AN INVESTIGATION OF THE BLOOD PRESSURE LOWERING AND CARDIOPROTECTIVE EFFECTS OF THE FREEZE DRIED EXTRACTS OF *Aloe secundiflora*

INVESTIGATOR

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Department of Medical Physiology,

College of Health Sciences, School of Medicine,

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DECLARATION

I hereby declare that this thesis is my original work and to the best of my knowledge has not been presented elsewhere for the award of a degree.

Sign _____________________________    Date ______________________

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H56/68826/2013

Supervisors Approval:

This thesis is being submitted with our approval as the University supervisors:

Sign _____________________________    Date ______________________

Dr. Peter M. Waweru, PhD.

Sign _____________________________    Date ______________________

Dr. Frederick O. Bukachi, MMED, PhD.
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I wish to acknowledge my colleagues (Awot Sunday, Denis Rono, Eliphas Gitonga, Faraj Alkizim and Rose Kamenchu) and lecturers for their continued advice and support throughout the study.
DEDICATION

I dedicate this work to my son, Bedan Mwaniki, may you grow to love knowledge.
ABSTRACT

Background: Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality worldwide. Hypertension is the main driver and its interaction with dyslipidaemia and diabetes mellitus accelerates onset and progression of cardiovascular diseases. Herbal products like Aloe secundiflora are used in traditional medicine to improve lifestyle and mitigate these risks.

Aim: To investigate the effects of freeze dried extracts of Aloe secundiflora on blood pressure, lipid profile and blood glucose in in-vivo and in-vitro animal models.

Setting: Department of Medical Physiology, University of Nairobi.

Study design: Experimental animal study.

Materials and methods: The chronotropic and inotropic effects of the freeze dried root and leaf extracts of Aloe secundiflora were investigated using the isolated rabbit heart mounted in a Langendorff system of (Power lab™ AD Instruments).

Forty (40) Wistar rats were randomly allocated into four groups; the positive control (Atorvastatin 25 mg/Kg), negative control (gavages with distilled water), low dose (40 mg/kg) test and high dose (80 mg/kg) test groups. They were treated and followed up for the five (5) week experimental period. The Blood Pressures were measured at the beginning of the experiment and thereafter at weekly intervals using a VET™ Doppler sphygmomanometer experimental recording system. The blood glucose and lipid profiles were measured at the end of the experimental period. The experimental data were expressed as mean ± Standard Error of Mean and analyzed by one-way ANOVA and Tukey post-hoc tests. The significance level was set at p < 0.05.

Results: The freeze dried crude root extract significantly reduced the contractility of the isolated rabbit heart, (p <0.001), but had no chronotropic effect at the test doses. There was no significant difference across the different doses. The freeze dried leaf extract reduced the heart rate and contractility, (p < 0.05), showing significant differences across the test doses.

The experimental group on a dose of 80 mg/kg of the freeze dried leaf extract had significantly lower systolic blood pressure than the control group by mean 13.9 ± 4.31 mmHg, p= 0.005. The lipid profile showed significantly lower LDL cholesterol in the experimental groups than both
the positive and negative control groups, p= 0.006. The 80 mg/kg dose experimental group also had significantly higher HDL cholesterol than the other groups. The test groups had significantly lower fasting blood glucose 7.1 ± 0.21 (c) vs. 7.06 ± 0.31 (c) vs. 6.39 ± 0.19 (t) vs. 6.35 ± 0.18 (t), (p = 0.033). There were no significant differences in weights or electrocardiographic features between the control and test experimental groups.

Conclusions: The Aloe secundiflora leaf extract had negative inotropic and chronotropic effects, caused a reduction in blood pressure, fasting blood glucose and improved the lipid profile (reduced the LDL and increased the HDL cholesterol) and should be further evaluated for active components for potential development as treatment or prophylaxis for cardiovascular diseases either as a drug or nutritional supplement.

KEY WORDS: Aloe secundiflora, Hypertension, Electrocardiogram, Lipid profile, Blood glucose.
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<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CVDs</td>
<td>Cardiovascular diseases</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>FELASA</td>
<td>Federation of European Laboratory Animals Science Association</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>ICIPE</td>
<td>International Centre for Insect Physiology and Ecology</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>QTc</td>
<td>Corrected QT interval</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
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<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
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<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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CHAPTER ONE

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide responsible for 17 million deaths annually, a third (30%) of all the deaths (WHO, 2013). Hypertension, the single most common modifiable risk factor of CVDs, accounts for 9.4 million deaths - half of all cardiovascular related deaths (WHO, 2013; Dennison-Himmelfarb and Handler, 2013). Hypertension complications resulting from uncontrolled hypertension include heart attacks, strokes, kidney failure, premature mortality and disability (WHO, 2013; Alwan, 2011).

The prevalence of hypertension is highest in Africa (46% of all adults aged above 25) (Carretero and Oparil, 2000). The high prevalence, weak health systems, late diagnosis, poor adherence, use of traditional herbal medicine and the deficient socio economic support structures from weak governance and generalized poverty have led to low and middle income countries bearing 80% of the global burden of hypertension (WHO, 2013; Opie and Seedat, 2005).

Alternative medicine has been used for management of chronic illnesses among the low and middle income countries. An estimated 80% of the world’s population relies on herbal medicine; the number is higher in Africa with Kenya having over 70% of the population relying on herbal medicine as a primary source of health care and over 90% having used herbal medicine at one time or another (Musila et al., 2004). Alternative medicine has been used in management of cardiovascular diseases with minimal or no evidence on their effectiveness and side effects profile (Chetty, 2010; Douri, 2014). This has led to some people getting no medicinal value at a cost of side effects, poor dosing and sometimes morbidity and mortality from complications arising from the non adherence to conventional medication (Musila et al., 2004). This calls for research into finding more scientific means of tapping the medicinal values of these herbs while negating the placebo use of herbs, adverse effects and poor dosing. This would help provide cheaper alternative medication for the populace of the low and middle income countries like Kenya and consequently reduce morbidity and mortality from chronic illnesses.
Aloe secundiflora is one of the herbal products that have been used for the management of several maladies with the medicinal value of Aloe species dating back to ancient Egyptian times (Kamau et al., 2013; Musila et al., 2001). The Aloe species have been used for skin conditions, cancers, diabetes mellitus, diarrhea, typhoid, post partum hemorrhage, malaria and many other conditions in both man and animals (Musila et al., 2004; Nanyingi and Mbaria, 2008).

The present study investigated the cardioprotective effects of freeze dried extracts of Aloe secundiflora their effects on blood pressure, lipid profile and blood sugar so as to explore its potential use in further research in drug development to reduce cardiovascular morbidity and mortality.
LITERATURE REVIEW

ALOE SECUNDIFLORA

Aloe secundiflora is a fleshy stem-less rosette with green sickle shaped glossy leaves about 50 x 12 cm with spiny edges. It has a branched inflorescence arising from the rosette with secund flowers with minute translucent spots. The leaves ooze a brownish/yellowish bitter tasting juice when cut (Mukonyi and Oduor, 2008; Ondiaka and Lwande, 2011).

