Original Article

Effect of aqueous and alcoholic extract of *Sesbania sesban* (Linn) Merr. root on glycemic control in streptozotocin-induced diabetic mice

Abstract

Aim: The present study was carried out to investigate the hypoglycemic effects of the aqueous and ethanolic extracts of *Sesbania sesban* (SS) (Merr.) roots, which is widely used in inflammation, fever, ulcers, leucoderma and diabetes in various parts of India. **Materials and Methods:** SS extracts were administered orally at doses (500 and 1000 mg/kg) to normal and streptozotocin (STZ) induced Type-2 diabetic mice. The fasting blood glucose (FBG), biochemical parameters in serum viz., blood glucose, serum insulin, cholesterol, triglyceride (TG), high-density lipoprotein (HDL) cholesterol, urea, creatinine and total protein, change in body weight, internal organs weight, food intake, water intake and glycogen level in liver were performed for the evaluation of hypoglycemic effects. **Results:** Both doses of aqueous and ethanolic SS extracts caused a marked decrease of FBG in STZ induced Type-2 diabetic mice. Both extracts decreased the cholesterol, TG, urea, creatinine level and increased the insulin, HDL cholesterol and total protein level. Decrease in body weight and glycogen level induced by STZ was restored. Increase in water and food intake induced by STZ was decreased. **Conclusions:** The results suggest that aqueous and ethanolic extracts of SS may have hypoglycemic potential for the Type-2 diabetes and support the traditional use of the roots of plant as a hypoglycemic agent.

Key words:
Fabaceae, hyperglycemia, Sesbania sesban, streptozotocin

Introduction

Diabetes mellitus is a metabolic disease, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels, such as lipids and proteins. This metabolic disorder is the result of a deficiency in insulin secretion or a resistance to insulin action, or both[1] which, further leads to several chronic complications such as diabetic neuropathy, retinopathy, nephropathy, cardiomyopathy and foot diseases.[2] Management of diabetic and its complications is a huge economic burden and one of the major global public health problem, rapidly getting worse particularly in the developing nations. The estimated diabetes prevalence in 2010 was 285 million, representing 6.6% of the world's adult population and it is predicted to be increased to 435 million by 2030.[3]

Several management strategies have been proposed for the early stages of hyperglycemia, with the aim of preventing the development of diabetes and associated complications. The current treatments of diabetes mellitus include diet, exercise, various oral anti-diabetic drugs, insulin therapy or even combination therapies.[4] The modern drugs, insulin and other oral hypoglycemic agents such as biguanides, sulphonylureas, glucosidase inhibitors have characteristic profile of adverse effects, which include frequent diarrhea,

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hypoglycemia, hepatotoxicity, lactic acidosis, dyslipidemia, hypertension and hypercoagulability.[5] Given the alarming increase in the world-wide diabetic population, there is a need for novel therapies, which are effective with minimal adverse events.[6] Hence, the search for a definitive cure for diabetes mellitus is being pursued vigorously by the scientific community. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them.[7] A number of investigations, of oral anti-hyperglycemic agents from plants used in traditional medicine, have been conducted and many of the plants were found with good activity.[3,8-11] This has lead an increasing demand for research on anti-diabetic natural products, which produce minimum or no side-effects.

The *Sesbania sesban* (SS) is a native wild tree of India and is widely distributed throughout in India, up to an altitude of 1200 m. It belongs to family *Fabaceae* (Leguminoseae). It is commonly known as Jayanti, Jayata and Egyptian pea. The root is hot and bitter, carminative, cures tuberculous glands, fever, ulcer, diabetes and lecoderma etc.[7]

The main objective of the present investigation is to ascertain the scientific basis for the use of this plant in the management of diabetes, using streptozotocin (STZ)-induces diabetic mice, particularly since no such report had been documented in the literature. Hence, the potential hypoglycemic effect of aqueous and ethanolic extracts of SS was evaluated using STZ-induced diabetic mice and compared with metformin (MT) as a reference standard.

Materials and Methods

All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Register Number: 562/GO /02/a/CPCSEA) and were in accordance with the CPCSEA guidelines, Government of India.

