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Hypoglycemic activity of *Sida spinosa* Linn. root extract in normoglycemic rats

Ibrahim Shaikh[1]*, Preeti Kulkarni[1], Ankur Patel[1], Venkatrao Kulkarni[1]

***Corresponding author:**

Ibrahim Shaikh

1.Department of Pharmacology, Soniya Education Trust's College of Pharmacy, S. R. Nagar, Dharwad – 580 002, Karnataka, IndiaTelephone: + 91 0836 2448540 (O) + 91 0836 2779764 (R)Fax: +91 0836 2467190

Email: i.ibrahimshaikh@rediff.com

Abstract

There is an increased demand of patients to use natural products with antidiabetic activity and ethnobotonical survey conducted revealed that *Sida spinosa* Linn. root is used in the treatment of diabetes. The present investigation aims to examine the hypoglycemic potential of *Sida spinosa* Linn. root in normal rats.

The root of *Sida spinosa* Linn. was extracted using ethanol and water and tested for acute toxicity according to OECD 423 guidelines. The ethanolic (SSE) and aqueous (SSA) extracts at 200 and 400 mg/kg b.w. were screened for blood glucose lowering capacity in normal animals (18h fasted rat model) and Glibenclamide (GLB) was used as a standard. Serum glucose (SG) levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer. The oral glucose tolerance test (OGTT) was carried out in normal rats.

The results of acute toxicity showed that the animals had good tolerance to single doses of SSE/SSA in doses as high as 4000mg/kg and were non-lethal. In OGT test, the normoglycemic rats treated with higher dose SSE, SSA and GLB (10mg/kg) showed 30.36%, 25.24% and 33.21% reduction (P<0.001) in SG level respectively over the period of 120 min compared to normal control group. In single dose one day study, higher dose of SSE, SSA and GLB showed significant (p<0.001) reduction in SG levels (22.3%, 18.2% & 28.6% respectively) at 3h after oral administration. The ethanolic extract (400 mg/kg) was more effective in reducing the SG level in OGTT compared to aqueous extract with improved glucose tolerance.

The root of *Sida spinosa* Linn. has potent hypoglycemic activity in normal rats justifying its use in indigenous system of medicine.

Keywords: Diabetes mellitus; Hyperglycemia; OGTT; Serum glucose; *Sida spinosa*.

Introduction

Diabetes mellitus (DM) is a disorder characterized by chronic hyperglycemia and impaired carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action [1]. The common characteristic feature in DM is persistent high glucose level in the blood [2]. Untreated hyperglycemia can lead to a reduced number of glucose transporters, down regulation in the number of insulin receptors as well as defect of tissue insulin signal transduction. Subsequent to these deteriorations, there is an absolute increase in hepatic glucose output which leads to fasting hyperglycemia [3] which itself manifests adverse effects on β-cell insulin secretion and insulin resistance [4]. This process leads to long-term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart, blood vessels etc. and creates a huge economic burden related to the management of diabetic complications [5].

India is one among the top 10 countries in the incidence of diabetes. The current treatment of DM includes diet, exercise, various oral antidiabetic drugs and insulin therapy. The oral hypoglycemic agents such as biguanides, sulphonylureas and α-glucosidase inhibitors have characteristic profile of adverse effects which include frequent diarrhea, hypoglycemia, hepatotoxicity, lactic acidosis, dyslipidemia, hypertension and hypercoagulability [6]. There is an increased demand of patients to use natural products with antidiabetic activity [7]. On the other hand, a large number of herbs have been reported to possess hypoglycemic/antihyperglycemic properties. They are easily available and comparatively safe. Based on an ethnobotonical approach, the plant *Sida spinosa* Linn. has been traditionally claimed to possess hypoglycemic property [8]. However, the scientific evidence to confirm this is still lacking, hence we thought it worthwhile to systematically investigate the effect of ethanolic and aqueous extracts of root of *Sida spinosa* Linn. on normoglycemic rats.

