

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

HYPOTENSIVE EFFECTS OF AQUEOUS EXTRACT OF *Moringa stenopetala* IN BOTH *in vivo* AND *in vitro* ANIMAL MODELS

Mekoya Mengistu

*A Thesis Submitted to the School of Graduate Studies of Addis Ababa University
in Partial Fulfillment of the Requirements for the Degree of Master of Science in
Medical Physiology*

August 2007

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Approved by the examining board:

Dr. Andualem Mossie
External examiner

Dr. Tewabech Zewdie
Internal examiner

Professor Yekoye Abebe
Principal advisor

Dr. Yalemtehay Mekonnen
Co-advisor

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other University and that all sources of material used for the thesis have been duly acknowledged.

M.Sc candidate: Mekoya Mengistu

Signature: _____

Date: _____

Supervisors: Prof. Yekoye Abebe
Principal Advisor

Dr. Yalemtehay Mekonnen
Co-Advisor

Signature: _____

Date: _____

Addis Ababa, Ethiopia

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LIST OF ABBREVIATIONS

AME	Apparent Mineralocorticoid Excess
AngII	Angiotensin II
AT1r	Angiotensin type I receptors
AU	African Union
BMI	Body Mass Index
BP	Blood Pressure
Ca (cyt)	Cytosolic Calcium
CAMH	The Conference of African Union Ministers of Health
CIPHIH	Commission on Intellectual Property, Innovation and Public Health
DBP	Diastolic Blood Pressure
ENaC	Epithelial Sodium Channel
ET	Endothelin
ETA	Endothelin Receptor A
ETB	Endothelin Receptor B
GRA	Glucocorticoid-Remediable Aldosteronism
<i>HF</i>	<i>Heart Failure</i>
<i>HR</i>	<i>Heart Rate</i>
i.p.	Intraperitoneal
iv	Intravenous
JNC	Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood pressure
<i>LVH</i>	<i>Left Ventricular Hypertrophy</i>
MABP	Mean Arterial Blood Pressure
MR	Mineralocorticoid Receptors
NAP	Natriuretic Peptide
NCX	Sodium–Calcium Exchanger
<i>NO</i>	<i>Nitric Oxide</i>
p. o.	Per os
PRA	Plasma Renin Activity

<i>PVR</i>	<i>Peripheral Vascular Resistance</i>
RAAS	Renin-Angiotensin- Aldosterone system
ROS	Reactive Oxygen Species
SA	Serum Aldosterone
SA-PRA	Serum Aldosterone - Plasma Renin Activity
SBP	Sytolic Blood Pressure
SNS	Sympathetic Nervous System
TGF- β	Transforming Growth Factor- β
WHO	World Health Organization
β HSD	Beta Hydroxysteroid Dehydrogenase

Abstract

Hypertension is sustained elevation of arterial blood pressure. It is the major cardiovascular risk factor contributing to myocardial infarction, cerebrovascular accidents, congestive heart failure, peripheral vascular insufficiency and premature mortality. Different medicinal plants have been used to treat hypertension. One such plant is *Moringa stenopetala*, a tree whose leaves, flowers, and fruits are used as vegetable in some areas of Southern Ethiopia. Different parts of *M. stenopetala* are also used to cure various diseases including hypertension. In this study, the *in vivo* and *in vitro* hypotensive properties of aqueous leaf extracts of *Moringa stenopetala* have been assessed on selected animal model. The crude aqueous leaf extract of *M. stenopetala* caused significant fall in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) at the doses of 10, 20, 30 and 40 mg/kg in normotensive anaesthetized guinea pigs (n = 12). The percent fall in SBP was $25.42 \pm 4.3\%$ (p = 0.00), $33.3 \pm 3.9\%$ (p = 0.00), $41.27 \pm 3.7\%$ (p = 0.00), and $51.73 \pm 3.9\%$ (p = 0.00) at the respective doses of 10, 20, 30 and 40 mg/kg. At the same dose the percent fall in DBP was $27.76 \pm 3.1\%$ (p = 0.00), $38.38 \pm 3.7\%$ (p = 0.00), $52.51 \pm 4.1\%$ (p = 0.00) and $59.91 \pm 3.7\%$ (p = 0.00). The percent fall in MABP was also $26.74 \pm 3.6\%$ (p = 0.00), $36.2 \pm 3.5\%$ (p = 0.00), $47.62 \pm 3.6\%$ (p = 0.00) and $56.39 \pm 3.5\%$ (p = 0.00) at the above respective doses. However, significant percent fall in pulse pressure was observed only at the cumulative dose of 40 mg/kg and the fall was $33.86 \pm 8.9\%$ (p = 0.01). The hypotensive effect might have been mediated by non-autonomic nervous system as its effect is not altered by atropine, which is muscarinic receptor blocker of acetylcholine, and propranolol, which is non-selective blocker of β -adrennergic receptors. The *in vitro* hypotensive study of *M. stenopetala* was carried out on isolated guinea pig aorta. High- K^+ (80mM) induced sustained contraction of the aortic tissue preparation. However, the aqueous leaf extract of *M. stenopetala* caused dose and time dependent inhibition (vasodilation) of the induced contraction. The significant inhibition of high K^+ -induced contraction was $36.55 \pm 8.64\%$ (p = 0.001), $67.76 \pm 9.55\%$ (p = 0.00), and $95.56 \pm 3.14\%$ (p = 0.00) at respective doses of 5, 6 and 7 mg/ml (n = 10 and data are expressed as M \pm SEM). Thus, the *in vivo* and *in vitro* studies justify traditional use of the leaves of *M. stenopetala* as antihypertensive agent by some localities of Southern Nations Nationalities and Peoples, Ethiopia. Furthermore, acute toxicity study of *M. stenopetala* showed that the extract was tolerable in mice when tested up to the oral dose of 10 g/kg with

no mortality and behavioral changes. This also signifies that the leaves of the plant can be a safe source of food.

Key words: Hypertention; *Moringa stenopetala*; Aqueous extract; *in vitro*; *in vivo*; Blood pressure; Acute toxicity; Phytochemical Screening

1. Literature Review

1.1 Clinical Background of Hypertension

Hypertension is a sustained elevation of the systemic arterial pressure. It is one of the major cardiovascular risk factors contributing to myocardial infarction, cerebrovascular accidents, end-stage renal disease, congestive heart failure, peripheral vascular insufficiency and premature mortality (Lifton *et al.*, 2001). In general, it is an important public health challenge in developed as well as developing countries (Rudd and Dzau, 1996, Chuang *et al.*, 2006). Blood pressure, like most physiologic variables, exhibits a bell-shaped distribution with no simple cut-off to demarcate “safe” from “dangerous” levels of blood pressure elevation (Rudd and Dzau, 1996).

Blood pressure can be categorized as optimal, normal, high normal and hypertensive. Optimal pressure is below 120/80 mmHg whereas normal is between 120/80 - 130/85 mmHg. High normal is considered to be between 130/85-139/89 mmHg whereas hypertensive is over 140/90 mmHg on repeated measurement and/or treatment with medication (WHO, 2001; Aberra, 2003; Jones *et al.*, 2004). Similar classification of hypertension according to WHO - European Society of Hypertension (2003) is: optimal (<120/80), normal (120-129/80-84), high normal (130-139/83-89), Grade 1 hypertension (mild, 140-159/90-99), Grade 2 hypertension (moderate, 160-179/100-109), and Grade 3 hypertension (severe, 180/110) (Zacharia *et al.*, 2003; Jones *et al.*, 2004; Sison, 2004). Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI) categorized as Optimum (<120/80), normal (<130/85), high normal (130 to 139/85 to 89), Hypertension as; stage 1 (140 to 159/90 to 99), stage 2 (160 to 179/100 to 109), stage 3 (\geq 180/110) (Padwal *et al.*, 2001).

Hypertension mediates most of its adverse effects via acceleration of atherosclerosis and vascular remodeling, especially in large to medium-size blood vessels. As such, hypertension most properly fits as a risk factor, among many others, rather than as a disease itself (Rudd and Dzau, 1996). Hypertension often keeps close company with a multitude of other cardiovascular risk factors including diabetes mellitus, dyslipidemia, hyperinsulinemia, and exogenous obesity (Rudd and Dzau, 1996; Hall *et al.*, 1999).

1.2 Types of Hypertension

1.2.1 Essential hypertension

Essential, or primary, or idiopathic hypertension is defined as high blood pressure (BP) in which secondary causes such as renovascular disease, renal failure, pheochromocytoma, hyperaldosteronism, or other causes of secondary hypertension are not present and accounts for 90 to 95% of all cases of hypertension (Carretero and Oparil, 2000). It tends to cluster in families and represents a collection of genetically based diseases or syndromes with several resultant inherited biochemical abnormalities and the resulting phenotypes can be modulated by various environmental factors, thereby altering the severity of blood pressure elevation and the timing of hypertension onset (Oparil *et al.*, 2003). It is referred to as essential, or primary, because the specific causes of hypertension are unknown. This is the common type, and can not be permanently cured. However, the level of BP can be substantially reduced by drugs and non pharmacological measurements such as sharp reduction of obesity (where present) and reduction of dietary salt (Chaudhuri, 2001; Aberra, 2003).

1.2.2 Secondary hypertension

Secondary hypertension is elevated BP that results from an underlying, identifiable, often correctable causes and is treatable or reversible. Only about 5 to 10% of hypertension cases are thought to result from secondary causes (Onusko, 2003). Kidney diseases are the most common causes particularly in older people. Other clinical conditions such as cushing's syndrome, hyperaldosteronism, pheochromocytoma, congenital adrenal hyperplasia, excess erythropoietin, and coarctation of aorta are also causative agents (Chaudhuri, 2001; Aberra, 2003). Certain medications such as cortisone, prednisone, estrogens and non steroidal anti-inflammatory drugs are also known to cause secondary hypertension (Chaudhuri, 2001; Aberra, 2003; Onusko, 2003).

1.3 Signs and Symptoms of Hypertension

Majority of patients with hypertension have no symptoms referable to their blood pressure elevation and are identified only in the course of examination (Braunwald *et al.*, 2005). The symptoms of hypertension can be due to elevated blood pressure, hypertensive vascular disease, and underlying disease of secondary hypertension (Braunwald *et al.*, 2005). Elevated blood pressure is associated with headache, dizziness, palpitations, and easy fatigability (Zampaglione *et al.*, 1996, Braunwald *et al.*, 2005). Symptoms due to vascular disease include epistaxis, hematuria, blurring of vision owing to retinal changes, episodes of weakness due to transient cerebral ischemia, angina pectoris, and dyspnea due to cardiac failure (Braunwald *et al.*, 2005). Furthermore, symptoms underlying disease in secondary hypertension can be polyuria, polydipsia, and muscular weakness secondary to hypokalemia in patients with primary aldosteronism, or weight gain and emotional lability in patients with cushing's syndrome (Braunwald *et al.*, 2005; Labinson *et al.*, 2006).

1.4 Prevalence of Hypertension

In developing countries, there is a rapid increase in hypertension prevalence, and in developed countries, the previous trend of an increase in hypertension prevalence is actually reversing (Das *et al.*, 2005; Hajjar *et al.*, 2006). The overall worldwide burden of hypertension in the year 2000 was estimated to be 972 million, 26.4% of the adult world population, with 333 million or 34.26% in developed and 639 million or 65.73% in developing countries (Hajjar *et al.*, 2006). It has been estimated that by the year 2025, 1.56 billion will have hypertension, an increase of 60% from the year 2000 (Hajjar *et al.*, 2006). As further stated by Tesfaye *et al.* (2007), globally high blood pressure is estimated to cause 7.1 million deaths, which is about 13% of the total cases.

The prevalence and hospitalization rates of heart failure, wherein the majority of patients have hypertension before developing heart failure, have continued to increase. Thus, hypertension is second only to diabetes as the most common antecedent for this condition (Chobanian *et al.*, 2003). The prevalence of hypertension increases with advancing age to the point where

more than half of people aged 60 to 69 years old and approximately three-fourths of those aged 70 years and older are affected (Chobanian *et al.*, 2003). The age-related rise in systolic blood pressure (SBP) is primarily responsible for an increase in both incidence and prevalence of hypertension. Framingham Heart Study Investigation recently reported the lifetime risk (long term risk) of hypertension to be approximately 90% for men and women who were nonhypertensive at 55 or 65 years of age and survived to age 80 to 85 (Jones *et al.*, 2004). The prevalence of systolic hypertension increases with age, and above the age of 50 years, systolic hypertension represents the most common form of hypertension. Diastolic blood pressure (DBP) is a more potent cardiovascular risk factor than SBP until age 50; thereafter, SBP is more important (Chobanian *et al.*, 2003).

