Effect of petroleum ether extract of *Sesbania sesban* (Merr.) roots in streptozotocin (STZ) induced diabetes in mice

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**ABSTRACT**

**Objective:** The present study was carried out to investigate the hypoglycemic effects of the petroleum ether extract of *Sesbania sesban* (SS)(Merr.) roots, which are widely used in inflammation, fever, ulcers, leucoderma and diabetes in various parts of India. **Methods:** SS was administered orally at different doses (250, 500 and 1000mg/kg) to normal and streptozotocin (STZ) induced type–2 diabetic mice. The fasting blood glucose (FBG), biochemical parameters in serum, change in body weight, internal organs weight, food intake, water intake and glycogen level in livers were performed for the evaluation of hypoglycemic effects. **Results:** All the doses of SS caused a marked decrease of FBG in STZ induced type–2 diabetic mice. SS decreased the cholesterol, triglyceride (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 SS may have hypoglycemic potential for the type 2– diabetes and support the traditional use of the roots of plant as a hypoglycemic agent.

**ARTICLE INFO**

**Article history:**
Received 25 August 2012
Received in revised from 15 September 2012
Accepted 7 December 2012
Available online 28 December 2012

**Keywords:**
Diabetes
Hypoglycemic agent
*Sesbania sesban*
Streptozotocin

**1. Introduction**

Diabetes mellitus is a complicated, chronic disorder characterized by either insufficient insulin production by pancreatic β–cells or by cellular resistance to insulin. Such a deficiency results in increased concentrations of glucose in the body, which in turn, damages many of body’s system, in particular the blood vessels and nerves[1]. The number of people in the world with diabetes has increased dramatically over recent years. Indeed, by 2010 it has been estimated that the diabetic population will increase to 221 million around the world[2]. The diabetes Control and Complications Trial (DCCT) Research Group (1993) stated that tight control of blood glucose is an effective strategy in reducing clinical complications of diabetes mellitus significantly, but even optimal control of blood glucose could not prevent diabetes, suggesting that alternative treatment approach is needed[3].

Currently available therapies for diabetes include insulin and various oral anti–diabetic agents such as sulfonyl ureas, etc. These drugs are used as monotherapy or in combination to achieve better glycemia control[4]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times there has been a renewed interest in the plant remedies[5,6].

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history but India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants and is known worldwide for its Ayurveda treatment[7].

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

The beneficial effects on glucose homeostasis of other species of the Leguminosae family popularly used as
anti-diabetic have been verified\cite{8}. The present study was performed to determine whether there was any scientific justification for the traditional uses of SS as a hypoglycemic agent in India, particularly since no such report had been documented in the literature. Hence, the potential hypoglycemic effect of petroleum ether extract of SS was evaluated by using STZ–induced diabetic mice and compared with metformin (MT) as a reference standard.

2. Materials and Methods

All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (IAEC) (Register Number: 536/02/a/CPCSEA) and were in accordance with the CPCSEA guidelines, Government of India.

2.1. Animals

Healthy adult Swiss albino mice (25–30 gm) of either sex of Wistar strain 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 mice feed and were maintained under standard conditions of temperature (25℃±5℃), relative humidity (55±10%), and 12/12 h light/dark cycle. They were housed in standard polycarbonate cages with wire mesh top and husk bedding.

2.2. Drugs and chemicals

Streptozotocin (Himedia), Metformin (gift sample from Affy Pharma Baddi), Petroleum ether (Sd Fine Chemicals), Total protein, Serum triglyceride, cholesterol, HDL cholesterol, Serum urea and creatinine estimation kits (ERBA Diagnostic Mannheim GmbH Mallaustr.). Any other chemicals used were of AR grade.

2.3. Plant material

The roots of SS were collected from the nursery of Kurukshetra University and from fields nearby Kurukshetra University during Sept–Oct, 2008. Then, collected roots were positively identified at the Environment Society of India, Chandigarh, India. A voucher specimen of the plant (Ref. No. ESI/15/2009) has been preserved there for future references.

2.4. Extraction method

Roots 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. Thus, the obtained coarse powder was subjected to Soxhlet Extraction for 48 hrs using petroleum ether solvent. The extract was distilled and last traces of solvent were removed by rotary evaporator under reduced pressure. %age yield of extract was 2.15%. The resulted crude extract was collected and preserved in airtight glass container at 4℃ – 8℃.

