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Methanolic fruit extract of *Sesbania grandiflora* exhibits anti-hyperglycemic activity in experimental type-2 diabetes mellitus model

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ABSTRACT

Type-2 diabetes mellitus (T2DM) is considered as a chronic metabolic disorder. Sesbania grandiflora (Family: Fabaceae; SG) is known for its antidiabetic activity however, there is no report for its fruit on such activity. Hence, the present study explores the anti-hyperglycemic potential of methanolic extract of SG (MSG) in a rat model of T2DM. T2DM was induced in male rats by a single injection of streptozotocin, 15 min after nicotinamide administration. MSG at both dose levels attenuated the plasma glucose level in rats subjected to glucose load similar to that of metformin. However, either dose of MSG did not alter the level of plasma glucose in normal rats. MSG (200 and 400 mg/kg) significantly attenuated the plasma glucose level in T2DM rats. Further, MSG at both dose levels attenuated T2DM-induced increase in the level of total cholesterol, triglyceride and low density lipoprotein, and decrease in the level of high density lipoprotein in the plasma of the rats. MSG (200 and 400 mg/kg) reduced the T2DM-induced increase in the level of lipid peroxidation and decrease in the activities of superoxide dismutase and catalase in both plasma and liver tissues. In addition, MSG at both dose levels reduced the T2DM-induced decrease in the level of expression of GLUT-4 in the liver. These results cumulatively suggest that the anti-hyperglycemic activity of MSG against T2DM condition could be due to the improvement in the aberrant lipid profile, oxidative stress and insulin sensitivity. Hence, MSG could be a potential candidate in the management of T2DM.

Key words: Type-2 diabetes mellitus; *Sesbania grandiflora*; Oxidative stress; GLUT-4; Hypertriglyceridemia.

INTRODUCTION

Diabetes mellitus is considered as a chronic metabolic disorder of multiple etiologies, sharing a common underlying feature of hyperglycemia [1]. Type-2 diabetes mellitus (T2DM) is categorized as non insulin-dependent type of diabetes mellitus, whose prevalence has been reported about 90% among diabetic population [2]. Currently available drugs for T2DM include sulfonylureas, biguanides, thiazolidinediones and alpha glucosidase inhibitors [3]. The use of most of the anti-diabetic drugs is limited in the management of T2DM due to their several adverse effects [4]. Recently, the therapeutic regimen prefers herbal to synthetic management due to their readily availability and minimal side effects in the pharmacotherapy of T2DM. Several indigenous Indian medicinal plants possess significant therapeutic activity against T2DM. Thus, the herbal drugs gain critical attention in the selection of suitable drug to attenuate the progression of T2DM.

Sesbania grandiflora (Family: Fabaceae) is an indigenous medicine in India, known as "Sesbania" and "agathi" in ayurvedic system of medicine. It is also known as "Agati Sesban" or "humming bird tree" in English. *Sesbania grandiflora* is a small, loosely branching tree that grows up to 8-15 m tall and 25-30 cm in diameter. The fruit is a thin pod which can be up to 60 cm long and contains about 15-50 seeds. Flowering and fruiting is almost throughout the year mostly during winter. Ethnobotanical study suggests its use in headache, swellings, anemia, bronchitis, pains, liver disorders and tumors [5]. Traditionally, its fruits are used for anemia, bronchitis, fever and tumors [6]. Experimentally, it exhibits significant antioxidant effect against alcohol and polyunsaturated fatty acid-induced oxidative stress models in experimental animals [7]. Two proteins, namely SGF60 and SGF90 isolated from the flowers of this plant have been shown to possess significant inhibitory effect on α-glucosidase, and hence has been speculated as a potential drug against T2DM [8]. Though, its anti-diabetic activity is well documented in experimental animal models of diabetes [9]. There is no such study for its fruit.

Therefore, the present study evaluates anti-hyperglycemic activity of methanolic extract of fruit of *Sesbania grandiflora* (MSG) in the streptozotocine-nicotinamide-induced animal model of T2DM. Further, to elaborate the mechanism of action, the extent of oxidative stress in the plasma and liver, and level of expression of glucose transporter 4 (GLUT-4) in the liver were estimated.

MATERIALS AND METHODS

2.1. Chemicals and reagents

Streptozotocine and nicotinamide was procured from HiMedia Pvt., Ltd., New Delhi, India. Metformin was provided as a gift sample by Ranbaxy Pvt., Ltd., Gurgaon, India. All other reagents and chemicals used were of analytical grade procured from local suppliers.

