

Full Length Research Paper

Effects of crude extracts and fractions of *Moringa stenopetala* (Baker f) Cufodontis leaves in normoglycemic and alloxan-induced diabetic mice

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***Moringa stenopetala* leaves are traditionally employed for the treatment of diabetes mellitus in Ethiopia. Previous studies confirmed that the aqueous crude extract of the leaves of this plant and fractions isolated from these extracts have hypoglycemic and antihyperglycemic effects. Whether the active components are also present in the ethanol extract and its fractions, however, have not yet been investigated. The aim of this study was to evaluate the effects of *M. stenopetala* leaves ethanol crude extracts and its fractions in healthy and alloxan induced diabetic mice. For single dose hypoglycemic test, normoglycemic mice were given 300 mg/kg of ethanol extract, aqueous extract, petroleum fraction, chloroform fraction, butanol fraction, aqueous residue, glibenclamide (0.66 mg/kg) and distilled water (10 ml/kg) intraperitoneally (i.p). Similarly, for single dose anti-hyperglycemic study, alloxan induced diabetic mice were given the same dose of test substances. For the repeated dose anti-hyperglycemic investigation, diabetic mice were given 300 mg/kg of ethanol extract, aqueous extract, chloroform fraction, butanol fraction, glibenclamide (0.66 mg/kg) and distilled water (10 ml/kg) through i.p route. The results of single dose hypoglycemic and antihyperglycemic studies indicated that extracts, chloroform fraction, butanol fraction and aqueous residue reduced blood glucose level significantly within the treatment period. The repeated dose of both extracts as well as chloroform and butanol fractions also reduced blood sugar level of diabetic mice significantly. The present findings, therefore, hints that the ethanol extract as well as its chloroform and butanol fractions might also have active principles responsible for antidiabetic effect which confirms the claim for the traditional antidiabetic use of *M. stenopetala*.**

Key words: *Moringa stenopetala*, diabetes mellitus, hypoglycemic effect, antihyperglycemic effect, normoglycemic, alloxan.

INTRODUCTION

Moringa stenopetala (Baker f.) Cufodontis belongs to the family *Moringaceae* that is represented only by a single gene *Moringa*. The genus is represented by 14 species to which *M. Stenopetala* (Baker f.) Cufodontis belongs. It is a branched tree that grows 6 to 10 m tall, thick at base

bark with white to pale gray or silvery coloration (Abuye et al., 2003).

It grows abundantly in Southwestern Ethiopia where the leaves are eaten as vegetable. The species is known by different vernacular names such as "*Shiferaw*" in Amharic, "*Aleko*" in Wollaytegn and Gamugna and "Cabbage tree" in English. It grows widely at an altitude range of 1000 to 1800 m. *M. stenopetala* (Baker f.) Cufodontis is a multipurpose plant. The leaves are one of the best vegetable foods that can be found in the locality

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(Abuye et al., 2003). The flowers are good nectar sources for honey; the seeds are used in clearing muddy water; the wet or dried root part chopped and mixed with water is also used to treat malaria (Mekonnen and Gessesse, 1998).

It has been reported that *M. stenopetala* (Baker f.) Cufodontis has medicinal value for stomach pain and to expel retained placenta following birth (Mekonnen, 1999), antileishmanial activity (Mekonnen and Gessesse, 1998), antitrypanosomal activity (Mekonnen et al., 1999) and antimicrobial activities (Biffa, 2005). Study made on the crude aqueous extract of the leaves of *M. stenopetala* (Baker f.) Cufodontis on rabbit (Mekonnen et al., 1997) and mice (Mussa et al., 2008) indicated the hypoglycemic activity of the plant. Moreover, the fractions isolated from the aqueous extract of the plants were also shown to have both hypoglycemic and antihyperglycemic effects (Mussa et al., 2008). The antidiabetic effects of the ethanol extract and its fractions, however, were not investigated. The aim of this study was, therefore, to evaluate the hypoglycemic and antihyperglycemic activity of ethanol extract of *M. stenopetala* leaves as well as its chloroform, butanol and petroleum fractions.

MATERIALS AND METHODS

Plant material collection

Leaves of *M. stenopetala* (Baker f.) Cufodontis were collected from Arbaminch town, 502 km away from Addis Ababa, in the Garden of Arbaminch Health Science College on 07 July, 2008. The plant material was authenticated by the National Herbarium, Department of Biology, Addis Ababa University, where a voucher number 001 was given and specimen of *M. stenopetala* (Baker f.) Cufodontis was deposited.