Animals

Healthy adult Swiss albino mice (25-30 g) of either sex of were obtained from a disease free animal house of Chaudhary Charan Singh, Haryana Agriculture University, Hisar, Haryana (India). The animals were housed in the animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana (India). Animals were fed with commercially available autoclaved standard pelleted laboratory animal diet and were maintained under standard conditions of temperature (25°C ± 5°C), relative humidity (55% ± 10%) and 12/12 h light/dark cycle. The animals were provided free access to purified water (purified through reverse osmosis during the study period. All the animals were housed individual during the entire study period, in each polycarbonate cage (290 mm × 220 mm × 140 mm) with rice husk bedding and stainless steel metal tops. All the animals were acclimatized for a period of 5 days prior to initiation of the treatment. Each animal during acclimatization and study period was identified with Study Name, Cage Number, Species and Strain, Sex, Animal Number, Acclimatization/study Start and End date. Body weights of all the animals were recorded once on the day of receipt, on the day of randomization and on the day of treatment. Color-coded cage cards were used to identify each cage throughout the study. During treatment period each cage card was identified with at least Study Number, Cage Number, Species and Strain, Sex, Dose Group, Dose Level, Route, Animal Number, Date/Fate of Death, Dosing Start Date and End Date.

Chemicals

STZ (Himedia Lab. Pvt. Ltd.), MT (gift sample from Affy Pharma Pvt. Ltd. Baddi), total protein, serum triglyceride (TG), cholesterol, high-density lipoprotein (HDL) cholesterol, serum urea and creatinine estimation kits (ERBA Diagnostic Mannheim GmbH Mallaustr). Any other chemicals used were of AR grade.

Plant material

The roots of SS were collected from the nursery of Kurukshetra University and from fields nearby Kurukshetra University during September to October, 2008. Then, collected roots were positively identified at The Environment Society of India, Chandigarh (India). Voucher specimen (No.-ESI/15/2009) has been deposited in the herbarium of the Institute of Pharmaceutical Sciences, Kurukshetra University.

Extraction method

One kilogram roots of SS were washed and cleaned thoroughly so as to remove any type of contamination. Then washed roots were air dried in shade, powdered in grinder and passed through sieve of mesh size no-40. Half of the coarse powder was subjected to Soxhlet extraction for 48 h using ethanol and remaining half was macerated with distilled water for 72 h. The extracts were distilled and last traces of solvents were removed by rotary evaporator under reduced pressure. The yield of crude extracts from ethanol and water were 4.2% and 12.75%, respectively. The resulted crude extracts were collected and preserved in airtight glass container at 4-8°C.

Phytochemical studies

Various chemical tests were carried out on aqueous and ethanolic extracts for the qualitative determination of phytochemical constituents.[12]

Normoglycemic study

The effects of SS extract were evaluated in the normal animals. The percentage reduction in the basal glucose level was measured at the various time intervals after single oral administration of extracts. A total of 36 mice

were selected based on the body weight and randomly assigned into 6 groups, such that there was no statistically significant difference amongst the group mean body weight. Each group consisted of 6 animals and allocated as Group I: Vehicle control, Group II-III: Ethanolic extract at doses (500, 1000 mg/kg), Group IV-V: Aqueous extract at doses (500, 1000 mg/kg), Group VI: MT (0.5 mg/kg, p.o.). Mice were fasted overnight and water was provided *ad libitum*. Both ethanolic and water extracts were suspended in distilled water. Blood samples were collected from tail vein at 0, 30, 60, 120 min after administration of the treatments, for glucose analysis by using one touch electronic glucometer, by using glucose strips.

Oral glucose tolerance test

The effects of alcoholic and aqueous extract of roots of SS were evaluated on the glucose loaded normal mice. The blood glucose levels were monitored at various time intervals after single administration of extracts. Mice were fasted overnight and randomly assigned in 6 groups of 6 animals each. Different groups received aqueous and alcoholic extracts at the dose of (A) 500 (B) 1000. Normal control group received tween 80 suspension (0.5%) and glucose 2 g/kg, Positive control group received MT followed by 2 g/kg of oral glucose. Blood glucose levels were analyzed using glucose strips at 0, 30, 60, 120 and 180 min after administration of the glucose at the dose of 2 g/kg to each group.

STZ induced diabetic study

Induction of diabetes

After fasting for 18 h, mice were injected 150 mg/kg STZ i.p. after dissolving it in freshly prepared ice-cold citrate buffer pH (4.5). Animals had free access to feed and water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock. Fasting blood glucose (FBG) levels were determined on 12th day to confirm stable hyperglycemia. Animals having FBG levels >150 mg/dl were selected for the experiment. The diabetic animals after confirmation of stable hyperglycemia were divided into different groups of 6 animals each. That day was considered as 0th day and further experimental procedure was followed.