Figure 1: Aloe secundiflora growing with and without flowers (Mukonyi & Oduor 2008).

Taxonomical classification:

Aloe secundiflora is taxonomically classified into Kingdom Plantae, Class Liliopsida, Order Liliales, Family Aloeaceae/Asphodelaceae/Liliaceae and Species Secundiflora. (Aloe secundiflora). There are two varieties; Aloe secundiflora var. secundiflora found in Kenya, Ethiopia, Northern Tanzania and Uganda, and Aloe secundiflora var. tweediae found in Northern Kenya, Northern Uganda and South Sudan (Kamau et al., 2013; López et al., 2013; Wabuye and Kyalo, 2008).

Geographical distribution:

The Aloe plants are found throughout Sub-Saharan Africa including the Indian Ocean islands. They grow in alluvial soils of arid and semi arid areas at 1200 – 1800 m above sea level and a
few at the Coast region (Mukonyi and Oduor, 2008; Wabuyele and Kyalo, 2008). The herb is widely distributed in the Machakos, Samburu and Baringo counties of Kenya and in parts of other Eastern African countries of varied species, subspecies and varieties (Mukonyi and Oduor, 2008; Wabuyele and Kyalo, 2008).

Figure 2: Distribution of indigenous Aloe species in Kenya (Mukonyi & Oduor 2008).

The local names and uses:
In the Kamba community the sap of *Aloe secundiflora* has been used as remedy for poor appetite, vomiting, malaria (‘Ndetema’ – fever), headache, respiratory tract infections, conjunctivitis and chest pain. They also use it for the management of hypertension by either drinking the sap/water concoction or by licking the ash derived from burning the roots or inflorescence (Kokwaro, 2009).
In the Kikuyu community a boiled sap/water concoction is taken 3 times daily for a week to ‘restore the uterine position in pueperium’. They also use it in the management of eye problems and other undefined maladies of the spleen, liver and rheumatism (Kokwaro, 2009).

In the Maasai and Samburu communities the sap is used to ferment alcoholic beverage and give local brews the bitter taste (Kokwaro, 2009; Ondiaka and Lwande, 2011).

The vernacular names are (Kiluma (Kamba), Kiruma/Mugwanugu (Kikuyu), Turkos (Pokot), Tangaratwe (Tugen), Suguroi (Maa & Samburu), Kipapa (Taita), Ejichuka (Turkana), Kolonje (Duruma), dahr (Somali) and Isale la njofu (Kichagga)(Mukonyi and Oduor, 2008; Njoroge, 2012; Ondiaka and Lwande, 2011).

Scientific Profile:
Aloe species are rich in anthraquinones and anthracenones of different composition (Karl-h et al., 2003). Chemical constituents of plants vary in composition from part to part, species to species and even geographically. The root extract of Aloe mainly contains anthracenones and some polysaccharides while the leaf exudates mainly the sap has anthraquinones (Saleem et al., 2001). The gel contains mainly non polar compounds (fatty acids, their esters and hydrocarbons) which have been previously investigated for reducing oedema, gastric secretion and ulcer prevention (Saleem et al., 2001). Acemannan, an immunostimulant is also derived from the inner leaf gel (Simões et al., 2012).

The main constituents of the leaf exudates are phenolic compounds mainly Aloenin, Aloenin B, Isobarbaloin, Barbaloin, Isoaloesin, Aloenside A and Aloenside B with lower contents of polysaccharides and aliphatic compounds (Karl-h et al., 2003; Patel and Patel, 2013; Saleem et al., 2001). The anthraquinones have been postulated to possess mutagenic, antimutagenic, leukemia inhibition, cathartic, antiviral (against Herpes Simplex Virus), tuberculostatic activity and laxative properties. These are mainly linked with Aloeemodin, the aglycone of aloin (Karl-h et al., 2003; Saleem et al., 2001). The wound healing properties have been ascribed to the polysaccharides. Aloenin, a non-anthraquinone σ-glucopyranoside has been compounded in skin care products (Karl-h et al., 2003; López et al., 2013; Sahu et al., 2013).
Figure 3: Structures of Isoaloesin, Aloenin and Aloenin B (Karl-h et al. 2003).

Research has validated the ethno-veterinary uses of *Aloe secundiflora* in the management of fowl typhoid, Newcastle disease and *Ascaridia galli* in poultry (Kaingu et al., 2013; Waihenya and Mtambo, 2002; Waihenya et al., 2002).

Despite close taxonomic relationship, Aloe species do not have the same activity as shown by their different antibacterial medicinal potentials from *Aloe barbadensis* (Mpala et al., 2010).

Kamau et al (2013) demonstrated diuretic and laxative effects of *Aloe secundiflora* on rabbits. However there were concerns that toxicity could lead to nausea, vomiting, tachycardia, arrhythmias, albuminuria and headache (Kamau et al., 2013). The extract also led to hypokalaemia which was feared could potentiate the arrhythmias despite the low toxicity profile previously described (Kamau et al., 2013; Mpala et al., 2010). Prior unpublished research has demonstrated negative chronotropicity and inotropicity of the leaf extract in-vitro (Mwaniki, 2008).
HYPERTENSION

Definition and classification
Hypertension is sustained elevated blood pressure (BP) of systolic pressure equal to or higher than 140 mmHg and diastolic pressure equal to or higher than 90 mmHg. Hypertension is either primary (essential) hypertension or secondary hypertension that has an identifiable direct cause which once removed can eliminate the hypertension (Daugherty et al., 2012; Dennison-Himmelfarb and Handler, 2013). Essential hypertension forms the bulk of the hypertension burden (Carretero and Oparil, 2000).

The seventh and eighth Joint National Committee on hypertension (JNC VII and JNC VIII) grades the level of hypertension with particular guidelines on how to manage each with either lifestyle modification alone or in combination with drug therapy. These grades are:

<table>
<thead>
<tr>
<th>Blood pressure classification</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>And &lt;80</td>
</tr>
<tr>
<td>Pre Hypertension</td>
<td>120 – 139</td>
<td>Or 80 – 89</td>
</tr>
<tr>
<td>Stage 1 Hypertension</td>
<td>140 – 159</td>
<td>Or 90 – 99</td>
</tr>
<tr>
<td>Stage 2 Hypertension</td>
<td>≥ 160</td>
<td>Or ≥ 100</td>
</tr>
</tbody>
</table>

Table 1: Grading of blood pressure by JNC VIII (James et al. 2013).

Blood pressure control
Blood pressure lowering medications target the intrinsic blood pressure regulation aspects such as heart rate, contractility and peripheral vascular resistance by use of sympatholytics, beta blockers, vasodilators, calcium channel blockers and diuretics. A majority of patients receive combination therapy of two or more drugs; each drug targeting different aspects of blood pressure regulation (Chow et al., 2013; Dennison-Himmelfarb and Handler, 2013). The diagram below illustrates the factors that interfere with the BP regulation equation.
Hypertensive patients usually have related co-morbidities. Forty percent have hypercholesterolaemia and hypertension is twice as common in patients with diabetes mellitus compared to the non diabetic population (Oparil et al., 2003; Vikrant and Tiwari, 2001). Hypertension management therefore often has to include adequate blood glucose control and correction of dyslipidaemia.

