

In vivo and *in vitro* hypotensive effect of aqueous extract of *Moringa stenopetala*

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Abstract

Background: *Moringa stenopetala*, Baker f. (*Moringaceae*) is used for food and medicine in Southern Ethiopia.

Objective: To substantiate the hypotensive effect of *M. stenopetala* *in vivo* and *in vitro*.

Methods: An *in vivo* experiment was carried out on male guinea pigs anaesthetized with pentobarbital. The arterial blood pressure was recorded from a carotid artery filled with heparinized saline via an arterial cannula connected to a pressure transducer. For the *in vitro* experiment the descending thoracic aorta was removed and kept moistened in Krebs-Henseleit solution and then mounted in a 20ml tissue bath maintained at 37°C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide.

Results: 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 doses of 10, 20, 30 and 40 mg/kg in normotensive anaesthetized guinea pigs (n = 12). The effect might have been mediated by non-autonomic nervous system as the effect is not altered by atropine and propranolol. The extract also caused significant dose and time dependent inhibition of K⁺ induced contraction on guinea pig aorta.

Conclusion: *M. stenopetala* has blood pressure lowering effect substantiating the use of the plant in traditional medicine.

Key words: *Moringa stenopetala*, hypotensive, aqueous extract

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Introduction

Moringa stenopetala Baker f. (*Moringaceae*)¹ is commonly grown in Southern parts of Ethiopia at an altitude of 1 100 to 1 600m above sea level. The leaves of *M. stenopetala* are cooked and eaten as vegetables and the leaves and roots are used to treat malaria, hypertension and gastrointestinal problems². It has been reported that *M. stenopetala* leaves and roots showed antitrypanosomal activity³. The antispasmodic effects of the leaves on smooth muscle tissues⁴ and antibiotic properties of the seeds^{5,6} were also reported previously. The isolated fractions from the aqueous extract of this plant were also observed to have hypoglycemic and antidiabetic effect in mice⁷. To further substantiate the claimed use of the plant, the present study reports the hypotensive effect

of the aqueous extract of the leaves of *M. stenopetala* both in *in vivo* and *in vitro* experiments.

Methods

Plant material collection, extraction and laboratory animal preparation

Fresh and uncrushed leaves of *M. stenopetala* were collected from Arbaminch about 505 kms south of Addis Ababa, at an elevation of 1 285 meters above sea level and between 6°30'N to 6°08'N latitude and 37°33'E to 37°37'E longitude Ethiopia during the month of December 2005. The fresh leaves were placed in Erlenmeyer flasks and placed in continuous hot water orbital shaker (GFL, model 3 020, 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.

Male guinea pigs were kept in the animal house of the Faculty of Medicine, Addis Ababa

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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*. The research was conducted in accordance with international accepted principles of laboratory animal use and care.

In vivo experiment on Guinea pigs

The *in vivo* experiment was carried out according to the method described by Gilani *et al.*⁸ and Ghayur *et al.*⁹ 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 inserted into the external jugular vein followed by saline flush (0.2 ml). Pulse pressure was obtained by subtracting diastolic blood pressure (DBP) from systolic blood pressure (SBP) 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. To study the 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.

In vitro experiment preparation on guinea-pig aorta

The *in vitro* experiment was conducted according to the method described by Ghosh¹⁰, Gilani *et al.*¹² and Ghayur *et al.*¹⁰. 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 a short strip (2 to 4 cm) that was prepared to be used for the experiment. The strip 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.

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 15 minutes. 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).

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 tests using SPSS 10 statistical software package. The values at $p < 0.05$ were regarded as statistically significant. All data were expressed as mean \pm standard error of the mean (M \pm SEM).

Results

The effect of aqueous leaf extract of *M. stenopetala* on SBP, DBP, pulse pressure, MABP in anesthetized guinea pigs ($n = 12$, $p < 0.05$) is shown in table 1. The *iv* administration of aqueous leaf extract of *M. stenopetala* at doses of 10 mg/kg, 20 mg/kg, 30 mg/kg and 40 mg/kg showed statistically significant reduction of SBP, DBP and MABP. On the other hand, table 1 also shows that the decline in pulse pressure was statistically significant only at the dose of 40 mg/kg. A typical tracing of the *in vivo* experiment is shown in figure 1. The duration of action was longer; that is, about 10 minutes as compared to the one 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*.

