

The effects of *Moringa stenopetala* on blood parameters and histopathology of liver and kidney in mice

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Abstract

Background: *Moringa stenopetala* and related species are commonly used in folk medicine for various human diseases such as antimalarial, antihypertensive, antidiabetic and as antispasmodic.

Objective: The aim of the study is to evaluate the effects of aqueous extract of *M. stenopetala* on blood parameters, and histopathology of liver and kidney in experimental mice.

Methods: Fresh leaves of *M. stenopetala* were collected from Arbaminch area, Southwest Ethiopia, in November 2005. The leaves were dried and extracted with water. Three month-old Swiss albino male mice, which were kept under uniform laboratory conditions, were randomly divided into four groups (one group of controls and three experimental). (The control group was orally given 0.5 ml of distilled water, and groups II, III and IV were given the aqueous leaf extract of *M. stenopetala* using intragastric tube to achieve the required doses of 600, 750 and 900 mg/kg body weight, respectively once a day at 24 hours intervals for six weeks and then sacrificed). Blood sample was collected from each mouse and examined for hematological and biochemical parameters. Liver and kidney were removed, stained and examined for histopathological profiles. The effects of treatment with aqueous extract of *M. stenopetala* on hematological, biochemical and histopathology features were compared with control group following standard procedures.

Results: Mice treated with 900 mg/kg of the extract per kg of body weight showed a significant increase in body weight compared to the controls ($P=0.014$). Neither a significant change in the weight nor in histopathology of liver and kidney were observed in the animals treated with aqueous extract of *M. stenopetala* compared to those of the controls. Serum glucose level ($P=0.034$) and serum cholesterol level ($P=0.016$) decreased significantly after six weeks treatment.

Conclusion: The aqueous leaf extract of *M. stenopetala* is shown to increase body weight and reduce serum glucose and cholesterol level in mice. This indicates nutritional and medicinal values, but we cannot yet recommend its therapeutic use before more and complete studies are done. [*Ethiop J Health Dev.* 2011;25(1):51-57]

Introduction

Moringa stenopetala, a smooth barked deciduous tropical plant, is a traditional medicinal and nutritional plant in Ethiopia (1). It is widely distributed in the southwestern part of Ethiopia at an altitude range of about 1100 to 1600 meters. The major growing areas are Arbaminch, Negelle and Wellayta Sodo. *M. stenopetala* is commonly called *Shiferaw* in Amharic (2). *M. stenopetala* and related species (*M. oleifera*) are commonly used in folk medicines as antimalarial, antihypertensive, against stomach pain, antidiabetic, anticholesterol, antispasmodic and to expel retained placenta during birth (1 - 6).

The active constituents in the leaves of *M. stenopetala* and related species such as *M. oleifera* (Behen) are glucosinolates; e.g., 4-(alpha -L-rhamnosyloxy) benzyl glucosinolate which yield 4-(alpha-L-rhamnosyloxy) benzyl isocyanate following enzymatic degradation with myrosinase. Phenol carboxylic acids and fatty acids including oleic acid (60 to 70%), palmitic acid (3-12%)

stearic acid (3-12%) as well as eicosanoic acid and lignoceric acid in addition to mustard oil are other constituents. The antimicrobial effect of *M. stenopetala* leaves and of related species is related to the secondary metabolite constituents (7, 8).

Documentation on the use of *M. stenopetala* in the southern regions of Ethiopia has shown that the leaves of *Shiferaw* are cooked and eaten, for example, with the traditional *kurkufa* (cereal preparation from maize and sorghum). The people of Konso and the surrounding communities in southern Ethiopia rely on the plant both as food and to treat various ailments (2, 9). Fresh leaf extracts of *M. stenopetala* showed some oxytocic-like activity on guinea-pig ileum and mouse uteri (1). To date, the effect of *M. stenopetala* aqueous leaf extract on blood parameters and on the histopathology of liver and kidney have not been studied. Therefore, the aim of the present study is to evaluate the effects of *M. stenopetala* on some

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blood parameters, and histopathology of liver and kidney in laboratory-bred mice.

Methods

The study was conducted in the Department of Anatomy, Faculty of Medicine and Akililu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University (AAU).