1.4.1. Prevalence of hypertension in Ethiopia

There are few reports on the prevalence of hypertension in Ethiopia. According to the health and health-related indicators of MOH (2000–2001), hypertension was the seventh leading cause of death in the country in 2001 (WHO, 2004). The prevalence of hypertension amongst bank employees in Addis Ababa was 11% with 13% in males and 5% in females (Teklu, 1983). This was lower prevalence than Zulus of South Africa and American blacks (Teklu, 1983). A study on the hypertension prevalence and age-related changes in blood pressure in semi-nomadic and urban Oromos showed prevalence of 0.40% in the semi-nomadic and 3.15% in the urban population (Pauletto *et al.*, 1994) (according to the old WHO criterion of hypertension at SBP/DBP \geq 160/95). As studied by Parry (1968), 16% of patients with cardiovascular disease seen at Tuberculosis Center in Addis Ababa were hypertensive (according to the old WHO criterion of hypertension as SBP/DBP \geq 160/95). Lester (1973) also noted that 11.6% of Ethiopian outpatients were hypertensive (according to the old WHO criterion of hypertension at SBP/DBP \geq 160/95).

1.5 Pathogenesis of Hypertension

Many pathophysiologic factors have been implicated in the genesis of hypertension. Major factors are:

1.5.1 Molecular mechanisms of hypertension

Epidemiological (or classical genetics) studies have indicated that BP is a multifactorial trait with about 30% to 60% of the phenotypic variation being attributed to genetic factors (Kato, 2002). Evidence for genetic influence on blood pressure comes from various sources. Twin studies documented greater concordance of blood pressure in monozygotic than dizygotic twins, and population studies showed greater similarity in blood pressure within families than between families (Lifton *et al.*, 2001). The latter observation is not attributable to only a shared environment since adoption studies demonstrate greater concordance of blood pressure among biological siblings than adoptive siblings living in the same household (Carretero and Opari, 2000; Lifton *et al.*, 2001; Ausiello *et al.*, 2003). Furthermore, single genes can have major effects on blood pressure, accounting for the rare Mendelian forms of high and low blood pressure. Although identifiable single-gene mutations account for only a small percentage of hypertension cases, study of these rare disorders may elucidate pathophysiologic mechanisms that predispose to hypertension and may suggest novel therapeutic approaches (Ausiello *et al.*, 2003).

The genetic mutations responsible for rare forms of Mendelian (monogenic) hypertensive syndromes such as glucocorticoid-remediable aldosteronism (GRA), Liddle's syndrome, and apparent mineralocorticoid excess have been identified, whereas, in autosomal dominant hypertension with brachydactyly, the gene is not yet identified but has been mapped to chromosome 12 (12p) (Carretero and Opari, 2000; Lifton *et al.*, 2001).

1.5.1.1 Glucocorticoid-remediable aldosteronism

This is an autosomal dominant form of monogenic hypertension in which aldosterone secretion is regulated by adrenocorticotrophic hormone but not by angiotensin II (Ang. II) (Carretero and Opari, 2000). It is featured by early onset of hypertension with normal or elevated aldosterone levels despite suppressed plasma renin activity (Lifton *et al.*, 2001). Glucocorticoid treatment causes BP to decrease and gives the syndrome its name, and most of the patients first described as having GRA showed severe hypertension and died prematurely from stroke (Carretero and Opari, 2000). Hypokalemia and metabolic alkalosis are variable associated findings. However, the hallmark of this disease is that exogenous glucocorticoids completely suppress aldosterone secretion (Lifton *et al.*, 2001). GRA is caused by a gene

duplication arising by unequal crossing over between two closely related genes involved in adrenal steroid biosynthesis. These genes encode aldosterone synthase (the rate limiting enzyme for aldosterone biosynthesis in adrenal glomerulosa) and steroid 11 β -hydroxylase, an enzyme involved in cortisol biosynthesis in the adrenal fasciculata whose expression is regulated by ACTH (Carretero and Opari, 2000; Oparil *et al.*, 2003). The resulting chimeric gene encodes a protein with aldosterone synthase enzymatic activity whose expression is regulated by ACTH. Aldosterone synthase activity is thus ectopically expressed in the adrenal fasciculata under control of ACTH rather than AngII, the normal hormonal regulator. As a consequence, aldosterone secretion becomes inexorably linked to cortisol secretion, and maintenance of normal cortisol levels results in constitutive aldosterone secretion, which leads to expanded plasma volume and hypertension (Lifton *et al.*, 2001; Oparil *et al.*, 2003). The expanded plasma volume suppresses secretion of renin, but this fails to diminish secretion of aldosterone (Lifton *et al.*, 2001).

1.5.1.2 Liddle's syndrome

This is an autosomal dominant form of monogenic hypertension that results from mutations in the amiloride-sensitive epithelial sodium channel leading to increased channel activity (Carretero and Opari, 2000; Lifton *et al.*, 2001; Oparil *et al.*, 2003). The mutations reported to date result in the elimination of 45 to 75 amino acids from the cytoplasmic carboxyl terminus of β - or γ -subunits of the channel. Thus, Liddle's syndrome is genetically heterogeneous (Carretero and Opari, 2000; Oparil *et al.*, 2003). These mutations result in increased epithelial sodium channel (ENaC) activity, largely attributable to an increase in the number of channels at the cell surface and hence, increased channel activity. The increased number of channels is due to reduced clearance of ENaC from the cell surface with dramatically longer half-life leading to increase in net renal salt balance sufficient to produce hypertension in humans (Lifton *et al.*, 2001). It is characterized by the early onset of hypertension with hypokalemia and suppression of both plasma renin activity and aldosterone, the latter differentiating this syndrome from primary aldosteronism (Oparil *et al.*, 2003).

1.5.1.3 Apparent mineralocorticoid excess (AME)

There are several diseases in which steroids other than aldosterone activate mineralo-

corticoid receptors. One of these is the syndrome of AME (Lifton *et al.*, 2001). This is an autosomal recessive form of monogenic juvenile hypertension that results from a mutation in the renal-specific isoform 11 β -hydroxysteroid dehydrogenase (11 β HSD) gene (Carretero and Opari, 2000). Cortisol circulates at concentrations up to 1000-fold higher than aldosterone (Freel and Connell, 2004). However, almost all mineralocorticoid receptor (MR) activations are mediated by aldosterone. This specificity of MR for aldosterone is mediated indirectly, with 11 β HSD “protecting” MR from cortisol by metabolizing it to cortisone, which has no affinity for the MR (Lifton *et al.*, 2001; Freel and Connell, 2004). Thus, in AME, the enzymatic deficiency allows the mineralocorticoid receptors in the nephron to be occupied and activated by cortisol, causing sodium and water retention, low renin, low aldosterone, and more importantly, a salt-sensitive form of hypertension mediated by increased ENaC activity (Carretero and Opari, 2000; Lifton *et al.*, 2001; Luft, 2001; Freel and Connell, 2004).

1.5.1.4 Autosomal dominant hypertension with brachydactyly

In this monogenic syndrome, hypertension and brachydactyly are always inherited together (100% cosegregation) (Carretero and Opari, 2000; Luft, 2001). The affected persons are shorter than non-affected relatives and with abnormal skeletal development in the hand and wrist. The gene for hypertension in this disorder has been mapped to the short arm of chromosome 12 (12p) in a large Turkish kindred (Carretero and Opari, 2000; Lifton *et al.*, 2001). Unlike the other three autosomal forms of hypertension, BP is not affected by volume expansion and the underlying mechanism is not known. Thus identification of the gene responsible may help clarify some of the genetic alterations in essential hypertension (Carretero and Opari, 2000).

Polymorphisms and mutations in other genes such as angiotensin-converting enzyme, β_2 -adrenergic receptor, α -adducin, angiotensinase C, renin-binding protein, G-protein β_3 -subunit, atrial natriuretic factor, and the insulin receptor have also been linked to the development of essential hypertension (Carretero and Opari, 2000)

1.5.2 Renin-Angiotensin-Aldosterone system

Under normal conditions, the increased renin secretion is quickly corrected by negative feedback mechanisms that tend to suppress renin release and bring circulating renin level back

to normal level (Carretero *et al.*, 1971; Cotran *et al.*, 1999). However, patients with renal hypertension and approximately 15% of patients with essential hypertension have elevated plasma renin level (Carretero *et al.*, 1971; Cotran, *et al.*, 1999; Braunwald *et al.*, 2005). Therefore, increased angiotensin formation caused by an increase in renin activity can be postulated to be a contributory factor in the development and maintenance of renal hypertension (Carretero *et al.*, 1971). Ang. II increases blood pressure by various mechanisms, including constricting resistance vessels, stimulating aldosterone synthesis and release and renal tubular sodium reabsorption, stimulating thirst and release of antidiuretic hormone, and enhancing sympathetic outflow from the brain (Oparil *et al.*, 2003).

In accelerated or malignant hypertension, irrespective of its etiology, the renin and angiotensin levels in plasma are consistently elevated, suggesting that the renin-angiotensin-aldosterone system (RAAS) mediates the accelerated increase in blood pressure (Carretero *et al.*, 1971). Ang. II also amplifies the response to sympathetic stimulation by a peripheral mechanism, that is, presynaptic facilitatory modulation of norepinephrine release (Oparil *et al.*, 2003). Furthermore, Ang. II induces cardiac and vascular cell hypertrophy and hyperplasia directly by activating the angiotensin II type 1 (AT₁) receptor and indirectly by stimulating release of several growth factors and cytokines. Activation of the AT₁ receptor stimulates various tyrosine kinases, which in turn phosphorylate the tyrosine residues in several proteins, leading to vasoconstriction, cell growth, and cell proliferation (Oparil *et al.*, 2003). There is local production of Ang. II in various tissues, including the blood vessels, heart, adrenals, and brain. The activity of local RAAS and alternative pathways of ang. II formation may make an important contribution to remodeling of resistance vessels and the development of target organ damage (including left ventricular hypertrophy, congestive heart failure, atherosclerosis, stroke, end-stage renal disease, myocardial infarction, and arterial aneurysm) in hypertensive persons (Oparil *et al.*, 2003).

1.5.3. Sympathetic activation and hypertension

Evidence drawn from a number of sources, utilizing both electrophysiologic and neurochemical techniques, provides compelling evidence that overactivity of the sympathetic nervous system is commonly present in patients with essential hypertension (Mancia *et al.*, 1999; Esler, 2000). This increased sympathetic nervous system activity increases blood

pressure and contributes to the development and maintenance of hypertension through stimulation of the heart, peripheral vasculature, and kidneys, causing increased cardiac output, vascular resistance, and fluid retention (Ausiello *et al.*, 2003). In addition, autonomic imbalance (increased sympathetic tone accompanied by reduced parasympathetic tone) has been associated with many metabolic, hemodynamic, trophic, and rheologic abnormalities that result in increased cardiovascular morbidity and mortality (Ausiello *et al.*, 2003).

Although the mechanisms responsible for the sympathetic activation occurring in essential hypertension have not been conclusively determined, genetic influences are evident, obesity, and behavioral and lifestyle factors appear to be involved (Mancia *et al.*, 1999; Esler, 2000; Ausiello *et al.*, 2003). The heritability of sympathetic overactivity in primary human hypertension has been little studied (Esler, 2000). However, in monozygotic twins, sympathetic nerve firing rates of skeletal muscle blood vessel were found to be almost identical in individual pairs, unlike in randomly paired groupings of unrelated subjects in whom a wide range of nerve firing rates was evident. Similarly, twin studies investigating the heritability of plasma norepinephrine concentrations have attributed approximately 50% of the variance to genetic factors (Esler, 2000). Normotensive young men with a family history of hypertension do have higher rates of norepinephrine spillover to plasma than young men with a negative family history of hypertension (Esler, 2000). Exposure to stress increases sympathetic outflow, and repeated stress-induced vasoconstriction may result in vascular hypertrophy, leading to progressive increases in peripheral resistance and blood pressure. This could partly explain the greater incidence of hypertension in lower socioeconomic groups, since they must endure greater levels of stress associated with daily living (Ausiello *et al.*, 2003).

1.5.4. Obesity and hypertension

Overweight is defined as a BMI $> 25\text{kg/m}^2$ and obesity as a BMI $> 30\text{kg/m}^2$ (Aneja *et al.*, 2004). Obesity, and especially abdominal obesity, is the main hypertensinogenic factor (Carretero and Oparil, 2000). It was estimated that each 10% weight gain is associated with a 6.5 mm Hg increase in systolic BP (Carretero and Oparil, 2000). Data from the Framingham Heart Study shows that approximately 78% of essential hypertension in men and approximately 65% of essential hypertension in women can be directly attributed to obesity

(Smith *et al.*, 2006). Several central and peripheral abnormalities that can explain the development or maintenance of hypertension in obesity have been identified (Rahmouni *et al.*, 2005). These factors include:

1.5.4.1 Overactivation of the sympathetic nervous system

Overactivity of the sympathetic nervous system is a common feature of obesity in humans and in animal models and recent works have highlighted the key role of increased sympathetic activity in obesity-hypertension (Aneja *et al.*, 2004; Rahmouni *et al.*, 2005; Narkiewicz, 2006). Long-term sympathoactivation could raise arterial pressure by causing peripheral vasoconstriction and by increasing renal tubular sodium reabsorption (Rahmouni *et al.*, 2005).

