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Evaluation of Antidiabetic Activity of *Momordica balsamina* Linn Seeds in Experimentally-induced Diabetes

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ABSTRACT

The present study was design with an aim to evaluate the antidiabetic potential of Momordica balsamina (MB) seeds. The study was performed on methanolic and aqueous extracts of MB seeds in oral glucose tolerance test (OGTT) and STZ-induced diabetes models in rats. In OGTT model, aqueous extract (AE) at 500 mg/kg dose had shown the significant ($P<0.05$) check in the rise of blood glucose, at par to metformin (500 mg/kg). Hence, AE (500 mg/kg) was selected for further study in STZ-induced diabetes model. Single dose of STZ (45 mg/kg i.p.) was used for diabetes induction, which had shown significant rise in blood glucose level, and significant decrease in body weight as compared to normal control rats. Three weeks treatment of diabetic animals with AE (500 mg/kg) showed significant check in rise of blood sugar compared to untreated diabetic rats along with improved complete lipid profile and body weight. On the basis of analysis of data obtained during the study, it may be concluded that AE of MB seeds is having significant antihyperglycemic potential and can be further fractionated in order to get a responsible molecule for this vary action.

Keywords: *Momordica balsamina*; Metformin; Streptozotocin; Wistar rats.

INTRODUCTION

Momordica balsamina Linn (MB) commonly known as Balsam apple (English), Junglee karela (Hindi) is an annual wine native to the tropical regions of Africa and was introduced in Asia, Australia and Central America. Leaves and fruits are used as vegetable. The activity based review of MB fruits indicate that it possess activities like antimicrobial, antispasmodic, anti-inflammatory, analgesic, anti-HIV, hypoglycemic, antidiahorrial, hepatoprotective, antimalarial, anti-oxidant, anticancer wound healing etc. [1-5]. The fruit is reported to contain momordicin, vitamin C, resin acids, fixed oil, carotene, aromatic volatile oil, alkaloids, cucurbitacins and saponins [6].

The allied species of *Momordica* genus are well reported for antidiabetic potential [7, 8] and antidiabetic activity of MB fruit pulp has already been proven in our laboratory (unpublished data). Hence, the present work on MB seeds was designed in continuation to the previous study.

MATERIALS AND METHODS

STZ was procured from Sigma Chemicals, USA and metformin from Zenlabs, India. The kits for glucose estimation, total cholesterol, triglycerides and HDL-C were purchased from Coral, India. Animal feed was obtained from Aashirwad Industries, Ropar, India. All other chemicals and reagents were of the highest commercial grade available. Cooling Centrifuge (REMI) and Rotary evaporator (Equitron, Roteva) were used during the study.

Collection of plant material

The MB fruits were purchased from the local market, Moga and got identified by Dr H B Singh, Director, Department of Raw Material Herbarium & Museum, National Institute of Science communication and Information Resources (NISCAIR), New Delhi, India vide number (NISCAIR/RHM 1062/93). The specimen is preserved and kept in our laboratory for future reference.

Preparation of extracts

Dried coarse powdered seeds (900 g) were defatted with petroleum ether (60-80˚c) and the marc was extracted with 90% methanol using Soxhlet apparatus (18 h) to obtain ME. The AE was prepared by triple maceration of the marc remained after methanolic extraction.

Identification of Phytoconstituents

Chemical tests were carried out on AE in order to determine the presence of phytochemicals, which are responsible for the activity.

Animals

Wistar rats (either sex) weighing 180-220 g were procured from the animal house of ISF College of Pharmacy, Moga (Reg. No. 816/04/c/CPCSEA). The animals were kept in polypropylene cages (3 in each cage) at an ambient temperature of 25 ± 2°C with 55-65% relative humidity at 12-12 h light and dark schedule throughout the study. The rats had free access to water and were fed with commercially available feed. The experimental protocol was approved by the institutional animal ethical committee.

Pharmacological studies

Anti hyperglycemic effect of prepared extracts was first observed in OGTT to find out effective extract and the dose. STZ-induced diabetes model was used to evaluate only extract, shown maximum anti-hyperglycemic effect in OGTT model.

