Preclinical evaluation of the anti-hyperglycemic activity of ethanolic extract of leaves of *Schefflera arboricola* in an alloxan-induced diabetic animal model of rats.

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ABSTRACT

Globally, both developed and developing nations are seeing a dramatic increase in the prevalence of Diabetes mellitus patients. The currently available treatments, particularly synthetic pharmaceuticals, often fail to restore normal blood glucose levels without side effects. Many herbal remedies are being investigated as complementary treatments for diabetes. The primary objective of this research was to test the ethanolic extract of leaves of *Schefflera arboricola* on alloxan induced diabetic rats. Acute oral toxicity was performed according to OECD guidelines 425. Animals were divided in to 6 groups. Normal control and toxic control group animals were administered with normal saline (1ml/100g), Std. group animals were treated with Glibenclamide (10 mg/kg), three diferent doses of ethanolic extract (100 mg/kg, 200 mg/kg, and 400 mg/kg bodyweight) were administered to test group diabetic animals. Body weight was measured every week. On 0,7,14,21 and 28 day blood was withdrawn by retro orbital plexus for estimation of blood glucose level. While lipid profile and antioxidant parameters were estimated at on 28th day . A significant decrease ($p<0.05$) in blood glucose and lipid profile was seen at 200 mg/kg level as compared to 100 and 400 mg/kg. The level of oxidative stress was also decreased at 200 mg/kg. The antihyperglycemic effect of *S. arbicola* was may be due to the presence of saponin and terpenoids and may also be due its antioxidant property. Our research indicates that *S. arboricola's* ethanolic extract may effectively restore blood glucose as well as lipid profile in diabetic wistar rats.

Keywords: Alloxan, Anti-hyperglycaemic activity, Diabetes, Ethanolic extract, *Schefflera arboricola*

INTRODUCTION

Diabetes mellitus refers to a form of hyperglycemia,[1] that may be brought on by insulin resistance or an inadequate synthesis of insulin . Resistance of insulin and its inadequate production from ß-cells of the pancreas are the major causes for the development of type 2 diabetes mellitus (T2D)[2], often known as adult-onset diabetes. Risk factors for diabetes include being obese or overweight, being physically sedentary, having a family history of the disease, and having high cholesterol or high blood pressure[3].

It is one of the most significant public health issues becoming a global pandemic[4]. The chronic metabolic condition, affecting around 150 million individuals worldwide, will expand to 300 million by 2025[5]. India has surpassed 20 million diabetics, projected to reach 57 million by 2025, making it the world's diabetes capital[6].

Changes in the molecules involved in insulin signalling may affect insulin resistance in people with T2D[7]. Recent research has shown that a specific intracellular signalling system triggers glucose absorption and translocation of the glucose transporter 4 (GLUT4). Another mechanism is triggered by exercise or muscular contraction through the activation of AMPK[8]. The two routes cause members of the MAPK family to become phosphorylated and active, with p38 MAPK being particularly important for the full activation of GLUT4[9]. Diabetes patients have been shown to have deficiencies in the insulin signal transduction pathway, which are also associated with low levels of PI3K and IRS-1. It has also been shown that PI3K inhibition blocks the absorption of glucose-induced by insulin. Also, since skeletal muscle is where glucose and fatty acid intake occurs most often, insulin resistance related to T2D is typically seen there. AMPK inhibition, brought on by glycolipotoxicity, results in several metabolic problems[10].

Synthetic oral anti-diabetic medications and insulin used to address diabetic complications have several adverse effects and do not manage the issues themselves[11]. Traditional medicinal herbs are utilized to treat various diabetic problems . Older traditional literature has detailed several herbal medications and minerals for treating diabetes mellitus. Compared to synthetic pharmaceuticals, herbal medications are safe and have fewer adverse effects . To provide humanity with a safer alternative to synthetic medications, it is crucial to investigate the hypoglycemic potential of medicinal plants. The primary objective of the current study was to investigate the antihyperglycemic potential of ethanolic extract of leaves of *Schefflera arboricola* in alloxan-induced diabetic rats.

