Original Article

Antidiabetic Activity of Aqueous Leaves Extract of *Sesbania sesban* (L) Merr. in Streptozotocin Induced Diabetic Rats

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Abstract

The aqueous leaves extract *of Sesbania sesban* (L) Merr. (Family: Fabaceae) was evaluated for its antidiabetic potential on normal and streptozotocin (STZ)-induced diabetic rats. In the chronic model, the aqueous extract was administered to normal and STZ- induced diabetic rats at the doses of 250 and 500 *mg/kg* body weight (b.w.) *p.o.* per day for 30 days. The fasting Blood Glucose Levels (BGL), serum insulin level and biochemical data such as glycosylated hemoglobin, Total Cholesterol (TC), Triglycerides (TG), High Density Lipoproteins (HDL) and Low Density Lipoproteins (LDL) were evaluated and all were compared to that of the known anti-diabetic drug glibenclamide (0.25 *mg/kg* b.w.). The statistical data indicated significant increase in the body weight, liver glycogen, serum insulin and HDL levels and decrease in blood glucose, glycosylated hemoglobin, total cholesterol and serum triglycerides when compared with glibenclamide. Thus the aqueous leaves extract of *Sesbania sesban* had beneficial effects in reducing the elevated blood glucose level and lipid profile of STZ-induced diabetic rats.

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Introduction

Sesbania sesban (L) Merr. is a small perennial tree with woody stems, yellow flowers and linear pods belongs to the family Fabaceae. Seed and bark are used as astringent, emmenagogue, in menorrhagia, spleen enlargement and diarrhea. The pods and leaves contain campesterol and beta-sitosterol. Flowers contain cyanidin and delphinidin glucosides. Pollen and pollen tubes contain alpha-ketoglutaric, oxaloacetic and pyruvic acids [1,2]. Leaves are used as antihelmintic and also useful in diabetes, colic and skin diseases. Seeds are stimulant, emmenagogue, astringent and also useful in diarrhea [3].

Reports suggest that previous phytochemical investigations of the plant led to the isolation of oleanolic acid, stigmasta-5,24(28)-diene-3-ol-3-0-β- D- galactopyranoside, fatty acids and amino acids [4]. Various types of lignins are composed of guaiacyl, syringyl and P-hydroxyphenylpropane building units [5] and also anti-tumor principal, kaempferol trisaccharide [6]. However, the literature indicates that there is no specific evidence to support the antidiabetic effect of *Sesbania sesban*. The present study investigates the action of aqueous extract of *Sesbania sesban* leaves in the STZ-induced diabetic rats to ascertain the scientific basis for the use of this plant in the treatment of diabetes.

Material and Methods

Collection of plant material

The leaves of *Sesbania sesban* were collected during July 2008 from the Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India. The leaves were identified by Dr. P.G.Diwakar, Joint Director, Botanical Survey of India, Pune. A voucher specimen (KS GSS12) has been kept in herbarium, in Botanical Survey of India, Pune Maharashtra.

Preparation of test sample

Sesbania sesban leaves were cut into small pieces and were allowed to dry in the shade. About 100 *g* of the dried powdered material was hot extracted at 60^oC for 6 *hr* using 1 *L* of water. The water extract was filtered and evaporated for dryness under vacuum, which yielded a sticky material (yield: 7.5% w/w) [7,8].

Preliminary phytochemical screening

The preliminary phytochemical screening of aqueous extract of the *Sesbania sesban* leaves was carried out for qualitative identification of type of phytoconstituents present. The presence of various phytoconstituents viz. steroids and terpenoids (Leibermann Burchard test), alkaloids (Dragendroffs test), tannins and phenolics (Ferric chloride test), flavonoids (Shinoda test), Sugars (Fehling solution test), amino acids (Ninhydrin test), etc. was detected by usual methods prescribed in standard texts [9,10].