Hypertension control remains below 50% in developed countries and is even poorer in developing countries despite the advancement in knowledge of pathophysiology and available treatment strategies (Daugherty et al., 2012; Dennison-Himmelfarb and Handler, 2013). Challenges in hypertension management range from lack of valid blood pressure measurement, target and threshold blood pressures, therapy in elderly patients, resistant hypertension, side effects profile, poor adherence, clinical inertia to pill burden all leading to the undesired complications and eventually cardiovascular mortality (Carretero and Oparil, 2000; Chow et al., 2013; Daugherty et al., 2012; Dennison-Himmelfarb and Handler, 2013).
Pathophysiology of hypertension

Hypertension occurs as a result of complex interaction of genetic and environmental factors (Oparil et al., 2003). Secondary hypertension results as a consequence of an underlying medical condition like chronic kidney disease and pheochromocytoma and can be addressed by treating the underlying cause. Essential (primary) hypertension has an unclear mechanism for more than 90% of the cases. Mechanistic theories explaining the interaction of genetic and environmental factors explain clustering of hypertension in families and associations with other factors like diabetes mellitus, insulin resistance and hypercholesterolaemia (Carretero and Oparil, 2000; Oparil et al., 2003; Vikrant and Tiwari, 2001).

Genetic susceptibility (inherited BP) is the leading cause of essential hypertension and has additive effects with other hypertensinogenic factors such as obesity, hypercholesterolaemia, diabetes mellitus, insulin resistance, high alcohol intake, high salt intake (in salt sensitive patients), ageing and sedentary lifestyle, stress, low potassium intake and low calcium intake. These factors in addition to genetic susceptibility and when two or more factors are present they have an additive effect (Carretero and Oparil, 2000; Dennison-Himmelfarb and Handler, 2013; Labeit et al., 2012).

Inherited BP (genetic BP) has been shown in 25% pedigree studies and up to 65% in twin studies usually involving more than ten gene mutations as opposed to single gene mutations. Gene mutations resulting in hypertension include those related to the Glucocorticoid Remediable Aldosteronism (GRA), Liddles syndrome, Apparent Mineralocorticoid Excess (AME) and those on the chromosomes related to familial hypercholesterolaemia. Most of the mutations affect renal sodium and water handling (Vikrant and Tiwari, 2001).

The sympathetic nervous system and the rennin-angiotensin-aldosterone system explain the major pathophysiologic mechanisms of hypertension (Vikrant and Tiwari, 2001). Endothelial dysfunction with dysregulation of the vasodilator activities of nitric oxide and prostacyclin has been shown to be a cause rather than a consequence of hypertension (Carretero and Oparil, 2000; Oparil et al., 2003; Vikrant and Tiwari, 2001). The diagram below shows the various factors that interact in the pathophysiology of hypertension.
BLOOD PRESSURE RECORDING IN MAN

The non-invasive sphygmomanometric technique for auscultatory blood pressure measurement is the gold standard. The blood flow occlusion and return of flow with the auscultated Korotkoff sounds to determine systolic and diastolic blood pressure is used for clinical and therapeutic studies and practice (Dieterle, 2012). Oscillometric blood pressure is more convenient, safer and enables self BP measurements but has engineering challenges hence has limited use (Dieterle, 2012).

Blood pressure measurement in infants, children and patients with very faint Korotkoff sounds may utilize ultrasonic techniques which follows the principle of detection arterial wall motion increase (for systolic blood pressure) and diminution (for diastolic blood pressure). The doppler may also use the blood occlusion and return of blood flow detection to determine systolic and diastolic blood pressure (Dieterle, 2012). Finger cuff connected to a plethysmograph is another non-invasive blood pressure measurement technique which utilizes the intra-arterial pressure...
waves to estimate the systolic and diastolic blood pressure. The Doppler and plethysmorgraph techniques have been applied in measuring blood pressure in laboratory animals (Dieterle, 2012).

**BLOOD PRESSURE RECORDING IN RATS**

The blood pressure of rats can be measured directly via indwelling catheters or radio telemetry or indirectly by use of the tail cuff method. The direct methods are the most accurate and are used as the gold standard to validate other methods (Pauline et al., 2011).

**DIRECT/ INVASIVE BLOOD PRESSURE MEASUREMENT**

Indwelling catheters are inserted into one of the major vessels (aorta, carotid or femoral) and then exteriorized. The exteriorized catheter is connected to a pressure transducer from which the mean arterial pressure (M. A. P.) can be directly recorded (Pauline et al., 2011). However it is difficult to use for repeated blood pressure measurements (Molčan et al., 2009; Pauline et al., 2011).

The radio telemetry method involves surgical implantation of a radio transmitter in the rat which is connected to a remote receiver that records minute to minute blood pressure and heart rate changes. It is used when continuous long term monitoring is required (Molčan et al., 2009; Pauline et al., 2011).

These methods involve anaesthesia and surgery both of which affect blood pressure. They are also associated with surgical morbidity and are expensive to acquire and maintain. These disadvantages have led to validation of other methods that are non invasive (Kumar and Tiwari, 2014; Malkoff, 2005; Pauline et al., 2011).

**INDIRECT/ NON INVASIVE BLOOD PRESSURE MEASUREMENT**

The tail cuff method is the main indirect blood pressure measurement method used (Kumar and Tiwari, 2014). It involves the use of an occlusion tail cuff (or limb cuff), a blood pulse sensor connected distal to the occlusion, a standardized sphygmomanometer to inflate the cuff and a monitor to record the pulse. Once the occlusion tail cuff has been inflated, the blood flow to the tail is curtailed and the pulse not detected. Upon slow deflation the pulse will again be detected.
when the occlusion pressure falls below systolic blood pressure. The pressure required to completely occlude rat tail blood flow is noted by the point at which the pulse disappears or appears and that is the systolic blood pressure. The blood pulse sensors used are photoplethysmograph, piezoplethysmograph, doppler sensors and volume pressure recording (Krege et al., 1995; Kumar and Tiwari, 2014; Pauline et al., 2011).

The non-invasive tail cuff method is simple, economical and repeated measurements can be obtained in conscious animals with or without preheating. This method has been validated as accurate, reliable and gives similar results to those of direct blood pressure measurement (Malkoff, 2005; Pauline et al., 2011).

LIPIDS AND BLOOD GLUCOSE MEASUREMENT IN ANIMAL MODELS

The assay of lipids and blood glucose measurements are carried out by commercial laboratory machines similar to those used for human measurements. The principles used in the assay of lipids involve stepwise enzymatic reactions with spectrophotometric recording of the products of the enzymatic reactions with specific absorbance(Aguilar et al., 2011; Brăslaşu et al., 2007; Koh et al., 2012; Onyeike et al., 2012).