MATERIAL AND METHODS

Identification, authentication and Collection of Plant material: *S. arboricola* leaves were procured from nearby Meerut's region, Uttar Pradesh, India. By comparing the plant leaves to legitimate herbarium specimens and to the literature that is currently accessible, the leaves were recognized and verified [12,13] by Department of Botany, Chaudhary Charan Singh University, Meerut.

Preparation of ethanol extract: Plant leaves were cleaned and dried at room temperature in shade, crushed by hands and sieved using a 40 no. mesh sieve. The coarse leaf powder was kept in airtight container untill usage**.** This coarse leaf powder was subjected to defatting procedure by using petroleum ether. 1 kilogram of coarse leaf material was extracted using ethanol (at 50 °C) in a soxhlet apparatus. The extraction was continued until a colorless liquid emerged. A rotary flask evaporator was then used to concentrate the extract[14]. The extract's yield as a percentage was determined using air-dried powder as the standard. The percentage yield of *S. arboricola* was 2.4%.

Phytochemical analysis: *S. arboricola's* ethanolic extract was subjected to a preliminary qualitative phytochemical screening to identify the plant's phytoconstituents[15].

Animals: Six male Wistar rats (200–250 g) were kept per cage under conventional laboratory conditions (21°C, natural light/dark cycle, 12 hours day/night) Water and food were readily accessible. One week before the commencement of the experiment, animals were given a standard diet[16] and water ad-libitum.

Acute oral toxicity: The OECD guideline 425, stepdown method was followed. The ethanolic extract of leaves of *S. arboricola* (2,000 mg/kg) was administered to 3 animals. Initially the animals were observed for 24 hours

for any sign of toxicity and then continuously observed for 14 days for any sign of toxicity and mortality. No sign of toxicity and mortality was found till 14 days.[17] Therefor 1/10 of 2000 mg/kg i.e. 200 mg dose was considered as safe dose.

Induction of diabetes: Alloxan monohydrate, dissolved in 0.9% w/v cold normal saline, was injected intra-peritoneally into overnight-fasted Wistar rats to induce diabetes (12 hours later). 20% glucose solution was added in their water bottle to avoid severe hypoglycemia[18]. Following 72 hours after alloxan administration, fasting blood glucose levels were checked, and animals with 200 mg/dl or above were considered as diabetic [19].

Experimental design: Total 36 rats were equally divided into 6 groups:

i) **Group 1 (Normal control group):** Normal animals were treated with vehicle (1 ml/100 g body weight) through the oral route.
ii) **Group 2 (Diabetic control group):** Diabetes-induced animals were treated with vehicle (1 ml/100g body weight) through the oral route.
iii) **Group 3 (Standard group-Standard drug):** Diabetes-induced animals were treated with the standard drug Glibenclamide (10 mg/Kg b.w) through the oral route.
iv) **Group 4 (Test group Low Dose):** Diabetes-induced animals were treated with ethanolic extract (100mg/Kg b.w) through the oral route.
v) **Group 5 (Test group Intermediate Dose):** Diabetes-induced animals were treated with ethanolic extract (200mg/Kg b.w) through the oral route.
vi) **Group 6 (Test group High Dose):** Diabetes-induced animals were treated with ethanolic extract (400mg/Kg b.w) through the oral route.

Pharmacological screening: Body weight and Blood glucose levels was measured every week. Lipid profile and antioxidant parameters were measured on the last day (28th day) by collecting the blood samples from Retro orbital plexus. Animals were sacrificed on day 28 for histopathology.

Statistical analysis: GraphPad Prism version 5 was used to do a statistical analysis on all the data. The data is represented as Mean±SEM. One-way analysis of variance (ANOVA) and a post-hoc test were used to examine hypotheses. Statistical significance of the test performed were considered at the $p< 0.05$.

RESULTS

The leaf of *S. arboricola* contains terpenoids and saponines according to a preliminary phytochemical examination. In diabetic rat, this extract has also been shown to repair damaged β-cells.

Body Weight: Body weight of diabetic control animals were significantly reduced. Group 6 (*Test group high dose*) showed the most significant results in comparison with the diabetic control group as shown in table 1.

