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Evaluation of Antidiabetic Potential of S*arcostemma brevistigma* Wight & Arn. Using Alloxan-Induced Diabetic Murine Model

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Abstract A multimillion-dollar boom is achieved every year by the ethnopharmaceutical companies, creating awareness around the globe to use herbal medicines to stay and live a healthy life. Nearly, two-thirds of the plants were discovered for herbal remedies, and some plants are even endangered to get extinct from the globe for their repeated utility. *Sarcostemma brevistigma* is one among the undiscovered medicinal plants which belongs to the family Asclepiadaceae found distributed in Indian states of Bihar, Bengal, Konkan, Tamil Nadu, and Kerala. This study was intended to determine the antidiabetic property of plant extract of *Sarcostemma brevistigma* evidenced by biochemical parameters, antioxidant activity, with the histopathological analysis in diabetic induced mice. Animals that were orally treated with the *S. brevistigma* extract showed blood glucose lowering effect when compared to the alloxan-induced mice, i.e., from 391.5 ± 6.3 to 193.6 ± 4.3 mg/dL. There is the significant increase in insulin level ($P < 0.05$) (27.97 ± 1.6 mIU/L) which is comparable to the metformin administered test group (30.35 ± 0.6 mIU/L). A significant difference ($P < 0.05$) of both ALT and AST levels were observed when compared to the other groups. A significant increase in antioxidant activity (reduced glutathione, malondialdehyde, superoxide dismutase) in plant extract administered group was observed when compared to the other treatment groups. Histopathological studies showed abnormalities in the liver, pancreas, kidney, lungs, heart, and spleen of alloxan-induced diabetic rats. The abnormalities were found to be normalized to a considerable extent after treatment with *S. brevistigma* extract. The results stood as evidence for *S. brevistigma* as an active antidiabetic herbal plant.

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Introduction

Diabetes mellitus (DM) is a grave metabolic ailment which occurs due to the deficit in insulin secretion and its function or both leading to a hyperglycemic condition. The increase of glucose molecule in the blood causes disturbances to metabolic processes inside the human body [1]. If left untreated on right time, it will result in irreversible damages to the eyes, nerves, kidneys, cardiovascular problems, and ulceration, and it ranks as the third causative reason of death all over the world [2]. This condition is mimicked in rodents by injecting alloxan, impairing the carbohydrate mechanism leading to hastened lipolysis, resulting in hyperlipidemia and hyperglycemia. Regardless of the currently identified and used antidiabetic drugs in the pharmaceutical arena, diabetes and its associated impediments sustained to be a key medical anomaly. Diabetes emerged as India's seventh biggest cause of early death in 2016, up from 11th in 2005, data shown from Institute of Health Metrics & Evaluation, India. According to International Diabetes Federation, India is one among the six countries of the IDF SEA (South East Asian) region. Around 425 million people have diabetes in the world and 82 million people in the SEA Region; by 2045, this will rise to 151 million. There were over 72 million cases of diabetes in India in 2017.

In recent times, few herbal plants like *Allium sativum*, *Gymnema sylvestre*, *Citrullus colocynthis*, *Trigonella foenum greacum*, *Momordica charantia*, *Ficus bengalensis*, and *Desmodium pulchellum* have been identified to be potential in treating diabetes and have been used empirically as antidiabetic and anti-hyperlipidemic medications [3]. Effective management of diabetes mellitus by using medicinal plants was reported in various studies. The antidiabetic potential of *Zaleya decandra* roots on alloxan-induced diabetes was evaluated in rats. Oral administration of ethanolic extract of the root (200 mg/kg body weight/day) for 15 days re-established the levels of glucose, cholesterol, triglycerides, total proteins, urea, creatinine, lipid peroxidation level, and antioxidant enzymes significantly in diabetic rats [4]. In an earlier study, the ethanol extract of *E. jambolana* seeds, water extract of *M. charantia* fruits, ethanol extract of *G. sylvestre* leaves, and water extract of *T. graecum* seeds have higher hypoglycemic and antihyperglycemic potential and may be used as complementary medicine to treat the diabetic population which can significantly reduce the dosage of standard drugs [5].

Sarcostemma brevistigma is generally known as moon plant, soma (Sanskrit) and somlata (Hindi) is a leafless sprawling shrub which belongs to the family Asclepiadaceae [6]. The plant contains chemical constituents like malic acid, succinic acid, reducing sugars, traces of tannin, alpha- and beta-amyrins, lupeol and lupeol acetate, and beta-sitosterol [7]. Esculentin, pregnane triglycosides, and cardenolide tetraglycosides have been isolated from the root. The phytochemical studies indicated the presence of bergenin, brevine, brevinine, sarcogenin, sarcobiose, and flavonoids in the aerial parts of the plant extract [3].