Preparation of test and standard drug material

The extracts and metformin were suspended in 1% w/v carboxy methyl cellulose (CMC) separately, in order to get the test solutions.

Induction of experimental diabetes

Diabetes was induced in the fasted rats by injecting freshly prepared STZ (45 mg/kg, i.p) in sodium citrate buffer (pH 4.3). After injection, animals were allowed to drink 5% glucose solution overnight. After one week of STZ injection the animals showed a fasting serum glucose level 250-300 mg/dl were considered as a diabetic for the present study.

Drug administration
In OGTT model, glucose load (1.5 g/kg) was given to the animals after 1 h of oral administration of test drugs. Blood samples were collected immediately after glucose administration and then at 1 and 2 h intervals.

In STZ-induced diabetic model, AE (500 mg/kg) and MTF (500 mg/kg) were administered orally at 24 h intervals during the entire period of the experiment. Blood was collected on the 0, 7^{th}, 14^{th} and 21^{st} day of the study.

Blood Collection
In STZ model, Blood samples were withdrawn by retro-orbital plexus under mild anesthesia. Blood was allowed to clot and then centrifuged at 3000 rpm for 15 min to get clear serum. The serum was analyzed for glucose level and for complete lipid profile [9].

Collection of tissue
Animals were sacrificed by an overdose of anesthetic ether at the end of experimental protocol. The liver was immediately excised and transferred into ice cold 0.9% sodium chloride solution and was stored at -20°C for further study.

Experimental Groups
In OGTT studies, the rats were divided into six groups with six animals in each. Group 1was considered as normal control, animals received 1% CMC. The rats of group 2 and 3 have treatment of ME at the doses (250 and 500 mg/kg, respectively), Group 4 and 5 were received AE (250 and 500 mg/kg, respectively) and the rats of group 6 were given MTF (500 mg/kg). In STZ-induced diabetes, four groups of rats with six animals in each had been incorporated. Group 1 rats were considered as normal control, received 1% CMC. Rats of group 2 were STZ treated received 1% CMC only served as diabetic control, group 3 received AE (500 mg/kg) and group 4 received standard drug MTF (500 mg/kg).

Body weight analysis
Animals were weighed on 0, 7^{th}, 14^{th} and 21^{st} days after diabetes induction to detect any changes in their body weights [10].

Biochemical analysis
Fasting serum glucose level, lipid profile and liver glycogen content were evaluated. Serum glucose level was estimated by GOD/POD method. Lipid profiles including total cholesterol (CHOD/PAP method), triglycerides (GPO/PAP method), HDL-C (PEG Precipitation method), LDL-C (Freidewald's method) and VLDL-C were determined [11]. Liver glycogen level was estimated according to standard procedure [12].

Statistical analysis
All the results are expressed as mean ± S.D and one way ANOVA was used for statistical analysis. The differences among the means were analyzed by Tukey's multiple comparison test using computerized program at 95% ($P<0.05$) confidence level.

RESULTS

The Phytochemical screening of AE had shown the presence of saponins, alkaloids and phenolic compounds.

OGTT Studies

In this study the serum glucose level was estimated in all groups of animals 1 h after glucose administration in comparison to 0 h serum glucose level. Vehicle control group showed 35% rise in blood glucose level. The AE at the doses of 250 and 500 mg/kg showed significant ($P < 0.05$) increase in serum glucose level by 22.47 and 17.82%, respectively. However, control, MTF and test drugs pretreated groups of animals normalized the serum glucose level within 2 h. The results are depicted in table 1.

Table-1 Effect of AE of MB seeds on serum glucose levels on glucose loaded normal rats

Groups	Serum glucose level (mg/dl)		
	0 min	60 min	120 min
Normal Control	92.64 ± 2.25	143.91 ± 2.96 (35.00%↑)	89.79 ± 6.74
Test I (ME 250 mg/kg)	90.50 ± 6.32	118.16 ± 6.70 (23.84%↑)[a]	96.33 ± 4.50
Test II (ME 500 mg/kg)	87.78 ± 4.00	111.11 ± 6.51 (21.62%↑)[a]	92.45 ± 4.32
Test III (AE 250 mg/kg)	90.83 ± 6.42	117.16 ± 4.98 (22.47%↑)[a]	95.60 ± 5.85
Test IV (AE 500 mg/kg)	83.65 ± 4.88	101.99 ± 3.99 (17.82%↑)[a]	90.49 ± 3.03
Test V (MTF 500 mg/kg)	78.01 ± 4.74	95.83 ± 5.52 (18.59%↑)[a]	91.36 ± 4.91

All values represent means ±SD, n=6; [a] p< 0.05 vs normal vehicle control group.

