Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:

Antihyperglycemic Effect on Chronic Administration of Butanol Fraction of Ethanol Extract of *Moringa Stenopetala* Leaves in Alloxan Induced Diabetic Mice

Alemayehu Toma[1*], Eyasu Makonnen[2], Asfaw Debella[3], Birhanu Tesfaye[3]

[1] *Pharmacology department, school of medicine, Hawassa University, Hawassa, Ethiopia*
[2] *Pharmacology department, school of medicine, Addis Ababa University, Addis Ababa, Ethiopia*
[3] *Department of Traditional and Modern Drug Research, Ethiopian Nutrition and Health Research Institute (ENHRI), Addis Ababa, Ethiopia*

ARTICLE INFO

Article history:
Received 7 September 2012
Received in revised form 15 September 2012
Accepted 11 November 2012
Available online 28 December 2012

Keywords:
Antihyperglycemic effect
Moringa stenopetala
Butanol fraction
Alloxan-induced diabetic mice
LD_{50}

ABSTRACT

Objective: The present study was conducted to evaluate the antihyperglycemic activity on chronic administration of the butanol fraction of the ethanol extract of *Moringa Stenopetala* leaves in alloxan induced diabetic mice. **Methods:** The mice were grouped in four groups; Normal control, Diabetic control, Butanol fraction treated and standard drug treated groups. The Diabetic mice received the butanol fraction of *Moringa stenopetala* daily for 28 days. **Results:** The butanol fraction of *Moringa stenopetala* treatment resulted in significant reduction of fasting blood glucose level, serum total cholesterol and triglycerides level. This fraction also showed a tendency to improve body weight gain in diabetic mice. Its oral LD50 was found to be greater than 5000mg/Kg indicating its safety in mice. **Conclusions:** Though the mechanism of action of *Moringa stenopetala* seems to be similar to that of sulfonylureas, further studies should be done to confirm its mechanism of antidiabetic action. Furthermore the active principle(s) responsible for the antidabetic effects should also be identified.

1. Introduction

Diabetes mellitus (DM) is a systemic metabolic disease characterized by hyperglycemia, hyperlipedemia, hyperaminoacidemia and hypoinsulinaemia, and leads to reduced insulin secretion, insulin action, or both [1]. It is frequently associated with development of micro and macrovascular diseases which include neuropathy, nephropathy and cardiovascular as well as cerebrovascular diseases [2]. The disease is associated with reduced quality of life and increased risk factors for mortality and morbidity. The long-term hyperglycemia is an important factor in the development and progression of micro and macrovascular complications [3].

The local communities residing in the biodiversity-rich areas of the southern region of Ethiopia have traditionally used and relied on plants for treating various ailments. In many cases, local knowledge of medicinal plants remains poorly documented in scientific literature. These plants have found a prime place in the indigenous system of medicine and are in focus for evaluation of their active ingredients. *Moringa stenopetala* is one of these medicinal plants which is widely used for antidiabetic purpose in the area and supported by Makonnen and coworkers for its hypoglycemic effect [4].

In the present study bioactivity guided fractionation and antihyperglycemic activity evaluation; acute oral toxicity test; change in body weight; serum triglyceride and total cholesterol were carried out in butanol fraction in long term use.

2. Methods and materials

2.1. Collections and preparation of plant materials

The leaves of *Moringa stenopetala* was collected from Wolaitta zone, South Nation's Nationalities Peoples Region, 400 kilometer south of Addis Ababa. After collection, the

*Corresponding author: Pharmacology department, school of medicine, Hawassa University, Hawassa, Ethiopia
Tel: 1560/fax +251462208755/tel +251913259141
E-mail: alexpharma99@yahoo.com

plant was identified and authenticated by Dr Getachew Addis, a taxonomist, and deposited in herbarium of Ethiopian nutrition and health research institute (ENHRI) with a voucher number AL-001. It was then dried under shade and crushed to powder for extraction. The study was carried out in EHNRI from November 2010 to April 2011

2.2. Chemicals and instruments

Alloxan(Sigma, Alderich, Germany), Ethanol(Alpha chemicals, India),n-Hexane(wagtech international Ltd, England), Glibenclamide(glitisol, Cyprus), n-Butanol(Blulex, India), Dichloromethane(Alderich, Germany), Humaster 80 automated chemistry analyzer (Humostar 80, Germany), Rota vapor (buchi rota vapor vac R-500, Switzerland), GLAB glucometer(Roche diagnostic, Germany) and GLAB active glucose test strip(Rochi diagnostic, Germany) were used in this study.