Plant Material Collection and Preparation

Fresh leaves of *M. stenopetala* were collected from Arba Minch area, about 500 km south of Addis Ababa, Ethiopia, in November 2005. Plant sample was identified by the National Herbarium of Addis Ababa University. The leaves were cleaned from any extraneous materials, dried at room temperature and ground to powder. The powder was mixed with water in Erlenmeyer flasks and placed in an orbital shaker at room temperature for 24 hours. The mixture was then filtered with cotton and Whatman filter paper (15.0 cm size). The filtrates were freeze-dried in a lyophilizer to yield a crude extract. From 388 g dry leaf, which was dissolved in a total of 1940 ml of distilled water, 40 g (10.31%) of crude extract was obtained. The crude extract was kept in a refrigerator at -20°C until used.

Experimental Animals and Treatment

Twenty adult (3 months old) Swiss albino male mice weighing 25-27 g were used. They were bred in the animal house of ALIPB, Addis Ababa University by placing one male to two female mice in a cage. They were kept under uniform laboratory conditions and exposed to 12 hrs of light and 12 hrs of darkness. The animals were provided with free access to pellets and allowed to drink tap water *ad libitum* throughout the duration of the experiment. The mice were randomly divided into four groups with each group consisting of five mice. Group I served as a control and each mouse was orally administered with 0.5 ml of distilled water. Groups II, III and IV were given the extract to attain the required doses of 600, 750 and 900 mg/kg body weight respectively, once a day at 24 hours intervals for six weeks. The standard WHO procedure for evaluation of herbal medicine for toxicity studies was adopted (10). The doses were selected based on the results of preliminary experiments. The extract was administered with intragastric tube according to the animal's body weight. The animal's weight was recorded at the beginning and end of the experiment.

Hematological and Biochemical Analysis

With light ether anesthesia, blood samples (2.0-3.5 ml) were withdrawn by cardiac puncture. Hematological analysis for white blood cell count, red blood cell count, platelet count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration and lymphocytes was made using a hematology analyser (Sysmex KX-21N Auto Hematology Analyzer, KOBE, JAPAN). Glucose, urea, uric acid, cholesterol, triglycerides and creatinine

were measured using an autoanalyser (Autoanalyser 900S^{plus} Human, Germany). Sodium, potassium, and chloride ions were determined by an electrolyte analyzer (9180 Electrolyte Analyzer, Roche, Germany). Total protein was analyzed by refractometry (Refractometry, American Optical Company, U.S.A).

Liver and Kidney Weights

Immediately after the blood withdrawal the mice were sacrificed and dissected. Liver and kidney were removed and weighed on a microbalance sensitive to 0.001mg (Precisa 125A, Switzerland) and recorded. Data were expressed per 100 g body weight.

Light Microscopic Preparation

For light microscopic preparation, the whole of the right kidney and the lower one centimeter long part of the right lobe of the liver in coronal and transverse pieces, respectively, were dissected, fixed in 10% formalin for 24 hours, dehydrated in a graded ethanol series, cleared in xylene, infiltrated and embedded in paraffin. Thin sections (5 µm) were cut using a microtome (Leica RM 2125, Leica Microsystems Nussloch GmbH, Germany) and stained with hematoxylin and eosin. The preparation was examined under light microscope (Leitz, Wetzlar, Germany). Photomicrographs of selected samples of the liver and kidney tissue were then taken with TMX 200 film under x10, x25 and x40 objective using a Leitz Dialux 20 Wild Photo Automat MPS 51. Following the evaluation of the control animals, the liver and kidney tissue in the remaining groups were evaluated blind to treatment.

Statistical Analysis

All the values in the test are presented as mean and standard error of the mean (mean ± SEM). Statistical differences between the means of various groups were evaluated by one-way analysis of variance (ANOVA) followed by Student's t-test using SPSS version 13 program. P-values <0.05 were considered significant.

Results

Mice treated with 900 mg of the extract per kg of body weight showed significant increase in their body weight compared to the controls (P=0.014). However, there was no significant change in the body weight of mice treated with doses of 600 and 750 mg/kg when compared to the control groups. No significant change in the weight of liver and kidney was observed in the entire group (Table1).

There was no significant difference in the hematological composition of the blood parameters between the control group treated with 600 mg/kg, 750 mg/kg and 900 mg/kg (P>0.05) (Table2). As shown in Table 3, glucose (P=0.034) and cholesterol (P=0.016) decreased significantly after treatment with 600 mg/kg, 750 mg/kg and 900 mg/kg *M. stenopetala* compared to that of the control group.