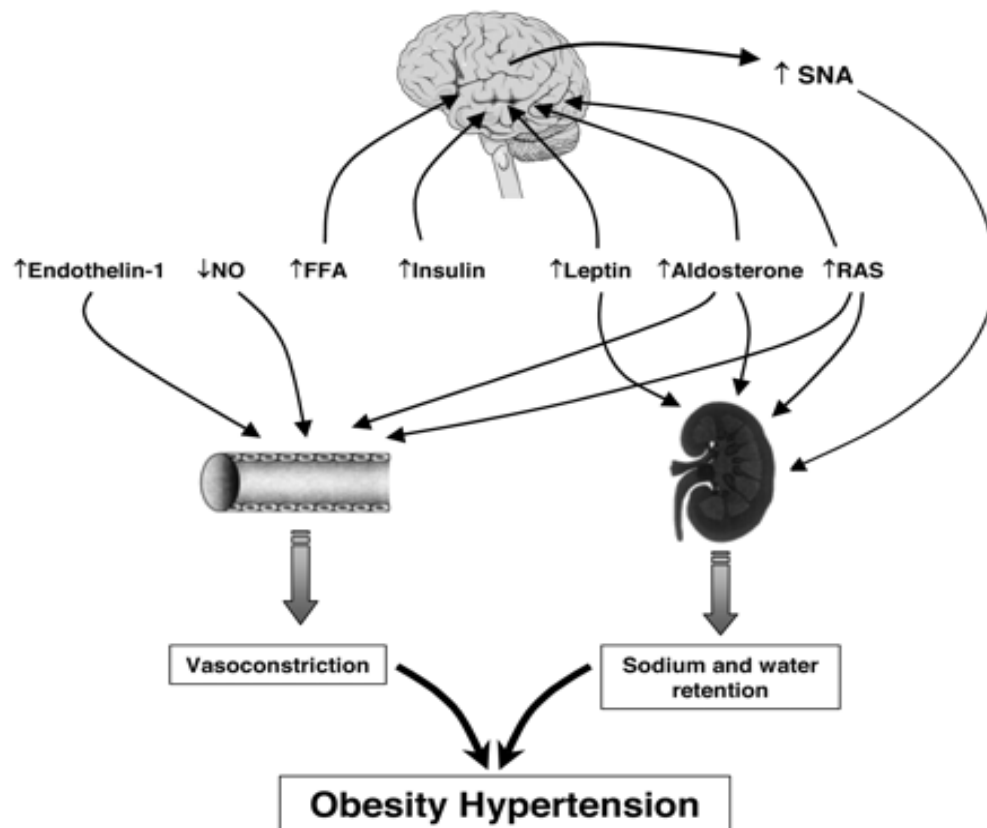


Fig. 1.1 Summary of mechanisms and hormonal systems involved in obesity-associated hypertension. FFA indicates free fatty acids; SNA, sympathetic nerve activity (Adopted from Rahmouni *et al.*, 2005).

Recent evidence indicates that leptin may represent a link between excess adiposity and increased cardiovascular sympathetic activity. Besides its effect on appetite and metabolism,

leptin acts in the hypothalamus to increase blood pressure through activation of the sympathetic nervous system (Rahmouni *et al.*, 2005). Hyperinsulinemia may also play a role in overactivity of the sympathetic nervous system associated with obesity (Hall *et al.*, 1999; Aneja *et al.*, 2004; Narkiewicz, 2006). In rats, insulin, like leptin, causes sympathoactivation to different tissues, including the kidney (Rahmouni *et al.*, 2005). Imazu *et al.* (2001) described the presence of hyperinsulinemia in the majority of individuals with hypertension. There is also high circulating level of free fatty acids in obese subjects and it appears to participate in activation of the sympathetic nervous system. The increased release of free fatty acids into the portal vein from lipolysis in visceral fat depots with increasing amounts of fat could therefore explain the strong association between visceral obesity and increased sympathetic nerve outflow and hypertension (Aneja *et al.*, 2004; Rahmouni *et al.*, 2005).

1.5.4.2 Activation of the Renin-Angiotensin- Aldosterone system in obesity

There is much evidence in support of an activation of the renin-angiotensin-aldosterone system (RAAS) in obesity (Aneja *et al.*, 2004; Rahmouni *et al.*, 2005; Narkiewicz, 2006). There is direct relationship between plasma renin activity (PRA), plasma angiotensin-converting enzyme (ACE) and plasma Ang. II levels with BMI in humans (Aneja *et al.*, 2004).

1.5.4.3 Obesity associated with endothelial dysfunction and renal function

Nitric oxide (NO) production by endothelial cells in the obese is impaired due to endothelial dysfunction (Aneja *et al.*, 2004). The mechanisms responsible for such endothelial dysfunction in obesity are not entirely clear. However, endothelin-mediated forearm vasoconstrictor tone is elevated and blockade of ET_A receptors restores endothelium-dependent vasodilatation in obese individuals to levels observed in lean controls (Davy and Hall, 2004). There is also increased prevalence of chronic renal disease in obese people, and this is not surprising as two of the most common causes of chronic renal failure, diabetes and hypertension, are closely associated with obesity (Hall *et al.*, 1999; Davy and Hall, 2004).

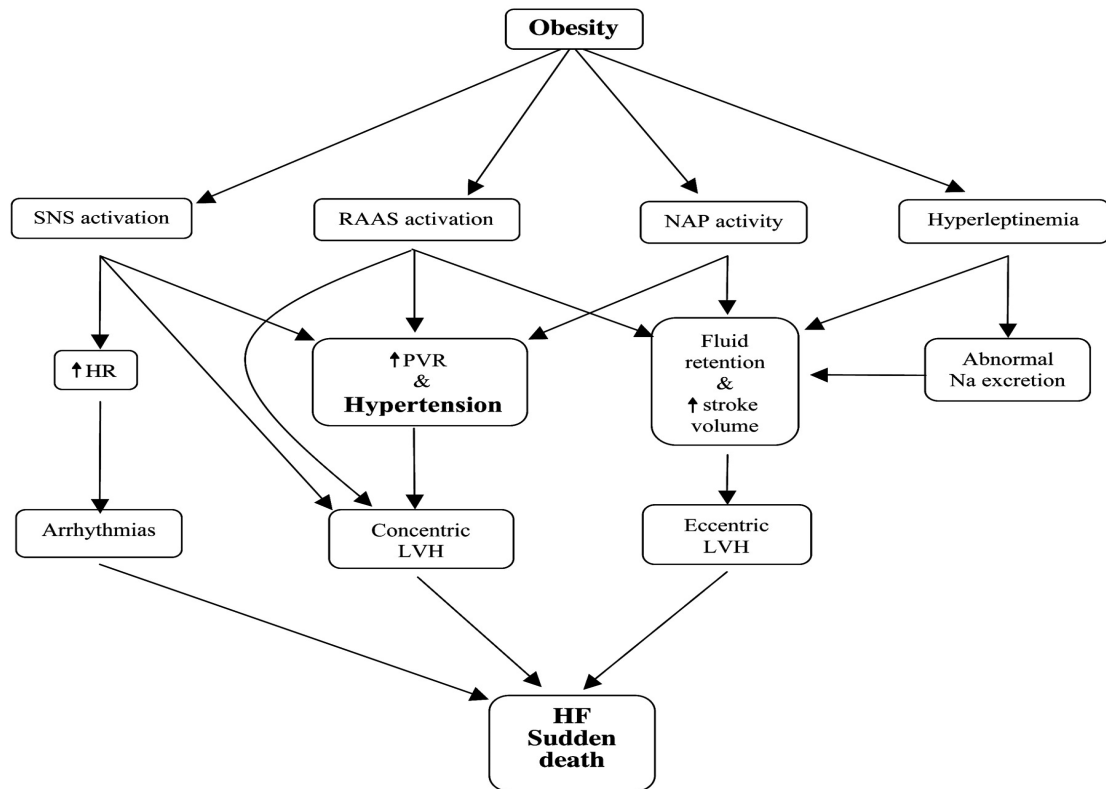


Fig.1.2 Summary of the mechanisms by which obesity may lead to excessive cardiovascular morbidity and mortality (Adopted from Aneja *et al.*, 2004).

1.5.5 Salt loading and hypertension

Epidemiological, migration, intervention, and genetic studies in humans and animals provide very strong evidence of a causal link between high salt intake and hypertension (Meneton *et al.*, 2003; Farquhar *et al.*, 2005). Yet the mechanism by which dietary salt increases arterial pressure are not fully understood, but seems related to the inability of the kidneys to excrete large amounts of salt (Meneton *et al.*, 2003; Iwamoto, 2005). Indeed, renal cross-transplantation studies between hereditary hypertensive and normotensive rats show that the abnormal kidney is ultimately responsible for the rise in arterial blood pressure. Consistently, when terminal nephrosclerosis patients with hypertension are transplanted with a kidney from a normotensive donor, the blood pressure drops to the normal range (Iwamoto, 2005). As pointed out by Farquhar *et al.* (2005), both neural and hormonal responses may also contribute to the sodium-induced change in BP, besides kidney playing a prominent role in long-term pressure homeostasis.

The pathophysiology of salt-driven hypertension was formulated more than 30 years ago, and the present day information on the mechanisms involved are shown in fig. 1.3. The side-by-side comparison illustrates how the theoretical construct of Guyton and co-workers has served as the framework upon which subsequent investigations have built today's understanding of salt-sensitive hypertension (Rodriguez-Iturbe and Vaziri, 2007).

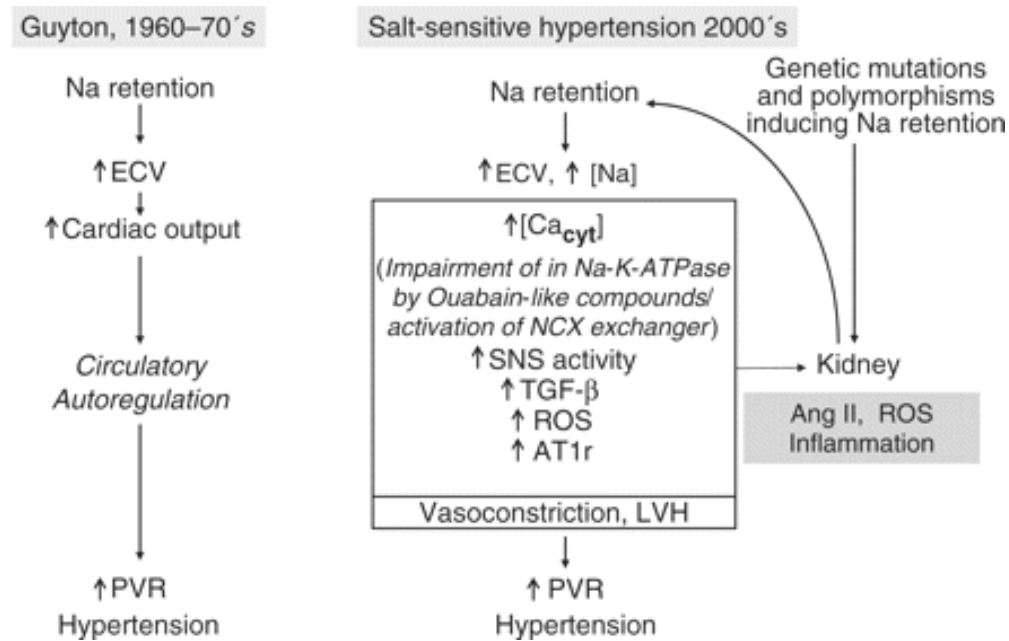


Fig 1.3 Aetiopathogenesis of salt-sensitive hypertension (Adopted from Rodriguez-Iturbe and Vaziri, 2007).

1.5.6 Primary hyperaldosteronism

There is growing awareness of primary hyperaldosteronism as a cause of secondary hypertension and manifests as hypertension and hypokalemia, or as resistant hypertension (Labinson *et al.*, 2006). Furthermore, it is described as a secondary, endocrine-mediated form of hypertension defined by an autonomous aldosterone overproduction, which is caused in most cases by adrenocortical adenoma or bilateral adrenal hyperplasia (Freel and Connell, 2004; Strauch *et al.*, 2006). It is also defined by Onusko (2003) as overproduction of aldosterone independent of its usual regulator, the RAAS. The resulting aldosteron induced retention of excess salt and water causing increased blood volume which is responsible for

the development of hypertension and is characterized by suppression of renin levels due to increased body fluid (as opposed to elevating renin levels, which causes secondary hyperaldosteronism) (Cotran, *et al.*, 1999; Onusko, 2003; Labinson *et al.*, 2006). As stated by Freel and Connell (2004), the prevalence of primary hyperaldosteronism in unselected individuals with hypertension to be between 5 and 15%. However, recent studies have demonstrated that primary aldosteronism is the most common form of secondary hypertension when determinations of serum aldosterone (SA), plasma renin activity (PRA), and the SA-PRA ratio are used as screening tools and the fludrocortisone, saline infusion, or captopril tests are used to confirm the diagnosis (Mosso *et al.*, 2003).