2.2. Plant material and extraction

The raw fruits of *Sesbania grandiflora* were harvested from Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, India. The plant was identified and authenticated by an expert Dr. K. Karthigeyan, Scientist-C, Central National Herbarium, Botanical Survey of India, Howrah, India. The specimen was deposited in herbarium of Central National Herbarium (Voucher no.: CNH/77/2012/Tech.II/920). Plant material was carefully washed with tap water and left to dryness in dark at room temperature and finally stored in well-closed cellophane bags. The shade-dried and coarse powdered fruit (2 kg) was subjected to defat with petroleum-ether (bp 40-60°C) using soxhlet extraction apparatus. The defatted sample was air dried in order to remove solvent residue. Extract was prepared by extracting the defatted powder with methanol solvent (80% v/v) for period of 72 hrs, which was then concentrated to a semisolid mass under reduced pressure in a rotary evaporator for 20 min at 70°C (yield: 5.6% w/w). Thereafter, the semisolid mass was mixed with a fresh suspension of 0.3% v/v carboxymethylcellulose (CMC) in distilled water.

2.3. Preliminary phytochemical screening and standardization to total phenolic content

The plant extract was subjected to identification tests for alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols [10]. The estimation of total phenolic content was carried out by Folin-Coicalteau calorimetric method as by Makkar et al, 2000 with some modifications[11]. The dried methanolic fruit extract of *Sesbania grandiflora* (200mg) was extracted with 10ml of 50% aqueous methanol in a water bath for 25 minutes. The extracted material of methanolic fruit extract of *Sesbania grandiflora* content was centrifuged under cooling condition at 4^0C for 10 minute and the clear supernatant was collected. The same process was repeated for the residue and both the supernatant were mixed and made up to 10 ml by 50% aqueous methanol as test sample. A series of standard tanic acid dilutions were prepared range from 1 to 20 µg/ml. Aliquots of standard tanic acid (1 to 20µg/ml) and extract fraction 0.05ml were initialy made upto 0.5ml with distilled water. To this solution, 0.25ml of folin-ciocalteu reagent 2N and 1.25ml sodium carbonate (20%) were mixed vigoriously and kept at room temperture for 45 minutes. The absorbance of all the tested samples were measured at 775 nm and the amount of total phenolic content as tannic acid equivalent was calculated from the standard curve of tanic acid. The total phenolic content was expressed as milligrams of tanic acid equivalent/ g extract.

2.4. Animals

Inbreed adult male Charles-Foster (CF) albino rats (180 ± 10 g), 2-3 months old, were obtained from Central Animal House, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi and were used in the study. The animals were grouped and housed in poly-acrylic cages lined with husk under standard condition (24 ± 2°C

temperature, 45-55% relative humidity and 12 h light: 12 h dark cycle) respectively. Animals were allowed to freely feed their standard pellet diet (Lipton India, Ltd., Mumbai) and water *ad libitum*. Animals were deprived with their food for 16-18 h before experimentations but allowed to water *ad libitum*. All the experimental procedures utilized were performed in accordance with the approval of the Institutional Animal Ethics Committee (Dean/12-13/CAEC/33) under strict compliance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the experimental studies.

2.5. Oral toxicity studies

An acute oral toxicity study was performed according to "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of methanolic extract (suspended in 0.3% CMC suspension). Albino male rats (N=6, 150-200 g), overnight fasted for 18 h were used for the study. The extract in increasing dose of 5, 50, 300 and maximum dose up to 2000 mg/kg was found tolerable in the experimental animals. Median dose: 200 mg/kg (One-tenth of the maximum lethal dose) and high dose: 400 mg/kg (Twice that of one-tenth dose) were selected for assessment of antidiabetic activity. Thereafter, the rats were continuously observed for first 3 h for any gross behavioral, neurological or autonomic toxic effects and for any lethality after 24 to 72 h till 14 days.

2.6. Induction of T2DM in rats

The T2DM was induced in overnight fasted male rats by a single injection of streptozotocin (65 mg/kg, i.p.), 15 min after nicotinamide (110 mg/kg, i.p.) administration. Streptozotocin was dissolved in 0.1M citrate buffer (pH 4.5) and nicotinamide was dissolved in physiological saline [12]. Blood glucose level was measured by using one-touch glucometer and diabetes was confirmed after 72 hr of injection of streptozotocine. Rats with fasting blood glucose level more than 250 mg/dl were considered to be diabetic and were selected for studies.