Experimental procedure

Diabetic mice were fasted overnight and randomly assigned in 6 groups of 6 mice each. Water was given *ad libitum*.

- Group I: Vehicle control
- Group II: Diabetic control
- Group III: Dose A (500 mg/kg) of aqueous extract
- Group IV: Dose B (1000 mg/kg) of aqueous extract
- Group V: Dose C (500 mg/kg) of EtOH extract
- Group VI: Dose D (1000 mg/kg) of EtOH extract
- Group VII: MT (0.5 mg/kg).

The effects of extracts were studied in all the groups, following 15 days of treatment. Blood samples were withdrawn from the tail vein from overnight fasted animals on 0th, 5th, 10th and 15th day following 2 h after vehicle/extract/standard administration. The blood glucose levels were determined by using one touch electronic glucometer, using glucose strips.

Physical parameters

The changes in body weight, food and water intake of animals were measured on 0th, 5th, 10th and 15th day of treatment.

Other parameters

On the 15th day, blood samples were collected from the retro orbital plexus (ROP) under mild anesthesia. Then blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at −20°C until analysis was performed. The animals were sacrificed by cervical dislocation and the following parameters were monitored.

Collection of organs

Liver, kidneys, pancreas, heart, lungs and spleen were isolated from animals and their weights were checked.

Biochemical parameters

Blood glucose, serum insulin, cholesterol, TG, HDL cholesterol, urea, creatinine and total protein were estimated by using various kits methods.

Estimation of liver glycogen level

Digestion of glycogen from tissues

About 0.1 g of liver skeletal muscle was weighed and minced. 0.2 ml of 30% KOH was added. Tubes were heated in boiling water bath for 15-20 min until clear solution was formed. Tubes were cooled and 0.24 ml of 95% ethanol was added in each tube to precipitate glycogen. Tubes were centrifuged at 3000 rpm for 10 min. The supernatant was discarded and tubes were kept on boiling water bath to remove any remaining ethanol. The precipitated glycogen was dissolved in 1 ml of distilled water.

Assay of glycogen

A total volume of 1 ml of unknown glycogen solution/standard solution/water was taken along with 50 ml of 80% phenol and 2.5 ml of concentrated sulfuric acid. The mixture was shaken after each addition. The test tubes were allowed to attain room temperature and absorbance was measured at 490 nm (UV spectrophotometer) and compared with the normal readings.

Statistical analysis

Data obtained from pharmacological experiments, are expressed as mean ± SD. Differences between control and treatment in these experiments were tested for significance using Analysis of Variance followed by Dunnett's *t*-test, with $P < 0.05$ were considered as significant.[13]

Results

Phytochemical studies

The ethanolic and aqueous extracts of SS consisted following chemical constituents viz., phytosterols, saponins, proteins, gums, mucilages and flavonoids.

Normoglycemic study

The administration of aqueous and ethanolic extracts at the dose of 500 and 1000 mg/kg of SS did not exhibit any significant changes in blood glucose level in normal mice [Table 1].

Oral glucose tolerance test

The effects of alcoholic and aqueous extract of roots of SS were evaluated on the glucose loaded normal mice. The blood glucose levels were monitored at various time intervals after single administration of extracts [Table 2]. A significant decrease in blood glucose level was observed at all the doses compared to vehicle control.

STZ induced diabetic study

The anti-hyperglycemic effect of the extracts on the FBG of diabetic mice is shown in Table 3. Administration of STZ (150 mg/kg, i.p.) led to approximate two fold elevation of FBG levels, which was maintained over a period of 15 days. Daily treatment of aqueous and ethanolic extracts at the dose of 500 and 1000 mg/kg led to dose dependent fall in FBG levels by about 50%. This decrease in glucose level was significant (*P < 0.05) in both the extracts at the dose of 1000 mg/kg, on 15th day.

Physical parameters

Table 4 shows the effect of administration of aqueous and ethanolic extracts of SS and MT on body weight of STZ-induced diabetic mice. Diabetic mice showed constant reduction in body weight during 15 days. Treatment with both the extracts reversed the body weight reduction, which was significant (*P < 0.05) at 1000 mg/kg in both the extracts on day 15th.