1.5.7 Coarctation of aorta

Coarctation of aorta is one of the most common cardiovascular causes of secondary hypertension (Manikoth *et al.*, 2005; Cay *et al.*, 2006). It is a congenital narrowing of the aortic lumen, most often occurring just distal to the origin of the left subclavian artery (Onusko, 2003). Rare coarctation can occur at any point from the aortic arch to the abdominal bifurcation. The condition is two to five times more frequent in males than in females and is often associated with other congenital cardiac abnormalities, most often a bicuspid aortic valve, patent ductus arteriosus, ventricular septal defect, or valvular aortic stenosis. It occurs in 35% of patients with Turner syndrome (XO) (Tadmouri and Ali, 2005). The clinical hallmark of coarctation of the aorta is variance in blood pressure in the upper and lower extremities. The pressure gradient produced by coarctation causes hypertension proximal to the narrowed segment and occasionally, dilation of that portion of the aorta. Hypertension in the upper part of the body results in left ventricular hypertrophy and may produce dizziness, headaches, and nosebleeds (Tadmouri and Ali, 2005).

1.5.8 Pheochromocytoma

Pheochromocytomas are chromaffin cell tumors that can secrete epinephrine (adrenaline), norepinephrine (noradrenaline), and/or dopamine that are causing blood pressure elevation (Nguyen-Martin and Hammer, 2006). It is a life-threatening condition because catecholamine secretion is unpredictable, resulting in hypertension, arrhythmias, and/or hyperglycemia and because the tumor is malignant in 3% to 13% of cases (Plouin *et al.*, 1997). Pheochromocytoma usually occurs in or near the adrenal medulla but may

occasionally be found in other parts of the chromaffin system (Maier, 1949) in the abdomen, pelvis, chest, and the neck (Nguyen-Martin and Hammer, 2006). The incidence of this disorder increases with advancing age, with the prevalence approaching 0.1% in elderly persons, and its prevalence is between 4% and 6.5% in patients who have an incidental adrenal tumor. There is also genetic predisposition to pheochromocytoma in between 10% and 20% of patients diagnosed with an apparent pheochromocytoma (Nguyen-Martin and Hammer, 2006).

1.6 Racial Differences and Hypertension

The prevalence of hypertension in blacks is much higher than that in whites (Ergul *et al.*, 1996; Ergul, 2000; Treiber *et al.*, 2000). For example, the prevalence of essential hypertension in the U.S. black population is among the highest of any group in the world and it is approximately twice that of white Americans (Anderson, 1989). The pathogenesis of hypertension appears to be different in black patients (Ergul, 2000; Treiber *et al.*, 2000).

1.6.1 Racial differences and sympathetic reactivity

It has been proposed that blacks experience chronic sympathetic system activation due to more recurrent exposure to social and environmental factors (Oparil *et al.*, 2003). Consistent with this hypothesis, a number of studies have demonstrated that black Americans display greater cardiovascular reactivity to a number of physical and mental stressors (Ergul, 2000). Normotensive blacks manifest greater stress-induced increases in blood pressure and muscle sympathetic activity than normotensive whites, suggesting that enhanced sympathetic reactivity may predispose blacks to the development of hypertension (Lang *et al.*, 1997). It has also been demonstrated that black children with a family history of essential hypertension manifest greater increases in total peripheral resistance, leading to greater increases in blood pressure in response to cold pressor test and the mental stress of playing a video game (Ergul, 2000). It is stated that physical stressors do not result in the significant differences in heart rate or blood pressure responses between black and white hypertensives but psychosocial stressors result in increased muscle and skin vascular resistance in black but not in the whites, suggesting enhanced vascular resistance among blacks (Anderson, 1989). Exposure to stress (particularly psychosocial stress) increases sympathetic outflow, and repeated stress-induced

vasoconstriction may result in vascular hypertrophy, leading to progressive increases in peripheral resistance and blood pressure. This could partly explain the greater incidence of hypertension in lower socioeconomic groups, since they must endure greater levels of stress associated with daily living (Oparil *et al.*, 2003).

1.6.2 Racial differences and salt sensitivity

In general terms, salt sensitivity is defined as an increase in blood pressure in response to relatively high sodium intake. Both normotensive and hypertensive black individuals are known to be more salt sensitive than white Americans (Ergul, 2000). It has also been demonstrated that there is marked racial differences in sodium homeostasis, including a greater prevalence of sodium sensitivity and diminished ability to excrete a sodium load acutely in blacks compared with whites (Parmer *et al.*, 1994). Another aspect of salt sensitivity is that salt potentiates sympathetic nervous system induced vascular reactivity (Ergul, 2000).

1.6.3 Racial differences and endothelin (ET) system

The ETs are a family of 3 distinct 21-residue peptides, ET-1, ET-2, and ET-3, which are produced from precursor proteins via multiple cleavage steps (Ergul *et al.*, 1996). ET-1, the major isopeptide synthesized by endothelial cells, is a potent vasoconstrictor and is secreted abluminally toward the underlying smooth muscle (Ergul, 2000). In addition to its direct vasoconstrictor effect, ET-1 amplifies the contractile response to other vasoactive agents, including norepinephrine and serotonin and reciprocally, norepinephrine and serotonin can also potentiate the vasoconstrictor response to ET-1 (Ergul, 2000). Adult black hypertensives were found to have considerably higher ET-1 levels than normotensive blacks and whites regardless of hypertension status (Treiber *et al.*, 2000). In normotensive adults it was also found that black men exhibited higher basal ET-1 levels than white men (Treiber *et al.*, 2000). As described by Ergul and co-workers (1996), ET-1 levels in hypertensive blacks to be sevenfold to eightfold higher than in normotensive blacks and threefold to fourfold higher than in hypertensive whites. It is also stated that both female and male black hypertensive patients have significantly higher ET-1 levels than white hypertensive patients. Thus, these striking differences suggest that ET-1 might be a contributory factor to the development,

maintenance, and complications of hypertension in black population (Ergul *et al.*, 1996; Ergul, 2000).

2. The Role of Traditional Medicine in Health Care System

Man has always relied on resources within the environment to survive since creation. Plants, animals and minerals constitute the major natural resources used by man for promotive, preventive, curative and rehabilitative health (CAMH2, 2005). Traditional medicine has been defined by WHO (2003) and Patwardhan (2005) as diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral-based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness.

The use of traditional medicine is a worldwide reality in that it has been used since the existence of mankind in all nations. According to WHO, almost 65% of the world's population have incorporated traditional medicine (mainly herbs) into their primary modality of health care (Fabricant and Farnsworth, 2001). Many people in developing countries, particularly those in rural areas, have more access to traditional than modern medicines and use them more frequently for primary health care. African Traditional Medicine (ATM) is the mainstay of primary health care for the majority of those in the rural areas in Africa (CAMH2, 2005) and up to 80% of the population uses traditional medicine for primary health care (WHO, 2003). For instance, an estimated 90% of people in Ethiopia use traditional medicine to meet their primary health care needs, as do 70% of people in Benin, India, and Tanzania (Patwardhan, 2005). Traditional medicine has also been described by the WHO as one of the surest means to achieve total health care coverage of the world's population (CAMH, 2004).

The relative ratios of medical doctors in relation to the whole population in some African countries is low. For example: Ethiopia: 1:33,000, Kenya 1:7,142, Somalia 1:14,285, South Africa 1:1,639, Uganda 1:25,000, Zambia 1:11,000, Zimbabwe 1:6,250 (CAMH, 2004). Thus, traditional medicine has paramount importance in Africa. The advantage of traditional medicine includes its accessibility and relative cheapness (Patwardhan, 2005). They may have

been used by communities, and found to be efficacious through long experience. But their method of action may not be understood in modern scientific terms, and they often consist of mixtures of different active substances (Patwardhan, 2005).

2.1 Herbal Medicines and Hypertension

One of the important areas in which herbal medicines have contributed successfully is in cardiovascular research (Gilani and Atta-ur-Rahman, 2005). For the treatment of cardiovascular diseases, herbal medicines have been used in patients with hypertension, congestive heart failure, angina pectoris, atherosclerosis, cerebral insufficiency, and arrhythmia (Wu *et al.*, 1998). Franzoso and co-workers (1996) also described the role of Chinese herbal medicines in the therapeutic methods for the treatment of hypertension. Prevention and treatment of complications of hypertension have been shown (Franzoso *et al.*, 1996).

2.3 Moringa and Its Medicinal Values

Moringa stenopetala belongs to family *Moringaceae*, and the family consists of a single genus, *Moringa*, which has about 14 different species (Edwards *et al.*, 2002; Shibiru, 2002). Among the 14 species, *M. stenopetala* and *M. oleifera* are common to Southern part of Ethiopia at an altitude of 1100 to 1600m above sea level (Mekonnen and Gessesse, 1998). *M. stenopetala* is a tree with 6-10 m tall and its trunk is about 60 cm in diameter at breast height. Its bark is white to pale grey or silvery, and smooth. Its leaf is up to 55cm long, 2-5 pinnate each having 5 pairs of pinnae. Each pinna has 3-9 leaflets which are elliptic to ovate. The inflorescence is pubescent, dense many-flowered penicles up to 60 cm long. The flowers are strongly scented, regular and hypogynous. Petals are white, pale yellow or yellow green. Its length is up to 10mm, and width up to 3.5mm. Pods are elongated, reddish with grayish bloom, and at first it can be twisted but later it is straight. The seed is cream and brown,

spongy, smooth, elliptic-trigonous. Its body is 25-35 cm long and 14-20mm wide (Edwards *et al.*, 2002; Shibiru, 2002).

A.



B.



Fig.2.1 Photograph showing the *M. stenopetala* tree (A) and leaves of *M. stenopetala* (B). The picture is taken from Lante village, near Arbaminch, Southern Ethiopia. Dec. 2005

The major growing areas of *M. stenopetala* are Arbaminch and its surroundings, Mirab Abaya and its surroundings, Goffa and its surroundings, Negelle, Keffa and Wollayta Soddo areas which are about 400 to 550kms south of Addis Ababa. It also grow around Dessie. *M. stenopetala* is commonly called Shiferaw (Amharic) and Cabbage tree (English). In the Southern Region, *M. stenopetala* is known by different vernacular names of the areas such as Aleko, Aluko, Halako (Gamo Goffa), Kallanko (Benna), Haleko, Shalchada (Konso), Telahu (Tsemay), Haleko (Burgi, Derashe), and Halakwa (Wollayta) (Mekonnen and Gessesse,1998; Shibru, 2002).

The other species of the genus, which is common to the region, is *M. oleifera*. As described by Mughal *et al.* (1999) it is found widely in the Sub-Himalayan tracts, from Chenab east ward to Sarda. It is widely cultivated in Asia, Africa, and other tropical parts of the world for food (Faizi, 1995). It is also cultivated in plains of India, Pakistan and Burma for its leaves, flowers and fruits (pods) that have great medicinal importance in the Ayurvedic, Unani and

Allopathic systems (Mughal *et al.* 1999). As noted by Limaye *et al.* (1995), almost all the parts of the plant (root, bark, gum, leaf, flower and seeds) have been used for various ailments in indigenous medicine.

In Southern Ethiopia, around Arbaminch, Goffa, Konso, and Wollayta the leaves and flowers are eaten as vegetables. The local people cook the leaves of *M. stenopetala* tree and eat them with their traditional *kurkufa*, a cereal dish made with maize and sorghum (Mekonnen, 2005). As further noted, the leaves have distinctive strong, mustard-like taste, contain calcium, iron, and other trace minerals, and are eaten as a supplement to the major staple foods (Mekonnen, 2005). It is also described by Abyu *et al.* (2003) that raw leaves of *M. stenopetala* contain 9% dry matter as crude protein, higher percentage of carbohydrate, vitamins at nutritionally significant levels averaging 28 mg/100g of vitamin C and 160 µg/100g of beta-carotene, minerals such as potassium, iron, zinc, phosphorus and calcium in significant amount.

The different parts of *M. stenopetala* are also used for treating various diseases. As stated by Mekonnen (1999), the leaves are boiled as tea or chopped and mixed with water to cure malaria, hypertension, asthma, diabetes, stomach pain, and some times to expel retained placenta. The leaf ethanol extract showed some oxytocic activity on uterus strips of model animals which proved the traditional use of the leaves of *M. stenopetala* for stomach pain and to expel the retained placentae by women who have just given birth (Mekonnen, 1999). The ethanol extract of the leaves has shown 73.3% antifertility effect in swiss albino mice, and the leaves & root ethanol extracts have also shown dose dependent antileishmanial effect on *Leishmania donovani* promastigotes (Mekonnen and Gessesse, 1998). As described by Mekonnen *et al.* (1997), the aqueous extract of *M. stenopetala* exhibited hypoglycemic effect in rabbits after 6h of administration of 10 and 15 mg/kg where there was increased effect as the concentration raised from 10 to 15 mg/kg. However, initial rise in blood glucose level was noted at the concentration of 10 and 15 mg/kg after 1.5 hrs of administration and this was inline with the carbohydrate content justifying the use of the plant as food (Mekonnen *et al.*, 1997). It is further stated by Mekonnen and Gessesse (1998) that the rural people use wet or dried root part chopped and mixed with water to treat malaria. The seeds are traditionally used in cleaning muddy water or for water purification in Kola Shara village, near Arbaminch because the seeds serve as adherents to coagulate all the impurities in the turbid water

(Mekonnen and Gessesse, 1998). As described by Eilert *et al.* (1981), the aqueous extracts of seeds of *M. stenopetala* and its close relative, *M. oleifera*, contain bactericidal and fungicidal properties which suggest the use of seeds of both species for water purification.

