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Antihyperglycemic and Antihyperlipidemic Activity of *Sida spinosa* linn. Root in Streptozotocin-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author IS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors PK, BAM and BAAW managed the literature searches, analyses of the study performed the spectroscopy analysis and author IS managed the experimental process. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Based on ethnobotonical approaches the ethanolic extract of the plant *Sida spinosa* Linn. has been traditionally claimed to have hypoglycemic properties.
Aim: Evaluation of Antihyperglycemic and antihyperlipidemic activity of ethanolic (SSE) and aqueous (SSA) extracts of *Sida spinosa* Linn. root in Streptozotocin- induced diabetic rats.
Materials and Methods: SSE & SSA were subjected to acute toxicity studies (OECD guidelines). Diabetes was induced by Streptozotocin (45 mg/kg i.v). Normal and diabetic rats were divided into different groups and orally administered with SSE, SSA (200 and 400 mg/kg) and Glibenclamide

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(10 mg/kg) for 30 days. The study includes tolerance of oral glucose, estimation of serum insulin and insulin tolerance in diabetic rats, lipid profile and histopathological study.
Results: Ethanolic extract has reduced serum glucose levels to maximum of 40.73%. Oral glucose tolerance test (OGTT) showed SSE, SSA (400 mg/kg) and Glibenclamide caused a significant antihyperglycemic effect with a reduction of 57.34%, 46.77% and 60.77% respectively after 120 min of glucose load. Both extracts were efficient in reducing the lipid parameters such as serum triglycerides, serum total cholesterol, LDL-c and VLDL-c to normal values and there was a marked rise in HDL-c level as compared to diabetic control group.
Conclusion: Results indicate that *Sida spinosa* Linn. Root possesses potent antihyperglycemic and antihyperlipidemic activity.

Keywords: Antihyperglycemic; antihyperlipidemic; glibenclamide; Sida spinosa; streptozotocin.

1. INTRODUCTION

American diabetes association expert committee has defined DM as a group of metabolic disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins resulting from defective insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

The most frequent diabetes forms are Type 1 diabetes mellitus (T_1DM) and Type 2 diabetes mellitus (T_2DM). T_1DM is characterized by an immune-mediated, selective destruction of >90% of insulin-secreting beta cells. Individuals with T_1DM therefore require regular insulin injections to control blood sugar levels [2].

In India, approximately 31.7 million people suffered from diabetes in 2000 and it is estimated that about 79.4 million people will be diabetic by 2030 [3].

Plants have played a significant role in maintaining human health and improving the quality of life for thousands of years. According to the WHO, about three quarters of the world's population relies on traditional medicine for primary health care needs and most of this treatment involves the use of plant extracts or their active components [4]. The use of herbal remedies for diabetes treatment is well known since ancient times [5]. Many Indian plants have been reported by various authors to treat diabetes traditionally [6] *Sida spinosa* Linn. (Malvaceae) is an erect, branched small perennial herb (or) small shrub which grows abundantly on cultivated fields, waste areas, road sides and open clearing in India [7]. It is widely distributed in Bangalore, Belgaum, Chikmagalur, Dharwar, Hassan, Mysore, and North Kanara regions of Karnataka, India [8]. The root contains alkaloids- betaphenethylamine, ephedrine, si-ephedrine, vasicinol, vasicinone, vasicine, choline, hypaphorine, methyl ester, betaine [9], phytosterols, α-amyrin, starch and ecdysterone. Roots are used as nervine tonic and diaphoretic, in debility and fevers [8] Decoction given as a demulcent in irritability of bladder and genitourinary tract. Leaves are used as demulcent and refrigerant; used for scalding urine. Ethnobotanical survey conducted by C.P.Khare reveals that ethanolic extract of the plant *Sida spinosa* Linn. exhibits hypoglycemic activity [9]. Hence the present study is undertaken to evaluate antidiabetic potency of *Sida spinosa* root.

2. MATERIALS AND METHODS

2.1Chemicals and Reagents

Streptozotocin was purchased from Enzo Life Sciences, Switzerland, Insulin (Biocon, India), ERBA diagnostics Mannheim GMBH, Germany for estimation cholesterol, triglycerides, HDL and LDL are purchased.

2.2 Plant Material

Roots of *Sida spinosa* Linn. were collected from surrounding areas of Dharwad, Karnataka, and authenticated by Dr. Hebbar, 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 collected material was washed with running water. The roots were chopped into small pieces and shade dried. Dried roots were coarsely powdered and used for extraction.

2.3 Preparation of Extracts

Recent studies suggest that the ethanolic extract of the plant *Sida spinosa* Linn. has significant antidiabetic activity [10]. Based on ethnobotonical approaches and recent data ethanolic and aqueous extracts were selected. The powdered root was extracted in a Soxhlet apparatus with ethanol at a temperature of 500C for 12 h. The resultant extract was filtered. The filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 400C. The dried mass was stored in a desiccator and the yield was 3.15% [11,12]. This extract has been termed as "SSE". The aqueous extract was prepared by maceration of dried root powder in distilled water for 7 days, at room temperature. The extract was filtered; the filtrate was subjected to rotatory flash evaporator under reduced pressure to dryness. The yield of the aqueous extract was 6.51% w/w. The dried extract was stored in desiccator and termed as "SSA" [13].