Table 1: Effect of aqueous and EtOH SS extracts on blood sugar level of normal mice

Group (n = 6)	0 min	30 min	60 min	120 min
Vehicle control	82.00±12.5	81.21±18.6	80.00±12.2	79.56±15.4
Aqueous extract (A)	86.16±2.1	84.11±4.2	81.00±2.3	79.99±3.3
Aqueous extract (B)	86.33±2.8	85.00±2.2	80.77±3.5*	77.34±2.7*
EtOH extract (A)	80.00±3.6	84.17±3.3	79.41±3.5	75.67±4.2*
EtOH extract (B)	75.11±2.1	77.83±3.4	75.23±1.1	72.78±3.5*
MT	78.99±4.3	79.00±4.5	72.67±2.7*	68.66±4.2*

The values are mean ± SEM, parenthesis: n = Number of animals indicated. *P < 0.005 as compared to vehicle control group. A (500 mg/kg); B (1000 mg/kg); MT (0.5 mg/kg). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; EtOH – Ethanol

Table 2: Effect of aqueous and EtOH SS extracts on oral glucose tolerance test

Group (n = 6)	0 min	30 min	60 min	120 min	180 min
Vehicle control	77.25±0.90	140.90±1.76	156.55±1.60	135.30±1.33	120.22±2.5
Aqueous extract (A)	85.30±7.3	133.73±2.2	148.33±3.5	111.23±3.3[a]	106.44±2.1
Aqueous extract (B)	83.45±2.9	136.70±2.3	125.67±2.3[a]	113.20±2.3[a]	91.67±1.6[a]
Alcoholic extract (A)	82.30±7.3	123.73±2.2[a]	135.33±4.5[a]	110.23±2.3[a]	103.44±2.1[a]
Alcoholic extract (B)	81.45±2.9	136.70±2.3	128.67±3.3[a]	113.20±2.3[a]	95.67±2.6[a]
MT	73.70±4.9	103.80±4.8[a]	121.7±1.3[a]	91.56±2.2[a]	91.00±4.6[a]

The values are mean ± SEM, parenthesis: n = Number of animals. A (500 mg/kg); B (1000 mg/kg); MT (0.5 mg/kg). [a]P<0.005 versus normal control; (One way ANOVA followed by Dunnett's, multiple comparison test). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; EtOH – Ethanol

Table 3: Effect of aqueous and EtOH SS extracts on the FBG in STZ induced NIDDM in mice

Group (n = 6)	0th day (mg/dl)	5th day (mg/dl)	10th day (mg/dl)	15th day (mg/dl)
Diabetic control	179.33±11.6	184.00±9.2	182.19±9.6	182.40±7.6
Aqueous extract (A)	171.50±1.1	150.99±5.4	122.14±10.0	99.34±14.4
Aqueous extract (B)	183.23±0.45	152.91±15.6	128.13±9.5	97.31±0.06*
EtOH extract (A)	182.15±6.3	150.00±9.4	131.14±6.6	101.35±11.4
EtOH extract (B)	175.08±0.21	145.88±10.3	128.11±9.3	97.32±16.3*
MT	182.99±11.7	150.71±12.5	133.09±9.7	91.31±10.2*

The values are mean ± SEM, parenthesis: n = Number of animals indicated. *P < 0.05 as compared to diabetic control group. A (500 mg/kg); B (1000 mg/kg); MT(0.5 mg/kg). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; FBG – Fasting blood glucose; STZ – Streptozotocin; NIDDM – Non-insulin dependent diabetes mellitus; EtOH – Ethanol

In diabetic animals food and water intake increases due to polyphagia and polydipsia, that is caused by uptake of STZ. Animals treated with both the doses (500 mg/kg and 1000 mg/kg) of SS roots extracts showed decrease in food and water intake [Table 5] after 15 days of treatment. However, significant (* $P < 0.05$) reduction was observed only with dose 1000 mg/kg of both extracts when compared with diabetic control.

Organs weight

It is evident from Table 6 that STZ-induced diabetes increased the weight of liver, kidneys, pancreas, heart and lungs. The increase in weights of these organs was antagonized by administration of aqueous and ethanolic extracts and MT. The values of liver, kidneys and heart are significant with MT only and with SS extracts values are not significant. Both the extracts at dose (1000 mg/kg) induced significant (* $P < 0.05$) decrease in weight in case of pancreas and lungs only.

