test cassette showing positive results for D-U sample FNT2 spiked with HMs, from left to right- HM1, Control, HM4 and HM3. Note the false negative for THC in the HM4 sample.

16.3 Discussion

Of the six CHMs tested, five of them had no influence on the quantitative rapid urinalysis assay. The one CHM which showed different results was HM4-Ngoma™ Herbal Tonic and Immune Booster. This herbal mixture reported false-negative results for THC in the urine samples that were positive for THC. It contains Sutherlandia, Echinacea, dandelion, alfalfa-lusern, aloe ferox, harpagophytom (devil’s claw) and alcohol (13.5%). It is the only HM with an alcohol content. Alcohol was excluded as a possible cause of the false negative result. A plausible explanation for the false-negative result could be that, alcohol as a solvent extracted some lipophilic compounds from the plants used, which would not be extracted by water as a solvent. These compounds would have then interfered with the test reagents, resulting in the false-negative results.

For the test to be positive, the drug metabolites in the urine sample must compete for binding on the immobilised protein conjugate (antibody) with the colour coded drug antigen on the test cassette. If the drug is present and it binds to the antibody, then no colour line
will develop, which signifies a positive result. A negative test result occurs when the colour
coded drug antigen binds to the antibody. The compounds in the HM4 interacted with the
test reagents and the THC in the sample in such a way that the drug metabolite could not
bind to the antibody.

There are several ways that could cause false-negative results for a particular drug when
using immunoassays. The drug may be present in much lower concentration that could not
be detected by the immunoassay (Kapur, 2001; Reisfield et al., 2009). Most screening
tests have cutoff concentrations or concentration thresholds above which the results would
be reported as positive (Dasgupta & Bernard, 2006; Moeller et al., 2008; Reisfield et al.,
2009). Although the test used is a qualitative test, it however has a quantitative part due to
the cutoff levels, which give a guide to the possible concentration of the substance if
positive (Reisfield et al., 2007). Another way could be by specimen manipulation either in
vivo or in vitro. The specimen could be diluted, substituted or adulterated. Adulteration
includes addition of a chemical to interfere with the assay. Dilution causes reduced
concentration to below cutoff levels (Reisfield et al., 2009) which is not the case in this
study, since the control sample remained positive.

False-negative results may also be caused by cross-reactivity of the antibody in the assay
(Saitman, Park, Fitzgerald, 2014). As noted before, screening tests are designed to detect
groups or classes of drugs; hence they have low specificity (Reisfield et al., 2009). This
could be the explanation for the false-negative THC result. THC is a highly lipid soluble
compound. It could be that the antibody to THC in the assay has variable degrees of
reactivity or affinity towards drugs or metabolites with identical chemical structure as THC,
or with unrelated chemical structures. The antibody could have affinity for THC or other
unrelated plant compounds that are lipophillic like THC. Since THC is of plant origin, the
HM4 may contain a plant compound that is structurally related. Plant compounds that are
usually extracted in alcohol include glycosides by ethanol and sugars, amino acids and
glycosides by methanol (Houghton & Raman, 1998).

Whether these were the compounds that interfered with the test is speculation, at this
stage as there could be more compounds present. The concentration at which this HM
was tested was high (40% v/v), which cannot be extrapolated to physiological levels.
However it permits the possibility of the danger of its use as an adulterant.

Many substances have been confirmed to interfere with urine drug screening tests for
cannabinoids. These are usually assay specific, which means a substance may interfere
on one assay and not others. Schwarzhoff and Cody (1993) reported that the cannabinoid assay was the most susceptible assay to false-negative results due to adulterants. Some of the substances interfering with cannabinoid immunoassays include ibuprofen, tolmetin (Reisfield et al., 2009) and common household chemicals such as drain cleaner, ammonia and bleach (Schwarzhoff & Cody, 1993; Uebel & Wium, 2002).

This is the first report of a commercial herbal medicine used as an African traditional medicine being a potential adulterant to the THC urine screening test, using a quantitative lateral flow chromatography immunoassay.

16.4 Conclusion

Of the six herbal mixtures tested, only one showed the potential of interfering with a rapid, qualitative urine test for substances of abuse. Ngoma™ Herbal Tonic and Immune Booster, which was the only alcohol-based mixture, caused false negative results for THC. Although in vivo tests should be done for proper validation, these results indicate the potential of herbal mixtures interfering with rapid, qualitative tests. This is the first report of herbal medicine or ATM interfering with the test, therefore it opens up one route of further studies investigating whether the use of these medicines interfere with diagnostic tests in general.
Chapter 17: DISCUSSION OF THE COMMERCIAL HERBAL MIXTURES

This chapter provides an overview of each CHM, through the objectives of this study.

HM1 - *Intlamba zifo™*

<table>
<thead>
<tr>
<th>HM1</th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>+</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>↓</td>
<td>+</td>
<td>†</td>
</tr>
<tr>
<td>Hypertension</td>
<td>↓</td>
<td>+</td>
<td>††</td>
</tr>
<tr>
<td>Asthma</td>
<td>↓†</td>
<td>+</td>
<td>↓†</td>
</tr>
</tbody>
</table>

↓ weak inhibition; †† moderate inhibition; ††† potent inhibition
+ weak stimulation; † moderate stimulation; †† potent stimulation

*Intlamba zifo™* stimulated the activity of healthy whole blood phagocytes at high concentrations and inhibited their activity at low concentrations through both pathways. It inhibited the activity of the healthy isolated human neutrophils (IHNs) at all concentrations through both pathways. It inhibited the activity of platelets activated via the fMLP pathway at all concentrations, but inhibited and stimulated the platelets at high and low concentrations respectively, via the PMA pathway.

*Intlamba zifo™* had general similar effects on cells and platelets from diabetic, hypertensive and asthmatic patients - inhibition of their activity at high concentrations, stimulation at low concentrations. The exceptions were that it stimulated whole blood phagocytes activity of hypertensive and asthmatic patients through the PMA pathway at all concentrations.