In a study, in vitro assays conducted with methanolic extract of *S. brevistigma* for α-amylase activity, glucose diffusion assay, glucose uptake by yeast cells, and non-enzymatic

glycosylation assay indicated that the plant has potential antidiabetic activity [8]. The stem extract of *S. brevistigma* was reported to possess hepatoprotective activity against CCl_4-induced hepatic damage in rats. The extract was reported to lessen the CCl_4-induced raised levels of the enzymes, signifying the increase in structural integrity of hepatocytic cell membrane or rejuvenation of impaired liver cells [9]. The fraction of this plant extract has been found to have anti-allergic, anti-inflammatory activities and repressed the contraction induced by acetylcholine and histamine on isolated guinea pig ileum and its trachea [10]. Chloroform-soluble fraction of this plant possesses in vitro uterine relaxant activity and spasmolytic activity [11].

Considering these facts, this study aimed to test the influence of the medicinal *plant S. brevistigma* extract in controlling the diabetics by assessing blood glucose level, its secondary complications (serum biomarkers) associated with the disease, and antioxidant activity in Swiss albino mice.

Materials and Methods

The fresh stem (twigs) along with leaves of *S. brevistigma* was collected from Siruvadi Reserve Forest (coordinates 12°16′14″ N 79°22′39″ E) near Melachery Village, Gingee Taluk, Villupuram District, Tamil Nadu, and was authenticated by Prof. P. Jayaraman, Director, Institute of herbal Botany, Chennai (PARC No./2012/3302). The collected plant materials were cleaned with running water and then with sterile water, and dried under shade for 2 weeks.

Extraction of Plant Material

The successive extraction procedure was carried out with 10 g of finely powdered stem with leaves both mixed together. The powdered sample was immersed in 100 mL of respective solvent (1:10 *w*/*v*) and kept under overnight shaking and subjected to Soxhlet extraction using solvents of differing polarity such as ethyl acetate, hexane, and methanol [12]. The extracted solvents were filtered using Whatman No. 1 filter paper. The methanolic extract alone was used for the study. The filtrate was concentrated using rotary vacuum evaporator at 45 to 52 °C in vacuum, and the dried residue was obtained and stored at 10 °C in a refrigerator.

Animal Model

The experiment was carried out using healthy male Swiss albino mice (25–30 g). They were housed in standard laboratory condition at room temperature 22 °C (± 3 °C) with 12 h light/dark cycle. Investigations were carried out with them in a well-maintained animal house, Centre for Biomedical Research, VIT, Vellore, India. The animals were provided with standard pelleted diet (SPD; 4.1% fat, 22.2% protein, and 12.1% carbohydrates, as a percentage of total kcal) and water ad libitum. Handling of animals was in accordance with the recommendations of Institutional Animal Ethical Committee (IAEC), and the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA), Government of India. All investigational procedures to be followed on animals were declared and accepted by the Animal Ethical Clearance Committee of VIT (VIT/IEAC/09/July26/No.19).

Induction of Diabetes by Alloxan in Mice

Alloxan monohydrate (Sigma-Aldrich, India) was administered (150 mg/kg body weight), as a standard dose through single intraperitoneal injection with 3% glucose added in drinking water for 24 h post-injection. After 48 h, the fasting blood glucose was checked by tail vein method and blood glucose measured using an Accu-Check® glucometer (Roche Diagnostics GMBH, Mannheim, Germany) and OneTouch Surestep glucose strips (Lot No. 285368A, LifeScan Inc., Milpitas, CA., USA), and mice showing glucose levels more than 200 mg/dL were selected for randomization into groups.

Acute Toxicity Test and Pilot Study

Acute toxicity test was done according to the World Health Organization (WHO) guideline (WHO 2000) and the organization of Economic Co-operation and Development (OECD) guideline 420 for testing of chemicals (OECD 2001). A group of three Swiss albino mice were administered 2000 mg/kg bw of methanolic extract of *S. brevistigma* in four equally divided doses (500 mg/dose) during a period of 8 h. The plant extract was dissolved in 2 mL of distilled water (freshly prepared) and administered by oral gavage feeding. The animals were observed for the next 72 h for behavioral parameters like alertness, grooming, aggressiveness, touch response, tremor, sleep, convulsion, muscle spasm, analgesia, lacrimation, diarrhea, salivation, and number of deaths (mortality).

A pilot study was conducted two groups comprising three Swiss albino mice in each group to access the blood glucose changes and to fix the dosage. Two doses, viz 250 mg/kg bw (Group 1P) and 400 mg/kg bw (Group IIP) of the plant extract, were administered by oral gavage feeding. Three parameters were observed namely body weight, blood glucose, and insulin levels. The experimental period was set for 28 days.