3. Significance of the Present Study

Hypertension is one of the major cardiovascular risk factors contributing to myocardial infarction, cerebrovascular accidents, end-stage renal disease, congestive heart failure, peripheral vascular insufficiency and premature mortality (Lifton *et al.*, 2001). The worldwide burden of hypertension in 2000 was estimated to be 26.4% of the adult world population, with 34.26% in developed and 65.73% in developing countries, and it has been estimated that there will have an increase of 60% from the 2000 by the year 2025 (Hajjar *et al.*, 2006). According to the health and health-related indicators of Ministry of Health (2000–2001), hypertension was the seventh leading cause of death in Ethiopia in 2001 (WHO, 2004). Hence, studies on hypotensive (antihypertensive) effects of herbal medicines are important to enhance the health care of the country where an estimated 90% of people use traditional medicine to meet their primary health care needs.

M. stenopetala has been used to treat hypertension traditionally in some parts of Southern Nation, Nationalities and Peoples Region (SNNPR). However, there was no study carried out so far to analyze the possible effects. Therefore, this study is to assess the possible hypotensive activities of aqueous leaf extract of *M. stenopetala*. This would help to impart adequate and scientific justification for the traditional use of *M. stenopetala* to treat

hypertension. It is also to promote the quality of the country's traditional use of herbal medicine. Though *M. stenopetala* is drought resistant, nutritionally useful plant apart from its traditional medicinal values, it is limited to certain areas of Southern Ethiopia. Therefore, this study will have far reaching contribution for the diversification of the species to the rest of the country where erosion, drought and famine are the major problems given the appropriate microenvironment.

4. Objectives of the Study

4.1 General Objective

- To provide scientific justification for the traditional use of *M. stenopetala* as a possible hypotensive agent

4.2 Specific Objectives

- To investigate the *in vivo* hypotensive activity of aqueous leaf extract of *M. stenopetala*.
- To study the *in vitro* hypotensive effect of aqueous leaf extract of *M. stenopetala*.
- To suggest the possible mechanism of hypotensive action of the aqueous leaf extract of *M. stenopetala*.
- To identify the major secondary metabolites of the aqueous leaf extract of *M. stenopetala* via preliminary phytochemical screening.

5. Materials and Methods

5.1 Study Design: Laboratory based experiment involving quantitative and descriptive analysis of data.

5.2 Study Setting

Laboratory of Department of Physiology, Faculty of Medicine, Addis Ababa University; Laboratory of Biomedical Sciences, Department of Biology, Faculty of Science, Addis Ababa University; and Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.

5.3 Plant Material Collection

Fresh, undried and uncrushed leaves of *M. stenopetala* were collected from Arbaminch and surrounding villages 505 kms south of Addis Ababa, Ethiopia during the month of December 2005.

5.4 Extraction

The fresh, undried and uncrushed leaves were placed in separate Erlenmeyer flasks and placed in continuous hot water orbital shaker (GFL, model 3020, Germany) for 20 minutes. The extract was then filtered with cotton and Whatman filter paper (15.0 cm size) and then freeze dried in a lyophilizer (Vacuubrad, GMBH Germany). Accordingly, from 4kg of fresh leaves of *M. stenopetala*, 36g of crude extract was obtained and the crude extract was kept in a refrigerator at -20 °C until use for the experiments.

5.5 Phytochemical Screening

Preliminary qualitative screening of major secondary metabolites of the aqueous leaf extract of *M. stenopetala* was conducted according to the method described by Debella (2002). The method was based on chemical tests involving color changes through reaction with different standard reagents.

5.6 Laboratory Animal Preparation and Experimentation

Male guinea pigs (400 to 600g) were purchased from Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, and placed in the animal house of the Department of Pharmacology, Faculty of Medicine, Addis Ababa University. The animals were housed 5 per cage with water and food *ad libitum*, ambient temperature 21 °C and 12 light /12 dark cycles. Before the experiment, each animal was caged separately and deprived of food for 18 hrs but water *ad libitum*.

5.6.1 In vivo experiment

The *in vivo* experiment was carried out according to the method described by Gilani *et al.* (1994 and 2005) and Ghayur *et al.* (2005) on twelve male guinea pigs (500 - 600g)

anaesthetized with pentobarbital (60 mg/kg, i.p.). The trachea was exposed and cannulated to facilitate spontaneous respiration (Harvard ventilator, model 683 or SN-480). The arterial blood pressure was recorded from the carotid artery filled with heparinized saline via an arterial cannula (Portex cannulae, external diameter 1.02 mm, internal diameter 0.75 mm) connected to a pressure transducer. The aqueous leaf extract of *M. stenopetala* and the drugs were injected in the form of bolus injection via a cannula (Portex cannulae, external diameter 1.02 mm, internal diameter 0.75 mm) inserted into the external jugular vein followed by saline flush (0.2 ml). The exposed surface for cannulation was covered with cotton wool moistened in warm saline. The temperature of the animal was maintained at 37 °C by the use of a heated table and overhead lamp. The animal was then allowed to equilibrate for at least 30 min before administration of any drug or extract. The pressure transducer was connected to a polygraph or BBC recorder to measure systolic and diastolic blood pressure. Pulse pressure was obtained by subtracting diastolic pressure from systolic pressure and mean arterial blood pressure (MABP) was also determined from the sum of DBP plus one-third of pulse width. Changes in blood pressure were expressed as the Mean \pm Standard error of the means of control values, obtained before administration of test substances. In the study of mechanism of action of the test extract, acetylcholine (1 μ g/kg), atropine (1 mg/kg), adrenaline (2 μ g/kg), and propranolol (0.1 mg/kg) were used to check whether the extract mediates via cholinergic or adrenergic pathway. Atropine is a competitive blocker of acetylcholine at a muscarinic receptor site (Gilani *et al.*, 1994), and propranolol is β -adrenergic blocker (Gosh, 1984; Eno *et al.*, 2004).

5.6.2 *In vitro* experiment on guinea-pig aorta

The *in vitro* experiment was conducted according to the method described by Ghosh (1984), Gilani *et al.* (1994) and Ghayur *et al.* (2005). Ten guinea-pigs of either sex (400- 600g) were sacrificed by gentle cervical dislocation. The descending thoracic aorta was quickly removed and placed in Krebs-Henseleit solution. Excess fat and connective tissues were trimmed off and the whole length of aorta was then cut spirally resulting in long strip. From this strip a short strip (2 to 4 cm) was prepared to be used for the experiment. The tissue was kept moistened with Krebs-Henseleit solution during the whole procedure and the strip preparation

was mounted in a 20ml tissue bath containing Krebs–Henseleit solution, maintained at 37 °C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The composition of the physiological salt solution was (mM): NaCl 118.2, NaHCO₃ 25.0, CaCl₂ 2.5, KCl 4.7, KH₂PO₄ 1.3, MgSO₄ 1.2 and glucose 11.7 (pH 7.4). A resting tension of 1g was applied to the tissue and an equilibrium period of 1h was allowed to equilibrate before addition of any drug or the test extract. During this period the bath fluid was changed every 15minutes. Effect of extract was first determined on the resting baseline of the tissue to see if it had any vasoconstrictor effect. High K⁺ (80 mM) was added in the bath to induce sustained contraction of the tissue. The aqueous leaf extract of *M. stenopetala* was later tested for its ability to inhibit (relax) the contraction induced with high K⁺ (80 mM). Changes in isometric tension of the strip was measured via a force displacement transducer (FT- 03) using a grass model 7E polygraph (Grass Instrument Co., Quincy, Mass., USA)

5.6.3 Acute toxicity test

Swiss albino mice of both sexes (25-30g) were divided in to four groups of five mice each and fasted for 12hrs. The test was performed using increasing doses of *M. stenopetala* aqueous leaf extract (1, 5 and 10 g/kg), administered orally, in 10ml/Kg volume to different groups serving as test groups (Ghayur and Gilani, 2006). Thus, group one received 1 g/kg, group two received 5 g/kg and group three received 10 g/kg of *M. stenopetala* extract. To the fourth group of mice saline was administered (10 ml/Kg, p.o.) as negative control. The mice were allowed food and water *ad libitum* during a 24 hrs test period and kept under regular observation for mortality and any behavioral change (Ghayur and Gilani, 2006).

6. Statistical Analysis

The results were analyzed statistically using one-way ANOVA. Post hoc comparison between control and test groups was made with Dunnett's multiple comparison test using SPSS 10 statistical software package. The values at P < 0.05 were regarded as statistically significant. All data were expressed as mean ± standard error of the mean.

7. Results

7.1. Phytochemical Screening

The aqueous leaf extract of *M. stenopetala* was screened positive for the presence of chromophers, polyphenols, saponins, phytosteroides and withanoids, flavonoids, tannins, alkaloids and antraquinone glycosides as shown in Table 1. But it showed negative for the presence of cardiac glycosides, phenolic glycosides, cyanogenic glycosides, antranides, carotenoides, and free anthraquinones.

Table1. Results of preliminary phytochemical analysis of crude leaf extract of *M. stenopetala* with the characteristic color indicators

	Secondary metabolite	Chemicals /method	Indicators
1	Chromophers	10ml distilled water + heat	Yellow to red color

		for 30min	
2	Polyphenols	1%FeCl ₃ + 1ml of K ₃ Fe (CN) ₆	Green blue color
3	Saponins	30ml distilled water +heat(5min)	Formation of persistent honey comb froth
4	Phytosteroides and withanoids	CHCl ₃ + conc. H ₂ SO ₄	Red, reddish brown or violet color
5	Flavonoids	5 drops of 2% lead acetate	Yellow or orange color
6	Tannins	3drops of 1%K ₃ (FeCN) ₆ + 3drops of conc. NH ₃	Formation of color
7	Alkaloids	Dragendroffs reagent	Yellow orange
		Mayers reagent	White
		0.5% Tannin solution	Yellowish-white precipitate
8	Antraquinone glycosides	2N HCL, benzene, 10% ammonia	Red color

7. 2. In vivo Result

The effect of aqueous leaf extract of *Moringa stenopetala* on systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure, and mean arterial blood pressure (MABP) in anesthetized guinea pig (n = 12, p < 0.05) are shown in table 2

The *iv* administration of aqueous leaf extract of *M. stenopetala* decreased the SBP dose dependently from control value of 81.91 ± 2.73 to 76.69 ± 2.59 , 60.89 ± 3.95 , 54.65 ± 3.73 , 47.79 ± 3.02 , and 39.26 ± 3.10 mmHg at the respective doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, and 40 mg/kg. The fall in SBP observed at dose of 5 mg/kg of *M. stenopetala* was not statistically significant (p = 0.676). However, at doses of 10 mg/kg, 20 mg/kg, 30 mg/kg and 40 mg/kg the effect on SBP was statistically significant (p = 0.00).

Similarly, the test extract caused fall in DBP from the control value of 53.16 ± 2.70 to 48.22 ± 2.40 , 38.73 ± 3.19 , 32.62 ± 2.51 , 24.89 ± 2.13 , and 20.79 ± 1.56 mmHg at the respective doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, and 40 mg/kg. The effect of test extract on the DBP at dose of 5 mg/kg was not significant ($p = 0.485$) and significant decline of DBP was observed at the doses of 10 mg/kg, 20 mg/kg, 30 mg/kg, and 40 mg/kg ($p = 0.00$). Data are expressed as mean \pm Standard error of the mean

On the other hand, the decline in pulse pressure was from 28.74 ± 2.29 to 28.46 ± 2.43 , 22.15 ± 2.44 , 22.02 ± 2.59 , 22.89 ± 2.44 , and 18.47 ± 2.37 mmHg at the respective doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, and 40 mg/kg. However, the significant fall was observed only at the cumulative dose of 40 mg/kg ($p = 0.01$). The fall in MABP was also from the control value of 62.74 ± 2.49 to 57.71 ± 2.18 , 46.12 ± 3.27 , 39.97 ± 2.71 , 32.52 ± 2.18 , and 26.94 ± 1.89 at the respective doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, and 40 mg/kg, and like SBP and DBP, the fall in MABP at the doses of 10 mg/kg, 20 mg/kg, 30 mg/kg, and 40 mg/kg was statistically significant ($p = 0.00$). The duration of action was longer; that is, about 10 minutes as compared to the 1 minute action of acetylcholine, and the animals did not show any sign of cardiopulmonary distress after repeated doses of the aqueous extract of *M. stenopetala*.

In the investigation of mechanism of action on how *M. stenopetala* exerts blood pressure lowering effect, standard drugs were used to compare their mechanism of action with that of test extract. Acetylcholine at a dose of 1 μ g/kg produced a considerable drop in blood pressure and pretreatment of animals with atropine (1 mg/kg), the muscarinic blocker of acetylcholine, abolished the effect of acetylcholine on blood pressure. However, atropine pretreatment did not alter the hypotensive effect of the aqueous leaf extract of *M. stenopetala* in anaesthetized guinea pig as shown in figure 7.6. Similarly adrenalin at a dose of 2 μ g/kg produced a considerable rise in blood pressure and pretreatment of the animals with propranolol (0.1 mg/kg), non selective β -blocker, abolished the blood pressure raising effect of adrenalin. However, pretreatment of the animals with the aqueous leaf extract of *M. stenopetala* did not abolish the effect of adrenalin, and blocking the adrenergic mechanism with propranolol did not prevent the action of the test extract in anaesthetized guinea pigs.