Materials and methods

Chemicals

Streptozotocin and Glibenclamide were obtained from Himedia Laboratories and Aventis Pharma, Mumbai, India, respectively. Blood Glucose, total cholesterol, HDL cholesterol and triglyceride were determined by commercially available kits from ERBA diagnostics Mannheim GMBH, Germany. All other chemical were of analytical grade and used as received.

Plant material

Roots of Sida spinosa Linn. were collected from surrounding areas of Dharwad, Karnataka, and authenticated by Dr. Hebbar, Government P.U. College, Dharwad. A herbarium specimen of the plant is kept in the Department of Pharmacognosy (SETCPD/Ph.cog/herb/12/2010), SET's College of Pharmacy, Dharwad, Karnataka, India. The roots were chopped into small pieces, dried under shade, coarsely powdered and used for extraction.

Preparation of extracts

The ethanolic extract (SSE) was prepared by Soxhlet extraction method by taking 200 g of powdered root and extracting with 800 ml of ethanol. Aqueous extract (SSA) was prepared by cold maceration method using powdered root (100 g) soaked in 1000 ml of distilled water for 7 days. Both the extracts were filtered, and the filtrates were evaporated using a rotary flash evaporator under reduced pressure to dryness. Further, the suspensions of SSE/SSA were prepared by using tragacanth (2%) in distilled water and used for the experiment.

Animals

Male albino Wistar rats of 150-200g were used for the experiment. Animals were kept in the animal house of SET's College of Pharmacy,

under controlled conditions of temperature (23±2°C), humidity (50±5%) and 12 h light-dark cycle. Animals were fed pellet diet (Golden feed, New Delhi) and water ad libitum. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET's College of Pharmacy, Dharwad, India (REG.No.112/1999/CPCSEA) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All the animals were acclimatized for seven days before conducting the study.

Acute toxicity study

The acute oral toxicity study was carried out as per the guidelines set by OECD 423 [9]. Animals (n=3) were fasted overnight prior to dosing (food but not water was withheld). The dose level to be used as the starting dose was 5mg/kg b.w. The test substance was administered in a single dose by gavage using intubation canula. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days.

Hypoglycemic activity

Both the extracts SSE and SSA were evaluated for the hypoglycemic activity using following experimental models.

Oral Glucose Tolerance Test (OGTT) in normal rats

OGTT was performed in overnight-fasted (18 h) normal rats. The rats were given the standard drug and test extracts 30 min prior to glucose (2 g/kg) administration orally. Serum glucose (SG) levels were estimated before glucose load (0 min), at 30 min, 60 min and 120 min afterwards using a glucose oxidase-peroxidase reactive strips and a glucometer (SD Check Gold Blood Glucose Meter, Standard Diagnostics, Korea).[10]

The experimental rats were divided into six groups of five each: Group 1 was the normal control and received vehicle (water) (10 ml/kg, p.o), group 2 and 3 were treated with SSE (200 mg/kg, p.o.) and SSE (400 mg/kg, p.o.) respectively, group 4 and Group 5 were treated with SSA (200 mg/kg, p.o.) and treated SSA (400 mg/kg, p.o.) respectively, whereas Group 6 received Glibenclamide (GLB) (10 mg/kg, p.o.).

The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$) calculated by trapezoid rule

$$AUC_{glucose} = \frac{(C_1 + C_2)}{2} X (t_2 - t_1)$$

Single dose one day study (18h fasted rat model)

The experimental rats were divided into six groups of five each and treated as follows:

Group 1 was the normal control and received vehicle (water) (10 mg/kg, p.o), group 2 and 3 were treated with SSE (200 mg/kg, p.o.) and SSE (400 mg/kg, p.o.) respectively, group 4 and Group 5 were treated with SSA (200 mg/kg, p.o.) and treated SSA (400 mg/kg, p.o.) respectively, whereas Group 6 received Glibenclamide (GLB) (10 mg/kg, p.o.).