Animals

Healthy adult male albino wistar rats (150-200 *g*), in house breed at the animal house of M.E.S. College of Pharmacy, Sonai, India were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions. (temperature 25 ± 2^oC; relative humidity 55±10%; and 12:12 light:dark cycle,) The rats were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) *ad libitum* and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethical Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals.

Acute toxicity studies

Acute toxicity study was carried out for the *Sesbania sesban* by adapting fixed dose method of CPCSEA, OECD guidelines no 420. Thirty fasted male albino mice were weighed (25-30 *g*, 10 weeks old), grouped into A, B, C, D, E, and F with five animals each. Group A animals served as control and received distilled water, while groups B, C, D, E and F were orally administered 500, 1000, 1500, 2000, and 2500 *mg/kg* body weight of SSAE in distilled water, respectively, using orogastric tubes. The animals were observed at 2, 6, 24 and 48 *hr* after extract administration to detect changes in autonomic or behavioral responses. Mortality was observed for 24 *hrs* [11].

Effect of aqueous extract in normoglycemic rats

The rats were divided into four groups of 6 animals (n=6) each. Group I served as control and received distilled water. Group II served as standard control, received glibenclamide (0.25 *mg/kg* b.w.). Group III and IV received 250 and 500 *mg/kg* SSAE orally. Blood glucose levels were determined at 0, 1, 2, 3 and 4 *hr* following treatment by retro-orbital plexus of the eye under mild ether anesthesia.

Effect of aqueous extract on oral glucose tolerance test in STZ-induced diabetic rats (OGTT)

The rats were divided into five groups of 6 animals (n=6) each. Group I served as control and received distilled water. Group II served as diabetic control and received distilled water. Group III served as positive control, received glibenclamide (0.25 *mg/kg* b.w.). Group IV and V received 250 and 500 *mg/kg* SSAE orally. All the animals were given glucose (2 *g/kg*) 30 *min* after dosing. Blood samples were collected from the retro-orbital plexus of the eye just prior (0 *hr*) and 30, 60, 90, and 120 *min*. After the glucose loading, blood glucose levels were estimated.

Evaluation of antidiabetic activity

Induction of diabetes: Diabetes was induced in rats by single intra peritoneal (*i.p.*) injection

of steptozotocin (STZ, Sigma chemical Co. USA) at a dose 60 *mg/kg* b.w. freshly dissolved in 0.1 *M* cold citrate buffer of pH 4.5; 48 *hr* later blood samples were collected and blood glucose levels were determined to confirm the development of diabetes. Those animals which showed hyperglycemia (blood glucose levels >240 *mg/dl*) were used in experiment [12].

Chronic treatment model

The rats were divided into five groups of 6 animals (n=6) each as below:

Group I- Normal control (received distilled water 10 *ml/kg* b.w., *p.o.*)

Group II- Diabetic control untreated (received distilled water 10 *ml/kg* b.w., *p.o.*)

Group III- Diabetic treated with standard drug glibenclamide (0.25 *mg/kg/day, p.o.*)

Group IV- Diabetic treated with SSAE (250 *mg/kg/day*, *p.o.*)

Group V-Diabetic treated with SSAE (500 *mg/kg/day*, *p.o.*)

For 30 days blood glucose levels and body weights were measured on 1st, 10th, 20th and 30th day of the study. Finally on day 30, blood was collected to estimate various parameters [13].

Estimation of plasma glucose, body weight and lipid profile

Every week, following overnight fasting (16 *hr* fasting with free access to water), the blood samples were withdrawn from the animals by retro-orbital puncture under light ether anesthesia.

The plasma glucose estimation was done by the glucose oxidase/peroxidase (GOD/POD) [14] method using a standard kit obtained from Span Diagnostics, India. Body weight of all experimental animals was recorded using a digital weighing scale. The TG, TC and HDL levels were estimated [15] using standard kits obtained from Span Diagnostics, India

VLDL = TG/5

LDL = TC − (HDL + VLDL) [16-18]

Estimation of serum insulin

Serum insulin concentration was determined by radioimmunoassay kit done spectrophotometrically using standard kits (RIA kit provided by BRIT, BARC, India), The kit included human insulin as standard and 125I-labelled human insulin antibody, which cross-reacts similarly with rat insulin.