2.7. Experimental Design

2.7.1.Study on Oral Glucose Tolerance Test (OGTT)

Initially, hypoglycemic activity of plant extracts was carried out in overnight fasted normal rats, which were equally divided into four groups of six rats each. Normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and standard group received 1 ml of reference drug MET suspended in the vehicle (100 mg/kg, p.o.), while third and fourth group animals were administered with 1 ml of MSG (200 and 400 mg/kg, p.o.) respectively. Following 30 min post administration all the animals were fed with glucose (2 g/kg, p.o.). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diabnostics, GmbH, Germany).

2.7.2.Study on normoglycemic rats

Normoglycemic studies were carried out in overnight fasted normal rats, which were equally divided into four groups of six rats each. Normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and 1 ml of standard group received reference drug MET suspended in the vehicle (100 mg/kg, p.o.), while third and fourth group animals were administered with equal volume of MSG (200 and 400 mg/kg, p.o.) respectively. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level.

2.7.3.Evaluation of anti-diabetic activity in T2DM rats

In the third set of experiment, diabetic rats were selected on seventh day after streptozotocine and nicotinamide injection based on the blood glucose level, were equally divided into three groups of six rats each. Diabetic control group received only vehicle (1 ml of 0.3% CMC; p.o.) and standard diabetic group received 1 ml of reference drug MET suspended in the vehicle (100 mg/kg, p.o.), while group from third to four were administered with 1 ml of MSG (200 and 400 mg/kg, p.o.) respectively once a day regularly up to 21st day. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level. The body weight, food and fluid intake of all groups of animals were monitored on a daily basis for 21 days at regular time. Fixed amount of rat chow and fluid was given to each rat and replenished the next day. For experimental purposes, only the weights before treatment and at the end of the study were used for analysis. At the end of 21st day, all the rats were euthanized by pentobarbitone sodium (60 mg/kg) and sacrificed by cervical dislocation. Blood sample was withdrawn from abdominal aorta into fresh centrifuge tubes and centrifuged at 2,500 rpm for 15 min to obtain plasma. Plasma samples were stored at -20°C until utilized for further biochemical

estimations. The liver also collected to analyze the level of expression of GLUT-4 protein and the markers for oxidative stress.

2.8. Biochemical estimations

Plasma insulin was assayed by using commercial enzyme-linked immuno sorbent assay kit (ELISA, Boerhringer Mannheim, Germany). The total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were estimated in plasma using the principle of absorbance in a spectrophotometer (BioTek, Switzerland) using commercial kit (HiMedia Pvt., Ltd.) following standard procedures.

2.9. Estimation of lipid peroxidase

The malondialdehyde (MDA) content was estimated using the method described by Uchiyama and Mihara, 1978 with minor modification by Sunderman et al., 1985 based on the thiobarbituric acid reaction (TBAR) test in both plasma and liver tissue [13,14]. The samples were added (1/10, v/v) to ice-cold 1.15% KCl solution and mixed with 0.1 ml of 8.1% sodium dodecyl sulfate (SDS), 0.75 ml of 20% acetic acid, and 0.75 ml of 0.8% TBA solution. The mixture was made up to 2.0 ml with distilled water and heated at 95 °C for 60 min. After cooling with tap water, 0.5 ml of distilled water and 2.5 ml of *n*-butanol/pyridine mixture (15:1, v/v) were added and the mixture shaken vigorously. The mixture was centrifuged at 4000×*g* for 10 min and the absorbance of the organic layer (upper layer) was measured at 532 nm. The TBAR reaction was standardized by the analysis of tetraethoxypropane standard solutions and was expressed as MDA in nmole/mg protein. The protein was estimated using standard protocol [15].

2.10. Estimation of superoxide dismutase (SOD)

The SOD activity was estimated by the method described by Ukeda et al., 1997 [16]. A 2.6 ml of 50 mmol/L sodium citrate buffer (pH 9.4) was added 0.1 ml each of 30 mmol/l xanthine (dissolved in 1 mol/L NaOH), 3 mmol/l EDTA, 0.8 mmol/L XTT (3-*{*1-[(phenylamino)-carbonyl]-3,4-tetrazolium*}*- bis(4-methoxy-6-nitro)-benzenesulfonic acid hydrate) dissolved in buffer at 50^0C and 0.05 ml of sample solution containing SOD or water. The reaction consists of the addition of 0.02 ml of xanthine oxidase solution (500 mU/ml) with the formation of a red formazan dye. The SOD activity was monitored for 30 s at 470 nm (25^0C) by the degree of inhibition of this reaction.