Animal Grouping and Experimentation

The mice were treated according to the following groups for an experimental period of 28 days. Each group consisted of five Swiss albino mice. The methanolic plant extract was administered by oral gavage feeding: group I euglycemic or control (without alloxan induction), fed with standard normal diet; group II Normal control group without alloxan treatment, fed with standard normal diet and co-administered with *Sarcostemma brevistigma* plant extract with a dose 250 mg/kg bw orally, once daily; group III alloxan-induced hyperglycemic mice with standard normal diet alone; group IV hyperglycemic mice given with standard normal diet and co-administered with *Sarcostemma brevistigma* plant extract (250 mg/kg bw) once daily; and group V alloxan-induced hyperglycemic mice fed with standard normal diet and co-administered with 250 mg/kg bw of metformin, once daily. The body weight and blood glucose levels are recorded from day 1 and at weekly intervals for 28 days (4 weeks). At

the end of the 4-week intervention, rats were fasted for 12 h and then sacrificed by cervical decapitation, and blood was collected as described previously [13].

Analysis of Serum Biomarkers and Enzymes

At the end of the experiment, biochemical parameters from the serum and enzyme levels were estimated. Alanine transaminase (ALT) and aspartate transaminase (AST) enzyme levels were measured. The insulin estimation was done by immuChem™ radioimmunoassay method using as a standard kit (MP Biomedicals, Germany). The biochemical analysis of the serum was performed using auto-analyzer (Prietest Touch Biochemistry auto-analyzer, Robotnik India Pvt. Ltd., India).

Estimation of Oxidative Enzymes in Liver Tissue

One gram of the liver tissue was homogenized in 10 mL of 0.1 M Tris-HCl (pH 7.4) buffer solution. The homogenate was centrifuged and the supernatant was used for the assay of superoxide dismutase (SOD) [14], reduced glutathione (GSH) [15], and malondialdehyde (MDA) [16]. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense against ROS by lowering the steady state level of oxygen. The SOD activity is determined by the ability of the enzyme to inhibit auto-oxidation of pyrogallol.

Histopathology Studies

The kidney, liver, lungs, spleen, heart, and pancreas were fixed in formalin (10%), clarified in xylene, and subjected to histopathological studies. The specimen was processed and embedded in paraffin wax. The tissues were sectioned using Rotary microtome HM325 (Thermo Scientific India Ltd., India). Sections of about 4–6 μm were made and stained with hematoxylin and eosin. The sections were observed under ×40 objective lens and were photographed [17].

Results and Discussion

Acute Toxicity and Pilot Study

In acute toxicity study, the animals did not exhibit any abnormal symptoms up to the dose of 2000 mg/kg bw of the plant extract. No deviations were noted in the normal behavioral pattern, and no signs and symptoms of toxicity were observed. The animals did not show any signs of tremor, convulsion, salivation, diarrhea, lethargy, sleep, coma, dyspepsia, nasal bleeding, etc., up to the dose of 2000 mg/kg bw after administering the methanolic extract of *S. brevistigma*. The mortality was zero at this dosage.

A pilot study with two groups of animals did not show significant variations in the preliminary observations recorded, viz body weight, blood glucose, and insulin level. There were no significant differences between alloxan-induced diabetic Group IP (250 mg/kg bw) and Group IIP (400 mg/kg bw) that were orally treated with the plant extract. Group IP recorded 192.68 ± 1.1 mg/dL and 26.32 ± 0.6 mIU/L of blood glucose and insulin levels,

respectively. Group IIP showed a slight increase in blood glucose level (193.82 ± 0.9 mIU/L) and a subtle difference in insulin level (26.02 ± 1.6 mIU/L) at the end of the study. Since there are no significant differences between the two treatment groups, the experimentation dosage was fixed as 250 mg/kg bw rather going for the higher dosage.

Body Weight, Blood Glucose, and Insulin Levels

The body weight and feed intake of *S. brevistigma* treated animals significantly ($p < 0.05$) increased in comparison to the diabetic control. In this study, it was observed that the alloxan-induced diabetic mice (group III) had a twofold upsurge in blood glucose level (391.6 ± 6.3 mg/dL) relative to the normal control animals (group I), 127.8 ± 1.7 mg/dL. Group IV animals that were orally treated with the extract showed blood glucose lowering effect when compared to the group III, i.e., from 391.5 ± 6.3 to 193.6 ± 4.3 mg/dL (Fig. 1). There is a significant reduction ($P < 0.05$) in blood glucose level of group IV when compared to group III (Table 1). This reduction in glucose data is supported by the insulin levels, group III treated with alloxan showed depletion of insulin level (7.5 ± 0.3 mIU/L) when compared to group I (35.86 ± 0.4 mIU/L). There is a significant increase in insulin level ($P < 0.05$) in group IV (27.97 ± 1.6 mIU/L) which is comparable to group V (30.35 ± 0.6 mIU/L).