STZ-induced diabetes decreased weight of spleen [Table 6]. Decrease in weight was restored by administration of SS extracts and MT. Values were significant (* $P < 0.05$) with MT and 1000 mg/kg dose of both aqueous and ethanolic extracts.

Biochemical parameters

Serum cholesterol, TG, urea and creatinine levels were increased with diabetes. Both extracts at dose 500 mg/kg induced significant (* $P < 0.05$) decrease in TG and creatinine only. Serum insulin, HDL cholesterol and total protein level were decreased in diabetic mice. These parameters were increased significantly (* $P < 0.05$) by SS extracts treatment [Table 7].

Table 4: Effect of aqueous and EtOH SS extracts on the body weight of STZ induced NIDDM in mice

Group ($n = 6$)	0th day (g)	5th day (g)	10th day (g)	15th day (g)
Diabetic control	25.44±0.02	20.55±0.06	17.11±0.11	15.66±0.22
Aqueous extract (A)	20.33±0.07	23.14±0.08	23.25±0.12	23.22±2.2
Aqueous extract (B)	20.45±0.09	21.51±0.10	22.36±0.14	22.11±2.7*
EtOH extract (A)	20.72±0.10	21.66±0.09	22.30±0.16	22.23±0.11
EtOH extract (B)	19.77±0.21	22.22±0.19	22.42±0.18	23.22±1.1*
MT	21.54±0.10	23.43±0.09	24.33±0.16	25.26±3.4*

The values are mean ± SEM, parenthesis: n = Number of animals indicated. * $P < 0.005$ as compared to diabetic control group. A (500 mg/kg); B (1000 mg/kg); MT (0.5 mg/kg). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; STZ – Streptozotocin; NIDDM – Non-insulin dependent diabetes mellitus; EtOH – Ethanol

Table 5: Effect of aqueous and EtOH SS extracts on the food and water intake of STZ induced NIDDM in mice

Group ($n = 6$)	Food intake (g)				Water intake (ml)			
	Day 1	Day 5	Day 10	Day 15	Day 1	Day 5	Day 10	Day 15
Diabetic control	65.50±3.3	62.52±3.1	69.10±2.9	70.78±1.6	45.50±4.3	47.52±2.1	49.10±3.9	50.78±1.6
Aqueous extract (A)	72.20±3.0	68.53±2.6	64.81±3.7	56.92±1.1	48.20±3.0	44.53±2.6	38.81±3.7	36.92±1.1
Aqueous extract (B)	70.66±2.5	65.31±2.3	61.64±2.3	56.58±1.4*	45.66±2.5	41.31±2.3	39.64±2.3	34.58±1.4*
EtOH extract (A)	71.22±2.0	63.34±4.3	59.74±2.3	55.12±1.4	45.22±2.0	42.34±4.3	39.74±2.3	35.12±5.4
EtOH extract (B)	69.99±3.5	62.62±2.6	57.44±3.3	53.82±4.1*	44.99±3.5	39.62±2.6	38.44±3.3	34.82±4.1*
MT	73.24±3.4	66.55±4.3	59.72±2.3	51.14±2.4*	46.24±3.4	40.55±4.3	38.72±2.3	32.14±2.4*

The values are mean ± SEM, parenthesis: n = Number of animals indicated. * $P < 0.005$ as compared to diabetic control group. A (500 mg/kg); B (1000 mg/kg); MT (0.5 mg/kg). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; STZ – Streptozotocin; NIDDM – Non-insulin dependent diabetes mellitus; EtOH – Ethanol

Table 6: Effect of aqueous and EtOH SS extracts on the weight of organs such as liver, kidney, pancreas, heart, lung and spleen of STZ induced NIDDM in mice

Group ($n = 6$)	Liver weight (g)	Kidney weight (g)	Pancreas weight (g)	Heart weight (g)	Lung weight (g)	Spleen weight (g)
Diabetic control	4.68±0.06	1.38±0.06	0.19±0.06	0.40±0.06	0.56±0.06	0.10±0.02
Aqueous extract (A)	3.50±1.1	0.99±0.34	0.14±0.01	0.34±0.04	0.44±0.01	0.12±0.04
Aqueous extract (B)	3.23±0.45	0.91±0.16	0.13±0.05*	0.31±0.06	0.43±0.05*	0.14±0.06*
EtOH extract (A)	3.15±0.30	1.01±0.44	0.14±0.01	0.35±0.04	0.45±0.01	0.13±0.04
EtOH extract (B)	3.08±0.21	0.88±0.23	0.11±0.03	0.32±0.03	0.41±0.03*	0.15±0.03*
MT	2.99±0.07*	0.71±0.15*	0.09±0.07*	0.31±0.02	0.39±0.07*	0.18±0.02*