2.5. Preliminary phytochemical studies

Chemical tests were carried out on petroleum ether extract for the qualitative determination of phytochemical constituents as described by Khandelwal et al\cite{9}.

2.6. Normoglycemic study

The effect of SS extract was 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 extract.

2.6.1 Experimental procedure

Mice were fasted overnight and randomly assigned into 5 groups of six animals each. Water was available ad libitum. SS extract and MT were suspended in tween 80 (0.5%).

Group I :  Vehicle control was given tween 80 suspension (0.5%, p.o.).

Group II–IV :  SS extract according to the body weight (250, 500, 1000mg/kg, p.o.).

Group V :  MT (0.5mg/kg, p.o.).

The animals were left in their individual cages. Blood samples were collected from tail vein at 0, 30, 60, 120 minutes after administration of the treatments, for glucose analysis by using one touch electronic glucometer, by using glucose strips.

2.7. Streptozotocin induced diabetic study

2.7.1 Induction of Diabetes

After fasting for 18 hrs, 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. FBS levels were determined on 12th day to confirm stable hyperglycemia. Animals having FBS levels more than 150mg/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.

2.7.2 Experimental procedure

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

- Group-I: Vehicle control: tween 80 suspension (0.5%)
- Group-II: Diabetic control: tween 80 suspension (0.5%)
- Group-III: Dose A (250mg/kg) of SS extract
- Group-IV: Dose B (500mg/kg) of SS extract
- Group-V: Dose C (1000mg/kg) of SS extract
- Group-VI: MT (0.5mg/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 two hours after vehicle/extract/standard administration. The blood glucose levels were determined by using one touch electronic glucometer, using glucose strips.

2.7.3. Physical parameters

The changes in body weight, food and water intake were calculated by checking the weights of individual animals on 0th, 5th, 10th and 15th day of treatment.

2.7.4. Other parameters

On 15th day, blood samples were collected from the Retro Orbital Plexus (ROP). 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 giving anesthesia with diethyl ether and the several parameters were monitored.

2.7.5. Collection of organs

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

2.7.6. Biochemical parameters

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

2.7.7. Estimation of liver glycogen level

2.7.7.1 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.

2.7.7.2 Assay of glycogen

1 ml of unknown glycogen solution / standard solution / water was taken along with 50 μl of 80 % phenol and 2.5 ml of concentrated sulphuric 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.

2.8. Histopathological parameters

On 15th day, animals were sacrificed and the pancreas of animals were excised and stored in 10% formalin after washing with normal saline. Histopathological parameters were analyzed in Medicose Laboratories, Chandigarh.

2.9. 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 ANOVA followed by Dunnet’s t-test, with P-value < 0.05 were considered as significant[10].

3. Results

3.1. Preliminary phytochemical studies

The SS extract was found to consist of the following chemical constituents’ phytosterols, fixed oils, fats, saponins, proteins, gums, mucilages and amino acids.

3.2. Normoglycemic study

The SS extract has been shown to decrease glucose level

<p>| Table 1 |
| Effect of SS extract on blood sugar level of normal mice |</p>
<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>0 minute</th>
<th>30 minute</th>
<th>60 minute</th>
<th>120 minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>82.00 ± 12.5</td>
<td>81.21 ± 18.6</td>
<td>80.00 ± 12.2</td>
<td>79.56 ± 15.4</td>
</tr>
<tr>
<td>SS (A)</td>
<td>78.00 ± 1.9</td>
<td>80.11 ± 4.3</td>
<td>78.33 ± 6.4</td>
<td>75.22 ± 1.1</td>
</tr>
<tr>
<td>SS (B)</td>
<td>82.50 ± 2.4</td>
<td>80.12 ± 3.0</td>
<td>77.67 ± 3.3</td>
<td>73.45 ± 5.1ab</td>
</tr>
<tr>
<td>SS (C)</td>
<td>79.67 ± 2.2</td>
<td>78.00 ± 2.2</td>
<td>74.34 ± 3.4a</td>
<td>70.23 ± 3.2a</td>
</tr>
<tr>
<td>MT</td>
<td>78.99 ± 4.3</td>
<td>79.00 ± 4.5</td>
<td>72.67 ± 2.7</td>
<td>68.66 ± 4.2</td>
</tr>
</tbody>
</table>