From the results obtained, it is quite evident that the extract of S. *brevistigma* can decrease hyperglycemia and regulate other biological parameters. Presence of phytochemicals in the plant extract may be a key factor that can be linked to the lowered blood glucose levels which were in accordance with the result reported earlier [18]. Above results also indicated that, in mice treated with plant extracts, normalization of insulin synthesis might be an indicator of decreased blood glucose level consequently that may stimulate fatty acid biosynthesis.

This consequently may inhibit the hormone-sensitive lipase in the adipose and might have resulted in utilization of fatty acid in adipose tissue by glucagons. As a result, free fatty acid levels get reduced in the plasma [19], and simultaneously, very low-density lipid (VLDL) and low-density lipid (LDL) concentrations may lessen, as there is an absence of the higher level of fatty acids [20, 21].

Alloxan induces hyperglycemia by a massive reduction of the β-cells of the islets of Langerhans [22] and also by reduction of the pancreatic β-cell population via formation of reactive

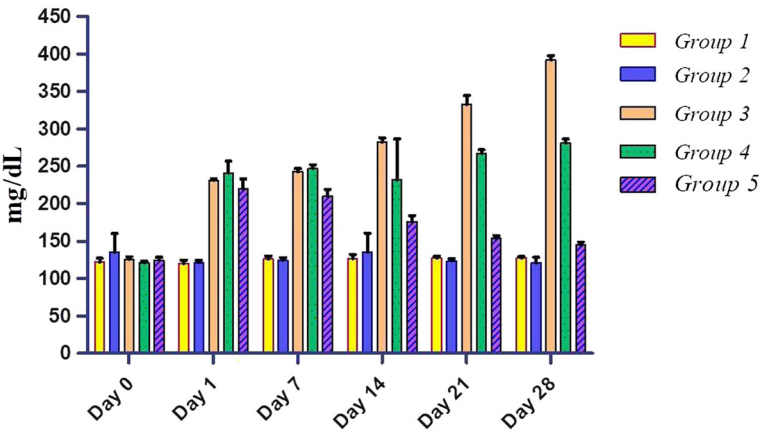

Fig. 1 Blood glucose level (mg/dL) at different durations among the experimental groups

Table 1 Insulin and glucose levels among the experimental groups

Study groups	Insulin (IU/L)	Glucose (mg/dL)
Group I	35.86 ± 0.4	127.80 ± 1.7
Group II	35.39 ± 0.4	121.40 ± 6.9
Group III	7.50 ± 0.3***	391.50 ± 6.3***
Group IV	27.97 ± 1.6***	193.60 ± 4.3**
Group V	30.35 ± 0.6***	145.00 ± 3.9*

*Significant ($P < 0.05$)

**Highly significant ($P < 0.01$)

***Very high significance ($P < 0.001$)

oxygen species like nitric oxide [23]. Group III treated with alloxan showed depletion of insulin level which was due to the destruction of β-cells of the islets of Langerhans. But, in alloxan-induced mice treated with *S. brevistigma* extract (group IV), the insulin level comparatively increased, indicating the regeneration efficacy of pancreatic β-cells. These effects are reversed by treating the mice with *S. brevistigma* and clearly evidenced with histopathological examination.

Biochemical Parameters

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most regularly used enzymes to assess hepatocellular damage. It was observed that in alloxan-induced mice with 150 mg/kg bw, the activities of ALT and AST in serum were elevated, i.e., 88.75 ± 5.2 and 80.21 ± 2.9 mg, respectively (Table 2). Notably, activities of ALT and AST in group IV treated with 250 mg/kg methonolic extract of *S. brevistigma* were reduced, i.e., from 88.75 ± 5.2 to 49.53 ± 0.9 and 80.21 ± 2.9 to 47.49 ± 2.4 mg, respectively. There is a significant difference ($P < 0.05$) of both ALT and AST levels in group IV, when compared to the other groups. The differences in the concentration of AST and ALT were directly correlated to variations in the metabolism in which these enzymes are involved (Fig. 2), and approximately onefold amount of decrease of the enzyme levels with the plant extract was noticed. Normal levels of ALT and AST that were found to be restored in group IV animals indicated the normal functioning of the liver. Moreover, normal mice fed with the plant extract did not show any increase in serum liver enzyme activities. These results indicated that the plant extract does not have any toxic effect on the liver and seems to be hepatoprotective.