Estimation of glycated hemoglobin

After 30 days experimental period, the 12*hr* fasted rats were sacrificed by cervical decapitation, blood was withdrawn by retro orbital puncture under light ether anesthesia and the glycated hemoglobin was estimated [19].

Estimation of liver glycogen

After 30 days experimental period, the 12*hr* fasted rats were sacrificed by cervical decapitation. The liver tissue (1 *g*) was collected, placed in a centrifuge tube containing 2 *ml* of KOH (300 *g/L*) after washing with saline water and heated for 20 *min* with occasional shaking. To this, a saturated solution of sodium sulphate (0.2 *ml*) was added and mixed thoroughly. The glycogen was precipitated by the addition of ethanol (5 *ml*).

The precipitate was removed and dissolved in 10 *ml* of water. One *ml* of this solution was added to 1 *ml* of HCl (1.2 *mol/l*) and boiled for 2 *hr*. After 2 *hr*, the solutions were neutralized by NaOH (0.5 *mol/l*) using phenol red as indicator. The neutralized solution was diluted to 5 *ml* and transferred to a calorimeter tube and read at 620 *nm* after adjusting the calorimeter with the reagent blank. The glycogen content was expressed as *mg/g* of liver tissue [20,21].

Statistical analysis

The results were expressed as mean± S.E.M. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. A difference in the mean p value <0.05 was considered as statistically significant.

Results

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of triterpenoids, carbohydrates, vitamins, amino acids, proteins, tannins, saponin glycosides and steroids.

Acute toxicity study

In the LD_{50} value determination, we observed that the SSAE was safe to use in animals and showed no mortality on 2500 *mg/kg* b.w. Therefore 2500 *mg/kg* dose was considered as a safe dose, 1/5th (500 *mg/kg* b.w.) and 1/10th (250 *mg/kg b. mg/kg* b.w.) of that was selected for all *in vivo* experiments as maximal dose.

Effect of aqueous extract in normoglycemic rats

The results from the study clearly indicated that there was no significant effect observed on normoglycemic rats when treated with the single dose of *Sesbania sesban* aqueous extract (Table 1).

Effect of aqueous extract on oral glucose tolerance test in STZ-induced diabetic rats (OGTT)

The results from the study clearly indicated that the aqueous extract of *Sesbania sesban* leaves at 250 and 500 *mg/kg* reduced the blood glucose level (hyperglycemia due to glucose load *2 g/kg p.o.*) significantly and glibenclamide (0.25 *mg/kg*) after 60 *min* of oral administration, when compared to diabetic control (Table 2).

Hypoglycemic effect of the aqueous extract

The results from the study clearly indicated that the aqueous extract exhibited significant hypoglycemic activity in STZ-induced diabetic rats, whilst there was no significant effect observed on normoglycemic rats. However, at the end of 30 days of treatment, there was a 70.12 %, 64.96% and 68.09% ($p<0.01$) decrease of serum glucose levels with the glibenclamide and aqueous extract (250 and 500 *mg/kg*) respectively when compared with diabetic control after 30 days (Table 3).

Changes in body weight

At the end of 30 days treatment, the body weight of normal rats, aqueous extract and standard drug treated group increased significantly; whereas body weight of diabetic control group decreased (Table 4).

Changes of serum insulin, liver glycogen and glycolsylated hemoglobin

After 30 days treatment period it was observed that animals treated with aqueous extract showed a significant increase in the serum insulin level, liver glycogen level and decrease in glycosylated hemoglobin level as

Table 1. Effect of aqueous extract of *Sesbania sesban* leaves in normoglycemic rats