It is stated on the package that *Intlamba zifo™* boosts the immune system and helps combat viruses and removes toxins (Table 3.2). Diabetes, hypertension and high blood pressure are listed among the many ailments in which *Intlamba zifo™* assists. The effects of this HM may be attributed to the action of the various components, either additively, synergistically or even antagonistically. The effects indicate that the HM has
immunomodulatory potential, which then confirms the rationale for their use, although it was not tested through clinical trials. The listed components/ingredients are aloe, water, assorted herbs and H₂O₂. Aloe is a known medicinal plant used for many diverse conditions affecting various systems, and is generally used for the treatment of pain, infections and infestations, skin complaints and in pregnancy (Grace et al., 2008; Amoo et al., 2014). The inhibitory and stimulatory effects of Intlamba zifo™ may be from the aloe component of the HM, since aloe has been proven, in vitro that it had antioxidant, anti-inflammatory and immunostimulatory activity, amongst other pharmacological activities proven (Steenkamp & Stewart, 2007; Fawole et al., 2010; Street & Prinsloo, 2013; Amoo et al., 2014). Many compounds have been isolated in aloe, that belong to classes such as the anthrones, flavonoids and alkaloids together with bioactive compounds such as aloin, aloesin and aloemodin, dithranol, magnesium lactate (Rodriguez-Fragoso et al., 2008; Amoo et al., 2014). As Spelman et al. (2006) stated, the pharmacological effects and therapeutic efficacies of medicinal plants are derived from several compounds acting in synergy, rather than from a single compound. Hence the activities in the study could be from the multiple components in aloe as well as those in the “assorted herbs”. Flavonoids are the plant compounds proven to have an inhibitory effect on the respiratory burst (ROS production) of neutrophils. They are also known as powerful antioxidants as they are able to scavenge a diverse range of ROS (Ciz et al., 2012).

The concentrations of the herbal components is not stated, hence the stated dose of 45 ml three times daily cannot be extrapolated to a specific amount of aloe or any of the components. From the expected pharmacokinetics that may occur in vivo, it is highly likely that the concentration in systemic circulation would be low; therefore the effects would be stimulation rather than inhibition of the neutrophils and platelets of diabetics, hypertensives and asthmatics. In healthy cells, the effect at low concentrations was inhibition of the cells and platelets. It is worth noting, therefore that Intlamba zifo™ had opposing effects on healthy cells versus diseased cells in asthma, hypertension and diabetes. Therefore, the inhibitory effects of the Intlamba zifo™ in this study may be indicative of its role as an antioxidant or scavenger of ROS, which may benefit the users as prophylaxis against the onset and progress of the various conditions mentioned before such as respiratory disease syndrome, asthma, RA, atherosclerosis, COPD, stroke, and myocardial infarction (Caldefie-Chezet et al., 2002; Tintinger et al., 2008). Since increased neutrophil activation was implicated in the pathogenesis of inflammatory and autoimmune disorders such as
systemic lupus erythematosus, RA, emphysema, atherosclerosis and Chron’s disease (Paula et al., 2009), \textit{Intlamba zifo}\textsuperscript{™} may also help in preventing such conditions.

However the inhibition of neutrophils and platelets may have serious implications in the health and wellbeing of individuals. The neutrophils and platelets may be unable to respond to stimuli as effector cells of the immune system. Therefore the intake of \textit{Intlamba zifo}\textsuperscript{™} may compromise the immune system, leading to individuals being more susceptible to infections. A possible adverse drug interaction may occur if there is need for use of antimicrobials as treatment of bacterial infections in a person taking this HM. The success of bacteriostatic drugs as treatment depends on a viable immune system. Therefore, in the presence of \textit{Intlamba zifo}\textsuperscript{™}, there may be a need to increase the dose or duration of treatment of the bacteriostatic agent, or use other antimicrobials. The inhibition of platelets may cause bleeding or increase the risk of prolonged bleeding which could be an unexpected complication in conventional therapy, such as in patients going for surgery.

Aloe has been noted to decrease platelet aggregation and prolong bleeding time (Yagi et al., 2002; Rodriguez-Fragoso et al., 2008). This adverse interaction may be worsened further by the presence of any antiplatelet drugs like aspirin through which people often self-medicate for minor ailments such as headaches. However, as reported for conventional antiplatelet therapy, platelet inhibition is not only associated with the risk of bleeding, but low levels of inhibition are associated with increased risk of ischaemic events (Ferreiro, Sibbing et al., 2010). Therefore the inhibition by the HM may also lead to inexplicable ischaemic events as a complication.

Stimulation of the neutrophils in DM by \textit{Intlamba zifo}\textsuperscript{™}, with the resultant increased ROS formation may improve the bactericidal activity and other functional impairments of the neutrophils. This would reduce the susceptibility and the severity of the infections and improve on the prognosis if infection is established in DM. However, as mentioned, the activity of NOX should be controlled well, as excessive ROS may cause unwanted damage to host tissue, reveal unexpected anti-inflammatory reactions and hasten the microvascular and cardiovascular complications of DM (Aydin et al., 2001; Roos et al., 2003; Tintinger et al., 2008). The platelet in DM is generally atherogenic and prothrombotic in nature (Ferreiro, Gomes-Hospital et al., 2010; Kakouros et al., 2011; Santilli et al., 2015). The stimulation of platelets in DM could worsen the already increased risk of cardiovascular disease such as CAD and ACS, due to the reported hyperaggregation of DM platelets and the formation of AGEs which lead to CVS complications (Kakouros et al.,
2011). Aloe has been used for diabetes due to its reported hypoglycaemic effect (Rodriguez-Fragoso et al., 2008; Amoo, et al., 2014). Therefore there might be interaction of *Intlamba zifo™* with the antidiabetic treatment being used, resulting in additive hypoglycaemic effect.

Stimulation of the neutrophils and platelets in HT by *Intlamba zifo™* may worsen the already existing oxidative stress in the condition, resulting in accelerated and heightened tissue damage including endothelial dysfunction, vasoconstriction and vascular damage (Lassegue & Griendling, 2004; Zuo, Rose, Roberts, He, Barnes-Bercelli, 2014). As stated by Ramasamy et al. (2010), the respiratory burst of neutrophils is altered by HT; therefore their increased SOA production may be a factor that aggravates the HT-related complications. The presence of *Intlamba zifo™* serves as a priming agent; hence any activating stimulus would cause a stronger response, with highly increased ROS production. This may worsen the HT since ROS contributes to the vascular damage and vasoconstriction and trigger the development and/or worsen the progress of many HT-associated complications. The interaction of stimulated platelets and neutrophils may cause an exaggerated or uncontrolled immune response to normal stimuli. This could result in or contribute to the pathogenesis of inflammatory and autoimmune disorders such as systemic lupus erythematosus, RA, emphysema, atherosclerosis and Chron’s disease and thromboembolic disorders (Ruf et al., 1992; Paula et al., 2009).

Platelets in HT and asthma are hyperactive and sensitive to agonists, their stimulation by *Intlamba zifo™* may cause exaggerated or uncontrolled inflammatory response and immune reaction. The increased ROS could lead to the development and progression of thromboembolic disorders such as myocardial ischaemia, infarction and atherosclerosis (Ruf et al., 1992), which are complications that occur in HT and in asthma. As reported, the ROS and granule enzymes cause damage to molecules, cells and tissues of inflammation and contribute to the pathogenesis of asthma (Parij et al., 1998).