The values are mean ± SEM, parenthesis: n = Number of animals indicated. * $P < 0.005$ as compared to diabetic control group. A (500 mg/kg); B (1000 mg/kg); MT (0.5 mg/kg). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; STZ – Streptozotocin; NIDDM – Non-insulin dependent diabetes mellitus; EtOH – Ethanol

Table 7: Effect of aqueous and EtOH SS extracts on serum profile in STZ induced NIDDM in mice

Group (n = 6)	Serum insulin	Serum cholesterol	Serum TG	Serum HDL cholesterol	Serum urea	Serum creatinine	Serum total protein
Diabetic control	14.46±1.11	329.00±0.91	190.18±9.5	51.00±1.5	63.00±1.9	1.35±0.1	6.0±0.5
Aqueous extract (A)	23.11±2.37	218.51±0.97	127.23±6.3*	115.50±1.3	46.50±1.3	0.83±0.3	10.6±0.3*
Aqueous extract (B)	22.16±3.22*	201.16±1.22*	108.36±5.2*	99.00±1.2*	37.00±1.2*	0.70±0.2*	11.3±0.2*
EtOH extract (A)	25.38±2.15	222.18±1.02	121.10±9.0*	111.00±2.0*	45.10±1.0	0.80±0.1	9.0±0.1
EtOH extract (B)	23.21±5.11*	190.26±1.11*	101.15±9.5*	101.00±1.5	38.00±1.0*	0.69±0.5*	11.1±0.5*
MT	22.11±4.11*	173.96±0.91**	91.96±9.1*	77.00±1.1*	34.00±1.1*	0.63±0.1**	12.3±0.1*

The values are mean ± SEM, parenthesis: n = Number of animals indicated. ** $P < 0.005$; * $P < 0.05$ as compared to diabetic control animals. A (500 mg/kg); B (1000 mg/kg); MT (0.5 mg/kg). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; STZ – Streptozotocin; NIDDM – Non-insulin dependent diabetes mellitus; EtOH – Ethanol; HDL – High-density lipoprotein

Liver glycogen assay

Diabetic control animals have decreased liver glycogen level, whereas animals treated with SS extract at dose 1000 mg/kg and MT prevent this decrease in liver glycogen level significantly (* $P < 0.005$) when compared with diabetic group [Table 8].

Table 8: Effect of aqueous and EtOH SS extracts on liver glycogen level in STZ induced NIDDM in mice

Group (n = 6)	Liver glycogen level
Diabetic control	65.3±5.5
Aqueous extract (A)	69.9±4.3
Aqueous extract (B)	72.3±2.2*
EtOH extract (A)	73.8±4.3
EtOH extract (B)	76.1±2.5*
MT	79.9±3.6*

The values are mean ± SEM, parenthesis: n = Number of animals indicated. * $P < 0.05$ as compared to diabetic control group. A (500 mg/kg); B (1000 mg/kg); MT (0.5 mg/kg). SEM – Standard error of mean; SS – *Sesbania sesban*; MT – Metformin; STZ – Streptozotocin; NIDDM – Non-insulin dependent diabetes mellitus; EtOH – Ethanol

Discussion

The present study reports the hypoglycemic effect of aqueous and ethanolic extracts of SS for the 1st time. In the present study, STZ produced significant increase in fasting hyperglycemia, which was antagonized by administration of either SS or MT. STZ selectively destroys pancreatic insulin-secreting β-cells by causing diabetes close to Type-2 diabetes of humans.[14] The elevated blood glucose levels in the diabetic mice used by us were in the range of 150-200 mg/dl which resembles Type-2 diabetes (150 to about 250 mg/dl) with partially functional pancreas. MT is a biguanide, decreases blood glucose concentration act by enhancing insulin sensitivity, inducing greater peripheral uptake of glucose and decreasing hepatic glucose output.[15]