Table 2. The effects of *iv* infusion of *M. stenopetala* aqueous extract in anaesthetized guinea pigs at doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg and 40 mg/kg on systolic blood pressure, diastolic blood pressure, pulse pressure, and mean arterial blood pressure. Control = the blood pressure obtained before administration of the test extract.

Doses of <i>M. stenopetala</i>	SBP	DBP	PP	MABP
control	81.91 ± 2.73	53.16± 2.70	28.74± 2.29	62.74± 2.49
5mg/kg	76.69 ± 2.59	48.22± 2.40	28.46± 2.43	57.71± 2.18
10mg/kg	60.89 ± 3.95**	38.73±3.19**	22.15 ± 2.44	46.12±3.27**

20mg/kg	54.65 ± 3.73**	32.62 ± 2.51**	22.02 ± 2.59	39.97±2.71**
30mg/kg	47.79 ± 3.02**	24.89 ± 2.13**	22.89 ± 2.44	32.52±2.18**
40mg/kg	39.26 ± 3.10**	20.79 ± 1.56**	18.47 ± 2.37**	26.94±1.89**

Data are expressed as the mean value ± standard error of the mean (n=12). ** denotes the significance at P values < 0.05, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, MABP = mean arterial blood pressure

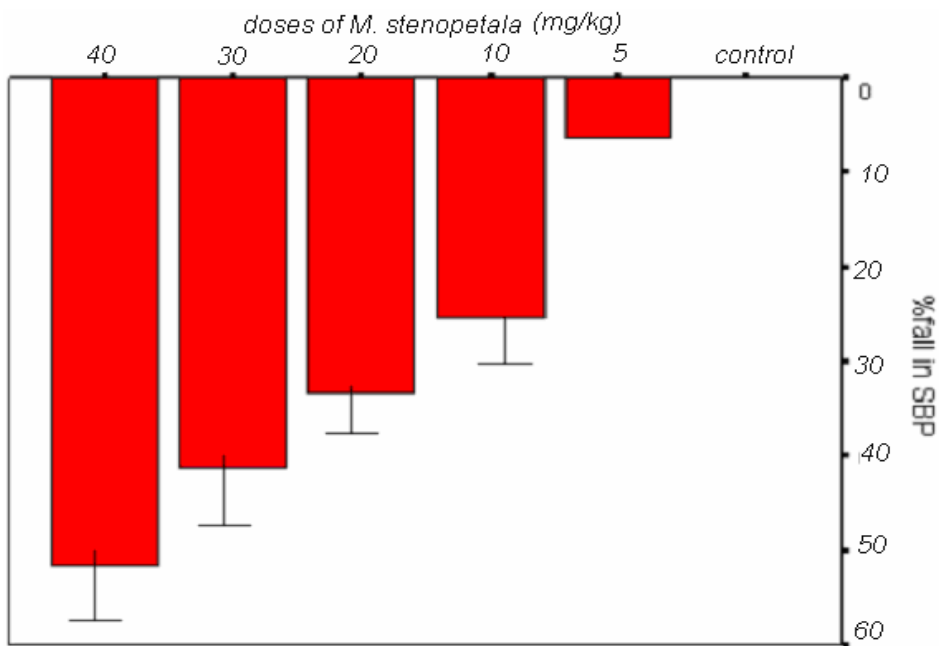


Fig.7.1 The percent fall in systolic blood pressure in response to aqueous leaf extract of *M. stenopetala* in anaesthetized guinea pigs (n = 12).

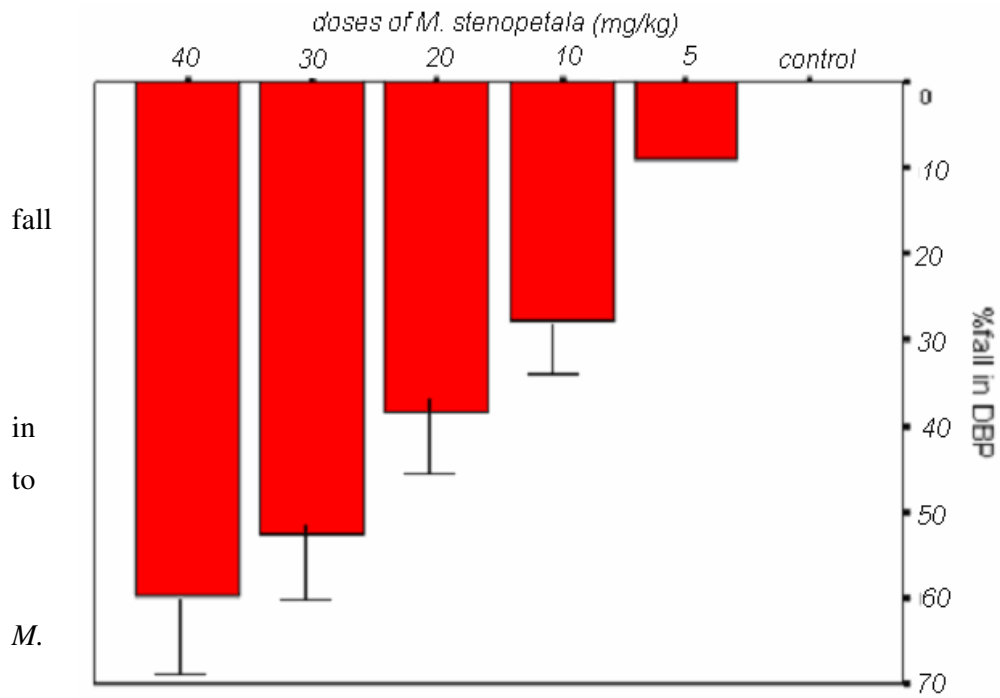


Fig.7.2
The percent in diastolic blood pressure response aqueous leaf extract of

stenopetala in anaesthetized guinea pigs (n = 12).

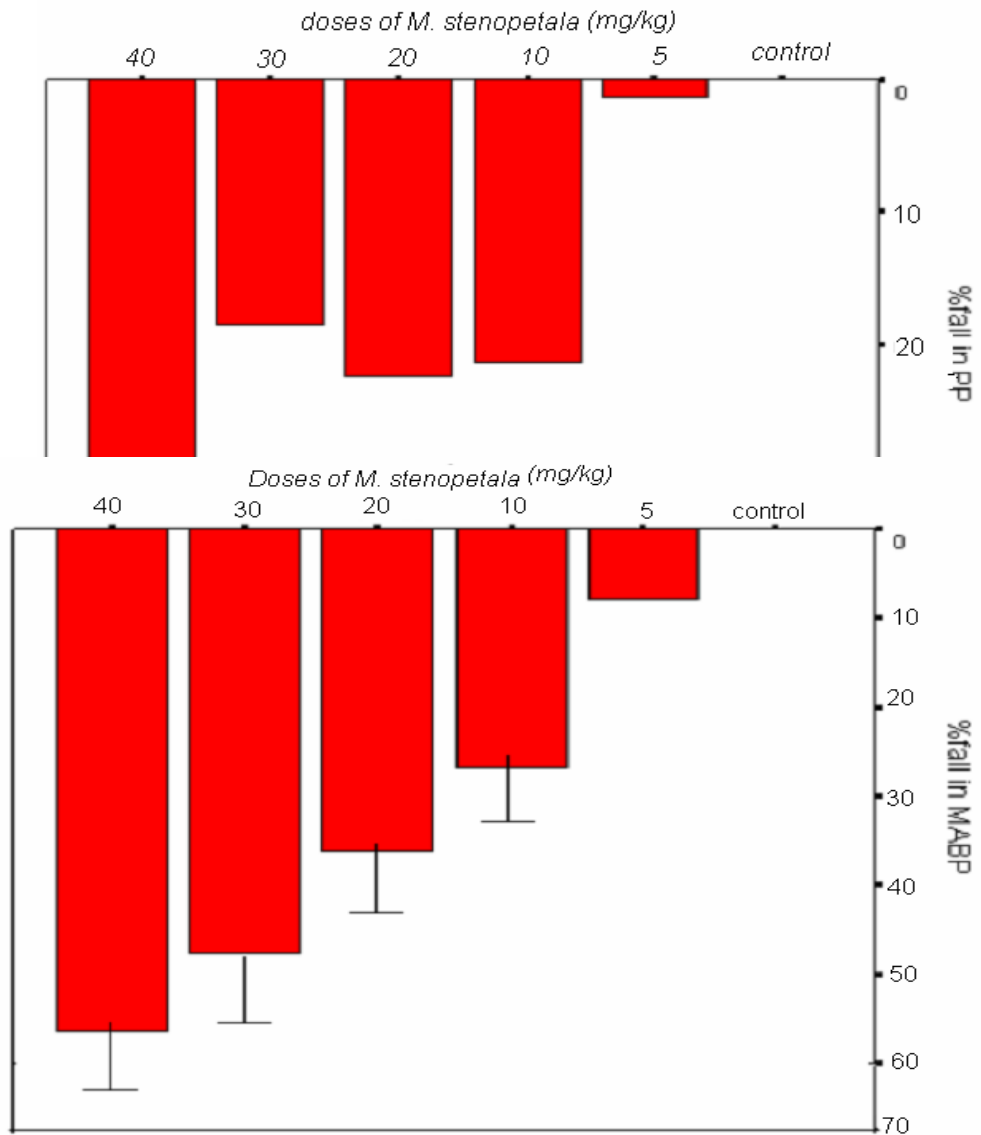


Fig. 7.3
The percent fall in

pulse pressure in response to aqueous leaf extract of *M. stenopetala* in anaesthetized guinea pigs (n = 12).

Fig.7. 4 The percent fall in mean arterial blood pressure in response to aqueous leaf extract of *M. stenopetala* in anaesthetized guinea pigs (n = 12).

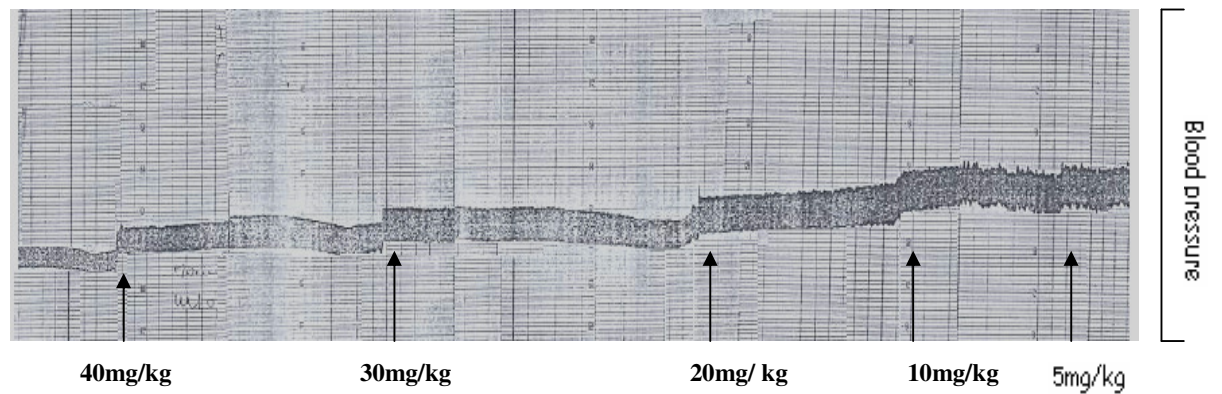


Fig. 7.5 Typical tracing showing the hypotensive effect of *M. stenopetala* crude extract in anaesthetized guinea pig. Arrows indicate the point at which the test extracts were administered.

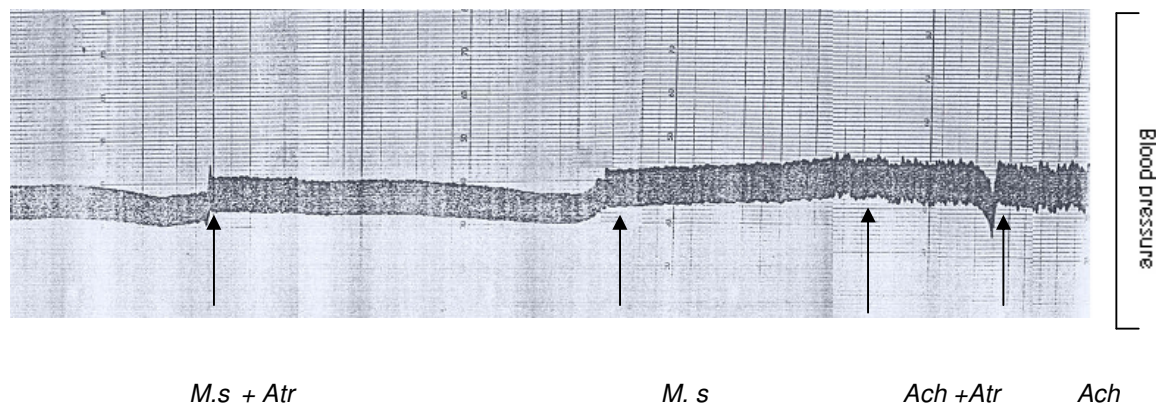


Fig. 7.6 Tracing showing the non-muscarinic mechanism of action of *M. stenopetala* in inducing hypotensive effect in anaesthetized guinea pig.

