EFFECTS OF THE CRUDE AQUEOUS EXTRACT AND ISOLATED FRACTIONS OF MORINGA STENOPETALLA LEAVES IN NORMAL AND DIABETIC MICE

Abiy Mussa[*], Eyasu Makonnen[1], Kelbessa Urga[2]

[*]Faculty of Veterinary Medicine, Haramaya University, Haramaya, Ethiopia, [1] Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia, [2] Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia

* For correspondence:
Faculty of Veterinary Medicine, Haramaya University,
P.O.Box 271
E-mail: abiymm@yahoo.com

Summary

The leaves of *Moringa stenopetalla* are traditionally employed for the treatment of diabetes mellitus in Ethiopia. The aim of this study was to evaluate the effect of the aqueous extract and isolated fractions of these plant materials on blood glucose levels in normal and diabetic mice. Non-diabetic and diabetic mice received 500mg/kg of the crude extract or chloroform fraction or n-butanol fraction or aqueous residue fraction or 0.66mg/kg glibenclamide (standard antidiabetic drug) or 10ml/kg distilled water (served as negative control). Diabetes was induced with alloxan. The blood glucose concentration was determined from the tail vein of each animal every 1.5 hours for 6 hours. The oral LD50 of the crude extract was also determined. In the non-diabetic mice, the crude aqueous extract showed significant reduction ($p<0.05$) in blood glucose level 6 hours after its administration; while the chloroform and n-butanol fractions showed significant reduction ($p<0.05$) 3 hours and 4.5 hours after their administration, respectively. The aqueous residue fraction did not significantly change the blood glucose level at any of the observation period. In alloxan induced diabetic mice, the crude aqueous extract reduced the blood glucose level significantly ($p<0.05$) at all periods of observation except at 6 hours after its administration; while the chloroform fraction reduced it throughout the observation period. For both n-butanol and aqueous residue fractions a significant reduction ($p<0.005$) in the blood glucose levels was observed at 1.5 hours of their administration. The present data showed that the crude extracts as well as the n-butanol and chloroform fractions of the leaves of M. stenopetalla have both hypoglycemic and antihyperglycemic effects, the later effect being more pronounced supporting the claim of the traditional use of the plant in diabetes mellitus.

Key words: *Moringa stenopetalla*, aqueous extract, isolated fractions, leaves, mice, hypoglycemic, anti-hyperglycemic

Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level. It could be insulin dependent, where there is absolute deficiency of insulin, and non-insulin dependent, where there is some insulin secretion though insufficient. People use different plants traditionally for the treatment of diabetes mellitus. *Moringa stenopetalla* is one among the many plants used, *M. stenopetalla* belongs to the family Moringacae represented only by a single genus Moringa. There are 14 species of Moringa., one of which is *M. stenopetalla*. This plant is known by different vernacular names like shiferaw in Amharic. This tree grows in Southern Ethiopia and Northern Kenya. The leaves are employed as food (1). The plant has medicinal values for stomach pain and to expel retained placenta following birth (2); antileishmanial activity (3); and antimicrobial activity (4). The previous study on the crude aqueous extract of the leaves of this plant on non-diabetic rabbits revealed the hypoglycemic activity of the plant (5). The objective of the present study was to evaluate the effect of the aqueous crude extract and some isolated fractions of *M. stenopetalla* leaves on diabetic and non-diabetic mice and determine the oral LD50 of the aqueous extract.

Materials and Methods

Plant materials preparation

Leaves of *M. stenopetalla* were collected from Arba Minch town, about 500 km south of Addis Ababa, Ethiopia in June 2006. The plant was identified at the National Herbarium of Ethiopia with a voucher number of 001. The leaves were air dried at room temperature and ground into powder. The powdered leaves were then mixed with distilled water in a ratio of 1:10 and left at room temperature for 24 hours. The extract was then filtered with whatman filter paper (N^o= 1) and lyophilized to get a dry powder. The dry powder was then suspended in distilled water and partitioned successively with chloroform and n-butanol. Each fraction was concentrated to dryness under reduced pressure on a rotary evaporator to give chloroform and n-butanol fractions. The remaining fraction was concentrated as aqueous residue fraction. They were all reconstituted to the required concentrations.

Screening for hypoglycemic effect

Non-diabetic albino mice of both sexes weighing 20-40g and fasted for 16 hours were divided into 6 groups (n=6), and received 500mg/kg crude aqueous extract or chloroform fraction or n-butanol fraction or aqueous residue fraction or standard hypoglycemic drug (0.66mg/kg glibenclamide from Cyprus) or a vehicle (10ml/kg distilled water). Blood was drawn from the tail vein of each animal at 0 time and every 1.5 hours after administration of the test substance or the vehicle or the standard for 6 hours. The blood glucose levels were then determined using a Hemocue Glucose 201 analyzer (6).