2.11. Estimation of catalase (CAT)

Catalase activity was measured by the method of Aebi, 1984[17]. The homogenate was prepared with 50 mmol/l phosphate buffer, pH 7.0, with a drop of TritonX100 and centrifuged at 15,000×*g* for 15 min at 4^0C. To 3.0 ml of phosphate buffer, 0.05 ml of 90 mmol/l hydrogen peroxide solutions and 0.02 ml of extract or water were added. The absorbance was read at 240 nm for 30 s.

2.12. Western blot analysis of GLUT-4 level in the liver

The liver tissue was homogenized in lysis buffer and centrifuged. Lysates (80 µg proteins) were electrophoresed on 10% sodium dodecyl sulfate (SDS)–PAGE gels, and then transferred to polyvinyldifluoridine (PVDF) membranes (Bio-Rad, USA). The membranes were incubated with rabbit polyclonal anti-GLUT-4 antibody (1:2000 Santa Cruz Biotechnology) overnight at 4 °C, and then with horseradish peroxidase–conjugated goat anti-rabbit IgG (1:3000, Santa Cruz Biotechnology) or horseradish peroxidase–conjugated goat anti-mouse IgG (1:3000, Santa Cruz Biotechnology) for 60 min at room temperature. Western blotting luminescent reagent was used to visualize peroxidase activity. Normalization was carried out by stripping films and reprobing with a mouse monoclonal antibody to the β–isoform of actin (1:10,000, Sigma). Films were scanned and subsequently analyzed by measuring optical densities of immunostained bands using an image processing and analysis system (ImageJ 1.37 software, NIH, USA).

2.13. Statistical Analysis

The results are expressed as mean ± S.E.M. Two-way Analysis of Variance (ANOVA) followed by Bonferroni Post-hoc test was employed for the analysis of temporal profile of plasma glucose level in OGTT, normoglycemic and T2DM study. All other statistical analysis was performed by using One-Way ANOVA followed by *Post-hoc* Student Newman Keuls test. All these statistical analysis were performed using GraphPad software (Version 4.0). $P < 0.05$ was considered to be statistically significant.

RESULTS

3.1. Effect of MSG on plasma glucose level in OGTT

Table-1 illustrates the effect of MSG (200 and 400 mg/kg) on OGTT at different time points of the experimental schedule. Repeated measures of two-way ANOVA revealed that there were significant differences among group [F (3, 100) = 38.35, P<0.05], time [F (4, 100) = 240.5, P<0.05] and there was a significant interaction between group and time [F (12, 100) = 3.55, P<0.05]. Post-hoc analysis showed that the plasma glucose level was significantly decreased in MET group animals compared to control rats at 30 min to the start of OGTT and this effect was maintained up to end of the OGTT schedule. MSG (200 and 400 mg/kg) significantly decreased the plasma glucose level at 30 min to oral administration of glucose compared to control group animals and these effects were persisted up to 120 min of the experimental schedule similar to that of MET group animals.

Table 1: Hypoglycemic effect of MSG (200 and 400 mg/kg) in oral glucose tolerance test

Group	0 min	30 min	60 min	90 min	120 min
Control	73.37±2.02	143.59±1.75	139.74±3.12	122.22±2.12	111.54±3.82
MET	75.67±0.81	125.64±1.44[a]	119.44±2.68[a]	100.02±3.06[a]	94.44±1.86[a]
MSG-200	75.48±0.87	129.53±4.48[a]	114.40±5.96[a]	102.98±2.74[a]	93.13±4.14[a]
MSG-400	75.12±1.23	123.50±0.71[a]	116.02±2.80[a]	101.79±2.89[a]	91.13±0.97[a]

All values are Mean±SEM (N=6). [a]P<0.05 compared to Control [Repeated measures two-way ANOVA followed by Bonferroni post-hoc test].