AST levels can be correlated to ALT activity with relevance to liver injury; nevertheless, AST might not be as much of important biomarker as elevated levels of the enzyme are found

Table 2 ALT, AST, total protein, insulin, and glucose levels among the experimental groups

Study groups	ALT (IU/L)	AST (IU/L)	Total protein (g/dL)
Group I	32.39 ± 0.6	30.52 ± 0.5	14.24 ± 2.5
Group II	32.28 ± 0.7	31.08 ± 0.3	13.05 ± 0.8
Group III	88.75 ± 5.2***	80.21 ± 2.9***	9.20 ± 0.2**
Group IV	49.53 ± 0.9***	47.49 ± 2.4***	10.06 ± 0.7*
Group V	41.55 ± 4.9**	40.22 ± 5.4***	11.77 ± 0.6*

*Significant ($P < 0.05$)

**Highly significant ($P < 0.01$)

***Very high significance ($P < 0.001$)

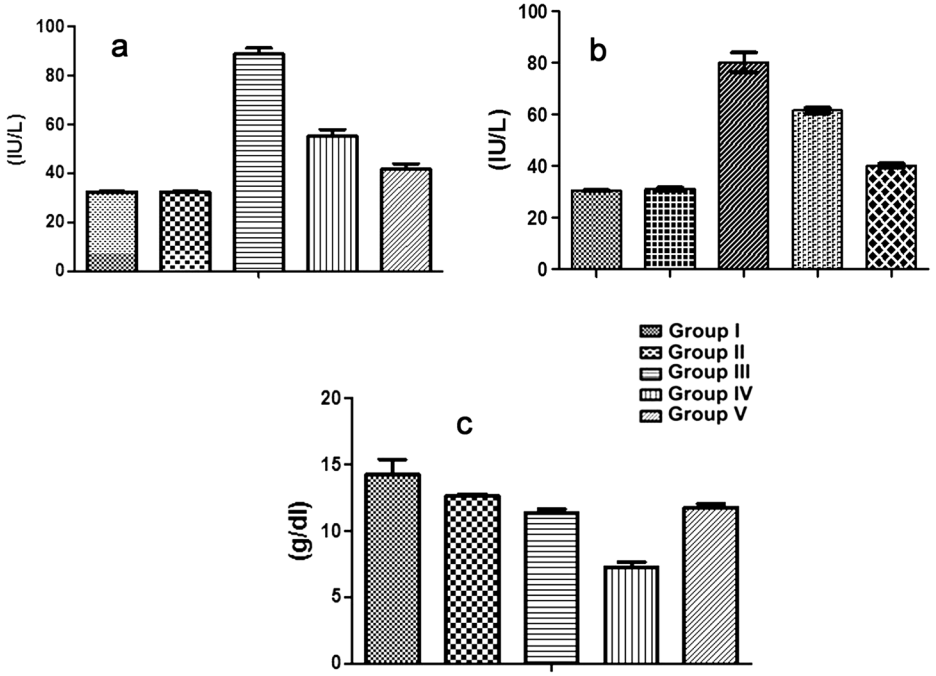

Fig. 2 Serum biomarker profile of the experimental and control groups. **a** Alanine aminotransferase (ALT). **b** Aspartate aminotransferase (AST). **c** Total protein levels

in a range of other tissues. Use of glucose or by stimulation of glucose uptake directly through normalization of insulin secretion may be considered, as a likely mechanism by which the plant extracts might have reduced the blood glucose levels can be hypothesized [24]. It may be also due to stimulation of β-cells in islet of Langerhans by the plant extract, which in turn increased serum insulin and reduced blood sugar level [25]. Another parameter like protein content was decreased overall which may be due to (hypo-albuminemia) in diabetes as reported earlier [18]. However, protein levels in group IV indicated reversing effect of *S. brevistigma* extracts on protein synthesis.

Serum lipid profile analysis of five groups of experimental animals was shown (Table 3). It can be inferred from the results that in alloxan-induced diabetic animals (group III), the total cholesterol, triglycerides, and VLDL and LDL levels in blood serum were found to be increased except HDL level, when related to group I. There was significant increase ($P < 0.05$) in the serum lipid content (TC, VLDL, LDL, and TG) in alloxan-induced treatment groups (III, IV, and V) when compared to the control group I, except that there was a less significant reduction in HDL level in group IV (Fig. 3).