*Intlamba zifo™* did not have any effect on the rapid screening tests done on urine, to test for substances of abuse.
HM2- *Maphilisa™* Herbal Medicine

Table 17.2. Summary of the overall effects of the HM2 on the cells and platelets from the highest to the lowest concentration

<table>
<thead>
<tr>
<th>HM2</th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>↓ +†</td>
<td>↓ +†</td>
<td>↓</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓↓</td>
</tr>
<tr>
<td>Hypertension</td>
<td>↓ +†</td>
<td>↓ +‡</td>
<td>↓ +</td>
</tr>
<tr>
<td>Asthma</td>
<td>↓ +</td>
<td>↓ +</td>
<td>↓</td>
</tr>
</tbody>
</table>

↓ weak inhibition; ↓↓ moderate inhibition; ↓↓↓ potent inhibition
+ weak stimulation; † moderate stimulation; ‡ potent stimulation

*Maphilisa™* Herbal Medicine generally inhibited the activity of healthy whole blood phagocytes, IHNs and platelets at high concentrations and stimulated their activity at low concentrations through both the fMLP and PMA pathways. At all concentrations, it inhibited the activity of the healthy IHNs and platelets through both pathways, the hypertensive platelets via the PMA pathway and the asthmatic IHNs through the fMLP pathway. The highest inhibitory effect (moderate) was on the diabetic IHNs, while the highest stimulatory effect (potent) was on the diabetic platelets and the hypertensive whole blood phagocytes via the PMA pathway.

It is stated on the package that *Maphilisa™* Herbal Medicine boosts the immune system and helps combat viruses and removes toxins. Diabetes, hypertension and high blood pressure are listed among the many ailments in which *Maphilisa™* Herbal Medicine assists (Table 3.2). The effects of this HM may be attributed to the action of the various components, either additively, synergistically or even antagonistically, namely African potato, aloe ferox, African ginger, citric acid, Sutherlandia and wild olive. It is stated on the package that *Maphilisa™* Herbal Medicine is an immune booster, used to boost energy, prevent sicknesses and help with appetite. The inhibitory and stimulatory effects observed in the study indicate that the HM has immunomodulatory potential, which then confirms the rationale for its use, although it was not tested through clinical trials. All the listed ingredients are known medicinal plants used for various conditions.
Aloe was discussed under HM1. African potato is commonly used in traditional medicine as an immune booster and for the treatment of many diverse conditions including diabetes (Ojewole, 2006; Nair, Foster et al., 2007; Nair, Dairam et al., 2007; Van Wyk, 2008; Street & Prinsloo, 2013). It was also used for HIV infection by patients before initiation of ART and while on ART (Peltzer et al., 2011). Pharmacological screening and in vivo animal studies have shown and confirmed among other activities, the antidiabetic and anti-inflammatory activities of African potato (Ojewole, 2006; Ncube et al., 2013). The main compounds isolated were hypoxoside and its biologically active metabolite, rooperol (Ncube et al., 2013). African ginger (Siphonochilus aethiopicus) is used in ATM to treat many conditions including asthma, diabetes and hypertension (Ali et al., 2007; Van Wyk, 2008; herballfrica.co.za). More than 100 compounds have been isolated, and the anti-inflammatory, anti-oxidant, immunomodulatory, and anti-hyperglycaemic actions were confirmed, among the many other pharmacological and toxicological properties of the plant (McGaw et al., 1997; Ali et al., 2007). Sutherlandia is the only plant claimed to be traditionally used for almost any disease, including asthma and diabetes (Street & Prinsloo, 2013; Van Wyk, 2008; Mills et al., 2005). Wild olive (Olea europaea L.subs Africana) is used traditionally for treatment of many conditions including diabetes, hypertension and asthma and as a tonic (Van Wyk et al., 1997; Joffe, 2002; Somova et al., 2003; Khan et al., 2007; Hashmi et al., 2015). Many of the compounds isolated from the plant parts were confirmed for various activities including the anti-inflammatory, antihypertensive, antioxidant, antidiabetic and immunomodulatory activities (Somova et al., 2003; Khan et al., 2007; Chebbi Mahjoub et al., 2011; Hashmi et al., 2015).

There are no quantities or concentration of the ingredients indicated about this HM, only the dosage of half a glass twice a day after meals, which is approximately 125 ml twice per day. The inhibitory and stimulatory effects on cells and platelets are attributed to the myriad of compounds present in the dose, acting in synergy and also possibly antagonistically. Inhibition of neutrophils and platelets could have negative impact in the health and wellbeing of individuals. The inhibition of neutrophils in healthy individuals means that the immune system would be compromised, which would then put individuals at risk of acquiring infections and diseases. As stated by McManus et al. (2001), neutrophils play a crucial role in the resistance of infections, and the reduced function thereof is linked to susceptibility to bacterial infections. The interaction between neutrophils
and platelets and the formation of P-N aggregates would be reduced. In a similar way to HM1, the efficacy of bacteriostatic antimicrobials may be compromised. Inhibited platelets increases the risk of bleeding in the healthy individuals which may be aggravated by the presence of any antiplatelet drugs as stated before. The inhibition of the neutrophils by Maphilisa™ HM would worsen the reduced neutrophil function and the increased susceptibility to microbial infections in DM. The reduced production of ROS and decreased bactericidal activity may be aggravated by the presence of this HM, further increasing the risk of acquiring infections. It therefore would require DM patients to be more vigilant in their care of feet, to avoid fungal and bacterial infections which may lead to ulcerations and gangrene. If infection is established the prognosis would worsen more and the severity of the infection would be increased.

Stimulation of the neutrophils in DM by Maphilisa™ HM, with the resultant increased ROS formation may improve on the functional impairments of the neutrophils. This would reduce the susceptibility and the severity of the infections and improve on the prognosis if infection is established in DM. However the stimulation and the increased ROS formation in HT and asthma would increase the oxidative stress already present, and increase the risk of the cardiovascular complications that occur with the diseases. The neutrophils in HT (whole blood) were much more stimulated, which means ROS production is much higher in these cells; hence the risks and complications may be more pronounced than in the other cells. Platelets in HT and asthma are hyperactive and sensitive to agonists and are aggregatory, atherogenic and prothrombotic in DM (Moritani et al., 1998; El Haouari & Rosado, 2009; Du & Kiriazis, 2013). Their stimulation by Maphilisa™ HM, resulting in increased ROS formation could enhance the development and progression of thromboembolic disorders and the risk of cardiovascular disease that occur as complications in these disorders. The ROS and released enzymes could cause damage to molecules, cells and tissues of inflammation and contribute to the pathogenesis of asthma and the vascular damage in HT. Special focus should be on the diabetic platelets which were stimulated to a greater extent than the others. It implies that the risks of thromboembolism and cardiovascular complications in the presence of this HM may be more pronounced or frequent in DM than in the other conditions.