Screening for anti-hyperglycemic effect

Diabetes was induced with a single ip injection of 120 mg/kg alloxan monohydrate (Sigma) in distilled water after an overnight fast. Seven days after alloxan administration, the diabetic mice were divided into six groups (n=6). Then the procedure on screening for hypoglycemic effect was repeated.

Determination of oral LD50

Healthy adult albino mice of both sexes were divided in to five groups (n=6) and received the crude extract at different doses by gavage. The mice were then observed for lethality for 24 hours (7).

Statistical analysis

Data were expressed as mean ± SEM and t-test was used to test for the level of significance. $P<0.05$ was considered significant. Percent reduction in blood glucose concentration was calculated with the following formula (8):

$$\frac{(Go - Gt)}{G0} \times 100$$

where Go is blood glucose level before administration
Gt is blood glucose level after administration

Results and Discussion

The crude aqueous extract showed a significant ($p<0.05$) reduction of blood glucose level at 6 hours after its administration (Table 1) in non-diabetic mice. This finding is consistent with the results of the previous study done in rabbits (5). The same table shows that the chloroform and n-butanol fractions reduced the blood glucose concentration significantly ($p<0.05$) starting 3 and 4.5 hours after administration of the fractions, respectively, while no significant reduction was observed with the aqueous residue fraction. The percent reduction in blood glucose level with time also shows the same observation (Table 2). These results could indicate the active component(s) responsible for lowering of glucose level is (are) present in larger concentrations in the organic solvents than in the aqueous solvent.

In diabetic mice, a significant reduction of blood glucose level ($p<0.05$) was observed after administration of the aqueous crude extract at observation periods except at 6 hours (Table 3). The chloroform, n-butanol and aqueous residue fractions significantly reduced the blood glucose level ($p<0.05$) at all observation periods (Table 2). The same observations can also be shown from the percent reduction in blood glucose level with time (Table 4). The blood glucose lowering effect of the n-butanol and aqueous residue fractions seems to be comparable to that of the standard in diabetic mice (Table 2). The effect, however, is much less as compared to the standard in non-diabetic mice. These results indicate the effect is greater in diabetic than non-diabetic mice.

Table 1: Effect of the crude aqueous extract and isolated fractions of the leaves of *M. stenopetalla* in non-diabetic mice

	Blood Glucose Concentration (mg/dl)				
Time	0 hr	1.5 hr	3 hr	4.5 hr	6 hr
Control	89.8±8.9	88.3±8.8	86.8±8.9	86.2±8.4	87.5±8.1
Standard	93.5±2.8	63.2±3.2[a]	41.0±5.1 [a]	38.0±4.5 [a]	43.8±5.6 [a]
Extract	86.4±8.0	86.0±5.4	78.8±6.3	82.8±4.3	67.4±5.9 [b]
Chlor. Fract.	96.0±7.5	90.4±4.3	78.8±4.8 [b]	69.0±6.1	66.0±7.0 [c]
But. Frac.	93.8±6.4	87.6±6.9	80.8±8.1	70.6±5.4 [b]	72.0±2.2 [c]
Aq. Res. Frac.	83.0±4.8	80.6±4.9	79.4±3.7	77.2±2.8	75.0±6.5

a= $p<0.005$, b= $p<0.05$, c= $p<0.01$ compared with initial level of blood glucose (0 hr) in the respective group. Chlor. Fract. = chloroform fraction; But. Frac.= n-butanol fraction; Aq. Res. Frac.= aqueous residue fraction. n=5 for the extract and isolated fractions, n=6 for the control and standard (glibenclamide)

Table 2: Percent reduction in blood glucose levels with aqueous extract and isolated fractions of the leaves of *M. stenopetalla* in non-diabetic mice

Time	0 hr	1.5 hr	3 hr	4.5 hr	6 hr
Control	0	1.7	3.3	4.0	2.6
Standard	0	32.4	56.1	59.3	53.2
Extract	0	0.5	8.8	4.2	22.0
Chlor. Frac.	0	5.8	17.9	28.1	31.2
But. Frac.	0	6.6	13.9	24.7	23.2
Aq.Res.Frac.	0	2.9	4.3	7.0	9.6

Chlor. Frac. = chloroform fraction; But. Frac. = n=butanol fraction; Aq. Res. Frac. = aqueous residue fraction. n=5 for the extract and isolated fractions, n=6 for the control and standard (glibenclamide)

Table 3: Effect of the crude aqueous extract and isolated fractions of the leaves of *M. stenopetalla* in alloxan-induced diabetic mice