2.4 Preliminary Phytochemical Investigation

SSE and SSA were subjected to preliminary phytochemical analysis to detect the presence of chemical constituents [11].

2.5 Animals

Male Wistar rats weighing 190–210 g were used. For acute toxicity studies female rats were selected. The animals were purchased from Sri Venkateshwara Enterprises, Bangalore, India. They were maintained in the animal house of S.E.T's College of Pharmacy, Dharwad, India for experimental purpose. The animals were maintained under controlled conditions of temperature (22±2°C), humidity (50±5%) and 12-h light-dark cycles. They were fed commercial stock diet and water, *ad libitum*. The animals were maintained separately in sanitized polypropylene cages which contain sterile paddy husk. Rats were taken to laboratory 48h prior to start of research work so as to minimize non-specific stress. Studies conducted according to prescribed guidelines by CPCSEA, Government of India, which was approved by Institutional IAEC of SET's College of Pharmacy, Dharwad-(REG.No.112/1999/CPCSEA). We selected male animals for all our studies, since females are shown to be protected from changes in lipid–induced insulin action [14].

2.6 Pharmacological Evaluation

The fresh suspensions of ethanol (SSE) and aqueous (SSA) extract were prepared using tragacanth (2%) in distilled water and administered orally to experimental animals. The extracts were administered at a constant volume of 10 ml/kg for each animal.

2.6.1 Determination of acute toxicity

The acute oral toxicity study was carried out as per the guidelines set by OECD 423 [15]. Animals (n=3) were overnight fasted prior to dosing. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/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. Animals were observed for following profiles.

1. *Behavioral profile.* Alertness, restlessness, irritability and fearfulness
2. *Neurological profile.* Spontaneous activities, reactivity, touch and pain response
3. *Autonomic profile.* Defecation and urination After a period of 24 h and 72 h animals were observed for any lethality or death.

2.6.2 Effect of extracts in STZ-induced diabetic rats

2.6.2.1 Induction of Diabetes

Diabetes was induced by single intravenous injection of Streptozotocin (STZ) (45 mg/kg). STZ was dissolved in ice-cold citrate buffer (pH-4.5) and injected immediately within few minutes to avoid degradation. Rats were provided with 10% glucose solution after 6 h of STZ administration for the next 24 h to prevent hypoglycemia as STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release [16] Serum glucose (SG) levels were estimated 48 h later using a glucose oxidase-peroxidase reactive strips and a glucometer (Tail clipping) to confirm the development of diabetes [17]. Only those animals which showed blood glucose level more than 250 mg/dl were used for the experiment [18,19]. Diabetic rats were

randomized into different groups based on their Serum Glucose levels.

2.6.2.2 Experimental design for Single-dose one-day study [20]

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

Group 1: Vehicle (water) (10 ml/kg, p.o.) treated normoglycemic rats
Group 2: Vehicle (water) (10 ml/kg, p.o.) treated diabetic rats
Group 3: SSE (200 mg/kg, p.o.) treated diabetic rats
Group 4: SSE (400 mg/kg, p.o.) treated diabetic rats
Group 5: SSA (200 mg/kg, p.o.) treated diabetic rats
Group 6: SSA (400 mg/kg, p.o.) treated diabetic rats
Group 7: Glibenclamide (GLB) (10 mg/kg, p.o.) treated diabetic rats

Blood samples were collected at 0, 1, 2 and 4 h after extracts/GLB administration [single-dose one-day study]. Serum glucose (SG) levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer. Percent reduction in glycemia was calculated with respect to the initial (0 h) level.

2.6.2.3 Experimental design for Multiple-dose thirty-day study [21]

The animals were treated with respective doses of SSE, SSA and GLB for thirty consecutive days [Multiple-dose thirty-day study].

Group 1: Vehicle (water) (10 ml/kg, p.o.) treated normoglycemic rats
Group 2: Vehicle (water) (10 ml/kg, p.o.) treated diabetic rats
Group 3: SSE (200 mg/kg, p.o.) treated diabetic rats
Group 4: SSE (400 mg/kg, p.o.) treated diabetic rats
Group 5: SSA (200 mg/kg, p.o.) treated diabetic rats
Group 6: SSA (400 mg/kg, p.o.) treated diabetic rats
Group 7: GLB (0.5 mg/kg, p.o.) treated diabetic rats

Oral glucose tolerance test (OGTT) in diabetic rats.

On 20th day, glucose tolerance of various groups was estimated by a simple OGTT. The rats were given the standard drug and test extracts orally 30 min prior to glucose (2 g/kg) administration. Blood samples were collected before glucose load (0 min), at 30 min, 60 min and 120 min afterwards. SG levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer (Tail clipping) [17].

Also, serum insulin was estimated at 0 (before glucose load), 30 and 60 min after glucose administration. Serum insulin (SI) was estimated by radioimmunoassay method using the kit from Cis Bio International, France. The results were expressed as integrated area under curve for insulin (AUC insulin), which was calculated by trapezoid rule [16].

2.6.2.4 Insulin tolerance test (ITT)

On 28th day, insulin (2 U/kg, i.v) was administered to 6hr fasted rats. Blood samples were collected before insulin load at 0 min and at 10, 20 and 30 min afterwards. SG levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer (Tail clipping) [17].