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 2. 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 (data not shown). 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 1: The effects of *iv* infusion of different doses of *M. stenopetala* aqueous extract in anaesthetized guinea pigs on systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure and mean arterial blood pressure (MABP)

Doses of <i>M. stenopetala</i>	SBP	DBP	PP	MABP
control	81.91 \pm 2.73	53.16 \pm 2.70	28.74 \pm 2.29	62.74 \pm 2.49
5mg/kg	76.69 \pm 2.59	48.22 \pm 2.40	28.46 \pm 2.43	57.71 \pm 2.18
10mg/kg	60.89 \pm 3.95**	38.73 \pm 3.19**	22.15 \pm 2.44	46.12 \pm 3.27**
20mg/kg	54.65 \pm 3.73**	32.62 \pm 2.51**	22.02 \pm 2.59	39.97 \pm 2.71**
30mg/kg	47.79 \pm 3.02**	24.89 \pm 2.13**	22.89 \pm 2.44	32.52 \pm 2.18**
40mg/kg	39.26 \pm 3.10**	20.79 \pm 1.56**	18.47 \pm 2.37**	26.94 \pm 1.89**

Data are expressed as Mean \pm SEM (n=12). ** P < 0.05

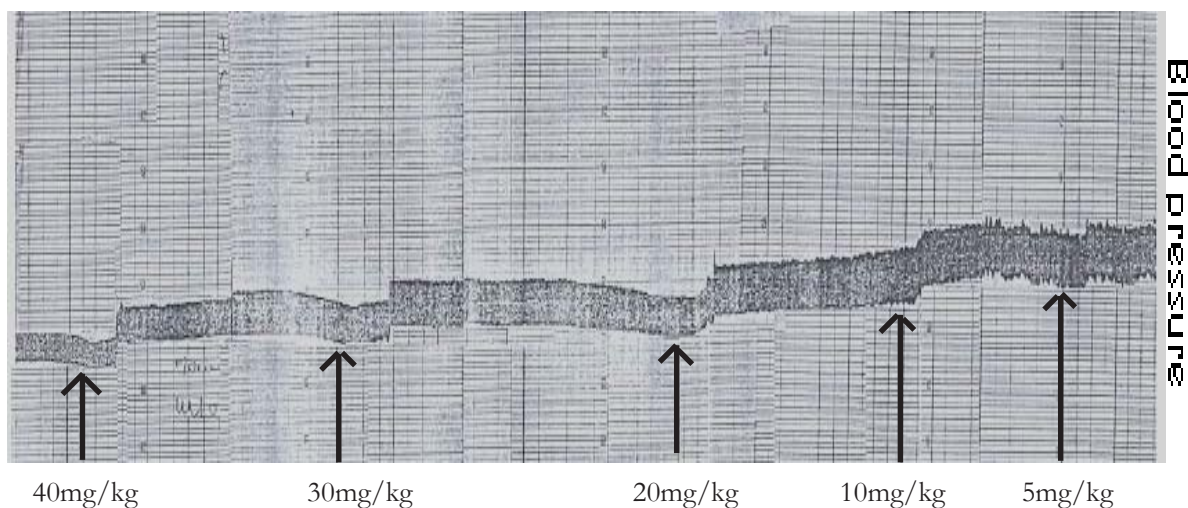


Figure 1: The hypotensive effect of *M. stenopetala* crude extract in anaesthetized guinea pig. Arrows indicate the point at which the test extracts were administered

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 2. 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 (data not shown). 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 percent inhibition of high-K⁺ (80 mM)-induced contraction by aqueous leaf extract of *M. stenopetala* on isolated aorta of guinea pig (n = 10)

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**	0.001
7 mg/ml	2.9 ± 2.9**	95.56 ± 3.14**	0.001