3.2. MSG (200 and 400 mg/kg) modulates the plasma glucose level in normal rats

The effect of MSG (200 and 400 mg/kg) on plasma glucose level in normoglycemic rats at different time points of the experimental schedule is depicted in Table-2. Repeated measures of two-way ANOVA revealed that there were significant differences among group [F (3, 80) = 15.83, P<0.05], time [F (3, 80) = 14.96, P<0.05] and there was a significant interaction between group and time [F (9, 80) = 2.79, P<0.05]. Post-hoc analysis showed that MET significantly decreased the level of plasma glucose in normal rats compared to vehicle treated animals on D-7 and this effect was sustained up to D-21. MSG (200 and 400 mg/kg) did not decrease the level of plasma glucose level in normal rats compared to vehicle treated rats on D-7. However, MSG (200 and 400 mg/kg) significantly reduced the plasma glucose level in normal rats compared to MET administered rats on D-7. These effects were maintained up to D-21.

Table 2: Time dependant effect of EMP (100, 200 and 400 mg/kg) on plasma glucose level in fasted normoglycemic animals

Group	D-0	D-7	D-14	D-21
Control	71.84±1.65	73.29±1.26	72.00±3.68	70.51±0.81
MET	75.67±0.69	55.40±3.48[a]	54.48±2.68[a]	54.26±2.24[a]
MSG-200	76.63±0.77	69.02±3.96[b]	66.02±3.10[b]	66.67±2.50[b]
MSG-400	74.71±0.73	67.95±3.10[b]	64.74±3.94[b]	64.10±2.79[b]

All values are Mean±SEM (N=6). [a]P<0.05 compared to Control and [b]p<0.05 compared to MET [Repeated measures two-way ANOVA followed by Bonferroni post-hoc test].

3.3. Effect of MSG on the plasma glucose level in diabetic rats

Table-3 illustrates the effect of MSG (200 and 400 mg/kg) on plasma glucose level at different time points of the experimental schedule in diabetic rats. Repeated measures of two-way ANOVA revealed that there were significant differences among group [F (4, 100) = 244.80, P<0.05], time [F (3, 100) = 355.20, P<0.05] and there was a significant interaction between group and time [F (12, 100) = 38.65, P<0.05]. Post-hoc analysis revealed that the plasma glucose level in DM group animals were elevated on D-7 compared to vehicle treated normal animals, and this effect was maintained upto D-21 of the experimental design. MET significantly reduced the STZ-induced increase in the plasma glucose level on D-7 and this effect was maintained upto D-21. Both doses of MSG did not alter the level of plasma glucose in diabetic rats on D-7. However, MSG (200 and 400 mg/kg) significantly decreased the level of plasma glucose in diabetic rats on D-14 and this effect was sustained upto D-21.

Table-3: Anti-hyperglycemic effect of MSG (200 and 400 mg/kg) at different time point of the experimental study in DM rats

Group	D-0	D-7	D-14	D-21
Control	76.24±1.92	75.43±2.21	75.42±1.38	75.21±2.00
DM	73.18±0.77	502.78±25.95 [a]	524.14±28.08 [a]	513.46±26.97 [a]
MET	75.09±0.71	331.62±2.24 [a,d]	284.61±4.30 [a,d]	233.33±12.56 [a,d]
MSG-200	76.24±0.92	491.88±24.00 [a,b]	308.12±10.58 [a,d]	303.85±9.33 [a,d,b]
MSG-400	75.86±0.84	489.53±22.51 [a,b]	275.85±25.91 [a,d]	269.44±17.53 [a,d]

All values are Mean±SEM. [a]P<0.05 compared to control, [d]P<0.05 compared to DM and [b]P<0.05 compared to MET (Repeated measures of Two-way ANOVA followed by Bonferroni Post-hoc test).

3.4. Effect of MSG (200 and 400 mg/kg) or MET on plasma lipid profile of diabetic rats:

The effect of MSG (200 and 400 mg/kg) on plasma TC, TG, LDL and HDL level in diabetic rats on D-21 of the experimental schedule is depicted in Fig-1. Statistical analysis by one-way ANOVA revealed that there were significant differences among groups in TC [F (4, 25) = 11.40, P<0.05], TG [F (4, 25) = 9.83, P<0.05], LDL [F (4, 25) = 35.58, P<0.05] and HDL [F (4, 25) = 18.05, P<0.05]. Post-hoc analysis showed that STZ injection significantly increased the level of TC, TG and LDL, and decreased the level of HDL in plasma compared to vehicle administered rats. MET and MSG (200 and 400 mg/kg) significantly reversed all these parameters in DM animals.