Blood glucose levels were estimated before administration of extracts and 1, 2 and 3 h afterwards.[11] The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$) calculated by trapezoid rule as mentioned above.

Statistical analysis

The data were analyzed statistically using analysis of variance (ANOVA) followed by Tukey's post test. Values are expressed as Mean ± Standard errors of mean (S.E.M).

Results

Acute oral toxicity studies

Acute toxicity study revealed that animals showed good tolerance (up to 4000 mg/kg b.w) to single doses of SSE and SSA extracts. Both extracts produced no noticeable effect on general behavior or appearance of the animals and all rats survived during and after the test period. Therefore, two non-lethal doses (200 and 400 mg/kg b.w) of SSE and SSA extracts were selected for screening of hypoglycemic activity in rats.

Oral glucose tolerance in normal rats

In simple OGT, administration of glucose (2g/kg) produced significant change in SG level of normal rats. As depicted in Fig.1, SSE (400 mg/kg), SSA (400 mg/kg) and GLB (10mg/kg) treated normoglycemic rats showed 30.36%, 25.24% and 33.21% reduction (P<0.001) in SG level respectively over the period of 120 min compared to normal control group. In addition, estimation of integrated $AUC_{glucose}$ indicated that treatment of normoglycemic rats with SSE, SSA and GLB significantly improved glucose tolerance over the period of 120 min. Evidently, treatment of normoglycemic rats with higher dose of SSE and SSA showed good tolerance to exogenously administered glucose.

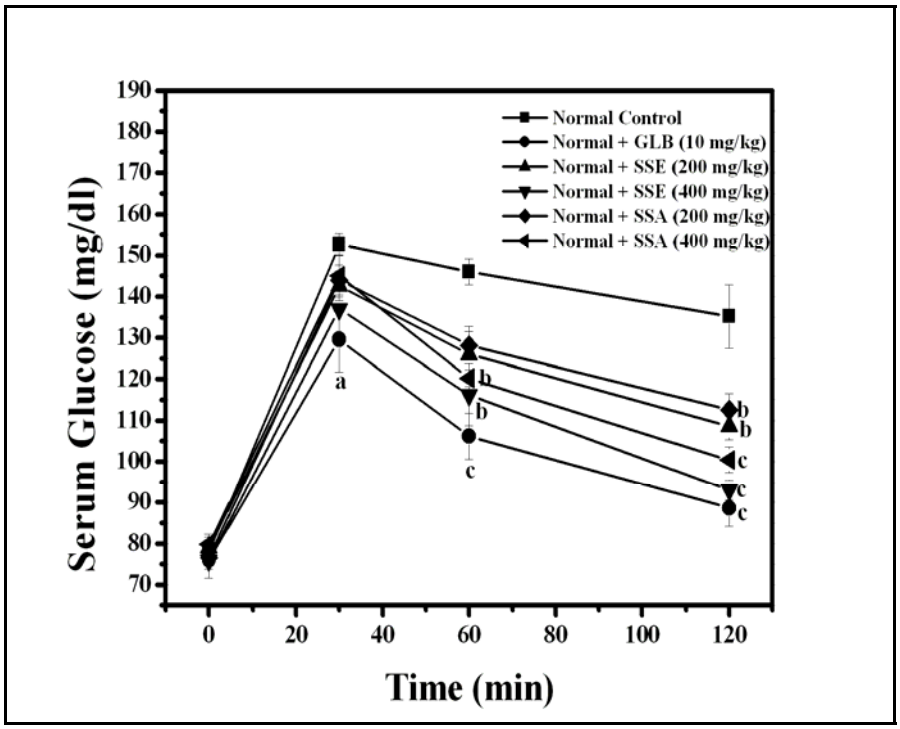

Figure. 1: SG levels were measured at 0 min, 30 min, 60 min and 120 min after p.o. administration of glucose (2g/kg b.w). Data represents mean ± S.E.M., for n=5. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}p < 0.001$ as compared with normal control.