2.6.2.5 Estimation of biochemical parameters

At the end of the treatment schedule, blood samples were collected from retro-orbital plexus. Serum was separated and analysed spectrophotometrically for triglyceride (STG), total cholesterol (STC), HDL-cholesterol (HDL-c), and using diagnostic reagent kit ERBA diagnostics Mannheim GMBH, Germany.

VLDL-cholesterol (VLDL-c) and LDL-cholesterol (LDL-c) in serum were calculated as per Friedewald's equation.

The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were also calculated [22].

$$\text{VLDL - c} = \frac{\text{Triglyceride}}{5}$$

$$\text{LDL - c} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL - c}$$

2.7 Histopathological Examination

The whole pancreas from each animal was removed after sacrificing the animals on the 30th

day, collected and preserved in 10% formalin. The samples were submitted to Jeevan Lab Pvt Ltd. (Belgaum, India) for histopathological examination.

2.8 Statistical Evaluation

The data were expressed as Mean±S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test.

3. RESULTS

3.1 Preliminary Phytochemical Investigation

Preliminary phytochemical investigation of SSE showed the presence of alkaloids, carbohydrates, phenolics, flavonoids, glycosides and tannins whereas; SSA contains carbohydrates, phenolics, flavonoids, glycosides and tannins.

3.2 Pharmacological Evaluation

3.2.1 Acute oral toxicity studies

Acute toxicity study revealed that animals showed good tolerance (up to a dosage of 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 $1/10^{th}$ and $1/20^{th}$ (200 and 400 mg/kg b.w) of SSE and SSA extracts were selected for screening of antidiabetic, antihyperlipidemic.

3.3 Evaluation of SSE and SSA in STZ-induced Diabetic Rats

3.3.1 Single-dose one-day study [20]

Single dose administrations of both the extracts have shown significant decrease in SG levels compared to base values at 0 h of same group.

There was a significant (P<0.01) decrease in levels of SG in SSE (400 mg/kg) and GLB (14.9% & 15.4% respectively) at second hour compared to diabetic control group. SSE (200 mg/kg) have also shown exhibited significant fall in SG level from 2^{nd} (P<0.05) to 4^{th} hour (P<0.001). SSE (200 & 400 mg/kg) treated groups have shown significant (P<0.001) fall in SG level (13.7% and 22.1% respectively) over 4^{th} hour whereas, GLB showed maximum reduction of 24.8% (P<0.001) at fourth hour post treatment with respect to their base values (Table 1).

3.3.2 Multiple-dose 30-day study in STZ-induced diabetic rats

Long term administration of the extracts and GLB, has shown drastic fall in SG levels compared to base values. SSE (400 mg/kg), SSA (400 mg/kg) and GLB showed reduction (40.73%, 30.39% and 49.63% respectively) of SG level. SSA (200 mg/kg) showed reduction (P<0.05) in SG level from 14th day which was maintained till 21st day (Table 2).

3.3.3 Oral glucose tolerance test in diabetic rats

On 20^{th} day there was no change in the SG levels and AUC for 120 min in case of Normal control group following oral glucose administration (2 g/kg) as shown in (Fig. 1A), however there was significant rise in fasting SG level (at time zero) and also significant impairment in glucose tolerance to orally administered glucose in diabetic control group compared to normal control. Higher doses of extracts and GLB have shown significant (P<0.001) improvement in glucose tolerance and marked fall in SG level (57.34%, 46.77% & 60.77% respectively) over the period of 120 min compared to diabetic control group. (Fig. 1B) shows the integrated AUC glucose for the extracts. AUC glucose for SSE (400 mg/kg), SSA (400 mg/kg) and GLB was significant (P<0.001) over the period of 120 min.

3.3.4 Estimation of serum Insulin

There was a high release of insulin in normal rats after oral glucose load whereas diabetic control rats showed no significant insulin release. Hence diabetic control rats resembled severe diabetic (type 1) condition in which maximum pancreatic damage will be there. (Fig. 2A) shows SSE and SSA treated diabetic rats have caused more release of insulin which is stimulated by glucose. This effect is comparable to GLB treated diabetic rats. In (Fig. 2B), treatment with SSE (400 mg/kg) and GLB has shown significant (P<0.001) rise in AU Cinsulin compared to diabetic control.

Table 1. Effect of SSE and SSA on SG levels in STZ-induced diabetic rats (Single-dose one-day study)

Group	SG levels [mg/dl]			
	0 hr	1hr	2hr	4hr
Normal control	77.4±3.6	79.8±1.4	79.6±2.2	78±1.9
Diabetic control	281±12.2	279±11.8	277.2±11.9	276.4±10.8
Diabetic + SSE [200 mg]	291.2±7.2	273±5.2 [6.0]	264.8±3.4[a] [8.8]	250.8±4.7[c] [13.7]
Diabetic + SSE [400 mg]	293.4±7.8	272.2±6.7 [7.1]	249.2±5.8[b] [14.9]	228±8.4[c] [22.1]
Diabetic + SSA [200 mg]	294±8.1	278±7.1 [5.4]	269.2±6.3 [8.4]	258±5.8[a] [12.1]
Diabetic + SSA [400 mg]	288±9.2	282±5.9 [1.7]	258.6±5.4 [9.8]	241.6±7.1[b] [15.8]
Diabetic + GLB [0.5 mg]	271.2±6.2	246.6±5.8 [9.0]	229.4±6.9[b] [15.4]	204±7.2[c] [24.8]