The values are mean±SEM, parenthesis: n=number of animals indicated
Vehicle control (0.5%(v/v),tween 80); A (250mg/kg), B (500mg/kg) and C (1000mg/kg)
a P < 0.005; b P < 0.05 significant from vehicle control group
(One way ANOVA followed by Dunnett’s, Multiple comparison test)
in normal mice (Table 1). Decrease in glucose level was started at 30 mins and it continued up to 120 mins. These changes in glucose levels were significant (P < 0.005, 0.05) when compared with vehicle control group. There was a decrease of glucose levels at all the doses (250mg/kg, 500mg/kg and 1000mg/kg) but significant decrease was only at doses 500mg/kg and 1000mg/kg. Increase of doses from 500mg/kg to 1000mg/kg didn’t show further decrease in glucose level as expected.

3.3. Streptozotocin induced diabetic study

The anti-hyperglycemic effect of the extract on the FBG of diabetic mice is shown in Table 2. Administration of STZ (150mg/kg, i.p.) led to approximate double elevation of FBG levels, which was maintained over a period of 15 days. Daily treatment of extract of SS led to dose dependent fall in FBG levels by about 50%. The decrease in glucose level was significant (P<0.05) at doses (500 and 1000mg/kg) of SS extract on 15th day.

3.4. Physical parameters

Table 3 shows the effect of feeding SS extract and MT on body weight of STZ–induced diabetic mice. Diabetic mice showed constant reduction in body weight during 15 days. STZ caused body weight reduction, which is reversed significantly (P<0.05) by various doses of SS extract after 15 days of treatment.

In case of diabetic animals food and water intake increases due to polyphagia and polydipsia, that is caused by uptake of STZ. Animals treated with doses (250mg/kg, 500mg/kg and 1000mg/kg) of SS roots extracts, showed significant (P<0.05) decrease in food and water intake (Table 4) after 15 days of treatment.

Table 2

Effect of the SS extract on the FBG in STZ induced NIDDM in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>0th Day (mg/dl)</th>
<th>5th Day (mg/dl)</th>
<th>10th Day (mg/dl)</th>
<th>15th Day (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>179.33 ± 11.6</td>
<td>184.00 ± 9.2</td>
<td>182.19 ± 9.6</td>
<td>182.40 ± 7.6</td>
</tr>
<tr>
<td>SS (A)</td>
<td>168.75 ± 5.8</td>
<td>151.10 ± 10.1</td>
<td>130.15 ± 9.8</td>
<td>101.38 ± 8.1</td>
</tr>
<tr>
<td>SS (B)</td>
<td>173.44 ± 8.4</td>
<td>150.98 ± 9.9</td>
<td>125.13 ± 7.6</td>
<td>96.37 ± 7.5a</td>
</tr>
<tr>
<td>SS (C)</td>
<td>166.11 ± 7.3</td>
<td>141.86 ± 10.5</td>
<td>120.11 ± 12.4</td>
<td>95.34 ± 11.5a</td>
</tr>
<tr>
<td>MT</td>
<td>182.99 ± 11.7</td>
<td>150.71 ± 12.5</td>
<td>133.09 ± 9.7</td>
<td>91.31 ± 10.2a</td>
</tr>
</tbody>
</table>

The values are mean ± SEM, parenthesis: n=number of animals indicated A (250mg/kg), B (500mg/kg) and C (1000mg/kg)
a P < 0.05 significant from diabetic control animals
(One way ANOVA followed by Dunnett’s, Multiple comparison test)

Table 3

Effect of SS extract on the body weight of STZ induced NIDDM in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>0th Day (gm)</th>
<th>5th Day (gm)</th>
<th>10th Day (gm)</th>
<th>15th Day (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>25.44 ± 0.02</td>
<td>20.55 ± 0.06</td>
<td>17.11 ± 0.11</td>
<td>15.66 ± 0.22</td>
</tr>
<tr>
<td>SS (A)</td>
<td>20.53 ± 0.11</td>
<td>21.11 ± 0.09</td>
<td>23.47 ± 0.15</td>
<td>23.27 ± 1.1</td>
</tr>
<tr>
<td>SS (B)</td>
<td>20.32 ± 0.05</td>
<td>22.18 ± 0.06</td>
<td>23.19 ± 0.11</td>
<td>23.33 ± 2.2</td>
</tr>
<tr>
<td>SS (C)</td>
<td>19.41 ± 0.09</td>
<td>21.24 ± 0.08</td>
<td>22.25 ± 0.15</td>
<td>22.00 ± 1.1a</td>
</tr>
<tr>
<td>MT</td>
<td>21.54 ± 0.10</td>
<td>23.43 ± 0.09</td>
<td>24.33 ± 0.16</td>
<td>25.26 ± 3.4a</td>
</tr>
</tbody>
</table>