Table. 1. Effect of SSE and SSA on Blood Glucose levels (mg/dL) in Normoglycemic rats. Each value represents Mean ± S.E.M., for n=5. [a] *P*<0.05; [b] *P*<0.01; [c] *P*<0.001 compared to baseline values i.e. 0 hr of the same.

Group	0 h	1 h	2 h	3 h
Normal Control(NC)	94.8±2.4	93 ±1.8	91 ±1.8	90.2 ± 1.8
GLB(10 mg/kg)	91.4±1.5	84.4±0.9[b]	78.8 ±1.5 [c]	65.2 ± 1.4[c]
SSE(200mg/kg)	96 ±1.2	91.8±1.8	87.2±1.2	82.2 ± 0.4[b]
SSE(400mg/kg)	93.6±2.0	90 ± 2.1	82 ±1.1[b]	72.6 ± 1.9[c]
SSA(200mg/kg)	92.6±1.3	91 ±1.4	87.8 ±1.6	81.4 ±1.3[b]
SSA(400mg/kg)	96.6±0.9	91.8±2.0	84.2±2.2[a]	79 ±1.8[c]

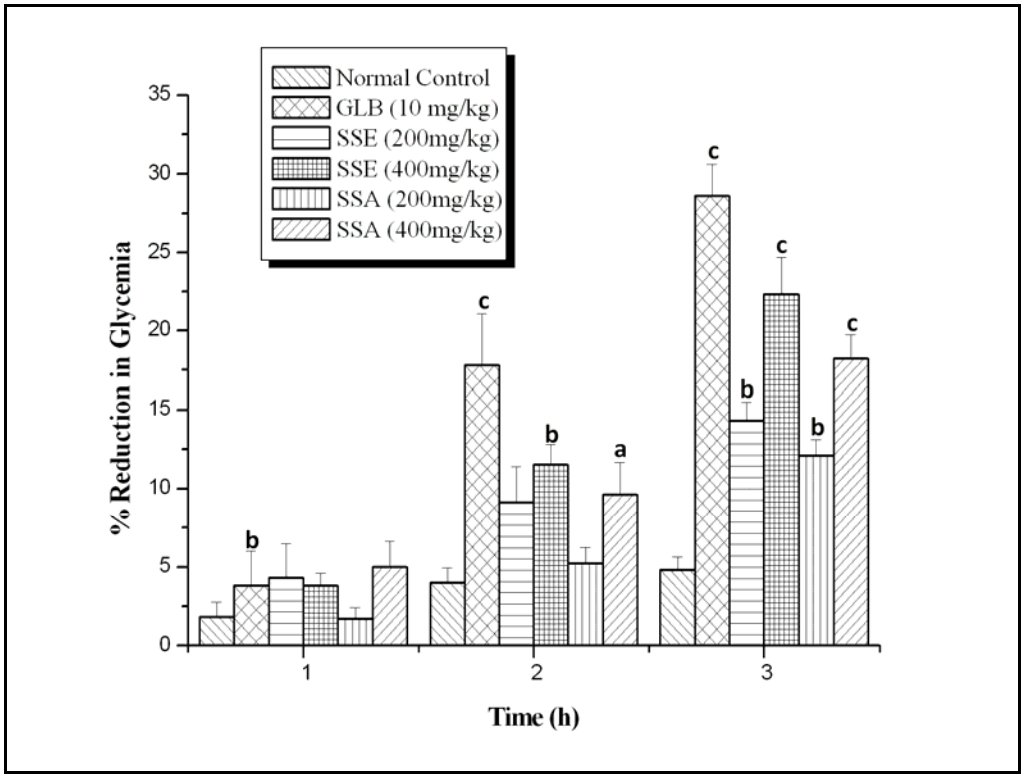

Figure. 2: SG levels were measured at 0 h, 1 h, 2 h and 3 h after single oral administration of SSE, SSA and GLB. Bar graph represents the percentage reduction in glycemia with respect to the initial (0 h) level. Each value represents Mean ± S.E.M., n=5. [a] *P* < 0.05; [b] *P*<0.01; [c] *P*<0.001 compared to normal control.