Each value represents Mean±S.E.M., for n=5. Values in parentheses indicates percentage reduction in glycemia and [a] P<0.05; [b] P<0.01; [c] P<0.001 compared to base values i.e. 0 hr of the same group

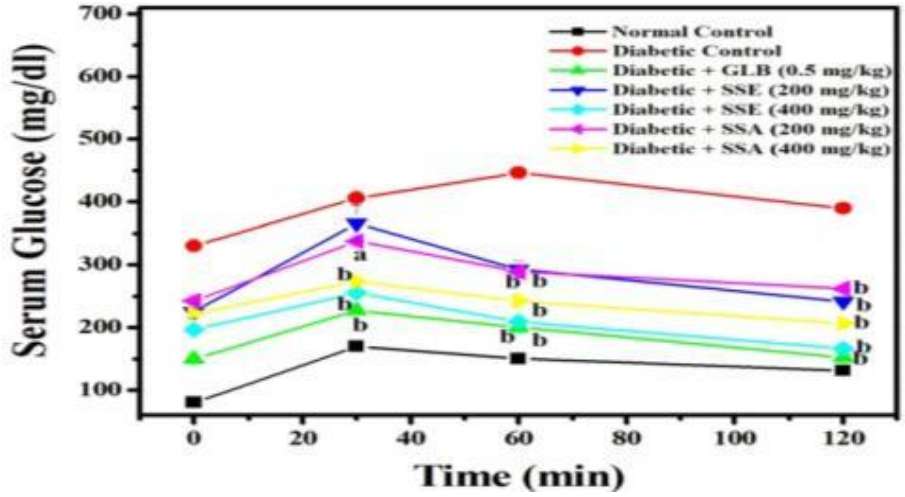

Fig. 1A. Effect of SSE and SSA on glucose tolerance in diabetic rats. SG levels were measured at 0 min, 30 min, 60 min and 120 min after p.o. administration of glucose (2 g/kg b.w)

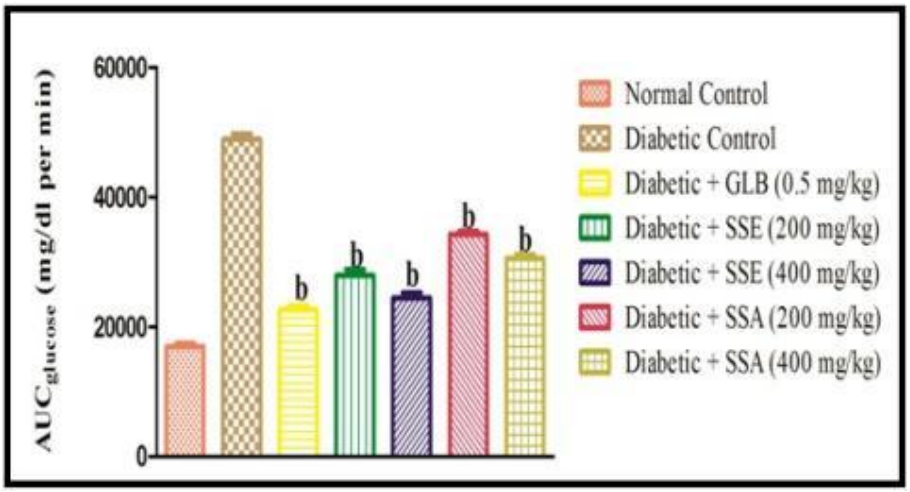

Fig. 1B. Effect of SSE and SSA on glucose tolerance in diabetic rats. Area under curve for glucose ($AUC_{glucose}$) values for 0-120 min post glucose load

Data represents the mean ± S.E.M., for n=5. [a] P < 0.01, [b] P < 0.001 as compared with diabetic rats

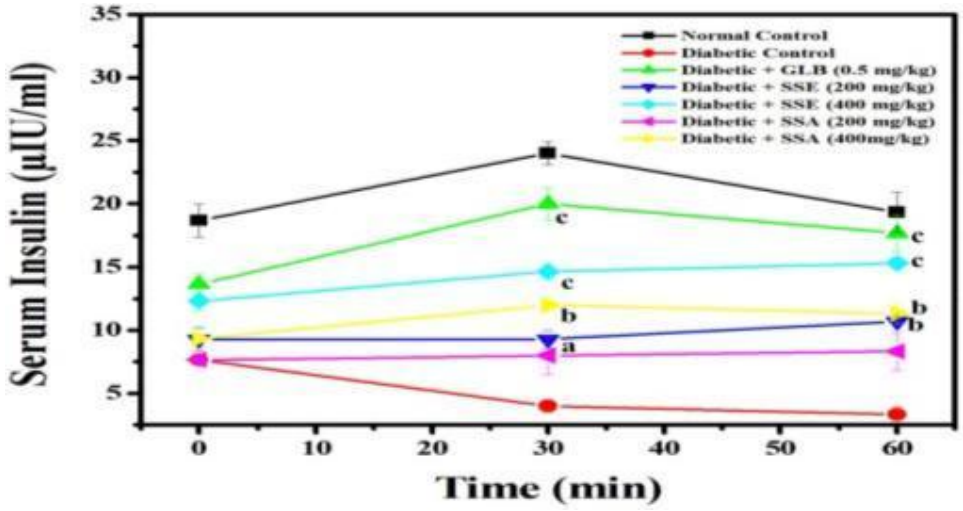

Fig. 2A. Serum insulin (SI) levels. Post glucose (2 g/kg b.w) challenge performed on 20th day of treatment with SSE, SSA and GLB