STZ-induced diabetes model

Diabetic control rats showed consistent rise in the serum glucose level up to 21 days of the study. AE (500 mg/kg) treated diabetic rats showed significant ($P < 0.05$) reduction in fasting serum glucose level by 24.11, 41.84 and 60.04%, respectively on 7th, 14th and 21st day of the study as compared to 0 day of the experiment. The fall in serum glucose level was consistent MTF (500 mg/kg) treated diabetic rats showed consistent fall in serum glucose by 22.66, 40.17 and 52.27%, respectively.

Table-2 Effects of three weeks treatment of AE (500 mg/kg) and MTF (0.5 g/kg) on serum glucose level in STZ-induced diabetes model

Groups	0 day	7 day	14 day	21 day
Normal Control	90.37 ± 5.20	91.35 ± 4.42	90.43 ± 5.23	91.70 ± 8.40
Diabetic Control	277.92 ± 8.45[a]	287.31 ± 6.26[a]	296.43 ± 6.91[a]	312.27 ±7.57[a]
AE 500 mg/kg	282.5 ± 3.93[b]	214.33 ± 4.22[b]	164.16 ± 5.26[b]	112.87 ± 6.60[b]
MTF 500 mg/kg	263.71 ± 5.68[b]	204.09 ± 6.17[b]	157.76 ± 5.90[b]	125.86 ± 6.84[b]

All values expressed are means ±SD, n=6; [a] P < 0.05 vs normal group; [b] P < 0.05 vs diabetic control group.

Body weight analysis

Table 3 shows the average weekly body weights of both control and treated groups. Reduction in body weight was observed in all the diabetic animals. Moreover, animals treated with MTF (500 mg/kg) registered a less gradual decrease in body weights on 14th and 21st days of the study. However, AE (500 mg/kg) treated group registered a less but significant ($P < 0.05$) check on decrease in body weight, comparison to MTF treated group on 14th and 21st day, compared to diabetic control group.

Table - 3 Effect of AE treatment at the dose of 500 mg/kg on body weight in STZ -induced diabetic rats

Groups	0 day	7 day	14 day	21 day
Normal Control	210.50 ± 7.12	215.00 ± 6.34	214.00 ± 4.85	217.00 ± 5.09
Diabetic Control	196.60 ± 6.53[a]	171.50 ± 9.16[a]	148.50 ± 9.85[a]	106.30 ± 8.89[a]
AE 500 mg/kg	202.60 ± 21.10[b]	184.0 ± 13.80	173.0 ± 15.47[b]	167.33 ± 15.60[b]
MTF 500 mg/kg	189.00 ± 11.15[b]	181.60 ± 11.44	177.80 ± 9.32[b]	175.00 ± 8.19[b]

All values expressed are means ±SD, n=6; [a] P < 0.05 vs normal group; [b] P < 0.05 vs diabetic control group

Biochemical analysis

Animals of the STZ-induced diabetic control group showed a significant rise in serum total cholesterol, triglycerides, LDL-C and VLDL-C levels, whereas significant reduction was seen in serum HDL-C and liver glycogen levels in comparison to normal rats. After 21 days treatment with AE (500 mg/kg), diabetic animals showed significant reduction in serum total cholesterol, triglycerides, LDL-C and VLDL-C levels with significantly elevated serum HDL-C and liver glycogen levels. MTF (500 mg/kg) treated group showed significant reduction in total serum cholesterol, triglycerides, LDL-C and VLDL-C levels. On contrary, elevated serum HDL-C and liver glycogen levels as compared to diabetic control group were observed.