Group treatment (n=6)	Fasting plasma glucose level (*mg/dl*) at (*hrs*)				
	0	1	2	3	4
I Normal	95.00±0.73	94.16±0.65	92.50±1.05	91.83±1.07	91.33±0.49
II Glibenclamide	95.16±0.70	92.50±0.67	89.16 ±0.47*	88.50±0.67*	85.33±0.95**
III Aqueous extract	95.66±1.05	94.16±0.60	90.00±0.57	89.33±0.33	88.50±0.42*
IV Aqueous extract	94.83±1.01	93.83±0.79	90.66±0.91	89.83±0.70	88.62±0.42*

* p<0.05, **p<0.01, Values are mean±SEM, n=6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test

Table 2. Effect of aqueous extract of *Sesbania sesban* leaves on OGTT in stz-induced diabetic rats

Group treatment (n=6)	Fasting plasma glucose level (*mg/dl*) at (*hrs*)				
	0	1	2	3	4
I Normal	95.00±0.73	123.17±2.72	134.83±1.35	144.83±1.35	154.83±1.35
II Diabetic control	259.17±1.16	269.50±0.95	279.50 ±0.95	289.50±0.95	297.83±0.83
III Positive control	255.17±1.01	265.17±1.01*	275.17±1.01*	285.17±1.01*	265.00±1.15**
IV Aqueous extract	259.67±1.02	266.33±1.30	276.33±1.30	286.33±1.30	274.67±1.17*
V Aqueous extract	258.50±2.04	265.17±1.13*	275.37±1.13*	285.47±1.13*	265.17±1.13**

* p<0.05, **p<0.01, Values are mean±SEM, n=6, when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test

compared to serum insulin levels in normal groups (Table 5).

Lipid profile

Lipid profile of animals treated with aqueous extract showed significant reductions ($p<0.01$) of 19.06% and 25.70% CHL (cholesterol), 50.34% and 60.44% LDL, 15.79% and 13.53% VLDL (Very Low density lipoproteins) and 27.84% and 32.21% TG after treatment with aqueous extract of *Sesbania sesban* leaves (250 and 500 *mg/kg),* respectively when compared with diabetic control rats. Also there was a significant ($p<0.05$) increase of HDL in the treated diabetic rats. In case of untreated diabetic rats, there was a fall in HDL level (Table 6).

Discussion

The present study was undertaken to evaluate the antidiabetic activity of aqueous leaves extract of *Sesbania sesban* (L) Merr. in normal, glucose-loaded hyperglycemic and STZ-induced diabetic rats. There was no lethality or no toxic reactions were found with the selected doses until the end of study period. The results of the study have shown that the aqueous extract of leaves at dose 500 *mg/kg* has a marked hypoglycemic activity by improvement of the glucose tolerance test in normoglycemic rats and by lowering the blood glucose levels in STZ-induced diabetic rats. The results of the study have shown a significant ($p<0.01$) difference between the initial and

Table 3. Effect of aqueous extract of *Sesbania sesban* leaves on serum glucose level

Group treatment (n=6)		Fasting plasma glucose level (*mg/dl*)			
		1st day	10th day	20th day	30th day
I	Normal control	94.50±2.07	94.33±2.10	96.33±1.89	95.16±2.02
II	Diabetic control	255.00±1.18	286.67±1.22	312.67±4.58	387.67±2.83
III	Diabetic + glibenclamide (0.25 *mg/kg*)	255.67±1.33	265.67±1.33**	210.67±2.84**	115.83±1.53**
IV	Diabetic + aqueous extract (250 *mg/kg*)	255.83±0.79	275.83±0.79**	235.83±0.79**	135.83±0.79**
V	Diabetic + aqueous extract (500 *mg/kg*)	256.33±2.65	268.67±1.02**	223.67±2.01**	123.67±2.01**

* $p<0.05$,**$p<0.01$, Values are mean±SEM, n=6, when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test

Table 4. Effect of aqueous extract of *Sesbania sesban* leaves on body weight in stz-induced diabetic rats