The values are mean ± SEM, parenthesis: n=number of animals indicated A (250mg/kg), B (500mg/kg) and C (1000mg/kg)
a P < 0.05 significant from diabetic control animals
(One way ANOVA followed by Dunnett’s, Multiple comparison test)
3.5. Organs weight

It is evident from Table 5 that STZ-induced diabetes increased the weight of liver, kidney, pancreas, heart, lung and spleen. The increases in weights of these organs were antagonized by administration of SS and MT. The values in case of liver, kidney and heart are significant with MT only and with SS extract values are not significant. SS at dose (1000mg/kg) induced significant (P<0.05) decrease in weight in case of pancreas and lungs only.

STZ-induced diabetes decreased weight of spleen (Table 5). Decrease in weight was restored by SS and MT administration. Values were significant (P<0.05) at doses (500mg/kg and 1000mg/kg) of SS and MT.

3.6. Biochemical parameters

Serum cholesterol, TG, urea and creatinine levels were increased with diabetes. 500mg/kg of SS extract 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 extract (Table 6).

3.7. Liver glycogen assay

Diabetic control animals have decreased liver glycogen level where as animals treated with SS and MT prevent this decrease in liver glycogen level significantly (P<0.005) when compared to diabetic mice.

4. Discussion

The present study reports the hypoglycemic effect of petroleum ether extract of SS for the first time. Administration of various doses of SS to euglycemic mice has shown to induce hypoglycemia. 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[11]. 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
Metformin is a biguanide that decreases blood glucose concentration. It lowers fasting plasma insulin concentrations and act by enhancing insulin sensitivity, inducing greater peripheral uptake of glucose, and decreasing hepatic glucose output. Administration of SS extract to STZ–induced diabetic mice showed significant and consistent decrease in FBG levels at different time intervals throughout the period of study as compared to the diabetic controls, indicating its potent antidiabetic activity.

Diabetic control mice showed no significant increase in the body weight over 15 days period of time. STZ administered animals are not able to gain body weight, has already been reported. SS treated mice’s gain significant body weight as compared to diabetic control.

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 decreased the urea level, water intake and food intake.

There are contradictory reports on the effect of liver weight in diabetes. Chen and Ianuzzo have shown an increase liver weight in animals where as Gupta et al have reported no change. In the present study, diabetic mice showed significant increase in liver weight.

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 restored the increased weight of organs.

Hypercholesterolemia and hypertriglyceridemia have been induced in STZ–induced diabetic rats. From the above results it was found that SS showed the effective depression of the cholesterol and TG levels in blood. STZ–induced diabetic mice have shown decreased in insulin and increase in TG levels. Because insulin activates lipoprotein lipase and hydrolyze TG under normal conditions, it fails to activate the enzyme and cause hypertriglyceridemia under insulin deficiency. SS treatment to diabetic mice increased the insulin level.

Former researchers have shown that there is a positive correlation between the risk of developing ischemic heart disease and raised plasma cholesterol and decreased plasma HDL. Researchers have shown that the concentrations of proteins have been reduced in STZ–induced diabetes. Treatment to diabetic mice with, SS extract increased the protein level. The diabetic hyperglycemia induces elevation of the serum levels of urea and creatinine which were considered as significant markers of renal dysfunction. After the treatment of STZ–diabetic mice with the SS extract, the level of urea was significantly decreased with the mean value of diabetic group. Similarly, the elevation of creatinine level caused by diabetes was declined after the administration of the SS, 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. The regulation of glucose metabolism by in vivo occurs by the multifunctional enzyme glycogen synthase and glycogen phosphorylase that play a major role in the glycogen metabolism. Since STZ selectively damage β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in liver tissue decreases as this depend on insulin influx of glucose. The reduced glycogen store in diabetic mice has been attributed to reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. In the present study the experimental diabetic mice treated with SS restored the glycogen level.

5. Conclusions

Petroleum ether extract 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.

Acknowledgements

The authors are highly grateful to Dr. Om Prakash Arora, Director of Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra for valuable guidance and for providing research facilities.

Conflict of interest statement
We declare that we have no conflict of interest.

References