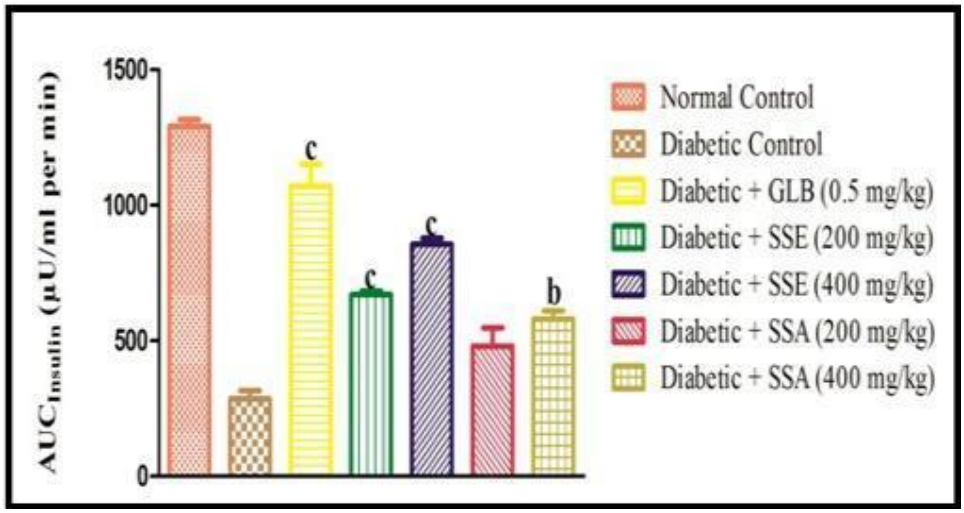

Fig. 2B. Serum insulin (SI) levels. Incremental $AUC_{insulin}$ values for 0-60 min

Data represent the mean ± S.E.M., for n=5. [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 as compared with diabetic rats

3.3.5 Insulin tolerance test (ITT) in diabetic rats

Insulin tolerance test is a measure of the extent of peripheral utilization of glucose. On 28th day, SG levels were measured following insulin challenge (2U/kg, i.v) and diabetic rats subjected to insulin challenge exhibited a marked fall in SG levels suggesting these diabetic rats were able to utilize the exogenously administered insulin & reduce the SG levels. As shown in (Fig. 3A), SSE (400 mg/kg) exhibited marked fall in SG level (50.77%) after 10 min of insulin administration. This reduction in SG level was comparable to GLB which showed 57.96% reduction as compared to diabetic control group at the same time period. (Fig. 3B.) depicts AUC glucose over 30 min time period of post insulin administration. Integrated AUC glucose for SSE (400 mg/kg) and SSA (400 mg/kg) treated diabetic rats over 30 min time period was found to be significant (P<0.001) compared to diabetic control group. These data suggest that the sensitivity to exogenous administered insulin was increased by SSE (400 mg/kg), SSA (400 mg/kg) and GLB.

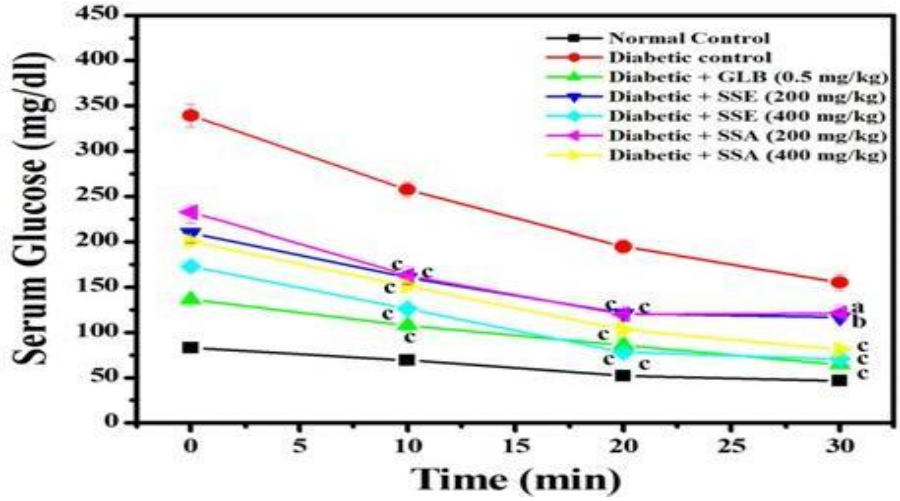

Fig. 3A. Effect of SSE and SSA on insulin tolerance in diabetic rats. SG levels were measured at 0 min, 10 min, 20 min and 30 min after i.v administration of insulin alone (2U/kg, b.w)