The individual ingredients of Maphilisa™ HM (African potato, aloe ferox, African ginger, Sutherlandia and wild olive) have antidiabetic, antioxidant, anti-inflammatory, antihypertensive and anti-asthmatic activities. However their interactions have not been
tested, and are thus unknown. It stems to reason to expect that the many components present would cause significantly high effects, whether wanted or unwanted. Therefore there could be additive effect resulting in hypoglycaemia, hypotension and excessive anti-asthmatic response in diabetic, hypertensive and asthmatic patients respectively, who use this HM. However, as explained by Cano and Volpato (2004), herbal mixtures are simply concoctions of two or three species with the same popular medicinal use, prepared jointly to enhance the known beneficial effects of each species. The main aim of the mixtures seems to be to potentiate the known therapeutic effects of the single-plant preparations. Therefore the HM components may truly have beneficial additive anti hypertensive, anti-asthmatic and antidiabetic effects. However care should be taken, as these components and hence the HM, may have interactions with conventional treatment of the conditions, with excessive hypoglycaemic, hypotensive and excessive anti-asthmatic responses.

*Maphilisa™* HM did not have any effect on the rapid screening tests done on urine, to test for substances of abuse.

**HM3- *Matla™* African medicine for all diseases**

**Table 17.3. Summary of the overall effects of the HM3 on the cells and platelets from the highest to the lowest concentration**

<table>
<thead>
<tr>
<th>HM3</th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>↓+†</td>
<td>↓+</td>
<td>↓†</td>
</tr>
<tr>
<td>Hypertension</td>
<td>↓+†</td>
<td>↓+</td>
<td>↓+</td>
</tr>
<tr>
<td>Asthma</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
</tbody>
</table>

↓ weak inhibition; ↓↓ moderate inhibition; ↓↓↓ potent inhibition
+ weak stimulation; † moderate stimulation; ‡ potent stimulation

*Matla™* African ‘medicine for all diseases’ has no information about the contents. All the information available about this HM is presented in Table 3.2. It is interesting to note though, that the pattern of effects *Matla™* HM has on cells and platelets is similar to the
one of Maphilisa™ HM. Matla™ HM generally inhibited the activity of healthy whole blood phagocytes, IHNs and platelets at high concentrations and stimulated their activity at low concentrations through both the fMLP and PMA pathways. At all concentrations, it inhibited the activity of the healthy IHNs through both pathways, the hypertensive platelets and the asthmatic IHNs through the fMLP pathway. The highest inhibitory effect (moderate) was on the diabetic IHNs, while the highest stimulatory effect (potent) was on the diabetic platelets followed by the hypertensive whole blood phagocytes (moderate) via both pathways.

While the components of Matla™ HM and their concentrations are unknown, the implications of the stimulation and inhibition of platelets would be the same as for HM1-Intlamba zifo™ and HM2-Maphilisa.

Matla™ HM did not have any effect on the rapid screening tests done on urine, to test for substances of abuse.

HM4- Ngoma™ Herbal Tonic Immune Booster

Table 17.4. Summary of the overall effects of the HM4 on the cells and platelets from the highest to the lowest concentration

<table>
<thead>
<tr>
<th>HM4</th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fMLP</td>
<td>PMA</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>↓+†</td>
<td>↓+†</td>
<td>↓†+</td>
</tr>
<tr>
<td>Hypertension</td>
<td>↓+‡</td>
<td>↓+‡</td>
<td>↓+</td>
</tr>
<tr>
<td>Asthma</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
</tbody>
</table>

+ weak stimulation; † moderate stimulation; ‡ potent stimulation
↓ weak inhibition; †† moderate inhibition; ††† potent inhibition

Ngoma™ Herbal Tonic Immune Booster generally inhibited the activity of healthy whole blood phagocytes, IHNs and platelets at high concentrations and stimulated their activity at low concentrations through both the fMLP and PMA pathways. At all concentrations, it inhibited the activity of the healthy IHNs through both pathways and the platelets through
the PMA pathway and the asthmatic IHNs through the fMLP pathway. The highest inhibitory effect (moderate) was on the diabetic IHNs, while the highest stimulatory effect (moderate) was on the diabetic platelets through the fMLP pathway followed by the hypertensive whole blood phagocytes (potent) via both pathways.

*Ngoma™* Herbal Tonic Immune Booster contains Sutherlandia, Echinacea, dandelion, alfalfa-lusern, aloe ferox, harpagophytom (devil's claw) and alcohol (13.5%). It is the only HM with an alcohol content. It is described on the package as “a powerful immune booster, made of a mixture of selected herbs traditionally used as a remedy against viruses, bacteria and fungal infections, also used to boost the immune system”. It is also stated that it provides relief for amongst others, symptoms of cold, influenza, inflammation, hypertension and stress (Table 3.2). It should be taken in a dose of one tablespoon (10 ml) in the morning after meals and one tablespoon in the evening after meals.

The two ingredients, Sutherlandia and aloe ferox have already been discussed as components of *Maphilisa™* HM and *Intlamba zifo™*. The other ingredients are also well-known medicinal plants and herbs. Devil’s claw is traditionally used as a decoction or infusion for many conditions including headache, arthritis, painful joints, for gall, liver, kidney and bladder problems; as a body tonic, detoxifying agent (Al-Harbi et al., 2013; Street & Prinsloo, 2013). Its anti-inflammatory, antioxidant and antidiabetic activities amongst others, were confirmed (Mncwangi et al., 2012; Al-Harbi et al., 2013; Street & Prinsloo, 2013). Echinacea is a herb well-known and used in Western herbalism for its immune boosting effects. It is used for upper respiratory infections, urinary tract infections, sore throat, herpes and candida infections among others. Topical preparations are used for treatment of wounds, skin infections, psoriasis, eczema and other inflammatory conditions (Leigh, 2001). Pharmacological tests have confirmed various effects including immunostimulant, anti-inflammatory and antioxidant activities (McCaleb, 1997; Wojdylo et al., 2007; Manayi et al., 2015). Alfalfa-luserne (*Medicago sativa*) is also a herb used in Western herbalism as a herbal remedy for many conditions, including arthritis, asthma, bladder disorders and gastrointestinal disorders (Hong et al., 2009; Kundan & Anupam, 2011). It contains a variety of compounds including alkaloids, enzymes, flavonoids, alkaloids and many other phytochemicals that confer a host of different activities. Pharmacological studies have shown its antiatherosclerotic, antioxidant and other activities which make it beneficial in the treatment of heart disease, stroke, atherosclerosis, diabetes and other conditions (Khaleel et al., 2005; Hong et al., 2009; Rana et al., 2010; Kundan &
Anupam, 2011). Dandelion (*Taraxacum officinale*) is also a medicinal herb used in Western herbalism for the treatment of many conditions including diarrhoea, liver and spleen complaints, kidney disease, diabetes, bronchitis and rheumatoid arthritis. The many compounds extracted and isolated from the herb include tannins, saponins, flavonoids, terpenoids, and phenolic compounds which were proven for the diuretic, anti-inflammatory, antioxidant, anti-allergic, antidiabetic and antithrombotic activities (Hu & Kitts, 2005; Schutz et al., 2006; Wright et al., 2007; Wojdylo et al., 2007; Clare et al., 2009; Yarnell & Abascall, 2009; Amin Mir et al., 2013; Ahmad, 2014). Phenolic compounds are a group of plant compounds that have variable structures. Many of the natural polyphenols have been studied and are known for their diverse effects on neutrophils, including reduction of ROS production, inhibition of PKC activation and reduction of phagocytosis and adhesion amongst others (Drabikova, Precko, Nosal, Harmatha, Smidrkal, Jancinova, 2012; Jancinova et al., 2012). Rahman (2008) asserted that polyphenolic compounds have therapeutic significance as antioxidans and anti-inflammatory agents in heritable chronic inflammatory diseases.