There was a significant increase in overall blood serum lipid content except for HDL, and there was a significant reduction. But, in alloxan-induced mice treated with plant extract, there is a moderate increase of HDL level. Similar results were reported earlier [26, 27]. Pancreatic lipase, a key mammalian enzyme, is considered to be responsible for hydrolysis of a majority of dietary fats. The increase in blood lipid content may be due to disruption in the regulation of the activity of the hormone-sensitive enzyme lipase, which might have been induced by insulin. The increase in blood lipid content especially (TGL, LDL, and VLDL) may be due to the insulin deficiency which in turn causes lipase deficiency in the alloxan-induced destruction of

Table 3 Blood lipid profile (total cholesterol, triglycerides, HDL, VLDL, and LDL levels) among the experimental groups

Study groups	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)
Group I	79.12 ± 0.6	85.1 ± 4.6	51.05 ± 5.7	16 ± 1.0	17.20 ± 3.2
Group II	85.40 ± 0.7	87.5 ± 6.6	49.91 ± 5.7	17.6 ± 1.0	22.50 ± 1.0
Group III	174.91 ± 5.2***	162.5 ± 5.6***	38.68 ± 5.9***	32.7 ± 0.8***	70.96 ± 4.5***
Group IV	122.57 ± 5.7***	144.5 ± 7.8***	45.57 ± 11.5*	28.2 ± 2.3***	32.00 ± 2.2***
Group V	109.00 ± 4.9***	136.4 ± 13.6***	51.40 ± 10.9NS	27.4 ± 1.7***	37.28 ± 1.3***

*Significant ($P < 0.05$)

**Highly significant ($P < 0.01$)

***Very high significance ($P < 0.001$)

islets of Langerhans. It is believed that hyperglycemia increases the generation of free radicals by glucose auto-oxidation and thus increases the number of free radicals that may lead to liver damage. Therefore, it can be inferred that the possible mechanism of action may be due to antioxidant activity of plant extract, which aid to recover from impaired metabolism of glucose.

Antioxidant Activity

Glutathione and superoxide dismutase were evidenced to be decreased in alloxan-injected animals when compared to the normal ones from 0.8 ± 0.6 to 0.2 ± 0.03 μg/g and from 86.8 ± 0.5 to 27.0 ± 1.1 U/mL, respectively (Table 4). In contrast, malondialdehyde level was found to be increased from 13.7 to 55.4 μg/g in animals treated with *S. brevistigma*, and concentrations of antioxidants were found to be reverted (Fig. 4).

Glutathione (GSH) is a main non-protein thiol in living life form, which plays a central role in coordinating the body's antioxidant defense processes. Decreased GSH concentration contributes to the pathogenesis complications associated with the diabetic state [21]. Reduced glutathione, synthesized mainly in the liver, is an important non-enzymatic antioxidant in the anti-oxidative defense system. A study indicated that there was a marked depletion of GSH

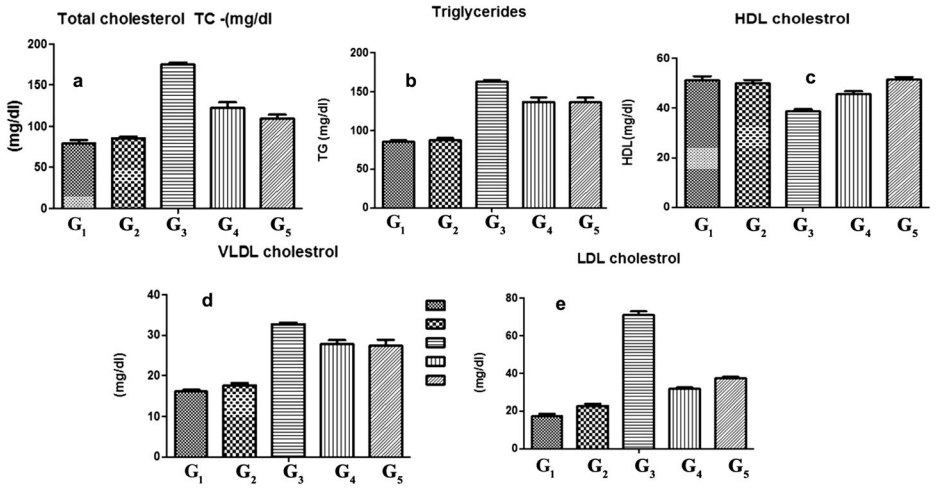

Fig. 3 Serum lipid profiles of the experimental groups under study. **a** Total cholesterol. **b** Triglycerides. **c** HDL cholesterol. **d** VLDL cholesterol. **e** LDL cholesterol. G_1 to G_5 represents group I to group V, respectively

Table 4 Anti-oxidant assay for reduced glutathione, malondialdehyde, and super oxide dismutase levels among the experimental groups

Study groups	Reduced glutathione (μg/g)	Malondialdehyde (μg/g)	Superoxide dismutase (U/mL)
Group I	0.8 ± 0.6	13.7 ± 0.7	86.8 ± 0.5
Group II	1 ± 0.09**	14.2 ± 0.6	86.2 ± 1.2
Group III	0.2 ± 0.03***	81.6 ± 1.7***	27 ± 1.1***
Group IV	0.4 ± 0.04***	55.4 ± 0.4***	54.1 ± 1.7***
Group V	0.7 ± 0.02***	30.7 ± 0.6***	75.6 ± 2.3***

*Significant ($P < 0.05$)

**Highly significant ($P < 0.001$)

***Very high significance ($P < 0.0001$)

observed in alloxan-induced diabetes mellitus mice and may be due to the utilization of this compound by two antioxidant enzymes, glutathione peroxidase (GPx), and glutathione-S-transferase (GST), as their substrate [28]. Our study indicated that there is moderate restoration or presence of reduced glutathione in group IV. In the enzymatic antioxidant defense system, SOD is one of the important enzymes and scavenges the superoxide radicals by converting them to H_2O_2 and molecular oxygen. In group IV, there was a moderate level of SOD synthesis, and there was an observed decrease of SOD activity in diabetic induced mice in this study.