Aqueous extract preparation

The powdered leaves of *M. stenopetala* (50 g) was soaked with warm distilled water and agitated for one day using a shaker. Then, the macerate was filtered with muslin cloth four times and was lyophilized. The aqueous extract yielded 27.86 % (13.93 g) and was stored in refrigerator for subsequent experiments (Salawu et al., 2009).

Ethanol extract preparation

Air dried powdered leaves of *M. stenopetala* (400 g) were soaked with 70% (v/v) ethanol for three days, successively. This was done three times, and was filtered with Whatman No. 1 filter paper and the filtrates were then mixed. The combined filtrates were concentrated using Rota Vapor (Büchi Rota Vapor vac R-500, Switzerland). The aqueous residues were dried in a steam bath at 40°C and kept in desiccators for future use. The ethanol extract yielded 20.42% (81.66 g) and was stored in refrigerator for subsequent experiments (Babu et al., 2003).

Solvent-solvent separation from the ethanol extract

Ten grams of the ethanol extract of the leaves of the plant was

dissolved in 100 ml of warm distilled water, and solvent-solvent separation was done according to Samsam-Sharjat (1992) method. The dissolved ethanol extract was separated in a separatory funnel with petroleum ether (40 to 60°C), chloroform and n-butanol, successively until extracting solvents became colorless. In all cases of separation, 150 ml of the solvents were used. After completing the separation process, the solvents were recovered by Rota Vapor. The separates and the aqueous residue were dried by steam bath at 40°C, and were kept in the refrigerator till when they would be used.

Animal preparations

Swiss albino mice (20 to 30 g) of both sexes were used for pharmacologic evaluations. The animals were obtained from the Animal House, Department of Pharmacology, Faculty of Medicine, Addis Ababa University. The institutional review board or ethical committee of the faculty approved the use of animals adhering declaration of Helsinki. All the animals used for this study were kept and maintained under laboratory conditions of temperature ($22 \pm 3^\circ\text{C}$), humidity and 12 h day-12 h light. They were exposed to free access of food (standard pellet diet) and water *ad libitum*. Prior to experimentation, the animals were acclimatized to the test environment for an hour (Ojewole et al., 2007).

Induction of diabetes in mice

After 16 h fasting, diabetes was induced to the mice by a single intraperitoneal (i.p.) injection of 120 mg/kg alloxan monohydrate dissolved in distilled water. Seven days after alloxan injection, diabetic mice were screened and used for the experiment (Frode and Medeiros, 2008).

Screening for hypoglycemic effect

Healthy 16 h fasted mice were divided into eight groups of five mice each. Extracts and control substances were administered by intraperitoneal injection to all groups. Groups 1 to 6 were given 300 mg/kg of 70% ethanol extract, aqueous extract, petroleum ether fraction, chloroform fraction, butanol fraction and aqueous residue. Groups 7 and 8 served as positive and negative control and they received glibenclamide (0.66 mg/kg) and distilled water (10 ml/kg), respectively. In order to choose the optimum dose causing hypoglycemia, different doses of aqueous ethanol extract (200, 300 and 500 mg/kg body weight) was tested as a pilot study and better effect was observed at 300 mg/kg.

Screening for antihyperglycemic effect

Diabetes was induced in mice with a single intraperitoneal injection of 120 mg/kg alloxan monohydrate dissolved in distilled water after overnight fasting. Seven days after the alloxan injection, diabetic mice were screened and divided into eight groups of five mice each. Same procedure was then used to administer the test substances as well as the standard and the vehicle.

Alloxan induced diabetic mice fasted for 16 h, and were selected and divided into six groups of six mice each for the repeated dose testing. Groups 1 to 4 were administered with 300 mg/kg of 70% ethanol crude extract, aqueous crude extract, chloroform fraction and butanol fraction respectively, daily for 8 days through i.p. route. Groups 5 and 6 served as control and received glibenclamide (0.66 mg/kg) and distilled water (10 ml/kg) daily for 8 days through intraperitoneal route, respectively.

Table 1. Hypoglycemic effects produced by single dose intraperitoneal administration of crude extracts and fractions of *M. stenopetala* in normoglycemic mice.