Total cholesterol estimation follows coupled enzymatic reactions that hydrolyze cholesterol esters and oxidize the OH group producing hydrogen peroxide as one of the by products. The hydrogen peroxide reacts in the presence of a peroxidase to produce a colour whose intensity is proportional to the cholesterol concentration measured spectrophotometrically (Onyeike et al., 2012; Otunola et al., 2010; Tende et al., 2015). The following reactions take place:

\[
\text{Cholesteryl ester hydrolase} \\
\text{Cholesteryl ester} + H_2O \quad \overset{\text{---}}{\rightarrow} \quad \text{Cholesterol} + \text{fatty acid}
\]
(Cholesterol oxidase)

\[
\text{Cholesterol} + \text{O}_2 \quad \rightarrow \quad \text{cholest-4-en-3-one} + \text{H}_2\text{O}_2
\]

(Peroxidise)

\[
2 \text{H}_2\text{O}_2 + 4\text{-aminophenazone} + \text{phenol} \rightarrow 4\text{-(p-benzoquinone- monoimino)henazone} + 4 \text{H}_2\text{O}
\]

Triglycerides are measured in a similar manner by hydrolysis to produce glycerol which is oxidized producing hydrogen peroxide as one of the by-products. The HDL-Cholesterol is measured spectrophotometrically after a series of reactions which stabilize the Apo B lipoprotein before the hydrolysis takes place. The LDL-Cholesterol is then calculated from the values of Total cholesterol, HDL-Cholesterol and triglycerides using this equation.

\[
[\text{LDL-C}] = [\text{Total Cholesterol}] - [\text{HDL-C}] - \frac{[\text{TG}]}{5} \quad (\text{mg/dL})
\]

\[\frac{[\text{TG}]}{5}\] estimates VLDL-cholesterol.

These reactions are automated in commercial laboratory machines and only the final processed values of the lipid profile and blood glucose are produced (Aguilar et al., 2011; Onyeike et al., 2012; Otunola et al., 2010; Tende et al., 2015).
JUSTIFICATION
The increased use of herbal medication to more than 70% in Kenya and the little knowledge available on the herbal remedies resorted to creates the need to research into the mechanisms of actions, dosing and predictable side effects of the herbs used. *Aloe secundiflora* is used in folklore medicine for hypertension management with little knowledge available on its effectiveness and side effects profile.

The present study therefore picks a leading killer disease, hypertension, and one of the commonly used herbal remedies, *Aloe secundiflora* and aims to investigate the blood pressure lowering and cardioprotective effects of this herb on laboratory animals hence set stage for future research on humans.

RESEARCH QUESTION
What are the blood pressure lowering and cardioprotective effects of the crude extract of *Aloe secundiflora*?

HYPOTHESIS

H₀: The crude extract of *Aloe secundiflora* has no blood pressure lowering and cardioprotective effects.

Hₐ: The crude extract of *Aloe secundiflora* has blood pressure lowering and cardioprotective effects.
OBJECTIVES

Broad objective:
To investigate the blood pressure lowering and cardioprotective effects of the crude aqueous freeze dried extract of *Aloe secundiflora*.

Specific objectives:
To investigate the chronotropic and inotropic effects of freeze dried leaf and root extracts on an isolated rabbit heart.

To investigate the effect of freeze dried leaf extract on the blood pressure in Wistar rats.

To describe the effect of freeze dried leaf extract on the lipid profile in Wistar rats.

To describe the effect of freeze dried leaf extract on the blood glucose in Wistar rats.

To describe the effect of freeze dried leaf extract on electrocardiographs in Wistar rats.
CHAPTER TWO:

METHODOLOGY

SETTING
The study was conducted at the animal house and main laboratory of the department of Medical Physiology Chiromo campus, College of Health Sciences, University of Nairobi.

STUDY DESIGN
This was an experimental animal study design.

MATERIALS AND METHODS

MODEL SELECTION
The present study utilized male Wistar rats 16-20 weeks old weighing 250-350 grams as the study animals. The objective was to assess the blood pressure lowering, lipid profile and electrocardiographic effects of the extract. The Wistar rats were obtained from the animal house of the Department of Medical Physiology- University of Nairobi, and kept under standard laboratory conditions of 15 – 25°C temperature, 45 – 65% relative humidity and a 12 hour light/dark cycle.

The in-vitro part utilized the isolated hearts of conventional laboratory rabbits- *Oryclolagus cuniculus*. The preferred breed was the New Zealand White rabbit due to its availability and are structurally bigger than most of the laboratory rodents. The big size of the rabbits made it easier to sacrifice them and remove the heart and subsequently mount it on the Langendorff apparatus available at the Medical Physiology Department.

The animals were handled in accordance to The Federation of European Laboratory Animal Science Associations (FELASA) guidelines (Convenor et al., 2002).

ETHICAL APPROVAL
Ethical approval was obtained from the department of Medical Physiology Postgraduate Committee before the use of the animals in the study.
**EXTRACTION**

The leaves and the roots of *Aloe secundiflora* were harvested from Machakos County stored and transported in polythene bags. The collected plants were identified and indexed in the University of Nairobi herbarium by a plant taxonomist.

The leaves were blended and the Aloe paste obtained reconstituted with three parts of water. The Aloe juice obtained was filtered with a Whatmanns size number one filter paper. The roots were dried, blended and reconstituted with three parts of water then filtered to obtain the root extract. The filtrates obtained were frozen, using the *Hotpoint*™ deep freezer. The frozen filtrates underwent lyophilization at the International Centre for Insect Physiology and Ecology (ICIPE) after which a brown powder (the root) and a green brown powder (leaf) were obtained. The powder was weighed and labeled then stored in the *Hotpoint*™ deep freezer. The extracts were reconstituted into different dosages daily before the experiment as previously described (Kamau et al., 2013; Waihenya and Mtambo, 2002; Waihenya et al., 2002). The process is summarized in the flow chart diagram below.

![Flow chart diagram](image)

**Figure 6**: The extraction process of the aqueous leaf and root extracts of *Aloe secundiflora*.  
17
EQUIPMENT

The following equipment was used in the study:

- Langendorff system of (Power lab™ AD Instruments), (Model ML865, AD Instruments, Dunedin, New Zealand).
- VET™ Doppler sphygmomanometer experimental recording system.
- SelectraProS Clinical Chemistry Analyzer (ELITech Group, France).
- On Call plus™ Glucometer (OneTouch SureStep, Milpitas, CA, USA).
- Calibrated beakers, syringes, needles, clear plastic containers, cello tape and strings.
- Ultrasonography gel, cotton wool, surgical spirit, masking tape, head phones.
- Diethyl ether, Ketamine, capillary tubes, vacutainers.
- Drug preparations (commercial preparations of acetylcholine, atropine, calcium chloride, atorvastatin).
- Physiological solution.

DRUG PREPARATIONS

Drugs used during the study were reconstituted into different dosages in laboratory glass beakers every morning before the experiment and used for that day only.