	Blood Glucose Concentration (mg/dl)				
Time	0 hr	1.5 hr	3 hr	4.5 hr	6 hr
Control	133.2±6.3	132.3±6.2	131.8±6.4	131.8±6.7	132.8±6.4
Standard	129.0±4.1	116.3±2.2[a]	82.5±3.3[b]	78.0±4.8[b]	75.5±4.8[b]
Extract	131.2±1.5	114.0±2.7[b]	108.8±4.2[b]	119.8±5.0[d]	120.2±7.9
Chlor. Frac.	160.8±4.2	123.6±5.4[b]	124.0±5.6[b]	131.2±6.5[a]	116.8±4.3[b]
But. Frac.	144.2±1.2	81.0±7.3[b]	83.2±5.7[b]	90.0±5.4[b]	91.4±8.4[b]
Aq.Res.Frac.	169.4±4.6	116±4.9[b]	101.6±4.1[b]	106.2±4.5[b]	95.0±3.5[b]

a= $p<0.005$, b= $p<0.0005$, d= $p<0.05$ compared with initial level of blood glucose (0 hr) in the respective group. Chlor. Frac. = chloroform fraction; But. Frac. = n=butanol fraction; Aq. Res. Frac. = aqueous residue fraction. n=5 for the extract and isolated fractions, n=6 for the control and standard (glibenclamide)

Table 4: Percent reduction in blood glucose levels with aqueous extract and isolated fractions of the leaves of *M. stenopetalla* in diabetic mice.

Time	0 hr	1.5 hr	3 hr	4.5 hr	6 hr
Control	0	0.7	1.1	1.3	0.3
Standard	0	9.8	36.0	39.5	41.5
Extract	0	13.1	17.1	8.6	8.4
Chlor. Frac.	0	23.1	22.9	18.4	27.4
But. Frac.	0	43.8	42.3	37.6	36.6
Aq.Res.Frac.	0	31.2	40.0	37.3	43.9

Chlor. Frac. = chloroform fraction; But. Frac. = n=butanol fraction; Aq. Res. Frac. = aqueous residue fraction. n=5 for the extract and isolated fractions, n=6 for the control and standard (glibenclamide)

The possible mechanism (s) of action of the extract as well as the fractions of *M. stenopetalla* leaves seem to be similar to that of sulfonyl ureas (stimulating insulin release) as they decrease the glucose level in normoglycemic mice.

The previous study on the chemical composition of the leaves of *M. stenopetalla* revealed the presence of rutin, 4-(4'-0-acetyl—L- rhamnosyloxy)-benzylisothiocynate and 4-(4'-0-acetyl—L- rhamnosyloxy) benzaldehyde and O- (rhamnopyranosyloxy) benzyle glucosinate (9). Alkaloids, saponins, glycoproteins, amino acids and proteins were found to reduce blood glucose levels (10). One or more of the aforementioned compounds might be responsible for the hypoglycemic and ant-hypergycemic effect of the plant.

The oral LD50 of *M. stenopetalla* leaves aqueous extract in mice was found to be > 50.6 g/kg which is over 500 times greater than the dose which produced the effect indicating the non-toxic nature of the extract. This might be the reason why the leaves are indiscriminately employed as food where the plant is widely grown.

The present study, therefore, supports the traditional use of the leaves of *M. stenopetalla* in both types of diabetes mellitus.

Acknowledgments

This study was carried out with the support of the Addis Ababa University and the Ethiopian Health Research Institute. We appreciate the assistance of the National Veterinary Institute for making mice available to us.

References

1. Abuye C, Urga K, Knapp H, Selmar D, Omwega AM, Imungi J.K, Winterhalter P. A compositional study of Moringa stenopetalla leaves. East African Medical Journal 2003; 80 (5): 247-252
2. Mekonnen Y. Effects of Ethanol extracts of Moringa stenopetalla leaves on guinea pigs and mouse smooth muscle. Phythotherapy Research 1999; 13: 442-444.
3. Mekonnen Y, Gessesse A. Documentation on the use of Moringa stenopetalla and its possible antileishmanial and antifertility effects. SINET: Ethiopian Journal of Science 1998; 21: 287-295
4. Biffa D. In vitro antimicrobial activities of bark and leaf extracts of Moringa stenopetalla against mastitis causing bacterial pathogens. Ethiopian Pharmaceutical Journal 2005; 23 :15-22
5. Makonnen E, Hunde A, Damecha G. Hypoglycemic effect of Moringa stenopetalla aqueous extracts in rabbits. Phythotherapy Research 1997; 11: 147-148.

6. Williamson EM, Okpako DT, Evans FJ. Selection, preparation and pharmacological evaluation of plant materials. Pharmacological Methods in Phythotherapy research 1996; I: 221
7. Weil C. Tables for convenient calculation of medial effective dose (LD50 or ED50) and instruction in their use. Biometrics. 1952; 8: 249.
8. Knapp RG, Miller MC. Clinical Epidemiology and Biostatistics. The National medical series for independent study, Harwal Publishing Company, Malverin, Pennsylvania, 1992; 275-292
9. Mekonnen Y, Gebreyesus T. Chemical investigation of the leaves of Moringa stenopetalla. Bulletin of the Chemical Society of Ethiopia 2000; 14(1): 51-56.
10. Wang HX, Ng TB. Natural products with hypoglycemic, hypocholestrolemic, antiatheroscelerotic and antithrombotic activities. Mini-review, Life Science 1999; 65: 2663-2677