Single dose one day study (18h fasted rat model)

Administration of single dose of both SSE and SSA in normal rats showed reduction in SG levels at different time intervals compared to baseline values i.e. at 0 h of the same group. As shown in Fig. 2 and Table. 1, oral administration of SSE (400 mg/kg) and GLB caused a significant (P<0.001) reduction in SG levels (22.3% & 28.6%) respectively at 3 h as compared to normal control group.

Discussion

Diabetes mellitus (DM) is a complex and multifarious group of disorders characterized by hyperglycaemia that has reached epidemic proportions in the present century. Several synthetic drugs being utilized have side effects and thus searching for a new class of compounds is essential to overcome these problems. Traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can counter the high cost and poor availability of the current medicines/present day drugs for many rural populations in developing countries. The present study was carried out to investigate the effect of SSE and SSA on the blood glucose levels normoglycemic rats.

Medicinal plants are widely used by the populations of developing countries as alternative therapy. Many Indian medicinal plants are reported to be useful in diabetes.[12] In India, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately only a few of such Indian medicinal plants have received scientific scrutiny. The present work was therefore designed to study the hypoglycemic effect of *Sida spinosa* Linn. root in normal rats.

In the present study, ethanolic extract of *Sida spinosa* Linn. root produced a significant decrease in the blood glucose level at a dose level of 400 mg/kg. From the results it is assumed that the root extract could be responsible for stimulation of insulin release and the observed restoration of metabolic activities. A number of other plants have also been shown to exert hypoglycemic activity through stimulation of insulin release.[13] Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide; they reduce blood glucose in normoglycemic animals.[14] Glibenclamide is reported to enhance the activity of β cells of pancreas resulting in secretion of larger amounts of insulin which in turn brings down blood glucose level.[15]

The present data indicates that higher dose of SSE and SSA significantly reduced blood glucose levels in normal rats. The efficacy of SSE and SSA were comparable to standard GLB. This effect could be due to increased insulin secretion from pancreatic β-cells of islets and/or due to enhanced transport of blood glucose to the peripheral tissue or by other mechanisms such as stimulation of glucose uptake by peripheral tissue or inhibition of endogenous glucose production.

Conclusions

The ethanolic and aqueous extract of *Sida spinosa* Linn. has potent hypoglycemic activity in normal rats. The present investigation aims at development of potent phytomedicine for treatment/management of diabetes mellitus from the title plant.

Authors' contributions

IS carried out the study. PK participated in the design of the study and performed the statistical analysis and helped to draft the manuscript. AP helped in carrying out the OGTT, single dose one day study and drafting the manuscript. VK participated in its design and coordination and helped review the manuscript.

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Declarations: Conflict of interest

The author(s) declare(s) that they have no conflicts of interest to disclose.

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Abbreviations

ANOVA	Analysis of variance
b.w	Body weight
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
DM	Diabetes mellitus
GLB	Glibenclamide
GLUT	Glucose transporter
h	Hour
IAEC	Institutional Animal Ethical Committee
i.v	Intravenous
IDDM	Insulin dependent Diabetes Melitus
min	Minutes
µg	Microgram
NIDDM	Non insulin dependent Diabetes Melitus
n	Number of animals
OECD	Organization for Economic Corporation and Development
OGTT	Oral glucose tolerance test
p.o	Per oral
S.E.M	Standard Error Mean
SG	Serum glucose
SSE	*Sida spinosa* Linn. root ethanolic extract
SSA	*Sida spinosa* Linn. root aqueous extract
STZ	Streptozotocin
WHO	World health organization

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