PHYSIOLOGICAL SOLUTIONS

Ringer-Locke solution was used to bathe the isolated rabbit heart during the study. It was aerated and maintained at a temperature of 37°C during the experiments. One litre of the Ringer-Locke solution was made by diluting the following solutes in one litre of distilled water (Locke and Rosenheim, 1904).

<table>
<thead>
<tr>
<th>Solute</th>
<th>Amount (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>9.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.02</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.42</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 2: The composition of the Ringer-Locke solution (Locke & Rosenheim 1904).
EXPERIMENTAL PROTOCOL
The study was conducted in two parts:

(i) An in-vitro assessment of the effects of the leaf extract and root extract on the heart rate and contractility of an isolated rabbit heart and

(ii) An in-vivo part using the more potent extract (leaf or the root extract) to assess the blood pressure lowering, lipid profile, blood glucose and electrocardiographic effects of the freeze-dried extract on the Wistar rats.

PART I: IN VITRO ASSESSMENT OF CHRONOTROPICITY AND INOTROPICITY OF THE FREEZE DRIED ROOT AND LEAF EXTRACT OF Aloe secundiflora
The Langendorff apparatus was set up and connected to the Power lab™ AD Instruments. The physiological solution was prepared, aerated and the flow rate set at 10 ml per minute. The solution and set up was warmed to 37 °C. The extracts and drugs were weighed and reconstituted to different dosages for use on each day of the study.

The rabbits were fasted overnight and sacrificed by stunning a blow at the back of the head. The heart was removed by severing the pulmonary vessels. A short aortic segment of 1-2 cm was used for reverse perfusion. The isolated heart was immersed in cold Ringer-Locke solution to reduce metabolic activity and the blood cleaned from the heart chambers to prevent intra chamber clots formation. The pericardial and perivascular fat were severed off and a small opening was made at the left atrium where the balloon (used as the pressure transducer) was placed to detect contractions. This was carried out quickly to avoid arrhythmias and loss of contractility by the isolated heart. The isolated heart was then mounted onto the Langendorff system of (Power lab™ AD Instruments) attaching the aortic segment with a 7 cm string to establish a reverse perfusion with the Ringer-Locke solution flowing into the heart through the aorta as previously described (Döring, 1990; Locke and Rosenheim, 1904; Skrzypiec-spring et al., 2007).

The contractions were then recorded until they were uniform. Cardiac massage was sometimes employed to help regularize the contractions. Baseline heart rate and contractility were recorded after five minutes of uniform contractions of the isolated heart. The baseline contractions were recorded for one minute before and after addition of the experimental drug. The contractions
were then recorded until the heart recovered from the treatment and the contractions returned to their pre-treatment level. The pretreatment recording (baseline recording) was used to calculate the baseline heart rate and contractility which was compared with the post treatment recording (experimental recording) as shown in the figures below.

Figure 7: Figure shows the abolition of heart contraction after addition of the extract.

Figure 8: Figure shows heart contraction before and after addition of the extract.

This was repeated for the negative control (normal saline), and the different dose preparations of the freeze dried roots and leaves extracts to have a set of five recordings from each dose. The maximum dose was used to evaluate the effect of atropine and calcium chloride on the effect of *Aloe secundiflora* root and leaf extract on the heart rate and contractility. The baseline recordings
were compared to the experiment recordings and the difference expressed as percentage change. The percentage change calculated from the effect of the different doses of root and leaf extract were expressed as dose response curves for the root and leaf extract respectively. The following is a flow chart of the experimental protocol of the freeze dried *Aloe secundiflora* leaf and root extract.

![Flow chart of the experimental protocol for the leaf and root extracts.](image)

**PART II: IN VIVO ASSESSMENT OF THE EFFECTS OF *Aloe secundiflora* FREEZE DRIED LEAF EXTRACT ON BLOOD PRESSURE, LIPID PROFILE, BLOOD GLUCOSE AND ELECTROCARDIOGRAM**

**ACCLIMATIZATION**

Forty (40) Wistar rats were obtained from the department of Medical Physiology. The rats were allowed to acclimatize, feeding and drinking ad libitum for two weeks before the study. The blood pressure measurements of the rats were taken before and after oral administration of distilled water simulating the treatment during the experiment. This was carried out to habituate
the rats to the blood pressure set up used later in the experiment. This was repeated for 4 days a week until consistent results were obtained as previously described (Tende et al., 2015; Ubota et al., 2006).

**GROUPING**

The male Wistar rats were randomly allocated to four groups of ten rats each. The baseline blood pressure and weight of the rats in each group were taken and recorded before beginning the treatment. The groups were then randomly allocated to different treatment groups as shown below:

- **Group A**: Negative control group (Normal saline)
- **Group B**: Low dose test group (40 mg/kg Aloe leaves freeze dried extract)
- **Group C**: Positive control group (Atorvastatin 25 mg/kg)
- **Group D**: High dose test group (80 mg/kg Aloe leaves freeze dried extract)

**TREATMENT**

The rats in Group A were treated (per oral administration using a flexible nasogastric tube) with one milliliter normal saline once a week for five weeks. The weights and blood pressure were recorded weekly (three readings per session and the average noted) for the five weeks. This was repeated for the rats in Group B with one milliliter of 40 mg/kg *Aloe secundiflora* leaf extract, Group C with one milliliter of 25 mg/Kg of Atorvastatin and Group D with one milliliter of 80 mg/kg of *Aloe secundiflora* leaf extract. The weight and blood pressure was taken weekly for all the four groups and recorded. Electrocardiograms of the different groups were taken at the end of the five weeks.

At the end of the five weeks the rats were fasted overnight and blood (4 milliliters) was drawn from the retro-orbital venous plexus for blood glucose and lipid profile analysis (Kamau et al., 2013; Karl-h et al., 2003; Maurya et al., 2010; Patel and Patel, 2013; Tende et al., 2015).
Acute blood pressure lowering effect was investigated on thirty (30) male Wistar rats which were randomly allocated to three groups of 10 rats each. The baseline blood pressure was taken and recorded. The rats in each group were treated with normal saline (negative control), 40 mg/kg freeze dried *Aloe secundiflora* leaf extract (low dose test group) and 80 mg/Kg freeze dried *Aloe secundiflora* leaf extract (high dose test group). They were allowed to rest for one and a half
hours then their blood pressures were taken again and recorded as described (Patel and Patel, 2013).

**BLOOD PRESSURE RECORDING**

The blood pressure was taken using the VET™ Doppler sphygmomanometer experimental recording system as follows:

1. The rats were restrained without anaesthesia in a transparent plastic bottle (modified restrainer).
2. The tails were warmed with 100 watt light bulbs for fifteen minutes to enhance blood flow at the tail hence improve the blood flow detection.
3. The tail cuff was tied on the tail and connected to the sphygmomanometer.
4. The Doppler flow detection probe was tied on the tail distal to the tail cuff after applying the ultrasonography gel and the probe adjusted to lie directly on the rat tail artery.
5. Head phones were connected to the VET™ Doppler sphygmomanometer experimental recording system to amplify the pulsation.
6. The tail cuff was inflated until rat tail blood flow was occluded and pulsations not heard. This corresponded to the disappearance of pulse during arm cuff inflation during blood pressure recording in humans.
7. The tail cuff was slowly deflated until the return of blood flow (pulsations) and that pressure noted. This corresponds to the Korottkoff sounds appearance during blood pressure recording in humans.
8. Steps 6 and 7 were repeated three times and the average pressure at which the blood flow (pulsations) returned was noted and recorded as systolic blood pressure.