Table 1: **Body and organ weights of mice after treatment with *M. stenopetala* extract**

Group	Treatment (mg/kg/bw)	Initial body weight (g)	Final body weight (g)	Relative organ weight (g/100g bw)	
				Liver	Kidney
I	Control (dis. water)	26.38 ± 0.69	28.64 ± 1.95	4.12 ± 0.10	0.71 ± 0.23
II	600	26.46 ± 0.52	32.44 ± 1.05	4.24 ± 0.35	0.74 ± 0.34
III	750	27.14 ± 0.72	33.88 ± 2.79	4.26 ± 0.32	0.77 ± 0.35
IV	900	26.46 ± 0.72	40.90 ± 2.97 [*]	4.34 ± 0.42	0.79 ± 0.41

Values are mean ± SEM. ^{*}P<0.05, N=5/group

Keys: SEM, standard error of the mean; g, gram; bw, body weight; mg, milligram; kg, kilogram; diss., distilled

Table 2: **Hematological analysis of mice after treatment with *M. stenopetala* extract**

Hematological parameters	Control (diss. water)	Treatment groups (mg/kg bw)		
		600	750	900
WBC x 10 ³ /μl	4.40 ± 0.21	5.10 ± 0.23	5.10 ± 0.71	5.66 ± 0.53
RBC x 10 ⁶ /μl	7.91 ± 0.92	8.52 ± 0.49	8.09 ± 0.52	8.55 ± 0.28
Platelets x 10 ³ /μl	396.75 ± 27.02	397.05 ± 12.05	389.07 ± 120.05	399.06 ± 112.67
HCT (%)	48.22 ± 1.58	48.62 ± 1.41	48.44 ± 1.96	48.04 ± 1.25
HGB (g/dl)	10.70 ± 1.00	10.62 ± 0.93	10.50 ± 0.60	10.80 ± 0.32
MCV (fl)	84.10 ± 1.47	82.56 ± 0.88	82.42 ± 0.51	85.78 ± 1.70
MCH (pg)	28.24 ± 0.55	28.20 ± 0.54	28.80 ± 0.22	28.70 ± 0.24
MCHC (g/dl)	32.74 ± 1.42	32.38 ± 1.10	33.44 ± 0.48	32.80 ± 0.39
Lymphocytes (%)	40.74 ± 3.70	39.74 ± 2.95	44.66 ± 3.42	46.18 ± 2.91

Values are mean ± SEM. P<0.05, N=5/group

Keys: WBC, white blood cell count; RBC, red blood cell count; HCT, hematocrit; HGB, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 3: **Biochemical analysis of mice after treatment with *M. stenopetala* extract**

Biochemical parameters	Control (DW)	Treatment groups (mg/kg bw)		
		600	750	900
Glucose (mg/dl)	152.30 ± 47.06	125.40 ± 12.07 [*]	121.80 ± 13.46 [*]	135.40 ± 8.78 [*]
Urea (mg/dl)	52.02 ± 4.83	47.60 ± 2.61	49.20 ± 2.99	49.60 ± 2.31
Creatinine (mg/dl)	0.78 ± 5.83	0.80 ± 5.47	0.80 ± 0.11	0.80 ± 5.48
Total protein (mg/dl)	6.80 ± 0.37	6.52 ± 0.32	6.72 ± 0.32	6.70 ± 0.46
Uric acid (mg/dl)	6.80 ± 0.37	6.52 ± 0.32	6.72 ± 0.32	6.70 ± 0.46
Cholesterol (mg/dl)	134.60 ± 15.09	119 ± 9.10 [*]	118.60 ± 8.50 [*]	113.20 ± 5.07 [*]
Triglycerides (mg/dl)	165.80 ± 26.50	163.40 ± 16.18	164.20 ± 40.76	167.40 ± 39.79
Na ⁺ (mmol/l)	125.80 ± 5.35	128.80 ± 5.30	133.00 ± 4.38	131.20 ± 5.48
K ⁺ (mmol/l)	8.30 ± 0.24	7.36 ± 0.78	7.36 ± 0.62	7.48 ± 0.62
Cl ⁻ (mmol/l)	123.00 ± 2.41	123.60 ± 2.91	121.80 ± 3.07	121.20 ± 3.93

Values are mean ± SEM. ^{*}P<0.05, N=5/group, Key: DW, distilled water

Light microscopic observation with the doses of 600 mg/kg, 750 mg/kg and 900 mg/kg of *M. stenopetala* showed no marked histopathological changes on the livers and kidneys of mice as compared to the control group (Figs. 1 and 2). The liver and kidney histology of the control mice showed characteristic features showing normal central vein lined by endothelial cells, radiating

hepatic cells and hepatic sinusoids (Fig. 1A) and Bowman's capsule lined with outer parietal layer (squamous capsular cells) and inner visceral layer (podocyte cells), urinary space, proximal convoluted tubules, distal convoluted tubules, macula densa, vascular pole, respectively (Fig. 2A).