2.3. Preparation of plant material extract

The powdered leaves (1.2 Kg) were extracted by percolation using 70 %(v/v) ethanol, and the mixture was then filtered using Whatmann filter paper no. 1. The extract was dried by evaporation using rotary vaporizers under reduced pressure at a temperature of 40-45℃. The residue filtrate obtained was then dried by steam bath at 40℃ and kept in refrigerator at 8℃. The yield of the extract was 19.6% in weight in weight (w/w).

2.4. Solvent-solvent fractionation of the total ethanol extract

The procedure for solvent-solvent separation was adopted from Ranjan (2002) with some modification. Ten percent (w/v) of ethanol extract of the plant was prepared with mild hot distilled water. The dissolved ethanol extract was partitioned with n-hexane (3 x 50), dichloromethane (3 x 50) and n-butanol (3 x 50) using separatory funnel successively until the extracting solvent become colorless. After completing the separation process, the solvents were recovered by Rota Vapor. The separates were dried by steam bath at 40℃ and kept in the refrigerator for the experiments. The yields of n-hexane, dichloromethane, and n- butanol were 1.6%, 0.4%, and 7.1% (w/w), respectively.

2.5. Pharmacological and Toxicological evaluation

2.5.1. Animals

The Swiss albino mice of both sexes weighing 18-25g each were used for the study. The animals were obtained from animal department of ENHRI, kept under standard conditions (at a temperature of 22 ± 2 ℃, and with 12 hr light/ 12 hr dark cycle) and provided with free access to standard pellet laboratory diet and water ad libitum. The experimental protocol was approved by the Institutional review board (IRB) of Addis Ababa University, School of medicine with protocol number 097/10/pharm.

2.5.2. Induction of experimental diabetes

Eight mice were randomly selected as normal controls; the remaining mice were fasted overnight with free access to water, and then injected intraperitonially with alloxan 150mg/kg body weight dissolved in normal saline solution. All the animals had free access to water and pellet diet after thirty minutes of administration of Alloxan. Seven days latter, the fasting blood glucose levels of mice were determined using glucose oxidase method with glucose analyzer. A blood glucose level greater than 200mg/dl was defined as DM. Alloxan induced diabetic mice were selected and divided in three groups; negative control, positive control, and test group.

2.5.3. Study on long-term effect of n-butanol fraction on blood glucose levels

The alloxan induced diabetic test group mice were administered with 500mg/kg body weight of butanol fraction of *Moringa stenopetala* daily for 28 days via oral gavage. The normal control mice were given 10ml/kg of body weight of normal saline via oral gavage. The negative control and positive controls among the diabetic mice were given 10ml/Kg body weight of normal saline and 0.66mg/kg of body weight of Glibenclamide via oral gavage, respectively. On days 0, 7, 14, 21, and 28 the blood samples were collected from tail vein following overnight fasting, and blood glucose levels were measured. The body weight of each mouse was also measured.

2.5.4. Assay of serum triglyceride (TG) and total cholesterol (TC) level

On day 29, the mice were fasted overnight, blood samples were collected in sterile tubes by cardiac puncture under ether anesthesia and left to stand at room temperature for 2h, then centrifuged at 1500xg for 15 minutes at 4℃. The supernatant was immediately separated from the whole blood to prepare serum samples in order to determine TG and TC using automated chemistry analyzer (Humostar 80, Germany).

2.5.5. Acute Toxicity studies with butanol fraction

Acute toxicity study was performed on Mice of either sex selected at random. The animals were kept fasting over night providing only water. They were divided in to four groups, six animals in each group (three males and three females), and then the fraction was administered orally in an increasing dose level of 300, 2000, 5000mg/kg via oral gavage according to the guidelines of the Organization for Economic cooperation and Development [5]. Animals were kept under close observation for 4 hours after administering the fraction for behavioral, neurological and autonomic profile and then they were observed for any change in the general behavior

and/or other physical activities and mortality were recorded with in 24 hours.

2.6. Statistical analysis

For antidiabetic activity study in mice, the results were expressed as mean + standard error of deviation. One-way analysis of variance (ANOVA) was used to analyze the changes in plasma glucose and other parameters. Then Tukey post-hoc comparisons were used to determine the source of significant differences where appropriate. The level of statistical significance was set at $P<0.05$. Statistical analysis was done using graphpad Instat-software.

3. Results

3.1. Effects of butanol fraction of Moringa stenopetala leaves on long term blood glucose level

During the four weeks fraction treatment, blood glucose levels were measured once weekly. The results are summarized in Table 1. Before induction of diabetes, there was no significant difference in blood glucose levels among the treatment groups ($P>0.05$). Blood glucose levels in the test groups showed no significant differences at the end of the first week of administration ($P>0.05$), but the mean blood glucose level in both the test and standard groups (positive control) were lower than that in diabetic control (negative control) group. Both standard and test groups showed significant reduction on the 21st day of treatment and beyond. On day 28, blood glucose levels in the butanol fraction and Glibenclamide treated groups reduced by 31.84 % and 26.70 %, respectively. There was no significant change in blood glucose level between the test group and positive control group ($P>0.05$). The normal control and diabetic control did not show any significant variation in blood glucose level through out the experimental period ($p>0.05$). The present results indicate that long term administration of butanol fraction of *Moringa stenopetala* decreases hyperglycaemia in diabetic mice.