Data are Mean ± SEM, ** p value < 0.05

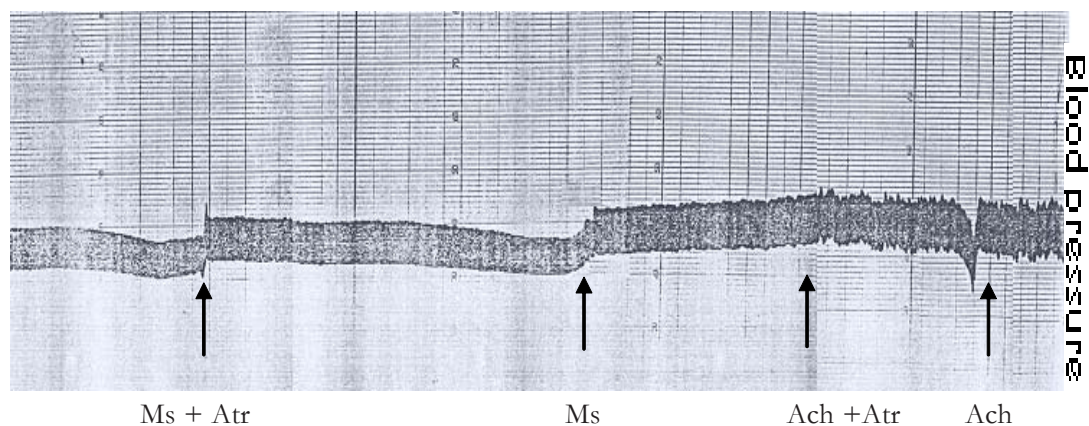


Figure 2: The non-muscarinic mechanism of action of *M. stenopetala* in inducing hypotensive effect in anaesthetized guinea pig. Ms = *Moringa stenopetala*; Atr = Atropine; Ach = Acetylcholine

The aqueous leaf extract of *M. stenopetala* did not exhibit any vasoconstrictor activity on the resting baseline of guinea pig aorta. The extract was then tested on high-K⁺ (80 mM) induced contraction. The results (figure 3) are expressed as the percentage contraction, taking the control K⁺-induced contraction before the application of the test extract as 100%. The test extract showed statistically significant dose dependent inhibition of high- K⁺ induced contraction at concentrations of 5mg/ml (n = 10, p = 0.001), 6 mg/ml (n = 10, p < 0.001) and 7 mg/ml (n = 10, p < 0.001). The inhibition of

contraction was within 15 minutes contact time after the application of each dose of the test extract. Thus, the inhibition of contraction was also time dependent (figure 4). The relaxant effect of the test extract was reversible as the tissue regained its spontaneous activity at least within two hrs after repeated washout.

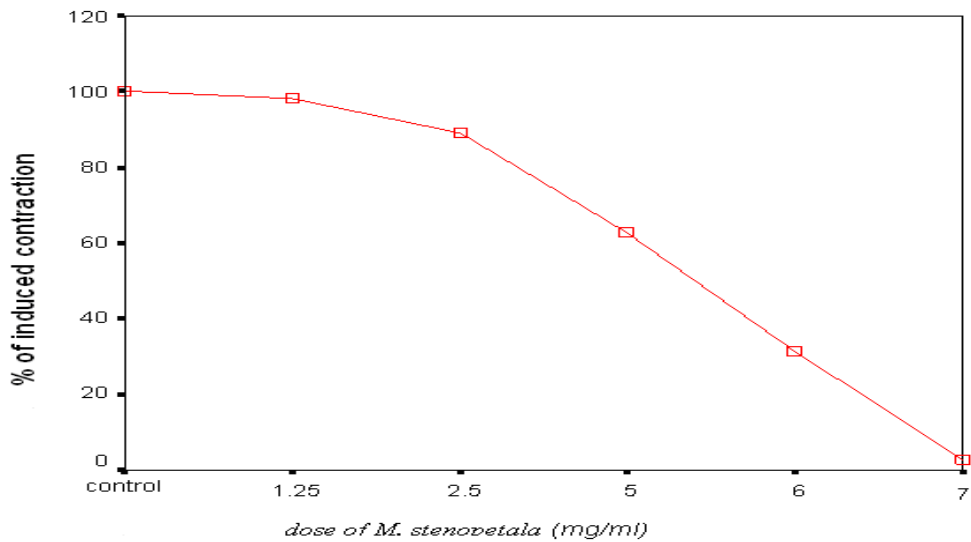


Figure 3: Dose response curve showing the inhibitory effect of aqueous leaf extract of *M. stenopetala* on high K⁺(80 mM)-induced contraction in isolated aorta of guinea pigs and values are % of induced contraction Mean \pm S.E.M