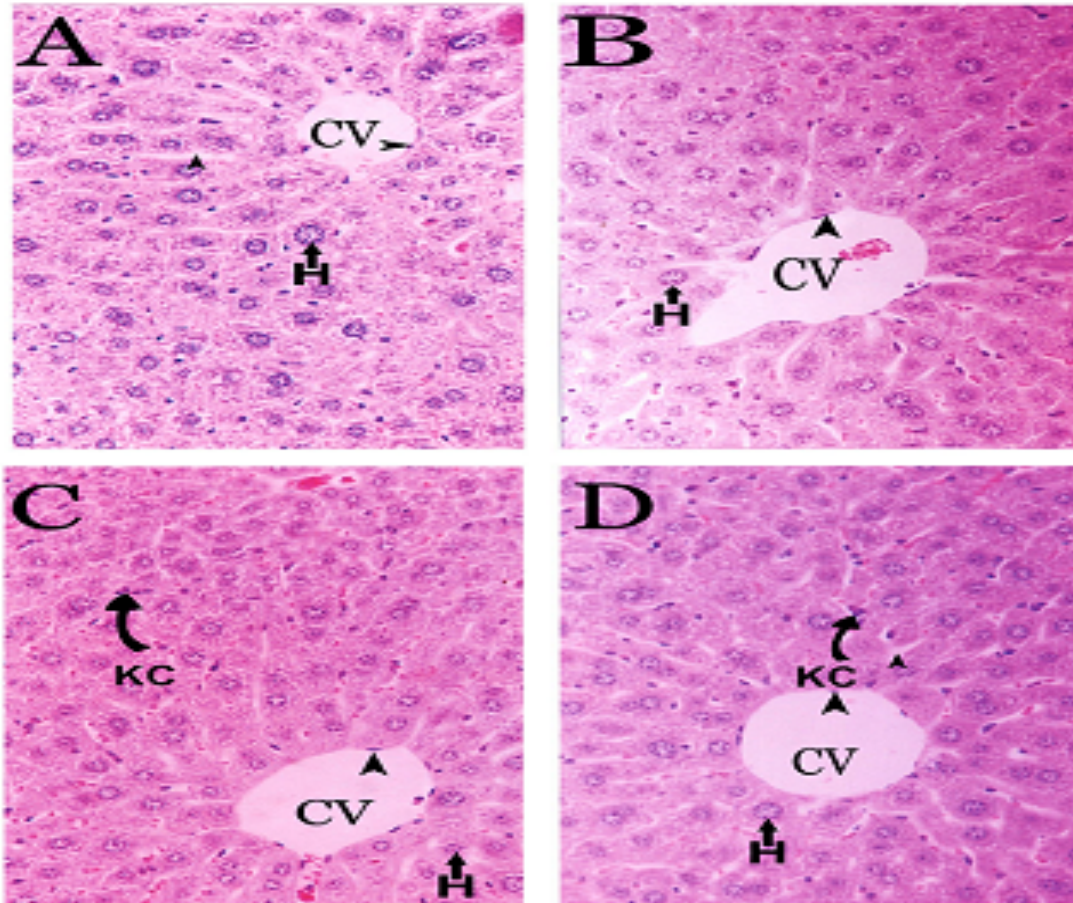


Figure 1: Photomicrographs of liver of untreated (control) mouse liver (A) (H and E, x25). (B) Mice treated with 600 mg/kg bw of *M. stenopetala* leaf extract. (H and E, x25). (C) Mice treated with 750 mg/kg bw of *M. stenopetala* leaf extract. (H and E, x25). (D) Mice treated with 900 mg/kg bw of *M. stenopetala* leaf extract (H and E, x25)

CV= Central vein, H= Hepatocytes, Large arrow head=Endothelial cells, Small arrow head= Sinusoids, KC= Kupffer cells