The same procedure was repeated with the pulse transducer connected to Power lab™ AD Instruments and the systolic blood pressure range compared to that obtained with the Doppler set up. Figure 11 below shows the pulsations observed during the blood pressure measurement using the pulse transducer connected to the Power lab™ AD Instruments. This recording was as previously described (Bunag and Butterfield, 1982; Daugherty et al., 2009; Ikeda et al., 1991; Okuniewski et al., 1998; Ubota et al., 2006; Whitesall and Hoff, 2004; Widdop and Li, 1997).
ELECTROCARDIOGRAM RECORDING

The rats were anaesthetized using an intra-peritoneal injection of 100 mg/kg of ketamine which took effect after ten to fifteen minutes. The limbs were cleaned with spirit soaked cotton wool, ultrasonography gel applied on the fore right limb (Positive lead), the left hind limb (Negative lead) and the right hind limb (Earthing lead) before attaching the ECG leads of the Power lab™ AD Instruments on the three limbs. The ECG recording (sample recording shown in figure 12 below) was taken for two minutes. The rats were then kept warm overnight with lamps till recovery (8 - 12 hours). This was repeated for all the rats in the four groups and analyzed using Power lab™ AD Instruments as previously described (Hampshire and Davis, 2001).
PHLEBOTOMY FOR LIPID PROFILE ASSAY

The rats were anesthetized for 7 – 10 minutes with diethyl ether soaked cotton wool in a glass bowl with a cover. The blood was drawn from the retro-orbital plexus of the rats. Using a non heparinized capillary tube the retro-orbital plexus was accessed by injecting at the nasal angle of the eye at 90° for about 2-4 mm then guiding the capillary tube upwards at 60° for 5-8 mm. This was used to draw blood (4 milliliters) from the retro-orbital plexus into a red top vacutainer (with no anticoagulant). A drop was used to measure the blood glucose using On Call plus™ Glucometer and the reading recorded as previously described (Herck et al., 2001; Hubbell and Muir, 1996).

RESULTS AND DATA ANALYSES

The data obtained were recorded in tables and expressed as mean ± S. E. M. and analyzed using one-way ANOVA with post hoc test (Tukey for the inter groups comparison). The p value was set at 0.05. The results were presented in tables, dose response curves and bar graphs. The computer statistics package of social sciences (SPSS) and Microsoft Excel were used in the analyses and results presentation.
CHAPTER THREE:

RESULTS

Introduction
This chapter presents the results of the study. The results presented are:

I. The root and leaf extract yielded from the extraction and freeze drying of the Aloe secundiflora.

II. The in-vitro effects of the freeze dried root and leaf extracts on the heart rate and contractility of an isolated rabbit heart.

III. The in-vivo effects of the freeze dried leaf extract on the blood pressure, electrocardiogram, lipid profile and blood glucose of male Wistar rats using four study groups; Negative control group (Normal saline), Positive control group (Atorvastatin 25 mg/kg), Low dose test group (40 mg/kg Aloe leaves freeze dried extract), and High dose test group (80 mg/kg Aloe leaves freeze dried extract).

Extract yield
The percentage yields from the Aloe secundiflora leaf and root extracts were as shown in table 3 below:

<table>
<thead>
<tr>
<th>Plant</th>
<th>Weight of grounded product</th>
<th>Weight of freeze dried extract</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe secundiflora leaves</td>
<td>2 kg</td>
<td>32.5 gm</td>
<td>1.625</td>
</tr>
<tr>
<td>Aloe secundiflora roots</td>
<td>500 gm</td>
<td>10.4 gm</td>
<td>2.080</td>
</tr>
</tbody>
</table>

Table 3: Percentage extracts yields of Aloe secundiflora.
The effect of the freeze dried root extract on Heart rate and Contractility

There was a percentage decrease in strength of contractility of 35.34 ± 4.05, p < 0.001. There was however no significant difference in contractility change among the root extracts doses of 10 mg, 20 mg, 30 mg and 40 mg as shown in table 4 and figure 13 below.

There was no significant change in heart rate p = 0.178.

<table>
<thead>
<tr>
<th>Root extract dose (mg)</th>
<th>Percentage change in contractility (Mean ± s. e. m.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline rate</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>-3.38 ± 4.05</td>
<td>0.580</td>
</tr>
<tr>
<td>10</td>
<td>35.34 ± 4.05</td>
<td>0.000</td>
</tr>
<tr>
<td>20</td>
<td>19.27 ± 4.05</td>
<td>0.001</td>
</tr>
<tr>
<td>30</td>
<td>13.85 ± 4.05</td>
<td>0.020</td>
</tr>
<tr>
<td>40</td>
<td>14.72 ± 4.05</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Table 4: Percentage change in contractility at different doses of the root extract.
The effect of the freeze dried leaf extract on Heart rate and Contractility

The freeze dried leaf extract significantly decreased the heart rate and contractility of the isolated rabbit heart in a dose response relationship. The dose of 20 mg caused a percentage decrease in the heart rate of $6.54 \pm 1.31 (p = 0.001)$ and in the contractility of $21.57 \pm 4.84 (p = 0.002)$. The following are the dose response curves for the changes in heart rate and the contractility respectively.

Figure 13: Percentage change in contractility at different doses of the Aloe secundiflora root extract.
Figure 14: Dose response curve of the heart rate change at different doses of *Aloe secundiflora* leaf extract.
Effect of *Aloe secundiflora* on blood pressure

The freeze dried leaf extract lowered the systolic blood pressure of Wistar rats. The systolic blood pressure in the experimental groups were significantly lower than in the control groups at the end of the studies i.e. 139 ± 3.07 (Negative control) vs. 137.6 ± 2.76 (Positive control) vs. 128.2 ± 2.45 (Low dose) vs. 125.1 ± 3.48 (High dose), (p = 0.005). The high dose experimental group (80 mg/kg leaf extract) having a significantly lower systolic blood pressure by 13.9 ± 4.31 (p = 0.014) compared to the negative control group. The difference in systolic blood pressure in the four groups is illustrated in the box plot below (Figure 16).
Figure 16: Box plot of systolic blood pressure of the four study groups.

There was however no significant reduction in the blood pressure of Wistar rats $134.4 \pm 3.0$ (Negative control) vs. $131.5 \pm 5.8$ (Low dose) vs. $132.3 \pm 3.8$ (High dose), ($p = 0.734$) upon acute administration of the leaves extract at similar doses that lowered blood pressure on chronic administration.
Figure 17: Acute systolic blood pressure before and after treatment.