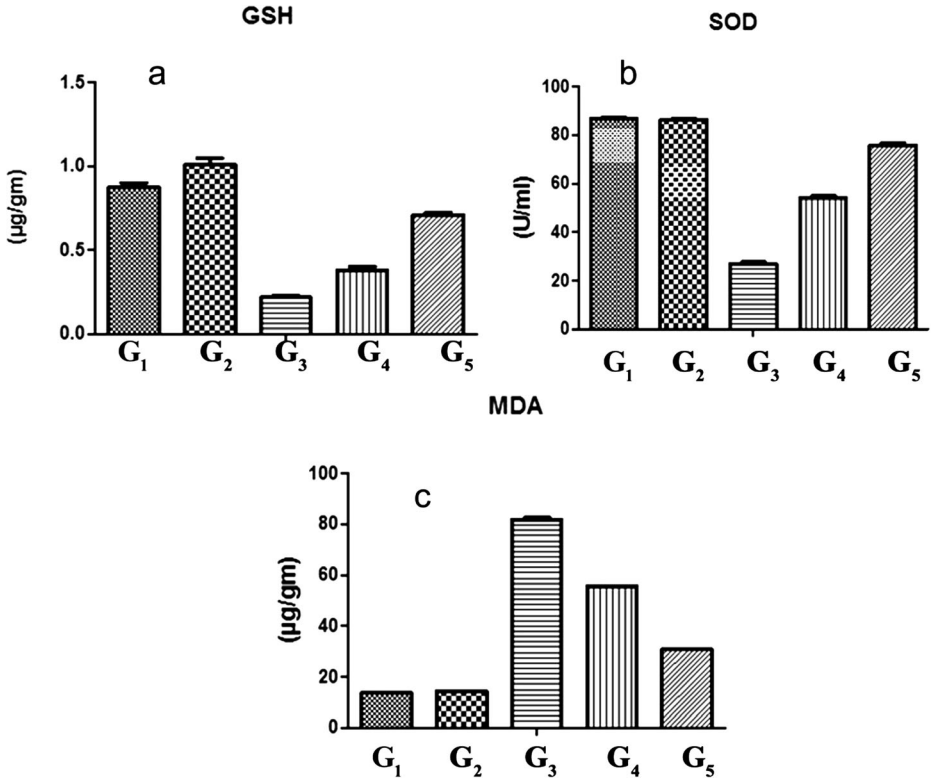

Fig. 4 In vivo antioxidant activity of different experimental groups. **a** Reduced glutathione. **b** Superoxide dismutase. **c** Malondialdehyde. G_1 to G_5 represents group I to group V, respectively

Lipid peroxide in the blood provides useful information for the prognosis of diabetes [29]. Blood MDA levels in alloxan-induced diabetic mice increased because of lipid peroxidation [30]. Previous studies, both in vitro and in vivo, have shown antioxidant activity to inhibit and reduce the degeneration of cells [31]. Our study indicated that there was a significant reduction in the level of blood MDA in group IV.

Histopathological Analysis

Histopathological observations of liver (Fig. 5) that displayed well-arranged cells and clear central vein with its portal and sinusoidal areas were almost normal in groups I and II, whereas in group III, it showed damages in hepatocytes, the disintegration of the central vein, fatty deterioration, and also injured hepatocytes with vacuoles. Diabetic mice treated with plant extracts (group IV) showed no significant abnormalities. In the case of the pancreas, no abnormalities were detected in groups I and II. Observations of pancreas (Fig. 5) indicated that the islets are unevenly shaped and comparatively small in group III. However, when compared to the untreated diabetic mice, histopathological examination of group IV revealed a rise in the number of pancreatic islets and more of β-cells. There was a decrease in the number of instigated lymphocytes and macrophages. In other words, the mice in group IV tend to restore to the healthy pancreas in due course of treatment.