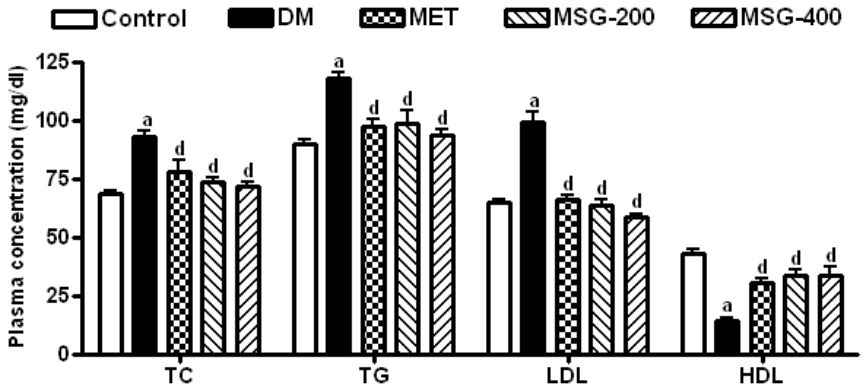

Fig. 1: The effect of MSG (200 and 400 mg/kg) on STZ-induced alterations in plasma TC, TG, LDL and HDL levels

All values are Mean±SEM. [a]P<0.05 compared to Control and [d]P<0.05 compared to DM (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

3.5. Effect of MSG (200 and 400 mg/kg) on plasma insulin level in DM rats

The effect of MSG (200 and 400 mg/kg) on plasma insulin level in diabetic rats on D-21 of the experimental schedule is depicted in Fig-2. Statistical analysis by one-way ANOVA revealed that there were significant differences among groups [F (4, 25) = 11.13, P<0.05]. Post-hoc analysis showed that STZ injection significantly decreased the level of plasma insulin level compared to vehicle treated rats. MET and MSG (200 and 400 mg/kg) significantly reversed the plasma insulin level in diabetic rats.

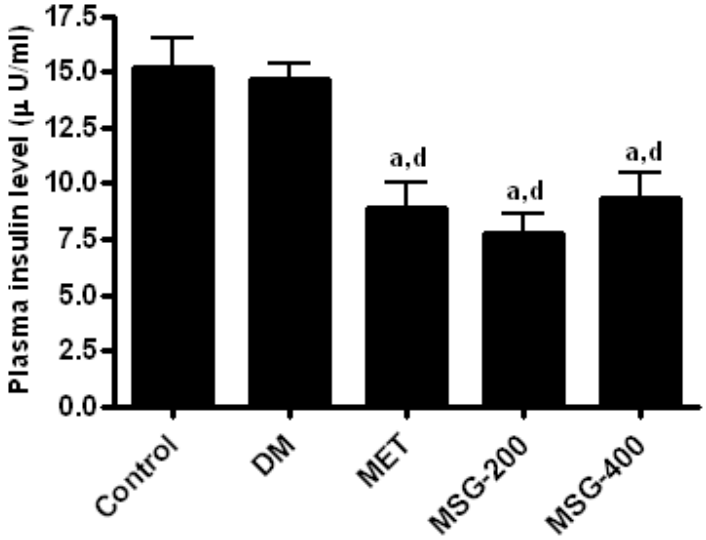

Fig. 2: The effect of MSG (200 and 400 mg/kg) on STZ-induced alterations in plasma insulin level.

All values are Mean±SEM. [a]P<0.05 compared to Control and [d]P<0.05 compared to DM (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

3.6. Effect of MSG (200 and 400 mg/kg) on oxidative markers in plasma and liver of DM rats

The effect of MSG (200 and 400 mg/kg) on plasma and liver LPO, SOD and CAT levels in DM animals on D-21 of the experimental schedule is depicted in Table-4. Statistical analysis by one-way ANOVA revealed that there were

significant differences among groups in plasma and liver LPO ([F (4, 25) = 15.13, P<0.05] and [F (4, 25) = 9.03, P<0.05] respectively), SOD ([F (4, 25) = 8.28, P<0.05] and [F (4, 25) = 5.43, P<0.05] respectively) and CAT ([F (4, 25) = 14.67, P<0.05] and [F (4, 25) = 14.92, P<0.05] respectively) levels. Post-hoc analysis revealed that STZ-injection significantly increased the level of LPO in both plasma and liver homogenate. MET and MSG (200 and 400 mg/kg) significantly reduced the STZ-induced increase in the LPO level in both plasma and liver homogenate. The levels of activity of SOD and CAT were significantly attenuated in DM rats by all the doses of MSG in both plasma and liver. All the treated groups showed significant amelioration in the level of activities of SOD and CAT in DM animals.