Table-4 The effect of 21 days treatment of diabetic rats with AE (500 mg/kg) and MTF (500 mg/kg) on serum cholesterol, triglycerides, HDL-C, LDL-C VLDL-C and tissue glycogen levels

Groups	TC	TG	HDL-C	LDL-C	VLDL-C	T Glycogen
Normal control	99.81±5.87	63.57±5.95	38.15±6.71	49.02±8.52	12.71±1.19	48.93±4.78
Diabetic control	253.09±8.8[a]	145.24±9.33[a]	21.84±4.58[a]	202.20±14.45[a]	29.04±1.86[a]	17.78±0.88[a]
AE 500 mg/kg	148.91±8.27[b]	107.13±8.02[b]	32.17±4.52[b]	94.67±8.82[b]	21.40±1.59[b]	37.54±5.83[b]
MTF 500 mg/kg	113.80±6.86[b]	81.37±6.28[b]	37.46±4.52[b]	60.27±7.24[b]	16.22±1.24[b]	42.28±2.77[b]

All values expressed are means ±SD, n=6; [a] P < 0.05 vs normal group; [b] P < 0.05 vs diabetic control group

DISCUSSION

In the present study MB seeds were investigated for their antidiabetic potential in STZ model. Preliminary antihyperglycemic activity was evaluated using OGTT study, most significant (*P<0.05*) blood sugar lowering effect was observed in animals treated with AE 500 mg/kg dose. Animals treated with MTF also showed a decrease in serum glucose rise.

Significant decrease in glucose level in normal animals can be attributed to the inhibition of α-glucosidase enzymes which reduce intestinal absorption of glucose [13] or may be due to a stimulating effect on the remnant β-cells or improvement in insulin action at the cellular level.

In STZ model AE (500 mg/kg) caused a significant check at par to MTF (500 mg/kg) in serum glucose rise of diabetic rats in comparison to untreated animals on 0 day (Table 2). The mechanism of action of the drug may also be similar to that of metformin since the pattern of changes in blood glucose level was similar. Metformin reduces glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. At the molecular level, these actions are mediated at least in part by activation of cellular kinase (AMP kinase) [14]. Like MTF, AE also had not produced hypoglycemia in normal rats. Hence, the test drug can also be regarded as antihyperglycemic and not hypoglycemic.

The AE had shown the presence of saponins, alkaloids and phenolic compounds which are well reported to possess antidiabetic activity [15].

After 21 days treatment of diabetic animal with AE (500 mg/kg) significant reduction in total cholesterol, triglycerides, and VLDL-C and LDL-C levels in serum was observed, whereas significant rise of HDL-C level was recorded, in comparison to diabetic control group. This anti hyperlipidemic effect can be attributed either due to up-regulation of peroxisome proliferator activator receptors (PPAR) activity or due to increased insulin secretion [16]. The results suggest

that AE inhibits the cholesterol synthesis pathway may be by inhibiting HMG-CoA reductase [17] or by reducing the NADPH required for fatty acids and cholesterol synthesis [18]. The lipid lowering activity of the extract in experimentally induced diabetic rats may also help in preventing associated atherogenesis and other secondary complications of diabetes mellitus [19].

The liver glycogen level was significantly elevated in both AE and MTF treated animals. This improvement may occur by two possible ways, one due to increased insulin level or due to up-regulation of GLUT - 4 and PPAR - γ activities which facilitates uptake of glucose by the peripheral tissues [20, 21]. This finding suggests that test drug possibly acts in similar fashion as that of metformin in normalizing the blood sugar level.

In diabetic control group, loss in body weight may be occurred due to some abnormality in carbohydrate metabolism such as lipolysis, glycogenolysis and acidosis, or it may be caused by disturbance in some metabolic pathways and results from protein deficiency [22-24]. However, diabetic rats treated with MTF and AE showed no significant change in body weight which is explainable by increased insulin secretion and increased food consumption [25, 26] .Hence, the drug may also possess pancreatic cells stimulating effect.

CONCLUSION

The present study confirms the antidiabetic property of MB seeds. The property may be due to saponins, alkaloids and phenolic compounds. It has also been observed that the drug did not lower down the sugar level below normal. Hence, it may play a good role in the management of diabetes. Therefore further study is required to find out responsible molecules for this action.

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