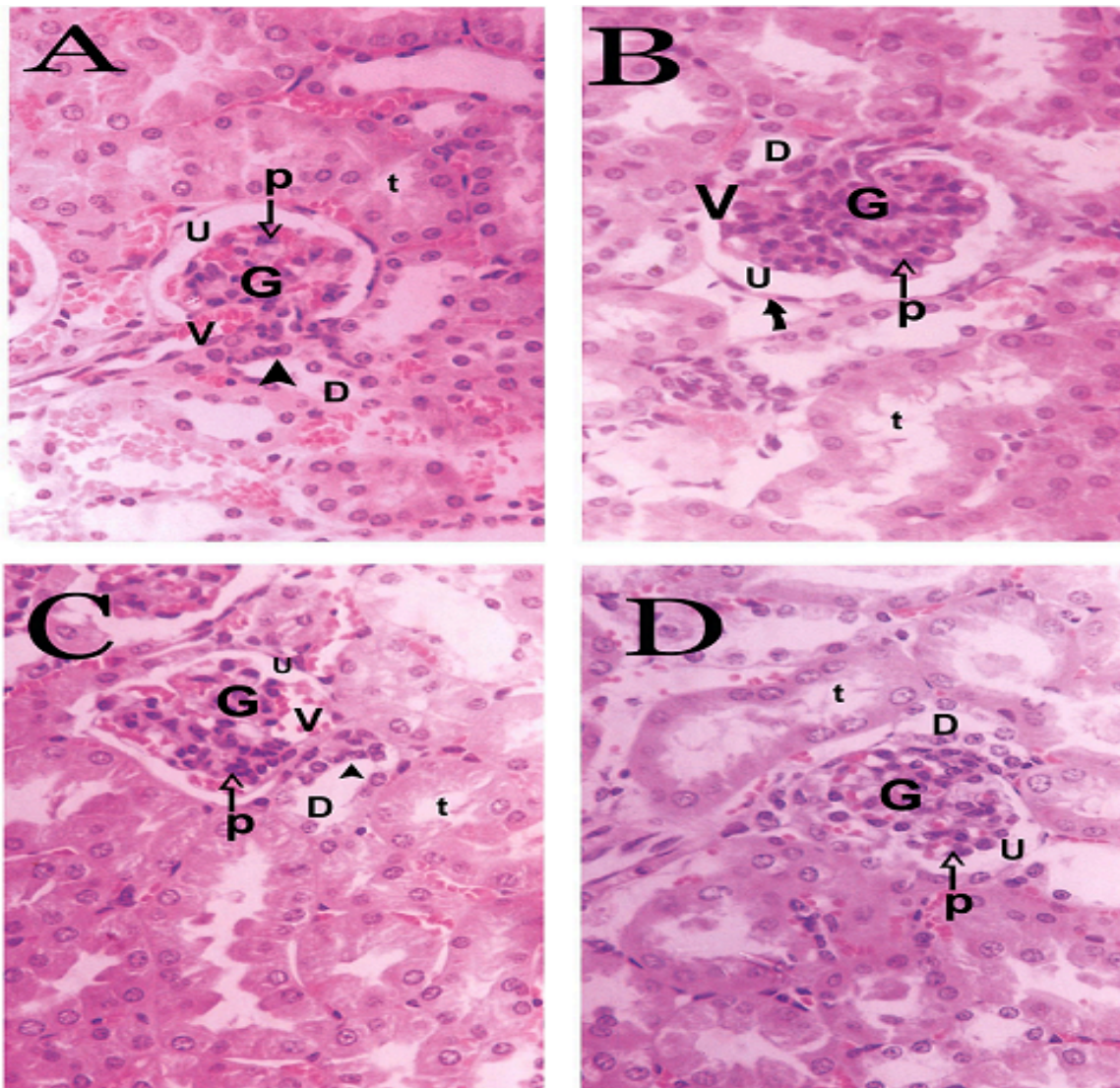


Figure 2: Photomicrographs of kidney of untreated (control) mouse kidney (A) (H and E, x40). (B) Mice treated with 600 mg/kg bw of *M. stenopetala* leaf (H and E, x40). (C) Mice treated with 750 mg/kg bw of *M. stenopetala* leaf extract (H and E, x40). (D) Mice treated with 900 mg/kg bw of *M. stenopetala* leaf extract (H and E, x40). G = Glomerulus, Curved arrow = Squamous cell, p= Podocytes, u= Urinary space, t= Proximal convoluted tubule, D= Distal convoluted tubule, arrow head= Macula densa, V= Vascular pole.

Discussion

In this study, the effects of long-term treatment with *M. stenopetala* aqueous leaf extract on some hematological and biochemical parameters of the blood, and histopathology of liver and kidney were investigated.