Table-4: The effect of MSG (200 and 400 mg/kg) on LPO, SOD and CAT levels in plasma and liver of DM rats

	Plasma			Liver		
Group	**LPO**	**SOD**	**CAT**	**LPO**	**SOD**	**CAT**
Control	0.66±0.04	0.89±0.04	5.73±0.23	0.68±0.05	1.00±0.08	6.42±0.56
DM	1.02±0.05 [a]	0.60±0.03 [a]	2.42±0.18 [a]	1.06±0.09 [a]	0.59±0.04 [a]	2.38±0.16 [a]
MET	0.67±0.04 [d]	1.02±0.05 [d]	6.40±0.29 [d]	0.71±0.05 [d]	0.93±0.11 [d]	6.12±0.57 [d]
MSG-200	0.80±0.03 [d]	0.88±0.05 [d]	6.54±0.70 [d]	0.74±0.01 [d]	0.88±0.07 [d]	5.59±0.62 [d]
MSG-400	0.68±0.03 [d]	0.88±0.08 [d]	5.87±0.56 [d]	0.70±0.04 [d]	1.13±0.11 [d]	7.32±0.37 [d]

All values are Mean±SEM. [a]P<0.05 compared to control and [d]P<0.05 compared to DM (Repeated measures of Two-way ANOVA followed by Bonferroni Post-hoc test).

3.7. Effect of MSG (200 and 400 mg/kg) on GLUT-4 expression in liver of DM rats

Fig-3 depicts the effect of MSG (200 and 400 mg/kg) on STZ-induced alterations in the level of expression of GLUT-4 in liver of DM rats. Statistical analysis revealed that there were significant differences in the level of expression of GLUT-4 [F (4, 10) = 15.35, P<0.05] in the liver among groups. Post-hoc analysis showed that DM decreased the level of expression of GLUT-4 in the cytosolic fraction of liver tissues. MET and both the doses of MSG ameliorated the STZ-induced decrease in the level of expression of GLUT-4 in liver.

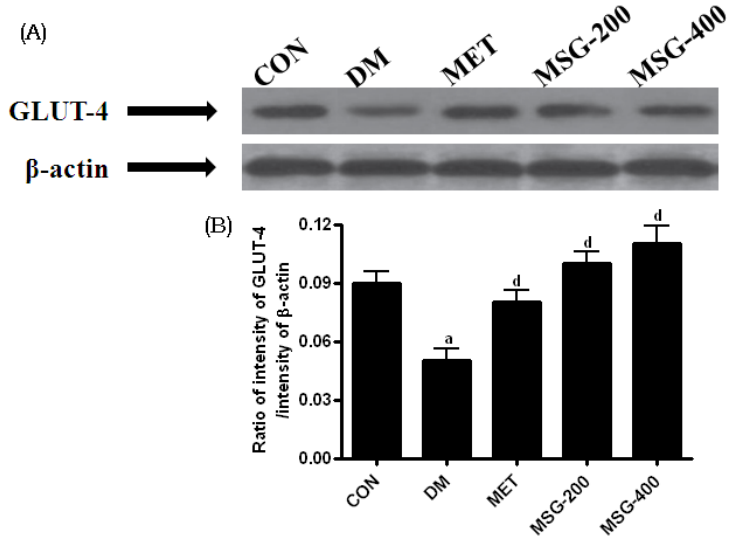

Fig. 3: The effect of MSG (200 and 400 mg/kg) on STZ-induced alteration in GLUT-4 expression level in liver

All values are Mean±SEM (n=3). [a]P<0.05 compared to Control and [d]P<0.05 compared to DM (One-way ANOVA followed by Student Newmann Keuls Post-hoc test).

DISCUSSION

In the present study, methanolic fruit extract of *Sesbania grandiflora* (MSG) (200 and 400 mg/kg) treatment exhibited anti-diabetic effect in T2DM rats. Further, MSG (200 and 400 mg/kg) reversed the T2DM-induced alterations in the levels of insulin, lipid profile and oxidative stress in plasma. In addition, MSG (200 and 400 mg/kg) attenuated the T2DM-induced increase in the oxidative stress and decrease in the level of expression of GLUT-4 protein in the liver of animals. These results emphasize the fact that MSG (200 and 400 mg/kg) could be a potential candidate in the management of T2DM.