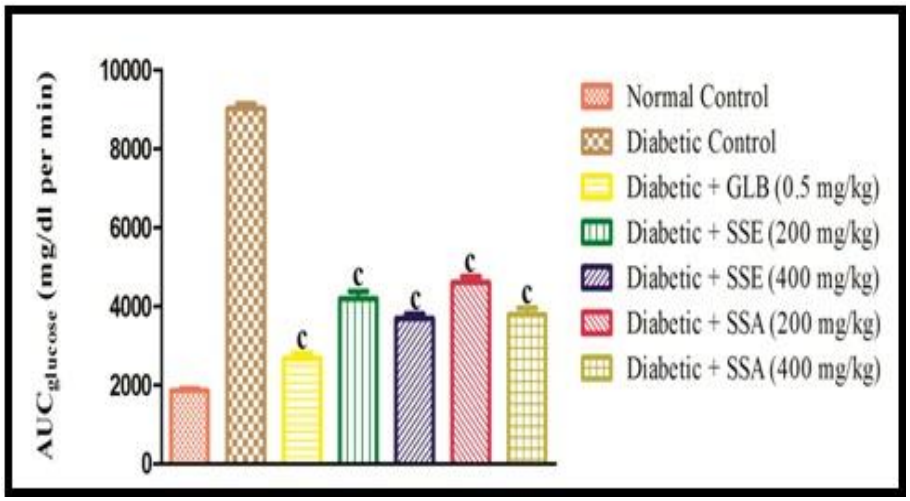

Fig. 3B. Effect of SSE and SSA on insulin tolerance in diabetic rats. Area under curve for glucose ($AUC_{glucose}$) values for 0-30 min post insulin injection

Data represents the mean±S.E.M., for n = 5. [a] P < 0.05; [b]P < 0.01; [c] P < 0.001 as compared with diabetic rats

3.3.6 Estimation of lipid parameters

Chronic administration of GLB and plant extracts to diabetic rats showed significant restoration in lipid parameters to normal values, when compared with diabetic control rats. As shown in Table 3, SSE (400 mg/kg) showed 23.27% reduction (*P*<0.001) in serum triglycerides (STG) and SSA (400 mg/kg) showed 27.26% reduction (*P*<0.001) in serum total cholesterol (STC) compared to GLB treated diabetic rats where 25.81% & 38% reduction in STG and STC was found. SSE (400 mg/kg) and GLB showed 32.60% & 29.82% reduction in VLDL-c level. There was 50.03% & 72.63% reduction in LDL-c level in SSA (400 mg/kg) and GLB treated groups. The level of HDL-c was significantly increased in GLB and ethanolic extract treated groups. Extract treated groups have reduced STG, STC, VLDL-c, LDL-c level and increased HDL-c level compared to diabetic control group.

3.5 Histopathological Examination

Fig. 4 depicts the histomorphological change in pancreas of different groups of animal.

Histopathological examination of pancreas in normal control group showed normal pancreatic acini and islet of langerhans with normal cellularity (4A). Whereas, decreased number and size of pancreatic islets, vacuolation, hydropic and necrotic cells, karyopyknosis, degranulation of cells and invasion of connective tissues were detected in the diabetic rats (Fig 4B). Both the higher doses of SSE (Fig 4E) and SSA (Fig 4G) and GLB (Fig 4C) markedly succeeded in amending the disrupted islets of langerhans of diabetic rats; resulting in the improvement of islet architecture and integrity. Also, the number and size o pancreatic islets increased significantly.

4. DISCUSSION

Sida spinosa Linn. exhibits hypoglycemic, antipyretic and diaphoretic activity [23]. The present study reports the effect of *Sida spinosa* has antihyperglycemic effect, thus scientifically validating the traditional claim for the first time. The potent antihyperglycemic effect exhibited by aqueous extract (comparable to glibenclamide) suggested that it may act by regenerating the β-cells in STZ-induced diabetic rats.

Streptozotocin is 1-methyl-l-nitrosourea attached to the carbon- 2 position of glucose that causes β-cell necrosis and induces ‘‘experimental diabetes’’ in many animal models [24] STZ action in β-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations. Two hours after STZ-injection, hyperglycemia is observed with a concomitant drop in blood insulin. Hyperglycemia can lead to a reduced number of glucose transporters, down regulation in the number of insulin receptors as well as defects of tissue insulin signal transduction. Subsequent to these deteriorations, there is an absolute increase in hepatic glucose output, which exceeds an increase in glucose utilization, and fasting hyperglycemia occurs [25].

An oral glucose tolerance test is a more sensitive measure of early abnormalities in glucose regulation than fasting plasma glucose [26]. Impaired glucose tolerance serves as a marker for the state of insulin resistance and predicts both large and small-vessel vascular complications [27]. SSE (400 mg/kg) and SSA (400 mg/kg) significantly reduced serum glucose level of STZ-induced diabetic rats. Likewise, the serum insulin levels also increased suggesting the possible mechanism by which SSE and SSA extracts bring about their antihyperglycemic action in diabetic rats, may be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from the existing β-cells or by its release from the bound form or by regenerating the β-cells in STZ-induced diabetic rats. Antihyperglycemic effects have been reported for some plants that contain flavonoids [28] and preliminary phytochemical results show that *Sida Spinosa* Linn. root contains flavonoids. The present study demonstrated that acute administration of the SSE/SSA has antihyperglycemic activity in STZ-induced diabetic rats (Table 1). In this study, SSE (400 mg/kg) showed significant fall in SG levels which was comparable to GLB. Further, the chronic administration of SSE (400 mg/kg) and SSA (400 mg/kg) led to marked antihyperglycemic activity (administered orally for 30 days) in STZ-induced diabetic rats (Table 2).