3.2. Effects of butanol fraction of Moringa stenopetala leave on body weight

Changes in body weights in control and experimental groups are shown in Table 2. There were no significant differences in the initial body weights among the four groups ($p>0.05$). After inducing diabetes, the difference in normal and diabetic groups were very significant ($P<0.01$). Butanol fraction of *Moringa stenopetala* leaves improved the weight gain compared with the diabetic control mice. By the end of the experiment, the body weight of the normal control group was significantly increased ($P<0.001$). In contrast, the mice in the diabetic control group had slightly increased body weight during the experimental period ($P>0.05$). Following butanol fraction administration for 4 weeks, the body weight of mice was significantly increased compared with that in the diabetic control group ($P<0.01$). There were no significant change in body weight between butanol fraction and standard drug treated groups ($P>0.05$).

3.3. Effects of butanol fraction of Moringa stenopetala leaves on serum total cholesterol and triglyceride levels

Serum total cholesterol (TC) and triglyceride (TG) levels were determined on day 29, and the results are summarized in Table 3. The serum total cholesterol and triglyceride levels were significantly higher in the diabetic control group than in the normal control group ($P<0.001$). TG and TC levels were significantly decreased with butanol fraction administration.

Table 1.
Antihyperglycemic effect of butanol fraction of Moringa stenopetala leaves and its percent reduction in alloxan induced mice.

Groups	Glucose level (mg/dl)					Percent Reduction
	Day 0	Day 7	Day 14	Day 21	Day 28	
Normal controls(without alloxan)	136.0±6.29	151.1±6.91	136.1±6.35	125.7±7.54	125.0±5.66	8.09
Diabetic controls(negative control)	256.2±13.34	286.9±12.10	293.0±45.61	268.2±25.33	285.3±17.43	−11.36
Glibenclamide(0.66mg/Kg) (positive control)	243.6±10.48	211.0±23.80*	178.6±32.60*	176.1±9.75**	178.7±8.93***	26.70
Butanol fraction (Test group)(500mg/Kg)	265.1±11.36	238.4±19.29	225.4±29.60	191.6±17.66*	180.7±16.66***	31.84

P=*<0.05, P=**<0.01, P=***<0.001 verses Diabetic controls
Mean±Standard error of deviation, n=8

Table 2.
Change in body weight after butanol fraction of Moringa stenopetala leaves in alloxan induced mice.

Groups	Body weight (g)					Before alloxan
	Day 0	Day 7	Day 14	Day 21	Day 28	
Normal control	21.7±0.75	24.7±0.80**	27.4±1.25**	31.0±1.30***	34.8±1.38***	36.7±1.59***
Diabetic control	21.3±0.76	21.2±0.77	20.8±1.07	21.5±1.43	23.3±061	24.1±1.22
Standard, Glibenclamide(0.66mg/Kg)	21.3±0.76	19.9±0.41	25.3±1.05*	26.6±0.76*	29.1±0.82*	29.9±1.43*
Butanol fraction(500mg/Kg)	21.3±0.76	20.7±0.79	23.8±0.91	26.1±1.50*	27.4±1.78*	30.1±1.47*

P=*<0.05, P=**<0.01,P=***<0.001 verses Diabetic controls Mean±Standard error of deviation, n=8

Table 3.
effect of butanol fraction of Moringa stenopetala in TG and TC after chronic administration in alloxan induced diabetic mice.

Treatment groups	Total cholesterol(mg/dl)	Triglyceride(mg/dl)
Normal control	184±7.48***	172.0±4.90***
Diabetic control	228.0±4,93	220.0±6.33
Glibenclamide	212.0±4.88	188.0±4.89**
Butanol fraction	192.0±4.94**	184.0±4.90***

P=**<0.01, *P*=***<0.001 verses Diabetic controls Mean±Standard error of deviation

3.4. Acute oral toxicity test

The present acute toxicity studies revealed that the administration of graded doses of butanol fraction of *Moringa stenopetala* (up to a dose of 5000 mg/kg) did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. No death was observed up to the dose of 5 g/kg body weight, indicating that the medium lethal dose (LD50) is greater than 5 g/kg body weight in mice. The mice were physically active. These effects were observed during the experimental period.