**Effect of leaf extract on lipid profile**

The results showed that the freeze dried leaf extract decreased LDL-C and increased HDL-C. The test groups had significantly lower LDL-C 1.0286 ± 0.09 (Negative control) vs. 0.7071 ± 0.05 (Positive control) vs. 0.8543 ± 0.08 (Low dose) vs. 0.7057 ± 0.06 (High dose), (p = 0.011). The LDL-C in the Low dose test group (40 mg/kg) being lower than the negative control group by 0.321 ± 0.1 (p = 0.019). The LDL-C in the high dose test group (80 mg/ kg) was also significantly lower than the negative control group by 0.323 ± 0.1 (p = 0.018). There was however no significant difference in the LDL-C in the high dose test group and in the positive control group (Atorvastatin 25 mg/Kg) 0.7057 ± 0.06 vs. 0.7071 ± 0.05 respectively, (p = 0.465).
Figure 18: The mean LDL-C of the four study groups. 

The high dose test group had a significantly higher HDL-C 0.1343 ± 0.01 (Negative control) vs. 0.1286 ± 0.03 (Positive control) vs. 0.1386 ± 0.02 (Low dose) vs. 0.3357 ± 0.07 (High dose), (p = 0.002), compared to the other groups being lower by:

- 0.201 ± 0.056 compared to the negative control group (p = 0.007)
- 0.207 ± 0.056 compared to the positive control group (p = 0.006) and
- 0.197 ± 0.056 compared to the low dose test group (p = 0.009).
Figure 19: The mean HDL-C of the four study groups.

The results showed no significance difference in the four groups in the Total cholesterol (p = 0.1145), Triglycerides (p = 0.603) and the Total Cholesterol: HDL ratio (p = 0.14) respectively.
**Effect of *Aloe secundiflora* on fasting blood glucose**

The results showed a difference in the fasting blood glucose of the four groups at the end of the study: 7.1 ± 0.21 (Negative control) vs. 7.06 ± 0.31 (Positive control) vs. 6.39 ± 0.19 (Low dose) vs. 6.35 ± 0.18 (High dose), (p = 0.033).

*Figure 20: Fasting blood glucose levels of the study groups.*
Effect of the freeze dried leaf extract on the Electrocardiogram

There were no significant changes shown on the electrocardiogram in the control groups and the test group. The following parameters were assessed:

<table>
<thead>
<tr>
<th>Electrocardiogram parameter</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR interval (ms)</td>
<td>1.302</td>
<td>.296</td>
</tr>
<tr>
<td>HR (BPM)</td>
<td>1.327</td>
<td>.290</td>
</tr>
<tr>
<td>PR (ms)</td>
<td>.591</td>
<td>.564</td>
</tr>
<tr>
<td>P duration (ms)</td>
<td>1.168</td>
<td>.333</td>
</tr>
<tr>
<td>QRS Interval (ms)</td>
<td>2.439</td>
<td>.116</td>
</tr>
<tr>
<td>QT Interval (ms)</td>
<td>.268</td>
<td>.768</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>.556</td>
<td>.583</td>
</tr>
<tr>
<td>P amplitude (μV)</td>
<td>.094</td>
<td>.911</td>
</tr>
<tr>
<td>Q amplitude (μV)</td>
<td>.132</td>
<td>.877</td>
</tr>
<tr>
<td>R amplitude (μV)</td>
<td>.402</td>
<td>.675</td>
</tr>
<tr>
<td>S amplitude (μV)</td>
<td>.216</td>
<td>.808</td>
</tr>
<tr>
<td>T amplitude (μV)</td>
<td>.053</td>
<td>.948</td>
</tr>
</tbody>
</table>

Table 5: The Electrocardiographic changes after treatment.
Effect on the weight

The weight of the Wistar rats in the four groups were not significantly different from the beginning to the end of the study 329.75 $\pm$13.5 (Negative control) vs. 334.7 $\pm$16.5 (Positive control) vs. 323 $\pm$16.7 (Low dose) vs. 327.65 $\pm$13.2 (High dose), (p = 0.958) as shown in the table below.

<table>
<thead>
<tr>
<th></th>
<th>Negative control group</th>
<th>Positive control group</th>
<th>Low dose test group</th>
<th>High dose test group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>276.5</td>
<td>213</td>
<td>345.5</td>
<td>354.5</td>
<td>.843</td>
</tr>
<tr>
<td>2.</td>
<td>259</td>
<td>384.5</td>
<td>339</td>
<td>312</td>
<td>.765</td>
</tr>
<tr>
<td>3.</td>
<td>368.5</td>
<td>369</td>
<td>358</td>
<td>304.5</td>
<td>.429</td>
</tr>
<tr>
<td>4.</td>
<td>394</td>
<td>290</td>
<td>247</td>
<td>270</td>
<td>.635</td>
</tr>
<tr>
<td>5.</td>
<td>354</td>
<td>333.5</td>
<td>377.5</td>
<td>263</td>
<td>.695</td>
</tr>
<tr>
<td>6.</td>
<td>298</td>
<td>372.5</td>
<td>359</td>
<td>345</td>
<td>.585</td>
</tr>
<tr>
<td>7.</td>
<td>356.5</td>
<td>385</td>
<td>229</td>
<td>312</td>
<td>.265</td>
</tr>
<tr>
<td>8.</td>
<td>312</td>
<td>320</td>
<td>377</td>
<td>372</td>
<td>.832</td>
</tr>
<tr>
<td>9.</td>
<td>349</td>
<td>335</td>
<td>310</td>
<td>385</td>
<td>.456</td>
</tr>
<tr>
<td>10.</td>
<td>330</td>
<td>344.5</td>
<td>288</td>
<td>358.5</td>
<td>.573</td>
</tr>
<tr>
<td><strong>Mean ± S.E.M.</strong></td>
<td><strong>329.75 ±13.5</strong></td>
<td><strong>334.7 ±16.5</strong></td>
<td><strong>323 ±16.7</strong></td>
<td><strong>327.65 ±13.2</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: The weight of the Wistar rats at the end of the study.
CHAPTER FOUR:

DISCUSSION

Hypertension control targets blood pressure regulation aspects in the short term (affecting the heart rate, contractility and vasodilatation) and long term (by altering the renal function mostly acting as diuretics or Renin angiotensin aldosterone system modifiers). The fight against cardiovascular morbidity and mortality targets modification of risk factors for cardiovascular diseases by blood pressure regulation, lipid lowering agents, and optimum blood glucose control as well as lifestyle changes. The present study investigated the effect of *Aloe secundiflora* on heart rate and contractility, blood pressure, electrocardiogram and lipid profile.

The present study showed the *Aloe secundiflora* extract had negative chronotropic and inotropic effects on cardiac muscles, decreased blood pressure and glucose levels as well as improved lipid profile by decreasing LDL-C and increasing HDL-C in Wistar rats.