Hyperlipidemia has been reported to accompany hyperglycemic states [29,30]. High levels of serum total cholesterol and more importantly LDL-cholesterol is a major coronary risk factor whereas, several studies showed that an increase in HDL-c is associated with a decrease in coronary risk. It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. Hence, STZ-induced diabetic rats have altered lipid profile. In this study, diabetic control rats exhibited significantly elevated cholesterol and triglyceride, LDL-c and VLDL-c levels as compared to normal control rats. Chronic administration of both doses of SSE, significantly reduced serum total cholesterol, serum triglyceride, VLDL-c and LDL-c levels, whereas, HDL-c level was significantly raised. Therefore, normalization of lipids in diabetic rats treated with *Sida spinosa* Linn. Root may be partly due to its stimulatory effect on insulin secretion from pancreatic β-cells (confirmed by serum insulin levels after oral glucose challenge (2 g/kg) performed on 20th day of treatment) (Table 3).

Histopathological examination showed that *Sida spinosa* Linn. has the potency to increase the size of islets, and relatively increases granulated and normal β-cells. However, the expansion was better with SSE (400 mg/kg) and SSA (400 mg/kg) which possibly regenerate β-cells. The increased β-cell mass would increase the secretion of insulin, which may increase the peripheral utilization of glucose. The observed antihyperglycemic and antihyperlipidemic activity of title plant may be attributed to the presence of bioactive principles and their synergistic properties.

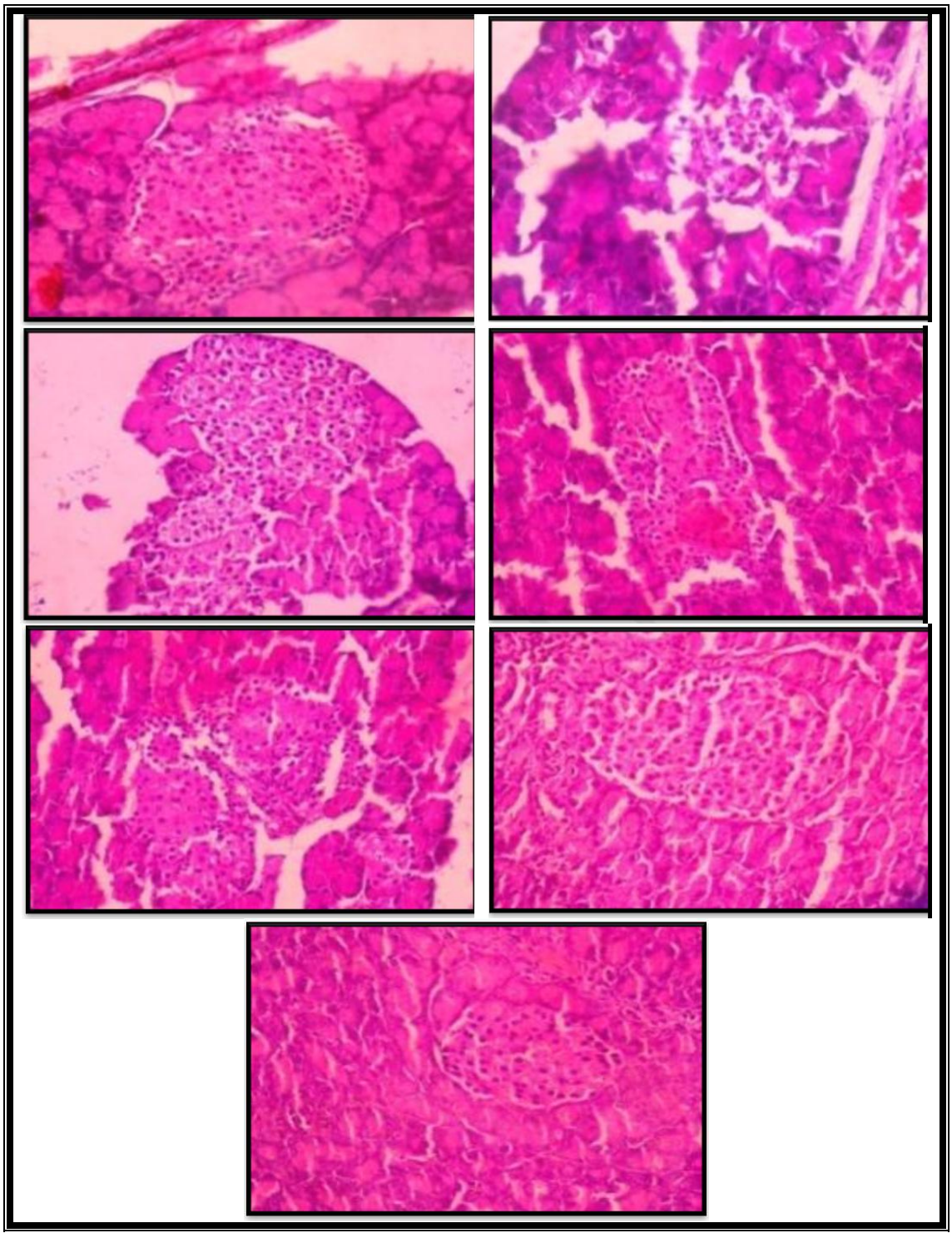

Fig. 4. Effect of SSE and SSA on histomorphological change in pancreas of different groups of animals