Group treatment (n=6)		Changes in body weight (*g*) at (days)			
		0	10	20	30
I	Normal	155.67±0.76	166.33±0.91	172.00±0.91	185.33±0.87
II	Diabetic control	158.17±0.70	153.50±0.56	148.67 ±0.66	139.00±0.77
III	Positive control	158.00±0.77	161.50±1.05**	163.33±0.80**	168.33±1.35**
IV	Aqueous extract	163.33±5.04	166.83±0.94**	168.50±0.34**	175.17±1.37**
V	Aqueous extract	158.67±0.49	165.33±1.70**	167.33±0.55**	173.50±1.14**

* $p<0.05$, **$p<0.01$, Values are mean±SEM, n=6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test

Table 5. Effect of aqueous extract of *Sesbania sesban* leaves on serum parameters after 30 days

Group treatment (n=6)		Serum insulin *µU/ml*	Glycosylated hemoglobin *mg/g Hb*	Liver glycogen *mg/g*
I	Normal control	18.16±0.55	0.22±0.007	14.66±0.33
II	Diabetic control	7.13±0.31	0.58±0.009	7.16±0.60
III	Diabetic + glibenclamide (0.25 *mg/kg*)	16.66±0.33**	0.25±0.007**	13.00±0.25**
IV	Diabetic + aqueous extract (250 *mg/kg*)	13.33±0.33**	0.27±0.006**	11.41±0.37**
V	Diabetic + aqueous extract (500 *mg/kg*)	15.33±0.33**	0.31±0.004**	12.58±0.23**

*$p<0.05$, **$p<0.01$, Values are mean±SEM, n=6, when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test

Table 6. Effect of aqueous extract of *Sesbania sesban* leaves on serum lipid profile after 30 days

Group	Cholesterol	LDL	HDL	VLDL	Triglycerides
Normal control	66.16±0.83	22.83±0.87	11.00±0.36	17.33±0.66	66.50±0.76
Diabetic control	95.33±0.71	97.33±0.49	9.83±0.30	22.16±0.30	114.33±1.47
Diabetic+glibenclamide (0.25 *mg/kg*)	69.66±0.66**	36.00±0.73**	13.83±0.47**	18.16±0.30**	76.50±0.76**
Diabetic+aqueous extract (250 *mg/kg*)	77.16±0.60**	48.33±0.42**	13.83±0.40**	18.66±0.21**	82.50±0.99**
Diabetic+aqueousextract (500 *mg/kg*)	70.83±0.60**	38.50±076**	14.83±0.30**	19.16±0.16**	77.50±0.84**

*p<0.05, **p<0.01, Values are mean±SEM, n=6, when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test

final fasting plasma glucose levels of aqueous leaves extract of *Sesbania sesban* and glibenclamide treated groups (Table 3). Induction of diabetes by STZ leads to loss of body weight due to increased muscle wasting and loss of tissue proteins [14,15]. The results obtained with the aqueous extract treatment in chronic diabetic model further clarified the antidiabetic effect of the extract. After 30 days of aqueous extract treatment, gain in body weight was observed in diabetic rats and the results were comparable with that of the standard drug glibenclamide.

Aqueous extract of *Sesbania sesban* showed significant increase in serum insulin level. A marked decrease in triglycerides, total cholesterol, LDL and VLDL was observed, while increase in HDL cholesterol has been observed in aqueous leaves extract treated diabetic rats, which suggest that HDL is inversely related to the total body cholesterol (Table 4) [19]. The possible mechanism of antidiabetic action of aqueous extract may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form.

Animals treated with aqueous extract indicated a significant decrease in the glycosylated hemoglobin level which could be due to an improvement in insulin secretion, whereas glycosylated hemoglobin level increased significant in untreated diabetic control group, which confirm the antidiabetic action of the extract [22]. The significant increase was observed in glycogen levels of the aqueous leaves extract treated diabetic rats. The extract did not produce any significant effects on normal animals (Table 6).

Conclusion

In conclusion, it can be stated that the aqueous leaves extract of *Sesbania sesban* has beneficial effects in reducing the elevated blood glucose level and lipid profile of STZ-induced diabetic rats, but has no effect on normal rats. Thus justifying the claim made by ayurvedic classics.

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