The kidney section (Fig. 5) of group I and II showed well-arranged cells with glomeruli and renal tubules, namely the proximal and distal convoluted tubules that exhibited normal

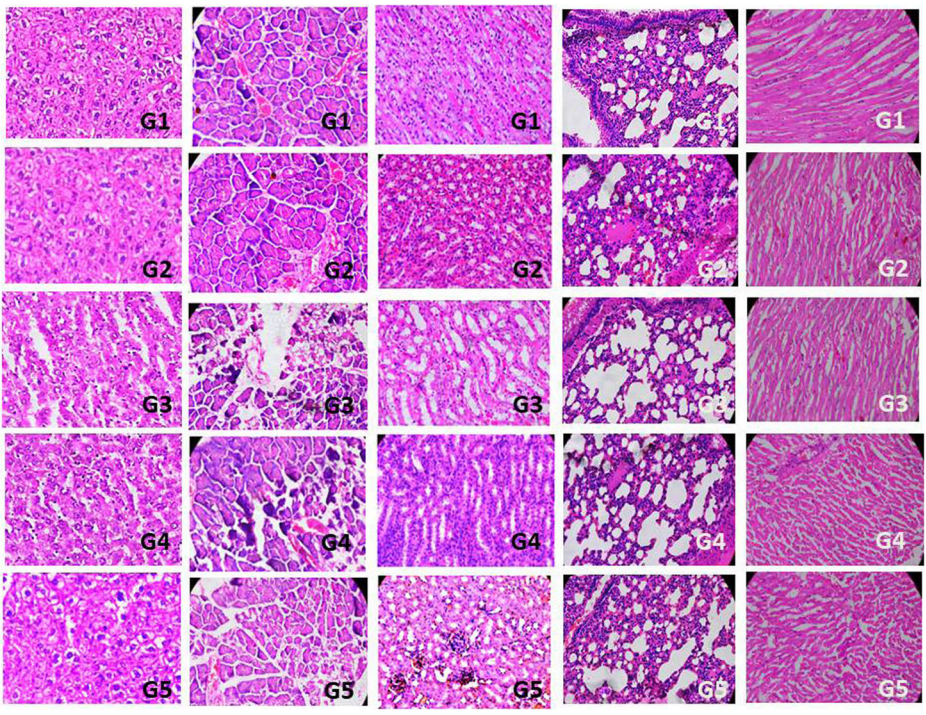

Fig. 5 Histopathological examination of hematoxylin and eosin stained sections of vital organs. From left to right, column 1 liver, column 2 pancreas, column 3 kidney, column 4 lungs, and column 5 cardiac tissue. G_1 to G_5 represent group I to group V, respectively

architecture, which indicates the absence of renal toxicity. In contrast, group III showed endocytic vacuoles particularly in the proximal tubules and the thickening of the glomerular basement with glomerulosclerosis, indicating severe tubular epithelial cell degeneration, whereas group IV showed reversing effect towards normality on kidney cells indicating mild tubular epithelial cell degeneration. Histopathological observations of the liver that displayed well-arranged cells and clear central vein with its portal and sinusoidal areas were almost normal in groups I and II, whereas in group III, it showed complete destruction of hepatocytes, degeneration of central vein, fatty degeneration, and also damaged hepatocytes with vacuoles. Diabetic rats treated with plant extracts (group IV) showed no significant abnormalities.

Animals treated with alloxan showed lesions and abnormal alveoli with large vacuoles (Fig. 5) that confirm destruction of lung cells (group III). Similar to other organs, diabetic rats treated with plant extracts (groups IV and V) showed regeneration process reverting to normalcy. No abnormalities were detected in groups I and II. The sections were obtained from cardiac tissues and observed for dilation and congestion of myocardial blood vessels, vacuolization of cardiac myocytes associated with intramuscular edema, necrosis of myocardial cells, and fragmentation of cardiac muscle bundles for all the study groups. There were no significant abnormalities detected among the groups.

The renewal of the β-cells of the alloxan-destructed islets is almost certainly due to the fact that pancreas contains stable (quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells [32, 33].

Conclusion

In conclusion, the methanolic extract of *S. brevistigma* can both decrease hyperglycemia and regulate other serum biological parameters. The plant extract also possessed moderate levels of in vivo antioxidant activity and reversal of abnormalities in the organs. This study suggests that *Sarcostemma brevistigma* might be considered as a potential source of natural antidiabetic agents. Further studies focusing on specific phyto-compounds and bioactive isolation can take *S. brevistigma* as a herbal remedy for treating diabetes.

Compliance with Ethical Standards All institutional and national guidelines for the care and use of laboratory animals were followed and compliance with ethical standards. Handling of animals was in accordance with the recommendations of Institutional Animal Ethical Committee (IAEC), and the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All investigational procedures to be followed on animals were declared and accepted by the Animal Ethical Clearance Committee of VIT (VIT/IEAC/09/July26/No.19).

Conflict of Interest The authors declare that they have no conflict of interest.

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