Cardiac and blood pressure effects of *Aloe secundiflora* extract

The freeze dried leaves extract of *Aloe secundiflora* demonstrated negative chronotropicity and inotropicity with the dose of 20 mg causing a percentage decrease in heart rate of 6.54% and in the contractility of 21.57%. There was a significant dose response relationship giving a sigmoid dose response curve causing a 100% effect at a dose of 40 mg as shown earlier. Blood pressure is a product of cardiac output and total peripheral resistance. The cardiac output is influenced by the heart rate and stroke volume which is determined by strength of myocardial contractility. The decrease in heart rate and contractility therefore showed that the freeze dried *Aloe secundiflora* leaf extract has a blood pressure lowering effect and as such prompted further investigation into the in vivo effect on blood pressure.

These findings are different from those of Verma et. al. (2002) who reported the leaf gel extract of *Aloe barbadensis* (*Aloe vera*) increased myocardial contractility but did not change the heart
rate at doses of 200 mg/L and 300 mg/L (Verma et al. 2002). Our findings also differ from Kumar et al. (2010) who when evaluating the effects of *Aloe barbadensis* on myocardial repolarisation reported a positive chronotropic effect low dose (100 mg/L) and no heart rate changes at higher doses of 200 mg/L and 300 mg/L (Kumar et al., 2010). This shows that unlike the *Aloe barbadensis*, the compounds in *Aloe secundiflora* leaves and roots extract have negative inotropicity and chronotropicity. The negative chronotropicity and inotropicity affects the blood pressure lowering axis and hence shows a blood pressure lowering potential.

The freeze dried leaf extract of *Aloe secundiflora* experimental group had significantly lower systolic blood pressure compared to the negative control group. This demonstrated that the extract lowered systolic blood pressure chronically upon once weekly administration as seen with *Aloe barbadensis* root extract on acute administration (Saleem et al., 2001). On the contrary there were no significant acute blood pressure changes when the systolic blood pressure was observed one and a half hours after extract administration. This results show that at the doses of 40 mg/Kg and 80 mg/Kg, the extract reduced blood pressure chronically and probably the reflex response prevented the expected short term decrease in blood pressure given the negative inotropicity and chronotropicity observed in vitro. The blood pressure lowering effect can be attributed to Aloin and Aloe emodin, anthraquinones found in the leaves extract of both *Aloe barbadensis* and *Aloe secundiflora* possibly through the long term blood pressure control axis by endothelial protection by antioxidant activity as well as from the diuretic effect of the gel extract *Aloe secundiflora* leaves demonstrated by Kamau et al.(Kamau et al., 2013; Patel and Patel, 2013; Rajasekaran et al., 2005; Saleem et al., 2001).

The following electrocardiographic parameters were assessed and showed no significant variation; RR, Heart rate, PR interval, P duration, QRS interval, QT interval, QTc and amplitudes of P, Q, R, S and T. These findings mirror the results of Kumar et. al. who when evaluating the effect of *Aloe vera* leaf extract on myocardial repolarisation reported no changes in the E. C. G. parameters at low doses but prolonged QTc interval at high doses of 300 mg/kg possibly due to interference with potassium channel activity by either β-adrenergic receptors or protein kinase stimulation (Kumar et al., 2010). This shows the predicted arrhythmias due to the *Aloe secundiflora* toxicity directly or via hypokalemia was not evident at the doses used for this study (Kamau et al., 2013).
**Aloe secundiflora** extract effects on blood glucose and lipid profile

*Aloe secundiflora* leaves extract showed a significant decrease in the LDL-C at the low dose (40 mg/Kg) and high dose (80 mg/Kg) of 0.321 ± 0.1 and 0.323 ± 0.1 compared to the negative control respectively. The decrease in LDL-C was not significantly different with that of the positive control group (Atorvastatin 25 mg/Kg).

The high dose test group (80 mg/Kg) had a significantly higher HDL-C than the negative and positive control by 0.201 ± 0.056 and 0.207 ± 0.06 respectively. The extract did not cause any significant changes in the total cholesterol, triglycerides and the total cholesterol/HDL-C ratio. This was different from the effect observed on *Aloe debrana* extract on streptozotocin induced diabetic rats at 300 mg/Kg where there was a decrease in total cholesterol, LDL-C, triglycerides and increased HDL-C (Suleyman et al., 2015). There was no significant difference in the weight changes of the Wistar rats in the four groups during the study duration whereas weight gain was observed in diabetic wasted rats on *Aloe debrana* extract at 300 mg/kg dose (Suleyman et al., 2015). The difference observed may be due to the use of non diabetic rats in this study while Suleyman et al. 2015 used diabetic rats.

The *Aloe secundiflora* leaves extract reduced the ‘bad cholesterol’ (LDL-C) and increased the ‘good cholesterol’ (HDL-C) which is the cardio protective effect targeted by most lipid lowering drugs used as adjuncts in management of cardiovascular diseases. The decrease in LDL-C and increase in HDL-C is comparable to the antihyperlipidaemic effect of *Aloe debrana* extract on streptozotocin induced diabetic rats (Suleyman et al., 2015). The antihyperlipidaemic effects of *Aloe secundiflora* may be due to the polysaccharides that increase insulin levels hence decreasing lipolysis and reducing serum lipids (Suleyman et al., 2015).

The significantly lower fasting blood glucose in the test groups reflect that *Aloe secundiflora* may potentially have similar hypoglycaemic effect of *Aloe vera* that has been attributed to the sterols and polysaccharides in the leaves extract which increase insulin levels as well as increased peripheral glucose utilization hence the hypoglycemic effect observed clinically and experimentally (Suleyman et al., 2015).
Antioxidant properties described in anthraquinones of Aloe species could be responsible for the improved lipid profile, blood glucose lowering and blood pressure lowering effects observed from the *Aloe secundiflora* freeze dried leaves extract (Rajasekaran et al., 2005). Endothelial damage has been implicated in development of cardiovascular diseases. The antioxidant properties of the extract could be playing a role in endothelial protection hence the improve blood pressure (Kispotta et al., 2012; López et al., 2013; Rajasekaran et al., 2005).

**STUDY LIMITATIONS**

The prolonged technically demanding process of taking blood pressure made it difficult to take all the blood pressure measurement at the exact same time for all the rats in each group. The diastolic blood pressure was not recordable in our experimental set up hence the effects of the *Aloe secundiflora* extract on diastolic blood pressure was not assessed. Some study rats died during anaesthesia, Electrocardiography and phlebotomy hence weekly Electrocardiograms and lipid profile changes were not assessed in all animals.

**CONCLUSION AND RECOMMENDATIONS**

The freeze dried leaf extract of *Aloe secundiflora* demonstrated blood pressure lowering effects possibly by the negative chronotropic and inotropic effects or diuretic effects without adverse electrocardiographic effects. The extract also lowered blood glucose and improved lipid profile by raising HDL-C and lowering LDL-C. These were observed with once weekly administration.

The weekly administration effects seen in normotensive rats shows there is a potential of using the extract for prophylaxis either as a drug or as nutritional supplement in the prevention of hypertension and for cardioprotection in laboratory Wistar rats. We recommend improved efforts in conservation of this medicinal plant and further research into the active components of the *Aloe secundiflora* extract and their potential use in human studies on hypertension, cardiovascular diseases and the non communicable realm of diseases that are on an exponential rise.
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