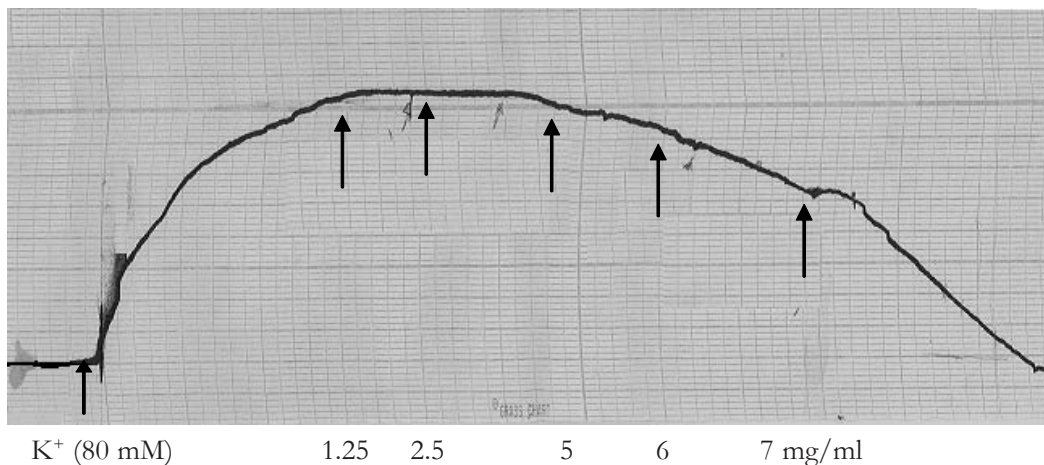


Figure 4: Concentration and time dependent inhibitory responses of aqueous leaf extract of *M. stenopetala* in isolated guinea pig aorta. Arrows show the addition of K⁺ (80 mM) and *M. stenopetala*

Discussion

An *iv* administration of aqueous extract of *M. stenopetala* showed a fall in SBP, DBP and MABP with significant fall in pulse pressure only at higher dose. This result is in agreement with previous *in vivo* hypotensive studies on leaves of *M. oleifera* in normotensive wistar rats¹³. 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^{14,15}. It was also noted by Gilani *et al.*¹² that the pure compounds isolated from *M. oleifera* caused dose dependent fall in SBP, DBP and heart rate in anaesthetized normotensive wistar rats. In another similar study, it was described that the aqueous extract of stem bark from *M. oleifera* produces a dose dependent hypotensive effect in anaesthetized mongrel dogs with a maximum effect at 20 mg/kg¹⁶.

Acetylcholine at a dose of 1 μ g/kg produced a considerable drop in blood pressure. This is in

agreement with the study made by Gilani *et al*² and a study done in anesthetized dogs¹⁶. Thus, the test extract of the present study mediates its hypotensive effect through mechanism(s) independent of muscarinic receptor activation. Similarly, the pure compounds isolated from leaves of *M. oleifera* did not cause contraction of guinea pig ilea unlike acetylcholine, ruling out the involvement of muscarinic receptor activation¹². *M. oleifera* 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 extract¹⁶.

On the other hand, adrenaline produced a considerable rise in blood pressure, and pretreatment of the animals with propranolol 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 evident from this study that the crude extract of leaves of *M. stenopetala* does not utilize the adrenergic mechanism because blocking the adrenergic mechanism with propranolol 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 *in vivo* study of the aqueous extract of *M. oleifera* on anesthetized dogs that produced dose dependent hypotensive effect and the response was not altered by propranolol and pheniramine¹⁶. Therefore, the hypotensive effect of *M. stenopetala* observed in the present *in vivo* study may be attributed to a direct action of the extract on the vascular system.

The present *in vivo* hypotensive property of the extract of *M. stenopetala* is substantiated by *in vitro* investigation on the isolated aorta of guinea pig where the test extract inhibited high K⁺-induced contraction. As noted by Gilani *et al.*⁸, K⁺ at high doses (>30mM) is known to cause smooth muscle contractions through opening of voltage dependent slow Ca²⁺ channels, thus allowing influx of extracellular Ca²⁺ causing a contractile effect. The percent dose-dependent inhibition of K⁺-induced contraction in isolated aortic preparation (table 2 and figures 3 & 4) 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 rabbit¹².

In the present study, the 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. As shown in other studies calcium channel blockers inhibit concentration dependent contraction induced by high K⁺^{10,17}. Thus, in this study the dose-dependent inhibition of high K⁺-induced contraction by *M. stenopetala* in isolated guinea pig aortic tissue preparation may be as a result of restricted Ca²⁺ entry through voltage-dependent slow calcium channels.

The *in vitro* study of *M. stenopetala* is also supported by previous *in vitro* experiments. The crude extract of the leaves of *M. stenopetala* resulted in time and concentration dependent decline in acetylcholine induced contractions of both guinea pig ileum and mouse duodenum tissues⁴. The extract also abolished rhythmic spontaneous contractions of both tissue preparations⁴. 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.

Conclusion

The results of this study demonstrated that the intravenous administration of fresh leaf extract of *M. stenopetala* exhibited blood pressure lowering effect in normotensive anaesthetized guinea pigs. The mode of action is 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 a non-selective blocker of β-

adrenergic receptors. In tissue preparation, the crude leaf extract of *M. stenopetala* exhibited vasodilatation effect on high K⁺-induced contraction of isolated aorta that may be acting through blockade of the voltage sensitive Ca²⁺ channels.

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