There are no quantities or concentration of the ingredients indicated in *Ngoma™* Herbal Tonic Immune Booster. The inhibitory and stimulatory effects on cells and platelets are attributed to the many compounds present in the dose acting in synergy, additively and possibly antagonistically. Inhibition of neutrophils and platelets with the resultant reduced ROS formation is expected since some of the components have antioxidant activities. The implications of stimulation and inhibition of cells and platelets in asthma, DM and HT would be the same as discussed under *Maphilisa™* HM. However from the specified dose, it is highly likely that HM would be in low concentrations in the body, hence the effects would be more stimulatory than inhibitory.

*Ngoma™* Herbal Tonic and Immune Booster is the only HM which showed the potential of interfering with a rapid, qualitative urine test for substances of abuse. At high concentration in urine, 40% v/v, it caused false-negative results for THC on a rapid immunoassay.
HM5- *Stametta™* Body healing liquid

Table 17.5. Summary of the overall effects of the HM5 on the cells and platelets from the highest to the lowest concentration

<table>
<thead>
<tr>
<th>HM5</th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>↓†+</td>
<td>↓†+</td>
<td>↓</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>↓+</td>
<td>↓+</td>
<td>↓↓</td>
</tr>
<tr>
<td>Hypertension</td>
<td>↓††</td>
<td>↓††</td>
<td>↓+</td>
</tr>
<tr>
<td>Asthma</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
</tbody>
</table>

↓ weak inhibition; ↓↓ moderate inhibition; ↓↓↓ potent inhibition
+ weak stimulation; † moderate stimulation; ‡ potent stimulation

*Stametta™* Body healing liquid (BHL) generally inhibited the activity of healthy whole blood phagocytes, IHNs and platelets at high concentrations and stimulated their activity at low concentrations through both the fMLP and PMA pathways. At all concentrations, it inhibited the activity of the healthy IHNs and the platelets through both pathways, the hypertensive platelets through the PMA pathway and the asthmatic IHNs through the fMLP pathway. The highest inhibitory effect (moderate) was on the diabetic IHNs, while the highest stimulatory effect (potent) was on the hypertensive whole blood phagocytes through both pathways followed by the healthy whole blood phagocytes (moderate) via both pathways.

*Stametta™* BHL is one of the popular CHMs, that one of the big retailers in SA has a similar in-house product. It contains aloe 1.667 g, ascorbic acid 1.667 g, aniseed oil 0.1817 g and magnesium sulphate 71.667 g in each 500 ml. It is said on the package that regular use will improve general well-being and strengthen the immune system and it is recommended to be used at a dose of 50 – 100 ml three or four times a week. It may also be used to assist in the treatment of or relief from painful and irregular menstruation, lower back pain, joint pain, digestive problems and strengthening of bones and muscle tissue (Table 3.2). Ascorbic acid is known for its antioxidant activities (Mainardi et al., 2009) and is an intracellular and extracellular scavenger of free radicals (Stamp et al., 2005). Aloe has antioxidant, anti-inflammatory and immunostimulatory activity, amongst its other
pharmacological activities (Steenkamp & Stewart, 2007; Fawole et al., 2010; Street & Prinsloo, 2013; Amoo et al., 2014). Therefore the inhibition of formation of ROS by the HM is expected since two of its components have antioxidant activity. In healthy cells this effect plays a protective role against tissue damage caused by oxygen free radicals. However the long-term stimulation of healthy cells and platelets may cause problems and complications similar to the other HMs, including an exaggerated or uncontrolled inflammatory and immune response, and the development and progress of thromboembolic and inflammatory conditions such as respiratory disease syndrome, asthma, RA, atherosclerosis, COPD, stroke, and myocardial infarction.

The implications of stimulation and inhibition of cells and platelets in asthma, DM and HT would be the same as discussed under *Maphilisa™* HM.

*Stametta™* BHL did not have any effect on the rapid screening tests done on urine, to test for substances of abuse.

**HM6-Vuka Uphile™ Herbal remedy**

**Table 17.6. Summary of the overall effects of the HM6 on the cells and platelets from the highest to the lowest concentration**

<table>
<thead>
<tr>
<th>HM6</th>
<th>Whole blood phagocytes</th>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fMLP</td>
<td>PMA</td>
<td>fMLP</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>↓+</td>
<td>↓+</td>
<td>↓</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>↓+</td>
<td>↓+</td>
<td>↓↓</td>
</tr>
<tr>
<td>Hypertension</td>
<td>↓+†</td>
<td>↓+</td>
<td>↓↓</td>
</tr>
<tr>
<td>Asthma</td>
<td>↓+†</td>
<td>↓+</td>
<td>↓↓</td>
</tr>
</tbody>
</table>

↓ weak inhibition; ↓↓ moderate inhibition; ↓↓↓ potent inhibition

+ weak stimulation; † moderate stimulation; ‡ potent stimulation

*Vuka Uphile™* Herbal remedy is said to be made from natural herbs which are not specified. “Diabetes, TB, joints and inflammation and high blood” are among the many conditions listed that this HM is used for (Table 3.2). Although the ingredients and their quantities are unknown, this HM has the effects similar to other HMs on the cells and
platelets. *Vuka Uphile™* Herbal remedy generally inhibited the activity of healthy whole blood phagocytes, IHNs and platelets at high concentrations and stimulated their activity at low concentrations through both the fMLP and PMA pathways. At all concentrations, it inhibited the activity of the healthy IHNs through both pathways, the healthy and hypertensive platelets through the PMA pathway and the asthmatic IHNs through the fMLP pathway. The highest inhibitory effect (moderate) was on the diabetic IHNs and the diabetic platelets through the PMA pathway, while the highest stimulatory effect (moderate) was on the hypertensive whole blood phagocytes through the fMLP pathway. The dosage of this HM is a ¼ of a glass after a meal, one in the morning and one at night.