Preliminary phytochemical screening revealed that MSG showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the MSG showed rich with total phenolic content (12.3±0.3 mg/g). It has been reported that phenolic compounds possesses several biological activities including hypoglycemic activity in experimental as well as clinical studies [18, 19, 20]. In the present study, MET and MSG at both dose level significantly lowered the plasma glucose level in the animals subjected to either exogenously administered glucose or T2DM induction. However, either dose of MSG did not show any effect on the plasma glucose level of normal animals suggesting the fact that the MSG lacks a hypoglycemic activity in normal animals. In addition to a hyperglycemic condition, we also report an insignificant change in the level of plasma insulin in the T2DM rats indicating the development of insulin resistance condition in the present study to validate the model. All the treatment groups showed a significant decrease in the level of plasma insulin level. It is well established that there is a loss in the glucose utilization through decrease in the level of GLUT-4 in several glucose sensing organs including liver [21]. Similar to earlier report, we also document a decrease in the level of GLUT-4 in the liver of T2DM induced rats [21]. MSG (200 and 400 mg/kg) attenuated the T2DM-induced decrease in the level of expression of GLUT-4 in the liver tissue. Therefore, it can be assumed that the anti-hyperglycemic activity of MSG could be due to the presence of phenolic compounds. Moreover, the anti-hyperglycemic effect may underlie the mechanism involving GLUT-4 translocation to reduce insulin resistance which has to be supported with further experimentation in the future.

It is well documented that there is a high risk for coronary heart disease in T2DM condition and the principal pathogenetic mechanism involves an aberration in the lipid profile in the plasma during this condition [22]. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. It has been observed earlier that the hypercholesterolemia and hypertriglyceridemia occurs in diabetic animals. Under normal conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides [23]. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Since lipid abnormalities accompanying with premature atherosclerosis, is the major cause of cardiovascular diseases in diabetic patient therefore ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. It has been systematically reported that cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes [24]. In the present study, MSG (200 and 400 mg/kg) significantly attenuated the T2DM-induced change in the lipid profile in terms of change in the level of TC, TG, HDL and LDL in the plasma of the animals. High levels of TC and more importantly LDL levels are major coronary risk factors [25]. It has been suggested that TG itself is independently related to coronary heart disease [26]. It has been evidenced that an increase in HDL levels is associated with a decrease in coronary risk [27] . Thus, it can be presumed that the improvement in the lipid profile in the T2DM condition could be due to the action on the enzymes involved in the secretion and regulation of these lipid profile markers, which has to be supported with further experimental report.

It has been established that oxidative stress plays a central role in the pathogenesis of diabetes [28]. Oxidative stress is the result of excessive free radical production and lipid peroxidation due to aberrant protection by the enzymatic and nonenzymatic anti-oxidative defense system. Oxidative stress is thought to be a direct concequence of increased production of reactive oxygen species (ROS). Degradation of lipid peroxides leads to the accumulation of toxic aldehydes, one of the most toxic being malondialdehyde (MDA), as an index of lipid peroxidation. It is well suggested that MDA is considered as one of the indicative marker of *in vivo* lipid peroxidation [29]. Further, it has been studied that lipid peroxidation is augmented during diabetes [30]. Oxidative stress, arising from an increase in ROS coupled with impaired antioxidant protection, has been suggested to be an important etiological factor for diabetes [31]. During oxidative stress LPO was significantly increased and the antioxidant enzymes such as SOD and CAT levels were significantly decreased. The decrease in SOD cause an increase in the generation of $H_2O_2^-$ and its accumulation due to decreased CAT level precipitate the extent of oxidative stress [32]. In our study, MSG at both dose levels attenuated the oxidative stress markers in plasma as well as liver tissue, suggesting its potential antioxidant activity.

In conclusion, MSG (200 and 400 mg/kg) exhibited a significant anti-hyperglycemic activity in T2DM rats. The T2DM-induced aberration in the lipid profile and oxidative stress markers were significantly altered with the pretreatment of MSG at both dose levels. MSG (200 and 400 mg/kg) attenuated the T2DM-induced decrease in the level of expression of GLUT-4 in the liver. These results cumulatively suggest that the anti-hyperglycemic activity of MSG against T2DM condition could be due to the improvement in the aberrant lipid profile, oxidative stress and insulin sensitivity. Hence, MSG could be a potential candidate in the management of T2DM.

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