Group	Treatment	Serum glucose (mg/dl) (min)				
		0 min	60 min	120 min	180 min	240 min
I	Ethanol extract	90.6 ± 3.7	74.6 ± 1.4**	64.8 ± 2.4***	59.4 ± 1.9**	62.8 ± 5.2**
II	Aqueous extract	88 ± 3.6	79.6 ± 1.8	68.8 ± 2.6**	63.6 ± 6.0*	64.4 ± 2.5*
III	Petroleum fraction	94.8 ± 7.9	89.6 ± 1.5	88 ± 2.2	82.4 ± 5.4	85.4 ± 2.4
IV	Chloroform fraction	84.4 ± 3.7	83 ± 4.2	68.4 ± 4.0**	63.8 ± 3.6*	67 ± 3.1*
V	Butanol fraction	91.6 ± 5.5	88.2 ± 4.3	82.2 ± 2.5	64.8 ± 3.4*	63.2 ± 3.2*
VI	Aqueous residue	87.2 ± 2.7	78.6 ± 1.2	77.4 ± 2.5	78 ± 3.9	74.4 ± 3.3
VII	Standard	87 ± 3.9	71.2 ± 2.9***	59.4 ± 3.2***	53 ± 4.2***	49.6 ± 4.9***
VIII	Control	90.8 ± 2.5	90.6 ± 1.8	88 ± 5.6	86 ± 3.8	87.6 ± 7.1

*, P < 0.05, **, P < 0.01 and ***, P < 0.001 as compared to the control. Results are means ± S.E.M. of n = 5. Dose of extracts and fractions = 300 mg/kg; Dose of glibenclamide = 0.66 mg/kg; Distilled water = 10 ml/kg.

Blood glucose level determination

For acute hypoglycemic and antihyperglycemic tests, blood samples were obtained from the tail vein of normoglycemic and alloxan induced diabetic mice, respectively, and glucose levels were determined at the beginning of the experiment (0 min), as well as at 60, 120, 180 and 240 min after administration using Hemocue Glucose 201⁺ Microcuvettes and Hemocue Glucose 201⁺ analyzer.

Acute toxicity and behavioral pattern studies

Healthy adult albino mice of either sexes that fasted for 16 h were divided into six groups (n = 6) and were administered the ethanol extract at the doses of 10, 15, 20, 30 and 50 g/kg of body weight and water for the control group orally. The animals were then observed continuously for 2 h for the following profiles:

1. Behavioral profile: alertness, restlessness, irritability and fearfulness.
2. Neurological profile: spontaneous activities, reactivity, touch response, pain response and gait.
3. Autonomic profile: defecation and urination.

After a period of 24 and 72 h, they were observed for any lethality (Shirwaikar et al., 2006).

Statistical analysis

Values were expressed as means ± S.E.M. Statistical differences between the treatments and the controls were tested by one-way analysis of variance (ANOVA), followed by Tukey/Kramer multiple comparison tests using the "Graph Pad Instat" statistic computer program. A difference in the mean values of P < 0.05 was considered to be statistically significant.

RESULTS

Effect of single doses of the test substances in normoglycemic mice

Administration of single doses of different extracts and fractions of *M. stenopetala* was found to reduce blood glucose level in normoglycemic mice. The ethanol extract

significantly lowered blood glucose level as compared to the control at 60, 180 and 240 min (P < 0.01) and 120 min (P < 0.001). Both aqueous and chloroform extracts resulted in similar reduction of blood glucose level at 120 min (P < 0.01), 180 min (P < 0.05) and 240 min (P < 0.05) after administration as compared to the control. Butanol fraction also showed significant reduction (P < 0.05) similar to both aqueous crude extract and chloroform fraction starting from 180 min after administration of the test substance. Petroleum and aqueous residue fractions, however, did not show any significant reduction of plasma glucose level within 4 h of treatment (Table 1). The reduction in blood glucose level with all test substances was observed to be more than that with the standard at the doses employed as shown in Table 1.

Effect of single doses of the test substances in alloxan induced diabetic mice

In alloxan induced diabetic mice, single dose of ethanol extract resulted in significant reduction of blood glucose level at 60 min (P < 0.05), as well as 120, 180 and 240 min (P < 0.001) after administration of the test substance. Aqueous extract also showed significant reduction in blood glucose level at 120 min (P < 0.01) as well as at 180 and 240 min (P < 0.001). The aqueous residue and chloroform and butanol fractions showed similar pattern of reduction in blood glucose level as that of the ethanol extract as shown in Table 2. The reduction in blood glucose level with all test substances was observed to be more than that with the standard at the doses employed as shown in Table 2.