Treatment with different doses of the extract was well tolerated by all the animals, as there were no toxic effects observed by direct visual observation of the animals throughout the experiment. There was no death and apparent behavioral changes recorded during the course of the experiment in all treatment groups as compared to the control group. This might suggest the non-toxic effect of the extract (11). The rate of food and water intake of the mice given 600, 750 and 900mg/kg doses of *M. stenopetala* aqueous leaf extract was comparable with those of the control group. In previous experiments it was

established that *M. stenopetala* was not toxic even at higher doses; showing why the plant is edible.

Mice treated with a dose of 900 mg/kg bw of *M. stenopetala* aqueous leaf extract showed statistically significant increase from 26.46 ± 0.72 g to 40.90 ± 2.97 g in their body weight ($P=0.014$). The increase in weight might be due to the fact that *M. stenopetala* is a good source of nutrition. This is in agreement with the previous studies done somewhere else (12, 13), who reported that *M. stenopetala* contains important nutrients such as vitamins, proteins, minerals, carbohydrates and fats. It was also reported that calcium is essential at all ages irrespective of physiological status of individuals and for the normal development of skeletal system (14, 15).

There was a slight increase in liver and kidney weight ratio of mice treated with doses of 600, 750 and 900mg/kg bw as compared to the control group although it was not statistically significant ($P>0.05$). This might be because of the presence of glucosinolate in *M. stenopetala* aqueous leaf extract (7, 16, 17), as has been described that rats fed with diets containing six-individual glucosinolates produce small increase in the weights of liver and kidneys.

In this study, blood glucose levels of mice treated with 600, 750 and 900mg/kg bw *M. stenopetala* decreased significantly from 152.30 ± 47.06 to 125.40 ± 12.07 , 121.80 ± 13.46 and 135.40 ± 8.78 , mg/dl respectively ($P=0.034$). This is in agreement with the findings of Jaiswal *et al.* (18), who reported that blood glucose level decreased after administration of *M. oleifera* aqueous leaf extract to rats. This may suggest that *M. stenopetala* may have an insulin-like effect on peripheral tissues either by promoting glucose uptake and metabolism or by inhibiting gluconeogenesis. It is likely that the aqueous extract of the leaves has some effect of increasing the tissue utilization of glucose (19, 20) by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues (21).

Similarly, mice treated with doses of 600, 750 and 900 mg/kg bw of *M. stenopetala* aqueous leaf extract had significantly decreased their blood cholesterol level from 134.60 ± 15.09 to 119.60 ± 9.10 , 118.60 ± 8.50 and 113.20 ± 5.07 , respectively, in a dose dependant-manner ($P=0.016$). This is in agreement with the study reported for *Moringa oleifera* leaf extract that showed hypocholesterolemic activity (22, 23). It was reported that the mechanism of cholesterol reduction is thought to be through the lowering of plasma concentrations of LDL by B-sitosterol, the bioactive phytoconstituent isolated from *Moringa oleifera* (22, 24, 25). Therefore b-sitosterol or a similar constituent in the leaves of *M. stenopetala* may be responsible for this effect as well. However, the extract did not show significant change ($P>0.05$) on urea, creatinine, total protein, uric acid, triglycerides, Na^+ , K^+ and Cl^- as compared to the control group. This might be an indication of the non-toxic action of *M. stenopetala* on the body metabolism of the mice.

In addition, mice treated with all the doses of *M. stenopetala* aqueous leaf extract did not show any morphological changes in the liver cells. This is in agreement with the *in vitro* cytotoxicity study done by Mekonnen *et al.*, (26), who reported that an aqueous extract of leaves from *M. stenopetala* on hepatocytes did not affect cell viability. Furthermore, no significant histopathological changes were observed in the kidneys of the mice treated with all doses. This might be substantiated by the results of biochemical parameters of the blood, such as urea and creatinine which are the main indicators of kidney damage (27-31). Since none of these pathological symptoms were observed in all the mice

investigated, *M. stenopetala* leaf extracts might not have adversely affected the kidneys.

In conclusion, this study showed that treatment of mice with the aqueous leaf extract of *M. stenopetala* showed that there was body weight increase in the mice and that there were no after adverse effects observed. From previous work on *M. stenopetala* showing the availability of important nutrients (12, 13) and medicinal value (1, 2), further studies are warranted to fractionate the active principle and find out the mechanism(s) of action of *M. stenopetala* leaf extract on blood glucose and cholesterol levels in animal models to ascertain its therapeutic importance.

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