The implications of stimulation and inhibition of healthy cells and platelets and in asthma, DM and HT would be the same as discussed under *Maphilisa™* HM.

*Vuka Uphile™* Herbal remedy did not have any effect on the rapid screening tests done on urine, to test for substances of abuse.
CHAPTER 18 CONCLUSION AND RECOMMENDATIONS

18.1 Conclusion

The six herbal mixtures tested caused inhibition and stimulation of the activity of the cells and platelets at different concentrations. Although these effects were *in vitro*, they provide an indication of possible actions of the herbal mixtures *in vivo*. The effects of the herbal mixtures were at times similar on healthy cells and diseased cells, however in several cases, the effects were opposite. Therefore it should not be assumed that what a herbal medicine does in healthy cells is the same in diseased cells, particularly those on chronic treatment.

Inhibition and hence reduced reactive oxygen species in healthy neutrophils and platelets may result in a compromised immune system, which could enhance the susceptibility to infections, with reduced antimicrobial capacity. It would also increase the risk of bleeding which may be a serious complication if such individuals would need conventional intervention such as surgery or lead to serious interactions with conventional drug therapy. It has been suggested that although surgeons and anaesthetists could be unaware of herbal medicine use by their patients, they should however, be familiar with the effects of herbal medicines and should enquire about their use. This would allow complete and truthful pre-operative assessment of the patient. Available data on common Western herbal medicines recommend that the use of such herbal medicines should be stopped two weeks before surgery and anaesthesia (Cheng, Hung, Chiu, 2002; Hodges & Kam, 2002).

Stimulation of the healthy cells and platelets, with increased production of reactive oxygen species, would in turn enhance the reactivity of these cells. The consequence would be exaggerated or uncontrolled inflammatory or immune reaction to stimulus; which could trigger unexpected or unwanted inflammatory conditions. It could also enhance the thrombotic state, leading to unexplained thromboembolic and cardiovascular conditions. Stimulation of neutrophils and platelets with increased formation of reactive oxygen species would aggravate both hypertension and asthma, and enhance the development and progress of associated complications. The inhibition of these cells may delay or
reduce the severity of the two conditions by lowering the oxidative stress; however it may result in a compromised immune system, and the increased risk of bleeding.

Stimulation of neutrophils in diabetes mellitus may enhance the functioning of the immune system, and lower the increased risk of infections in the disease while their inhibition would do the opposite and impair the immune system. The stimulation of the platelets which are already hyperactive in diabetes mellitus would greatly increase the risk of development and progress of atherothrombotic and cardiovascular complications. Their inhibition would lower the risks of these complications; however it would be paralleled by the increased risk of bleeding.

The herbal mixtures in general affected the two pathways of stimulation comparably. Since the mixtures are concoctions of various medicinal plants, the effects cannot be attributed to a particular component but is assumed to be the additive or synergistic interaction of the many present compounds in each of the components. It is also possible that the components interacted in antagonism, which would result in no effect or reduced effect on the cells. The common inhibition of the two pathways signifies that the herbal mixtures are scavengers of reactive oxygen species. It also means that they probably interact with some other element of the NADPH oxidase system directly, resulting in reduced formation of reactive oxygen species.

The stimulatory and inhibitory effects occurred at different concentrations, highlighting the importance of appropriate dosing. However with the six commercial herbal mixtures, dosing is diverse, ranging from 10 ml twice per day to ½ a cup twice daily. Therefore it may be difficult to predict what the actual effect would be in vivo. Also, in most of the commercial herbal mixtures, the stimulatory effect was inversely related to concentration, meaning the lower concentration had the higher stimulation effect. The inhibitory effects were generally directly related to the concentration, which meant that the inhibition effect increased as the concentration increased. This is in agreement with the study by Nosal et al. (2015), in which polyphenolic compounds had dose-dependent inhibitory effects on neutrophils. It seems apparent that for a user, starting with a low dose would stimulate the cells. Increasing the dose may eventually result in inhibition which may be an unwanted effect. The predicament in the dosing of the commercial herbal mixtures is therefore the cutoff line between stimulatory and inhibitory effects which may be a critical line between the “beneficial” effect and the unwanted or adverse effects. As reported by Ferreiro,
Sibbing et al. (2010), there is high variability in the degree of platelet inhibition by the same antiplatelet regimen in individuals; and that low levels of inhibition are closely linked with increased risk of ischaemic events while high levels are linked with the risk of bleeding. Therefore with regard to platelets, the inhibitory effect from the same dose of a herbal medicine may differ between users, and may lead to either bleeding or ischaemic events depending on whether it is low or high.

The ingredients of the herbal mixtures are quite diverse while others are not stipulated. Only one of the herbal mixtures, \textit{Intlamba zifo}™ is particularly recommended to complement conventional treatment. It is written on the label that the commercial herbal mixture will support the doctor’s prescription and is not meant to replace it. The possibility of interactions with drug treatment exists, whether prescription or non-prescription drugs. The therapeutic efficacy of bacteriostatic antimicrobials may be negatively affected, while complications may arise from concomitant intake of the HMs and antiplatelets such as aspirin or other NSAIDs.

In view of the observed effects on cells and platelets from healthy, diabetic, hypertensive and asthmatic volunteers, the possible drug interactions, and in recognition of the fact that users of African traditional medicines tend not to disclose their use of the medicines; it is therefore advisable to probe patients about the use of African traditional medicines and commercial herbal medicines. It would also be advisable for conventional health care providers to make patients aware that disclosure of traditional medicine use would benefit their conventional treatment as all possible risks and interactions would be considered.

\section*{18.2 Recommendations and future prospects}

In view of the growing trade and market for commercial herbal mixtures, there is a need for regulation and guidelines for production of the HMs. The information presented on the labels and packages of the commercial herbal mixtures is lacking, if not completely absent. Hence, this study serves to encourage a speedy progress for regulation and specifications on the labelling requirements. It is important that the information about the ingredients, the dose, storage conditions, clear instructions for use and duration of use, as well as date of manufacture and expiration is provided. In the same way that other complementary medicines are regulated in the amended Act 101 of 1965, specific requirements for
labelling, package inserts and patient information leaflets are necessary for commercial herbal mixtures.