Effect of repeated doses of the test substances in alloxan induced diabetic mice

As shown in Table 3, repeated intraperitoneal administration of the ethanol extract resulted in significant reduction

Table 2. Effects of a single dose intraperitoneal administration of crude extracts and fractions of *M. stenopetala* in alloxan induced diabetic mice.

Group	Treatment	Serum glucose (mg/dl) (min)				
		0	60	120	180	240
I	Ethanol extract	165.8 ± 5.0	127.8 ± 2.2*	101.4 ± 5.3***	100.6 ± 2.4***	85.6 ± 5.4***
II	Aqueous extract	146.4 ± 2.9	133.8 ± 2.8	115 ± 3.9**	94 ± 3.1***	89 ± 3.5***
III	Petroleum fraction	152 ± 2.8	150.6 ± 4.9	153.4 ± 4.4	138.8 ± 4.6	134.6 ± 2.0
IV	Chloroform fraction	141.8 ± 4.8	127.8 ± 4.0*	108.6 ± 5.6***	90.4 ± 7.2***	80.6 ± 5.6***
V	Butanol fraction	146.6 ± 6.7	129 ± 4.8*	109 ± 2.2***	79.2 ± 3.7***	84.6 ± 3.0***
VI	Aqueous Residue	134 ± 4.6	128.4 ± 3.2*	122.6 ± 3.1*	123.8 ± 2.6*	121 ± 1.8*
VII	Standard	146.6 ± 4.7	123.6 ± 3.4**	82 ± 6.7***	63.8 ± 3.7***	61.6 ± 2.5***
VIII	Distilled water	147 ± 2.8	146.6 ± 2.5	146 ± 2.6	148.2 ± 6.6	139.8 ± 2.8

*P < 0.05, **P < 0.01, ***P < 0.001 as compared to the control. Results are means ± S.E.M. of n = 5. Dose of extracts and fractions = 300 mg/kg; Dose of glibenclamide = 0.66 mg/kg; Distilled water = 10 ml/kg.

Table 3. Effects of repeated doses intraperitoneal administration of crude extracts and fraction of *M. stenopetala* in alloxan induced diabetic mice.

Group	Treatment	Serum glucose (mg/dl) (day)			
		1st day	3rd day	5th day	8th day
I	Ethanol extract	160 ± 4.9	142.2 ± 3.3***	127.8 ± 4.2***	108.5 ± 2.9***
II	Aqueous extract	155.8 ± 3.8	151.7 ± 2.9**	128.8 ± 3.4***	112.3 ± 4.3***
III	Chloroform fraction	165.2 ± 2.9	155.8 ± 2.9	145.5 ± 2.4**	119.8 ± 1.7***
IV	Butanol fraction	170 ± 2.2	158.5 ± 2.6	147.2 ± 2.6**	121.7 ± 3.2***
V	Standard	165.8 ± 5.7	124.3 ± 3.2***	112.2 ± 4.1***	103.7 ± 2.1***
VI	Diabetic control	164.8 ± 5.0	167 ± 1.8	162.5 ± 3.8	167.7 ± 1.5

*P < 0.05, **P < 0.01, ***P < 0.001 as compared to the control. Results are means ± S.E.M. of n = 5. Dose of extracts and fractions = 300 mg/kg; Dose of glibenclamide = 0.66 mg/kg; Distilled water = 10 ml/kg.

in blood glucose level (P < 0.001) starting from the 3rd day after administration. The aqueous extract resulted in significant reduction of blood glucose level on the 3rd day (P < 0.01) and on the 5 and 8th days (P < 0.001) after administration. For both chloroform and butanol fractions, significant changes were observed on the 5th day (P < 0.01) and 8th day (P < 0.001) after test substances administration. The reduction in blood glucose level with all test substances was observed to be more than that with the standard at the doses employed as shown in Table 3.

Acute toxicity of ethanol extract of *M. stenopetala* leaves

The acute toxicity study of the ethanol extract of the leaves of the plant as shown in Table 4 suggests that there was no acute toxicity since there was no observed fatality. Therefore, the oral LD50 of ethanol extract of *M. stenopetala* (Baker f.) in mice was found to be greater than 50 g/kg.