M.s = *Moringa stenopetala*; *Atr* = Atropine; *Ach* = Acetylcholine

7. 3. In vitro Result

The *in vitro* hypotensive effect of aqueous leaf extract of *M. stenopetala* was carried out on the isolated descending thoracic aorta of guinea pigs. When test was made on resting baseline of guinea pig aorta, the aqueous leaf extract of *M. stenopetala* did not exhibit any vasoconstrictor activity. The extract was then tested on high- K^+ (80 mM) induced contraction and the result was as shown in table 3 and (fig 7.7 and 7.8). The results are expressed as the percentage contraction, taking the control high K^+ - induced contraction before the application of the test extract as 100%. Taking Mean \pm SEM of percent contraction of 100.0 ± 0.0 induced by high- K^+ before the administration of aqueous extract of *M. stenopetala* as control, the extract exhibited inhibition of contraction by reducing from control value of 100 ± 0.0 to 98.3 ± 0.6 , 88.9 ± 2.7 , 62.8 ± 8.5 , 31.4 ± 9.7 , 2.9 ± 2.9 at the respective doses of 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml, 6 mg/ml, and 7 mg/ml. Therefore, the percent inhibition (vasodilation) of high K^+ -induced contraction of the aortic tissue was $1.47 \pm 0.49\%$, $11.12 \pm 208\%$, $36.55 \pm 8.64\%$, $67.76 \pm 9.55\%$, and $95.56 \pm 3.14\%$ at the above respective doses (n = 10 and data are expressed as M \pm SEM).

The test extract showed statistically insignificant percent inhibitory effect on high-K⁺ (80mM) induced contraction of isolated guinea pig aorta at concentration of 1.25 mg/kg (n = 10, p = 0.772) and 2.5 mg/kg (n = 10, p = 0.255). However, significant dose dependent inhibition of high-K⁺ induced contraction was observed at the concentrations of 5mg/ml (n = 10, p = 0.001), 6 mg/ml (n = 10, p = 0.000) and 7 mg/ml (n = 10, p = 0.000). The inhibition of contraction was within 15minutes contact time after the application of each dose of the test extract. Thus, the inhibition of contraction was not only dose dependent but also time dependent. The relaxant effect of test extract was reversible as the tissue regained its spontaneous activity at least within 2 hrs after repeated washout.

Table 3. The percent inhibition of high-K⁺ (80 mM)-induced contraction by aqueous leaf extract of *M. stenopetala* on isolated aorta of guinea pig (n = 10). Data shown are M ± SEM, and ** denotes the significance at p value < 0.05

Treatment	% of induced contraction	% inhibition of high K ⁺ (80 mM) induced contraction	Level of Significance
Control(K ⁺ of 80 mM)	100.0 ± 0.0	0.00 ± 0.00	
1.25 mg/ml	98.3 ± 0.6	1.47 ± 0.49	0.772
2.5 mg/ml	88.9 ± 2.7	11.12 ± 2.86	0.255
5 mg/ml	62.8 ± 8.5**	36.55 ± 8.64**	0.001
6 mg/ml	31.4 ± 9.7**	67.76 ± 9.55**	0.000

7 mg/ml	$2.9 \pm 2.9^{**}$	$95.56 \pm 3.14^{**}$	0.000
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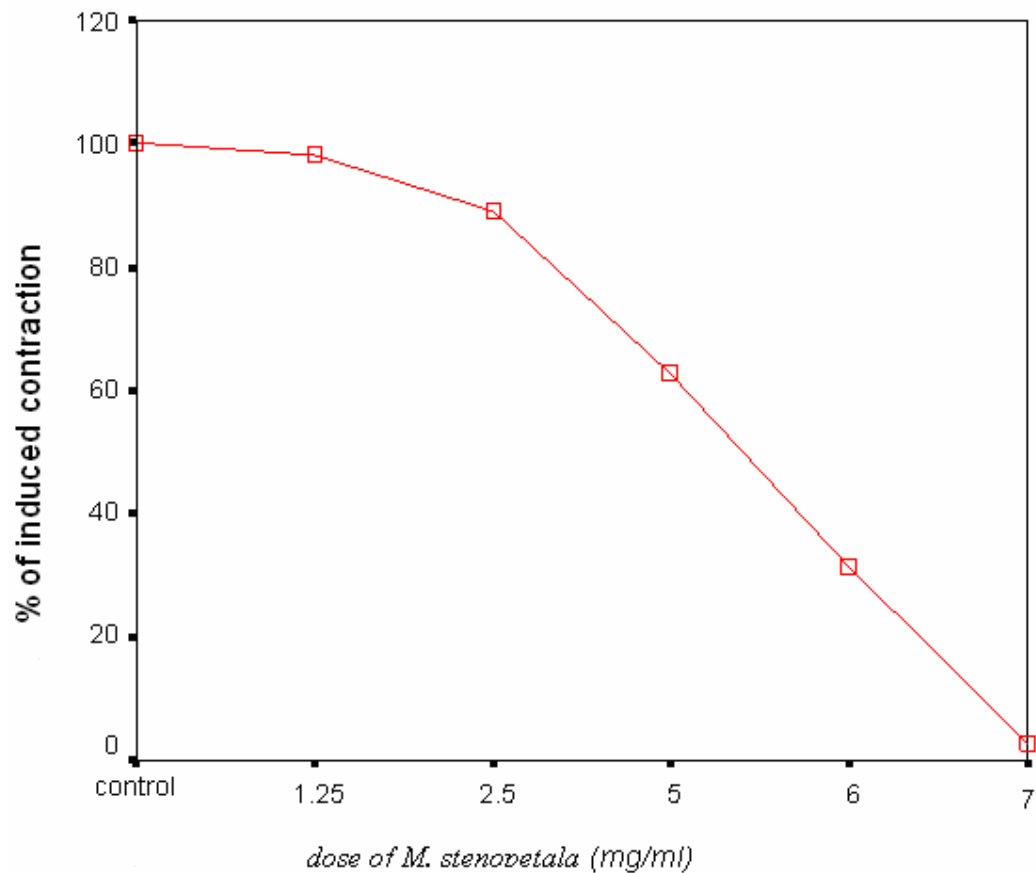


Fig. 7.7 Dose – response curve showing the inhibitory effect of aqueous leaf extract of *M. stenopetala* on high K^+ (80 mM)-induced contraction in isolated aorta of guinea pig (n=10 and values of % of induced contraction shown are $M \pm S.E.M$). Control = high concentration of K^+ (80 mM).

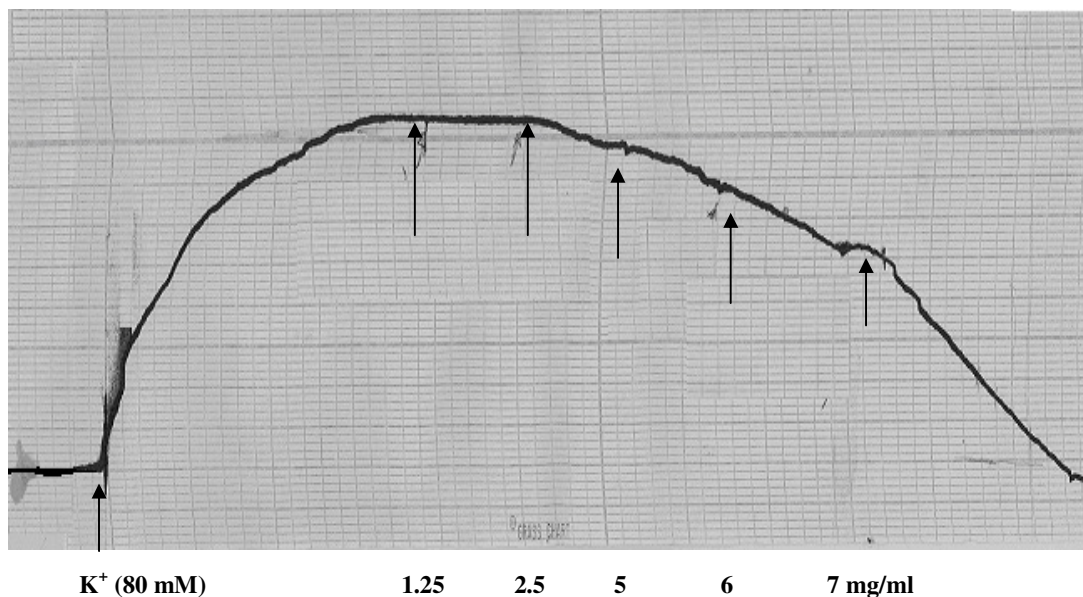


Fig. 7.8 Typical tracing showing concentration and time dependent inhibitory responses of leaf extract of *M. stenopetala* in isolated guinea pig aorta. Arrows show the point at which K^+ (80 mM), 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml, 6 mg/ml, and 7 mg/ml of leaf extracts of *M. stenopetala* were added.

7. 4. Acute Toxicity Result

There was no mortality in group I, group II, and group III mice that received oral dose of 1 g/kg, 5 g/kg and 10 g/kg (10 ml/Kg), respectively in the 24 hrs of regular observation. Also there was no any sign of behavioral change in all test groups as compared to negative control that received saline (10 ml/Kg, p.o.) in the 24 hrs of regular observation. Thus, the extract can be considered tolerable in mice when tested up to the oral dose of 10 g/kg with no mortality and behavioral changes.

8. Discussion

Blood pressure is product of the cardiac output and total peripheral resistance of arterioles. Therefore, the main organs concerned with BP maintenance are the heart and the blood vessels, although, these are under the influence of the central nervous system. Blood pressure measurement therefore, helps to reflect on the integrity of the cardiovascular system (Eno *et al.*, 2004). In this study, the blood pressure was measured to evaluate the hypotensive effect of *M. stenopetala* which is used traditionally for treatment of hypertension in some parts of Southern Nation, Nationalities and Peoples Region (SNNPR).

An *iv* administration of aqueous extract of *M. stenopetala* has showed no significant blood pressure lowering effect at the dose of 5mg/kg in anaesthetized normotensive guinea pig (n = 12, p > 0.05). Significant fall in systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure was observed at the doses of 10, 20, 30, and 40 mg/kg (n = 12, p = 0.00). However, significant fall in pulse pressure was observed only at the cumulative dose of 40 mg/kg (p = 0.01). This may be due to proportional fall in systolic and diastolic pressure, since pulse pressure is the difference of systolic and diastolic pressure. The decline in systolic blood pressure was from control value of 81.91 ± 2.73 to 76.69 ± 2.59 and 39.26 ± 3.10 mmHg; the diastolic blood pressure from 53.16 ± 2.70 to 48.22 ± 2.40 and 20.79 ± 1.56 mmHg; the pulse pressure from 28.74 ± 2.29 to 28.46 ± 2.43 and 18.47 ± 2.37 mmHg, and mean arterial blood pressure from 62.74 ± 2.49 to 57.71 ± 2.18 and 26.94 ± 1.89 at doses of 5mg/kg and 40 mg/kg, respectively (Table 2). Thus, the crude extract of the leaf of *M. stenopetala* caused a percent fall of systolic blood pressure by $6 \pm 0.8\%$ and $52 \pm 3.9\%$, diastolic blood pressure by $9 \pm 1.3\%$ and $60 \pm 3.7\%$, pulse pressure by $1.5 \pm 1.3\%$ and $34 \pm 8.9\%$, and mean arterial blood pressure by $8 \pm 0.9\%$ and $56 \pm 3.5\%$ at the doses of 5 mg/kg and 40 mg/kg, respectively in anaesthetized normotensive guinea pigs (n = 12) as shown in fig. 7.1 - 7.4.

This result is in agreement with different previous *in vivo* hypotensive studies on *M. oleifera*, the close relative of *M. stenopetala*, where the extract of *M. oleifera* showed fall in arterial blood pressure. There was 35–40% reduction in mean arterial blood pressure by isolated compounds such as carbamates and thiocarbamates from crude extract of leaf of *M. oleifera* at the dose of 3mg/kg in anaesthetized normotensive wistar rats (Faizi *et al.*, 1994). The P-

Hydroxybenzaldehyde (PBA) which is aglycone of α -1- rhamnosyloxybenzaldehyde isolated from the leaves of *M. oleifera* showed 40% and 75% decreases in blood pressure at a dose of 3 and 10mg/kg in anaesthetized normotensive wistar rats, respectively (Faizi *et al.*, 1998). It was the first report of hypotensive activity of PBA. Furthermore, glycosides isolated from leaves of *M. oleifera* caused dose dependent fall in systolic, diastolic, and mean arterial blood pressure in ansthaetized rats with the magnitude of fall in mean arterial blood pressure being 10-20% and 30-40% at a dose of 1mg/kg and 3mg/kg, respectively (Faizi *et al.*, 1995). It is also noted by Gilani *et al.* (1994) that the pure compounds isolated from *M. oleifera* caused dose dependent fall in systolic blood pressure, diastolic blood pressure, and heart rate in anaesthetized normotensive wistar rats. In similar study, it was described that the aqueous extract of stem bark from *M. oliefera* produces a dose-dependent hypotensive effect in anesthetized mongrel dogs (7-12 kg) with a maximum effect at 20 mg/kg (Limaye *et al.*, 1995). He further noted that the hypotensive effect of the extract justifies the use of the plant as a counter-irritant in folk medicine.