In view of the observed effects on cells and platelets from healthy, diabetic, hypertensive and asthmatic volunteers and the possible drug interactions, it is important for conventional health care providers to find out about the use of African traditional medicines from their patients. It would also be advisable for conventional health care providers to make patients aware that disclosure of African traditional medicines use is welcomed and would benefit their conventional treatment as all possible risks and interactions would be considered. As suggested by Jayasekera et al. (2005), when the use of herbal medicines is discovered, it should be investigated further to avoid any possible complications. Therefore it is recommended that patient assessment forms such as the pre-operative assessment forms include a request where patients indicate their previous or current use of African traditional medicines.

It would be interesting to investigate further

a) How the six commercial herbal mixtures affect cells and neutrophils of other cell types, eg of smokers, other conditions etc.

b) How these six commercial herbal mixtures affect other cellular activities- eg neutrophil chemotaxis, aggregation of platelets.

An in vivo study should be performed to see how it correlates with the in vitro findings

 Having observed that the commercial herbal mixtures inhibit production of reactive oxygen species, it should be investigated further, using other techniques to determine exactly where the NADPH oxidase system is affected within the cascade.
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MEdUNsA RESEARCH & ETHICS COMMITTEE

CLEARANCE CERTIFICATE

MEETING: 02/2011
PROJECT NUMBER: MREC/M/09/2011: PG

PROJECT:
Title: In vitro investigation of the toxicity of commercially available herbal mixtures used as African traditional medicine in Pretoria, Gauteng

Researcher: Mrs M Mothibe
Supervisor: Prof CP Kahler-Venter
Co-supervisor: Prof E Osuch
Hospital Superintendent: Dr P Shembe
Department: Pharmacology & Therapeutics
School: Medicine
Degree: PhD Pharmacology

DECISION OF THE COMMITTEE:
MREC approved the project.

DATE: 10 March 2011

PROF GA’OGUNBANJO 
CHAIRPERSON MREC

Note:
i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.

ii) The budget for the research will be considered separately from the protocol.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.
DEPARTMENT OF HEALTH
OFFICE OF THE CLINICAL DIRECTOR

To : Ms. M.E. Mothibe
      Department of Pharmacology & Therapeutic
      P.O.Box 225
      MEDUNSA
      0204

Date : 08 September 2010

RE : PERMISSION TO CONDUCT RESEARCH.

The Dr. George Mukhari Hospital hereby grants you permission to conduct research on
"Investigation of the toxicity of commercially available herbal mixtures used as African traditional
medicine."

This permission is granted subject to the following conditions:

☑ That you obtain Ethical Clearance from the Human Research Ethics Committee of the
relevant University.

☑ That the Hospital incurs no cost in the course of your research.

☑ That access to the staff and patients at the Dr George Mukhari Hospital will not interrupt the
daily provision of services.

☑ That prior to conducting the research you will liaise with the supervisors of the relevant
sections to introduce yourself (with this letter) and to make arrangements with them in a
manner that is convenient to the sections.

Yours sincerely

[Signature]

DR. P SHEMBE
DIRECTOR: CLINICAL SERVICES
INFORMATION TO VOLUNTEERS

Title of study: *In vitro* investigation of the toxicity of commercially available herbal mixtures used as African traditional medicine in Pretoria, Gauteng

One of the objectives of this study is to investigate the effects of herbal mixtures on samples being tested for drugs/substances of abuse. These herbal mixtures are those commonly bought over the counter, and people use them on their own without the permission or recommendation of a doctor or a nurse.

Therefore we request a urine sample from you, as a healthy volunteer. The sample will be collected under safe and appropriate conditions, and handed to the nurse at the Dept of Pharmacology Clinic, BMS N512. It will not be labeled. Your urine sample will be pooled together with other urine samples from healthy volunteers like you, to make a stock solution of urine. This is the stock from which we will take aliquots, spike them with standard amounts of herbal mixtures and test them for substances of abuse.

No routine or diagnostic blood tests will be done on your sample, except the dipstick test which is necessary to show the baseline chemistry of each sample before pooling. It is important that you are a non-smoker and you are not on any chronic medication. Do not take any self-medication such as painkillers (e.g. panado®, disprin®), vitamin supplements or any traditional or herbal medicines in the two weeks before the day of collection of your sample. If you have, please inform us.

We thank you for your assistance.

Researcher: ME Mothibe
Signature: __________________ Date: ______________
Title of study: *In vitro* investigation of the toxicity of commercially available herbal mixtures used as African traditional medicine in Pretoria, Gauteng

Maitlhomo a patlisiso e ke go batlisisa gore meriana ya setso e e dirisiwang, e na le ditlamorago tse di jang mo di-“cells” tse tshweu tsa madi. Meriana e ya setso ke e batho ba ithekelang yona fela, mme ba e dirise kwa ntle ga tumello kgotsa thotloetso ya ngaka gongwe mooki.

Re tlhoka madi a kana ka 30ml mo go wena, mme a tla tsewa ke ngaka kgotsa mooki, fa tlase ga maemo a bolokesegileng e bile a le matshwanedi. Madi ao a ya go dirisiwa mo dipatlisisong tsa saense. Ga go ne go diriwa ka one diteko dipe tse di tlwaegileng, kgotsa tse e leng tsa go supa bolwetsi bope. Re ya go ntsha di-“cells” tse tshweu mo go ona, re di bale, mme re di dirise mo patlisisong gona mo letsatsing le a tserweng ka lone. Masaledi a madi a gago g a ne a bolokiwa, mme a ya go latliwa ka mokgwa o o tshwanetseng.

Go bothlokwa gore o bo o le motho yo o sa gogeng motsoko, mme o se motho yo o phelang ka go nwa meriana ya leruri. O seke wa nwa meriana mengwe fela, jaaka ya ditlhabi (sekao- panado®, disprin®), di-vithamine kgotsa meriana ya setso mo dibekeng di le pedi pele re tsaa madi a gago. Fa o e nwele, tsweetswee re itsise.

Re tla buisana le wena ka mogala letsatsi kgotsa a le mabedi pele ga motlha wa go tsaa madi, gore re totobatse gore a o tla nna gone.

Boitshupo jwa gago bo tla sirelediwa, mme le madi a gago ga a kitla a dirisetswa mabaka mangwe a sele, kwantle ga a a tlhalositsweng fela.

Re lebogela go tsaa karolo ga gago.

Mmatlisisi: ___________________ Tshaeno: _______________ Letlha: __________
INFORMATION TO PATIENTS

Title of study:  *In vitro* investigation of the toxicity of commercially available herbal mixtures used as African traditional medicine in Pretoria, Gauteng

The aim of this study is to investigate the effects of herbal mixtures on white blood cells. These herbal mixtures are commonly bought over the counter, and people use them on their own without the permission or recommendation of a doctor or a nurse.