Chronic administration of SS extracts to STZ-induced diabetic mice showed significant and consistent decrease in FBG levels at different time intervals throughout the period of study when compared to the diabetic controls, indicating its potent anti-diabetic activity. The destruction of β-cells during diabetes ultimately causes physico-metabolic abnormalities such as a decrease in body weight gain and increase in food and water intake.[16] In addition, diabetic mice showed a clear muscle atrophy involving a decrease in both skeletal muscle mass and protein content. This was accompanied by a marked loss of total carcass nitrogen. These changes were related to important alterations in protein turnover in skeletal muscle.[17] Hence, a notable decrease in the body weight gain observed in the diabetic group of mice might be the result of protein wasting due to the unavailability of carbohydrates for energy metabolism and the loss or degradation of structural proteins.[18] The significant improvement in body weight gain in diabetic mice supplemented with SS extracts and MT highlight the blood glucose homeostasis which in turn promotes the body weight gain. In both types of diabetes mellitus polyuria, polydipsia and polyphagia symptoms develop. When the glucose concentration in the blood is raised beyond the renal threshold, reabsorption of glucose in the proximal renal tubule is incomplete and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits the reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells, causing dehydration and increased thirst. The hormone insulin is also responsible for stimulating hunger. In order to cope up with high sugar level in blood; body produces insulin which leads to increased hunger. In the present study, the treatment with SS and MT decreased the water intake and food intake.[19]

Insulin deficiency ultimately results in increased production of glucose by liver and decreased utilization of glucose in peripheral tissues.[20] The elevated blood glucose level observed in the diabetic mice was significantly decreased in SS extracts and the MT treated group of mice suggesting insulin stimulatory effect of SS extracts from the remnant β-cells. This was further evidenced from the observed increase in the level of plasma insulin in diabetic mice treated with SS extracts. An alteration in the internal organ weights

may primarily indicate toxicity or pathology occurring to these organs. In the present study, it was found that weights of all the organs studied, except the spleen weight, were increased by the diabetic state. Treatment with SS extracts and MT, restored the increased weight of organs.[21]

Hypercholesterolemia and hypertriglyceridemia have been induced in STZ-induced diabetic mice. Insulin activates lipoprotein lipase and hydrolyzes TG under normal conditions. In diabetic state it fails to activate the enzymes and cause hypertriglyceridemia under insulin deficiency.[22] From the above results, it was found that SS extracts and MT showed the effective depression of the cholesterol and TG levels in blood and increased the insulin level.

The diabetic hyperglycemia induces elevation of the serum levels of urea and creatinine which were considered as significant markers of renal dysfunction.[23] Insulin deprivation in diabetic state causes a profound increase in protein catabolism, especially in skeletal muscle. Moreover, this total muscle protein catabolism is due to a net increase in protein breakdown rather than a decline in protein synthesis.[24] Urea is the main end product of protein catabolism in the body. Accumulation of urea nitrogen in experimental diabetes may due to the enhanced breakdown of both liver and plasma proteins.[25] After the treatment of STZ-diabetic mice with the aqueous and ethanolic extracts and MT, the level of urea was significantly decreased and level of protein increased when compared with diabetic group suggesting the prophylactic role of SS in protein metabolism.

Creatinine is a byproduct of the breakdown of creatine and phosphocreatine and an elevation in creatinine usually occurs simultaneously with an increase in blood urea nitrogen.[26] Therefore, it may be concluded that the early renal changes occurred in the diabetic mice were significantly improved by the oral administration of aqueous and ethanolic extracts and MT, compared with the diabetic group.

The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and on the availability of insulin which stimulate glycogen synthesis over a wide range of glucose concentration.[27] Since STZ selectively damage β-cells of islets of Langerhans resulting in marked decrease in insulin levels, hence glycogen content of skeletal muscle and liver was markedly decreased in diabetic mice.[28] In the present study, the experimental diabetic mice treated with SS extracts and MT restored the glycogen level which may be due to the stimulation of insulin release from beta cells.

Conclusions

Aqueous and ethanolic ether extracts of SS roots exhibited significant antihyperglycemic activities in STZ-induced diabetic mice. The extract showed improvement in various body and serum parameters as well as regeneration of β-cells of pancreas and so might be of value in diabetes. However, further phytochemical investigations are required to isolate and identify the hypoglycemic principles in the plant as well as elucidating their mechanism of action.

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