In the present study, the duration of action of the *iv* administered crude extract of *M. stenopetala* in anaesthetized guinea pig was longer; that is, about 10 minutes as compared to the 1 minute of acetylcholine. The animals did not show any sign of cardiopulmonary distress after repeated doses. This is in line with the long duration of action of the test extract observed in the *in vitro* experiment where the high K^+ (80 mM)-induced contraction inhibition of the *M. stenopetala* in isolated aortic tissue was removed in about 2hrs after repeated washout. This is inconsistent with antihypetensive effect duration of action of only 1-2 minutes observed from crude extract of *M. oleifera* (Gilani *et al.*, 1994; Faizi *et al.*, 1997; Faizi *et al.*, 1998).

In the investigation of mechanism of action on how *M. stenopetala* exerts its hypotensive effect, acetylcholine at a dose of 1 μ g/kg produced a considerable drop in blood pressure, and pretreatment of animals with atropine (1 mg/kg), the muscarinic blocker of acetylcholine, abolished the blood pressure lowering effect. However, atropine pretreatment failed to abolish the hypotensive effect of the aqueous leaf extract of *M. stenopetala* in anaesthetized guinea pig (figure 7.6) suggesting non-cholinergic involvement. This is in agreement with the study made by Gilani *et al.* (1994) where acetylcholine at a dose of 1 μ g/kg produced a drop

in blood pressure and pretreatment with atropine (1 mg/kg) abolished this effect, but pretreatment with atropine did not alter the hypotensive effect of isolated compounds from leaves of *M. oleifera* in anaesthetized wistar rats. Similarly, in anesthetized dogs the aqueous extract of *M. oleifera* produced dose dependent hypotensive effect which was not altered by atropine (Limeya *et al.*, 1995). Thus, the test extract of the present study mediates its hypotensive effect through mechanism(s) independent of muscarinic receptor activation. This conclusion is supported by previous *in vitro* studies. In a study by Mekonnen (1999), unlike acetylcholine, the crude leaf extract of *M. stenopetala* failed to cause contraction of guinea pig ileum and even inhibited acetylcholine induced contraction. There was also inhibition of acetylcholine induced contraction of mouse duodenum by the aqueous extract of *M. stenopetala* (Mekonnen, 1999). Similarly, the pure compounds isolated from leaves of *M. oleifera* did not cause guinea pig ileal contraction unlike acetylcholine, ruling out the involvement of muscarinic receptor activation (Gilani *et al.*, 1994). In another study on *M. oleifera*, the aqueous extract caused dose dependent negative inotropic effect in isolated frog heart at concentration of 0.1-1 μg and atropine failed to block the negative inotropic effect of the extract (Limaye *et al.*, 1995).

On the other hand, adrenaline at a dose of 2 $\mu\text{g}/\text{kg}$ produced a considerable rise in blood pressure, and pretreatment of the animals with propranolol (0.1 mg/kg), non selective β -adrenergic blocker, abolished this effect. However, pretreatment with aqueous leaf extract of *M. stenopetala* did not abolish the effect of adrenalin. Thus, the extract does not act through the same mechanism as that of propranolol. It is also quite clear from this study that the crude leaf extract of *M. stenopetala* does not utilize the adrenergic mechanism because blocking the adrenergic mechanism with propranolol (β -adrenoceptor antagonist) did not prevent the action of the present test extract suggesting that the extract is acting at a different site (*i.e.* non-adrenergic). This is consistent with the effect of *Viscum album* on blood pressure where blocking the adrenergic mechanism with propranolol did not prevent the action of the extract (Eno *et al.*, 2004). Similarly, the *in vivo* study of the aqueous extract of *M. oleifera* on anesthetized dog produced dose dependent hypotensive effect and the response was not altered by propranolol and pheniramine (Limeya *et al.*, 1995). Therefore, the hypotensive effect of *M. stenopetala* observed in the present *in vivo* study may be attributed to a direct

action of the aqueous leaf extract of *M. stenopetala* on the vascular system and may be also by moderation of calcium availability to the myocardial cells.

The *in vivo* hypotensive property of crude leaf extract of *M. stenopetala* is substantiated by *in vitro* investigation on the isolated aorta of guinea pig. The test extract inhibited high K^+ -induced contraction. As noted by Gilani *et al.* (2005), K^+ at high doses ($>30\text{mM}$) is known to cause smooth muscle contractions through opening of voltage-dependent slow Ca^{++} channels, thus allowing influx of extracellular Ca^{++} causing a contractile effect. The percent inhibition of high K^+ -induced contraction by aqueous extract of *M. stenopetala* at the doses of 1.25 mg/ml and 2.5 mg/ml were statistically insignificant ($p < 0.05$). However, significant inhibition of high K^+ -induced contraction was $36.55 \pm 8.64\%$ ($p = 0.001$), $67.76 \pm 9.55\%$ ($p = 0.00$), and $95.56 \pm 3.14\%$ ($p = 0.00$) at respective doses of 5, 6 and 7mg/ml ($n=10$ and data are expressed as Mean \pm standard error of the mean). Thus, the test extract caused a dose-dependent inhibition (relaxation) of K^+ -induced contraction in isolated aortic preparation (Table 3 and fig. 7.7). This result is consistent with dose-dependent inhibition of high- K^+ (80 mM)-induced contraction by isolated compounds from crude extracts of *M. oleifera* on isolated aorta of rabbit (Gilani *et al.*, 1994). The present finding is also in agreement with the study described by Ghayur *et al.* (2005) where the ginger extract showed dose-dependent vasodilation (inhibition of contraction) of high K^+ (80 mM)-induced contraction on isolated rabbit aortic tissue preparation. In the present study, it was also observed that in isolated frog heart, the aqueous extract of *M. stenopetala* caused dose dependent depression on heart rate (negative chronotropy) and contractility (negative inotropy), however the values were not recorded because of technical problem of the device.

Smooth muscle relaxation can be achieved by various mechanisms such as potassium channel opening, calcium channel blocking and receptor antagonism (Suresh *et al.*, 2006). Opening K^+ channels via activating ATP-sensitive K^+ channels hyperpolarizes the smooth muscle, which closes voltage-gated calcium channels and decreases intracellular calcium. With less calcium available to combine with calmodulin, there is less activation of myosin light chain kinase and phosphorylation of myosin light chains. This leads to vasodilation (Keiichi *et al.*, 1997). The inhibition of high K^+ -induced contraction by the aqueous leaf extract of *M. stenopetala* in the present study indicates the present test extract could not act through opening the potassium

channels. Because the potassium channel openers do not inhibit high K^+ -induced contraction (Keiichi *et al.*, 1997; Suresh *et al.*, 2006).

In the present study, mechanism of the dose-dependent relaxation of high K^+ -induced contraction by *M. stenopetala* in isolated aorta preparation may be mediated through the calcium channel blockade. Because calcium channel blockers concentration dependently inhibit contraction induced by high K^+ (Keiichi *et al.*, 1997; Ghayur *et al.*, 2005). In similar study, Ghayur *et al.* (2005) described that the ability of ginger crude extract to relax K^+ (80mM)-induced contraction would indicate an L-type voltage-dependent calcium channel-blocking (CCB) mode of vasodilation. Thus, in this study the dose-dependent inhibition (vasodilation) of high K^+ -induced contraction by *M. stenopetala* in isolated guinea pig aortic tissue preparation may be as a result of restricted Ca^{++} entry through voltage-dependent slow calcium channels. The vascular smooth muscle relaxation leads to vasodilation, causing a decrease in peripheral resistance and as a result, reduction in blood pressure (Jones *et al.*, 2004).

The *in vitro* study of *M. stenopetala* is also supported by previous *in vitro* experiments. The crude leaf extract of *M. stenopetala* resulted in time and concentration dependent decline in acetylcholine induced contractions of both guinea pig ileum and mouse duodenum (Mekonnen, 1999). Acetylcholine, a neurotransmitter released by parasympathetic nervous system, mediates its spasmogenic action in the gut by stimulating muscarinic receptors (Gilani *et al.*, 2005). The extract also abolished rhythmic spontaneous contractions of both tissue preparations (Mekonnen, 1999). Thus, the direct depressant effect of the leaf extract of *M. stenopetala* on isolated frog heart, smooth muscle tissue preparation of guinea pig aorta, guinea pig ileum and mouse duodenum is probably responsible for its hypotensive effects observed in the *in vivo* study on anaesthetized animal model.

Even though detailed chemical identification and structural elucidation are lacking in the present study, some of major secondary metabolites from the crude leaf extract of *M. stenopetala* were observed while others were tested negative in the preliminary phytochemical analysis. Different literatures show that some of the secondary metabolites which are observed in the present phytochemical analysis are therapeutically active hypotensive agents

and some of secondary metabolites which are absent in crude leaf extract of *M. stenopetala* are cardiotoxic (Debella, 2002). These data are consistent with the present study on *M. stenopetala*. For instance, the alkaloids obtained by the fractionation of the aqueous extract of the leaves of *M. oleifera* were found to have a negative inotropic effect on the frog heart. This activity was further characterised by testing it on the isolated guinea pig ileum (Dangi *et al.*, 2002). It has also been found that flavonoid significantly lowered blood pressure in spontaneously hypertensive rats (Duarte *et al.*, 2001) whereas cardiac glycosides which are absent in the extract are characterized by highly specific and powerful cardiotoxic action they exert on the cardiac muscles (Debella, 2002). Thus, alkaloids and flavonoids may be important secondary metabolites for the hypotensive effects of crude leaf extract of *M. stenopetala* observed from the *in vivo* and *in vitro* study.

As observed from acute toxicity study, the aqueous leaf extract of *M. stenopetala* can be considered tolerable in mice when tested up to the oral dose of 10 g/kg with no mortality and behavioral changes. This result is in agreement with the toxicity study by Faizi *et al.* (1998) on *M. oleifera*, and according to their finding, the plant extract up to 3 g/kg administered subcutaneously was found devoid of any lethal effect and no apparent behavioral change or effect on locomotor activity. Furthermore, *in vitro* cytotoxicity study on aqueous extract of leaves from *M. stenopetala* on hepatocyte cells did not affect cell viability, unlike the ethanol extract suggesting the aqueous extract may be non-toxic (Mekonnen *et al.*, 2005). The present finding also agrees with toxicity study on crude extract of Raddish by Ghayur and Gilani (2006) which showed to be safe in mice when administered up to an oral dose of 10 g/kg with out any sign of behavioral change in 24 hrs after administration. This indicates that the leaves of the plant can be safe and therefore, the present study also justifies the use of the leaves of *M. stenopetala* as a source of food.

9. Conclusion and Recommendations

9.1 Conclusion

- The results of this study show that the intravenous administration of fresh leaf extract of *M. stenopetala* exhibited blood pressure lowering effect in normotensive anaesthetized guinea pigs.

- The crude leaf extract of *M. stenopetala* exerts its hypotensive effect in the normotensive guinea pig through neither cholinergic nor adrenergic pathway because its effect is not altered by atropine which is a muscarinic receptor blocker of acetylcholine and propranolol which is non-selective blocker of β - adrenergic receptors.
- In tissue preparation, the crude leaf extract of *M. stenopetala* exhibited inhibitory effect on high K^+ -induced contraction of isolated aorta. This may be acting through blockade of the voltage sensitive Ca^{++} channels.
- As it is shown in the *in vivo* and *in vitro* blood pressure lowering study from crude leaf extract of *M. stenopetala*, the present findings provide scientific justification for the traditional use of the leaves as antihypertensive agent by some localities of southern people of Ethiopia.
- Acute toxicity study showed that the aqueous leaf extract of *M. stenopetala* is tolerable in mice up to an oral dose of 10 g/kg. This signifies that the plant is safe and justifies the use of the leaves of the plant as a source of food by some localities of the Southern Nations, Nationalities and Peoples Region (SNNPR).

9.2 Recommendations

- Detailed phytochemical screening, further fractionation and isolation of active ingredient is required to identify the exact chemical compounds responsible for the activities observed in the present study from the crude leaf extract of *M. stenopetala*
- As the present study is focused on the hypotensive effects of *M. stenopetala* on normotensive animals, experimentally induced hypertensive animals should be

further worked on to verify the effect of leaf extracts of *M. stenopetala* in hypertensive animal models.

- Since the present study showed the blood pressure lowering effects of *M. stenopetala* on model animals, prevalence study of hypertension is recommended in the indigenous people where *M. stenopetala* is used as food to evaluate the effect of the plant on the blood pressure of that population.
- Priority should be given by government, NGOs, researchers and local communities to conserve medicinal plants in order to develop safe, effective, and accessible products since majority of Ethiopian population depend on medicinal plants for their primary health care.

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