We need 30ml of blood from you, which will be collected under safe and appropriate conditions by a doctor or nurse. This blood is needed for scientific research purposes. No routine or diagnostic blood tests will be done on your blood. We will isolate white blood cells and platelets, count them and use them for the study on the same day that it is collected. The remains of your blood will not be stored but will be discarded accordingly.

It is important that you are a non-smoker, and that you take only the medicines that are your regular treatment for your condition. Do not take any other medicines such as painkillers (e.g. panado®, disprin®), vitamin supplements or any traditional or herbal medicines in the two weeks before the day of collection of your blood sample. If you have, please inform us.

We will contact you telephonically to confirm your availability a day or two before the day of collection.

Your identity will be protected and your blood will not be used for any purpose other than what is stipulated.

We thank you for your participation.

Researcher: ___________________  Signature: _______________  Date: _______________
Title of study: *In vitro* investigation of the toxicity of commercially available herbal mixtures used as African traditional medicine in Pretoria, Gauteng

Maitlhomo a patlisiso e ke go batlisisa gore meriana ya setso e e dirisiwang, e na le ditlamorago tse di jang mo di-“cells” tse tshweu tsa madi. Meriana e ya setso ke e batho ba ithekelang yona fela, mme ba e dirise kwa ntle ga tumello kgotsa thotloetso ya ngaka gongwe mooki.

Re tlhoka madi a kana ka 30ml mo go wena, mme a tla tsewa ke ngaka kgotsa mooki, fa tlase ga maemo a bolokeseqileng e bile a le matshwanedi. Madi ao a ya go dirisiwa mo dipatlisisong tsa saense. Ga go ne go diriwa ka one diteko dipe tse di tlwaelegileng, kgotsa tse e leng tsa go supa bolwetsi bope. Re ya go ntsha di-“cells” tse tshweu mo go ona, le di “platelets”, re di bale, mme re di dirise mo patlisisong gona mo letsatsing le a tserweng ka lone. Masaledi a madi a gago ga a ne a bolokiwa, mme a ya go latlihiwa ka mokgwao o o tshwanetseng.

Go botlhokwa gore o bo o le motho yo o sa gogeng motsoko, mme o le motho yo o nwang fela meriana ya ka metliha ya bolwetsi ba gago. O seke wa nwa meriana mengwe fela, jaaka ya ditlhabi (sekao- panado®, disprin®), di-vitaminine kgotsa meriana ya setso mo dibekeng di le pedi pele re tsaa madi a gago. Fa o e nwele, tsweetswe re itsise.

Re tla buisana le wena ka mogala letsatsi kgotsa a le mabedi pele ga motlha wa go tsaa madi, gore re totobatse gore a o tla nna gone.

Boitshupo jwa gago bo tla sirelediwa, mme le madi a gago ga a kitla a dirisetswa mabaka mangwe a sele, kwantle ga a a tlhalositsweng fela.

Re lebogela go tsaa karolo ga gago.

Mmatlisisi: ___________________ Tshaeno:_______________ Letlha: ____________
APPENDIX 1
UNIVERSITY OF LIMPOPO (Medunsa Campus) ENGLISH CONSENT FORM

Statement concerning participation in a Research Project.

Name of Study*

Investigation of the toxicity of commercially available herbal mixtures used as African traditional medicine ..........

I have heard the aims and objectives of the proposed study and was provided the opportunity to ask questions and given adequate time to rethink the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way.

I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without supplying reasons. This will have no influence on the regular treatment that holds for my condition neither will it influence the care that I receive from my regular doctor.

I know that this study has been approved by the Medunsa Campus Research and Ethics (MCREC), University of Limpopo (Medunsa Campus) / Dr George Mukhari Hospital. I am fully aware that the results of this study will be used for scientific purposes and may be published. I agree to this, provided my privacy is guaranteed.

I hereby give consent to participate in this study.

............................................................ ........................................................
Name of volunteer Signature of volunteer.

............................................................ ........................................................
Place. Date. Witness

Statement by the Researcher

I provided verbal information regarding this study.
I agree to answer any future questions concerning the study as best as I am able.
I will adhere to the approved protocol.

............................................................ ........................................................
Name of Researcher Signature Date Place
APPENDIX 1

UNIVERSITY OF LIMPOPO (Medunsa Campus) SETSWANA CONSENT FORM

Seteitemente se se ka ga go tsaya karolo mo Porojeke ya Patlisiso.

Leina la Patlisiso

Investigation of the toxicity of commercially available herbal mixtures used as African traditional medicine .......... 

...........................................................

Ke utlwile maitlhomo le maikemisetso a patlisiso e e tshitshintsweng mme ke filwe tšhono ya go botsa dipotso le go fiwa nako e e lekaneng ya go akanya gape ka nthla e. Maitlhomo le maikemisetso a patlisiso e a thaloganyega sentle. Ga ke a patelediwa ke ope ka tsela epe go tsaya karolo.

Ke thaloganya gore go tsaya karolo mo patlisisong e ke boithaopo le gore nka ikgogela morago mo go yona ka nako nngwe le nngwe kwa ntle ga go neela mabaka. Se ga se kitla se nna le seabe sepe mo kalafong ya me ya go le gale ya bolwetsi jo ke nang le jona e bile ga se kitla se nna le thotholelelese epe mo thokomelong e ke e amogelang mo ngakeng ya me ya go le gale.

Ke a itse gore patlisiso e e rebotswe ke Patlisiso le Molao wa Maitsholo tsa Khampase ya Medunsa (MCREC), Yunibesithi ya Limpopo (Khampase ya Medunsa) / Bookelo jwa Ngaka George Mukhari. Ke itse ka botlalo gore dipholo tsa patlisiso di tla dirisetswa mabaka a saentifikile e bile di ka nna tsa phasaladiwa. Ke dumelana le seno, fa fela go netefadiwa gore se e tla nna khupamarama.

Fano ke neela tumelelo ya go tsaya karolo mo patlisisong e.

...........................................................

Leina ka moithaopi                                Tshaeno ya moithaopi.

...........................................................

Lefelo.                                Letlha.                                Paki

Seteitemente ka Mmatlisisi

Ke tlametse tshedimosetso ka molomo le/kgotsa e e kwadilweng malebana le Tekelelo / Patlisiso / Porojeke* e. Ke dumela go araba dipotso dingwe le dingwe mo nakong e e tlang tse di amanang le Tekelelo / Patlisiso / Porojeke* ka moo nka kgonang ka teng. Ke tla tshegetsa porotokolo e e rebotswe ng. 

...........................................................

Leina la Mmatlisisi                                Tshaeno                                Letlha                                Lefelo