4. Discussion

In this study the antihyperglycemic effect of butanol fraction was carried out in alloxan induced diabetic mice. Alloxan is cytotoxic to the pancreatic − cells and thus is an effective diabetogenic agent. It has been widely used to induce DM in experimental animal models, allowing the investigation of hypoglycemic agents in treatment of diabetes. According to the administered dose of these agents, syndromes similar to either type 1, type 2 diabetes mellitus or glucose intolerance can be induced [6, 7].

The acute antihyperglycemic effect of butanol fraction of *Moringa stenopetala* was shown after 2 to 4 hours of treatment in the previous study [8]. In our study, the diabetic mice were treated for 28 days with butanol fraction of *Moringa stenopetala* leaves, which exhibited significant antihyperglycemic effect on the 21st and 28th days after treatment, suggesting that the fraction has role in management of hyperglycemia on chronic administration. The over all percent reduction of butanol fraction of *Moringa stenopetala* leaves was found to be 31.84 which were higher than the standard drug after 28 days. This is in line with previous study (unpublished report) on its acute antihyperglycemic effect which showed higher blood glucose reduction than the standard drug after 4.5 hours.

Medicinal plants with various active principles and properties have been used from ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer. Beneficial multiple activities of plants used traditionally in DM like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring the integrity and function of β−cells, releasing insulin activity, improving glucose uptake and utilization, and the antioxidant properties present in medicinal plants, offer an exciting opportunity to develop them into novel therapeutics [9]. The antihyperglycemic activity of *Moringa stenopetala* may be attributed to one or more of the aforementioned mechanism′s. Moringa stenopetala contains several active principles [4, 8] which might contribute for the antidiabetic effect. The antihyperglycemic effect of the butanol fraction of the plant confirms the presence of some of the active components.

Several phytomolecules including flavonoids, alkaloids, glycosides, saponins, glycolipids, dietary fibres, polysaccharides, peptidoglycans, carbohydrates, amino acids and others obtained from various plant sources have been reported as potent hypoglycemic agent. Flavonoids are a heterogeneous group of ubiquitous plant polyphenols, which exhibit a variety of pharmacological activities, including the anti−atherogenic effect, lipoprotein oxidation, blood platelet aggregation and vascular reactivity [10]. Triterpenoid and steroidal glycosides, referred to collectively as saponins, are bioactive compounds present naturally in many plants and known to possess potent hypoglycemic activity [11]. Qualitative phytochemical analysis of *Moringa stenopetala* extract and its butanol fraction revealed presence of flavanoids, phenol′s, saponins, terpenoids, sugars and steroids [8]. Hence, the observed antidiabetogenic effects of butanol fraction of *Moringa stenopetala* may primarily be at the level of carbohydrate metabolism and secondarily at lipid metabolism.

In the present study, blood glucose level in glibenlamide treated as well as butanol fraction of *Moringa stenopetala* leaves treated group showed almost similar change throughout the study period. This may reveal that the mechanism of *Moringa stenopetala* on glycaemic control may be similar to that of Glibenclamide, a prototype sulfonylureas, which was supported by previous works [4].

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 [12]. It is also described by [13] that raw leaves of contain minerals such as potassium, iron, zinc, phosphorus and calcium in significant amount. The presence of these minerals may also contribute to the antihyperglycemic effect of the plant.

DM is a metabolic disorder that usually affects carbohydrate, fat, and protein metabolism, followed by multiorgan injury in the later period, and hyperipidimia is associated with hyperglycemia [14]. In the diabetic control group, the levels of TG and TC increased significantly compared with those in normal mice. In the butanol fraction treated groups, TG and TC levels were significantly decreased. The results indicate

that butanol fraction of *Moringa stenopetala* leaves not only have significant antihyperglycemic activity, but also have antihyperlipidemic effects in alloxan induced diabetic mice.

Another observation drawn from this study is the relative safety of the butanol fraction of *Moringa stenopetala* leaves at the graded dose of up to 5000 mg/kg. According to Clarke and coworker [14], any compound or drug with the oral LD50 estimate greater than 1000 mg/kg could be considered low toxic and safe. Arising from this documented fact, the butanol fraction at an oral dose of 5000 mg/kg could be considered relatively safe on acute exposure.

The present findings reveal that the buthanol fraction has antihyperglycemic and antihyperlipedemic effects with wide safety margin. Though the mechanism of action of *Moringa stenopetala* seems to be similar to that of sulfonylureas, further studies should be done to confirm its mechanism of antidiabetic action. Furthermore the active principle(s) responsible for the antidabetic effects should also be identified.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This study was supported by the School of Graduate Studies, Addis Ababa University and Traditional and Modern Drug Research Department of the Ethiopian Health and Nutrition Research Institute. We appreciate the assistance of W/ro Rekik Ashebir for her technical assistance during laboratory work.

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