[A] Normal control [B] Diabetic control [C] Diabetic + GLB (0.5 mg/kg) [D] Diabetic + SSE (200 mg/kg) [E] Diabetic + SSE (400 mg/kg) [F] Diabetic + SSA (200 mg/kg) [G] Diabetic + SSA (400 mg/kg)

Table 2. Effect of SSE and SSA on SG levels in STZ-induced diabetic rats (Multiple-dose thirty-day study)

Group	SG levels [mg/dl]				
	0 day	7 day	14 day	21 day	28 day
Normal control	77.4±3.62	85.2±3.85	90.8±1.52	86.2±3.76	83±2.62
Diabetic control	281±12.24	297.4±7.44	320.2±10.36	334.8±13.39	339.2±12.70
Diabetic + SSE [200 mg]	291.20±7.21	268.20±4.68[a] [7.80]	241.60±5.23[c] [16.75]	227.20±4.75[c] [21.75]	210.80±5.16[c] [27.40]
Diabetic + SSE [400 mg]	293.40±7.85	256.60±5.16[b] [12.49]	229.20±6.37[c] [21.72]	198.00±5.93[c] [32.38]	173.60±4.71[c] [40.73]
Diabetic + SSA [200 mg]	294.00±8.17	281.20±4.93 [4.21]	266.80±3.36[a] [9.07]	244.40±6.57[c] [16.68]	232.40±6.29[c] [20.78]
Diabetic + SSA [400 mg]	288.00±9.22	258.80±4.02[a] [9.93]	248.60±6.41[a] [13.57]	226.20±5.47[c] [21.34]	200.20±3.66[c] [30.39]
Diabetic + GLB [0.5 mg]	271.20±6.20	208.0±5.23[c] [23.21]	179.60±6.19[c] [33.76]	155.80±6.26[c] [42.49]	136.40±3.01[c] [49.63]

Each value represents Mean±S.E.M., for n=5. Values in parentheses indicates percentage reduction in glycemia and [a] P<0.05; [b] P<0.01; [c] P<0.001 compared to base values i.e. 0 day of the same group

Table 3. Effect of SSE and SSA on lipid profile in STZ-induced diabetic rats [Multiple dose thirty-day study]

Parameter	Normal control	Diabetic control	Diabetic + SSE (200 mg/kg)	Diabetic + SSE (400 mg/kg)	Diabetic + SSA (200 mg/kg)	Diabetic + SSA (400 mg/kg)	Diabetic + GLB (0.5 mg/kg)
STG(mg/dl)	91.82±2.58	145.89±4.42	117.51±4.17[b]	111.74±4.06[c]	126.75±6.36	119.32±4.85[a]	107.86±3.72[c]
STC(mg/dl)	60.77±3.60	103.54±4.36	88.52±3.74[b]	81.82±2.56	79.86±3.35[b]	74.66±1.91[c]	63.87±4.30[c]
HDL-c(mg/dl)	29.09±2.05	20.01±0.75	25.56±1.95[b]	28.44±1.84[c]	22.75±1.11[a]	24.31±1.96[b]	33.26±1.71[c]
VLDL-c(mg/dl)	18.36±0.51	29.15±0.88	22.31±0.97[b]	19.61±1.03[c]	21.99±1.71[b]	20.78±0.98[c]	21.57±0.71[c]
LDL-c(mg/dl)	13.31±4.96	54.37±4.80	33.79±2.40[c]	13.24±3.34[c]	35.85±3.35[b]	29.74±1.80[c]	9.03±3.21[c]
TC/HDL-c ratio	2.13±0.25	5.20±0.71	3.58±0.35[c]	2.75±0.24[c]	3.57±0.29[c]	2.81±0.20[c]	1.95±0.16[c]
LDL-c/HDL-c ratio	0.49±0.19	2.74±0.56	1.38±0.18[c]	1.09±0.14[c]	2.07±0.09[c]	1.25±0.11[c]	0.30±0.11[c]

Each value represents Mean±S.E.M., for n=5. [a] P<0.05, [b] P<0.01, [c] P<0.001 compared to Diabetic control rats

5. CONCLUSION

The *Side spinosa* Linn. root is beneficial in controlling diabetes by reducing blood glucose and increasing the level of insulin. Antihyperlipidemic efficacy is due to increase in secretion of insulin from pancreatic β-cells by the plant extracts. The present investigation has opened an excellent opportunity in the development of herbal formulation from *Sida spinosa* Linn. root to control diabetes.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments were examined, approved and conducted as per instructions given by Institutional Animal Ethical Committee (IAEC), Department of pharmacology, SET's College of pharmacy, Karnataka-580002.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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