DISCUSSION

Diabetes mellitus is known for many years as a serious complex chronic disorder which has been proved to be non-curable but controllable. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin (Alberti and Zimmet, 1998). Various types of modern drugs and plant medicines are known for their effectiveness in reducing the detrimental impacts that arise from the disease. Previous studies also confirmed the antihyperglycemic actions of different species of plants, such as *Abrus precatorius* L., *Bridelia micrantha* and *Ocimum gratissimum* L. (Deliorman et al., 2005; Sezik et al., 2005).

The hypoglycemic effects of the ethanol extract and its fractions observed in the present study are in agreement with that observed with the aqueous extract (Makonnen et al., 1997; Mussa et al., 2008) and its fractions (Mussa et al., 2008). The more pronounced effect of ethanol crude extract than aqueous extract might be due to its good leaching capacity of the intracellular components of

Table 4. Acute toxicity of ethanol extract of *M. stenopetala* in mice.

Group	Dose (g/kg)	Number of deaths	Death (%)	Probit value
1	10	0/6*	0.0	-
2	15	0/6*	0.0	-
3	20	0/6*	0.0	-
4	30	0/6*	0.0	-
5	50	0/6*	0.0	-
6	Control	0/6*	0.0	-

Values with superscript (*) are not significantly different as compared to the control at $P < 0.05$.

the plant material (leaves) which are inaccessible to water and has the potential to reduce blood sugar level.

Chloroform and butanol fractions also caused almost similar significant reduction in blood glucose level at 180 and 240 min, But the chloroform fraction showed more significant effect at 120 min which might be attributed to the more solubility of the active component(s) in chloroform than butanol. The petroleum fraction and aqueous residue did not produce significant change in blood sugar level. This might be explained in terms of the less solubility of the active component(s) in these solvents.

Many studies showed that the dose of alloxan for inducing diabetes depend on the animal species, route of administration and nutritional status (Frode and Medeiros, 2008). Based on the dose administered, syndromes similar to either type 1 or type 2 diabetes mellitus or glucose intolerance can be induced (Lenzen et al., 1996).

The diabetogenic mechanism of alloxan is through generation of a redox cycle with the formation of superoxide radicals which undergo dismutation to hydrogen peroxide and accumulate in the cytosol simultaneously with calcium to cause rapid destruction of pancreatic β -cells (Szudelski, 2001). The range of diabetogenic dose of alloxan is very narrow. In mice, the intravenous dose proposed to cause type 2 diabetes is in the range of 100 to 200 mg/kg (Machado et al., 2001; Miranda and Muriach, 2006). The dose of alloxan employed to induce type 2 diabetes in this study was 120 mg/kg.

Similar patterns of blood glucose reduction were observed with the extracts and most fractions in the diabetic mice as those in normoglycemic ones. Same explanation could be given for the differential potencies of the extracts and fractions. The chloroform and butanol fractions, however, exhibited similar reduction in glycemia like ethanol extract unlike the aqueous hypoglycemic effects. This might hint that both chloroform and butanol fractions might contain euglycemic active component(s). The duration of antihyperglycemic effect of the crude extracts and the fractions (chloroform and butanol) was extended until the end of the study. The fasting condition of the mice might also have contributed to this effect. The

aqueous residue also showed antihyperglycemic effect which could be due to the presence of euglycemic active component(s).

Antihyperglycemic evaluation on repeated dose administration was performed only with both crude extracts (ethanol and aqueous extract) and fractions, such as chloroform and butanol. These test substances were chosen since they showed significant reduction in blood glucose level in both normoglycemic and diabetic mice with single doses. Ethanol extract resulted in more reduction in blood sugar level than the aqueous extract on the 3rd day while both extracts showed similar level of reduction on the 5 and 8th days. Chloroform and butanol fractions also reduced blood sugar level starting from the 5th day. From this study, it seems that the plant material contains more euglycemic active components.

The observation that the ethanol extract did not show any toxic manifestations or lethality up to the dose level of 50 g/kg indicates the wide safety margin of the extract and supports the traditional use of the leaves of *M. stenopetala* for its food value.

Conclusion

Results of the present study showed that ethanol crude extracts and its chloroform and butanol fractions have both hypoglycemic and antihyperglycemic effects justifying the claimed traditional use of *M. stenopetala* for management of type 2 diabetes mellitus.

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