Table 1: Effect of ethanolic extract of leaves of *S. arboricola* on body weight

Groups→ Time↓	Normal group	Diabetic control group	Diabetic group (Standard drug)	Test group (low dose)	Test group (intermediate dose)	Test group (high dose)
0 day	222.55 ±3.5	224.31± 6.7	217.16 ±9.3	227.68 ± 4	231.26± 8.8	221.17±2.1

7 days	224 ± 5.3	215.35±7.9	219.68 ± 8.5	228.49± 7.5	233.38± 3.3	224.15 ± 8.7
14 days	228.83 ± 6	210.57 ±4.2	223.55 ±8.4	229.32±9.4	236.28± 5.5	229.32±7.1
21 days	230.17 ±7.4	206.31±3.3	226.68±7.7	230.39±8.6	238.15±6	233.54±4.7
28 days	231.17 ±7.8	202.39 ± 4	228.79±8.8	231.11±8.2	240.84±6.9*	236.24±6.7*

n=6, data is represented as mean±SEM, *p<0.05

Blood glucose level: Diabetic control group animals had elevated plasma glucose levels compared to group 1 (*normal control group*). A significant fall in Plasma Glucose level was evident in all treated animals (151.74±13.8, 132.35±10.7, 120.35±13.8 and 112.31±11.0 mg/dl) in group 3 (*diabetic group standard drug*), group 4 (*test group low dose*), group 5 (*test group Intermediate dose*) and group 6 (*test group high dose*) respectively, as compared to group 2 (*diabetic control group*) (269.18±18.6 mg/dl) at 28 days of treatment as shown in table 1.

Table 2: Effect of *S. arboricola* extract on blood glucose levels.

Groups→ Time↓	**Normal group**	**Diabetic control group**	**Diabetic group (Standarddrug)**	**Test group (low dose)**	**Test group (intermediate dose)**	**Test group (high dose)**
0 Day	78.67 ±11.3	280.48±35.4	237.64±21.0	225.98±26.6	245.38±15.6	243.83±34.79
7 Day	79.83 ±10.1	276.0±27.4	177.78±17.2***	217.63±22.4*	228.35±10.9**	223.12±18.4***
14 Days	77.0 ±13.0	274.20±25.3	144.54±14.9***	182.41±14.8**	183.51±13.0**	175.58±14.6**
21 Days	75.67 ±10.5	269.54±23.3	123.89±13.9***	162.37±10.7**	152.35±16.4**	143.28±16.7***
28 Days	75.33 ±14.5	269.18±18.6	112.31±11.0***	151.74±13.8**	132.35±10.7**	120.35±13.8**

n=6, data is represented as mean±SEM, *p<0.05, **p<0.01, ***p<0.001

Biochemical estimation: Group 5 (*test group Intermediate dose*) and Group 6 (*test group high dose*) showed a significant decrease in total cholesterol, triglyceride and LDL level, with increased HDL levels compared to Group 2 (*diabetic control group*) in Table 3.

Table 3: Effect of *S. arboricola* extarct treatment on lipid levels of diabetic rats.

Groups → Parameters ↓	**Normal group**	**Diabetic control group**	**Diabetic group (Standard drug)**	**Test group (low dose)**	**Test group (intermediate dose)**	**Test group (high dose)**
TC (mg/dl)	43.73±7.97	123.98±7.99	47.85±7.18***	95.37±7*	68.32±8.9**	55.12±8.74**
TG (mg/dl)	88.37±4.14	193.51±3.22	78.34±4.41***	111.61±4.75*	82.35±3.13**	72.11±4.37**
LDL(mg/dl)	58.23±1.05	134.21±1.33	63.58±1.29***	86.39 ±1.02*	74.39±1.14**	68.58±1.69**
HDL(mg/dl)	42.61±3.18	28.91±3.73	44.58±3.45*	27.73±3.94	35.92±4.57	39.54±4.4

n=6, data is represented as mean±SEM, *p<0.05, **p<0.01, ***p<0.001

Fig. 1: a) Total cholesterol b) Triglyecride c) Low density lipoproteins d) high density lipoproteins levels

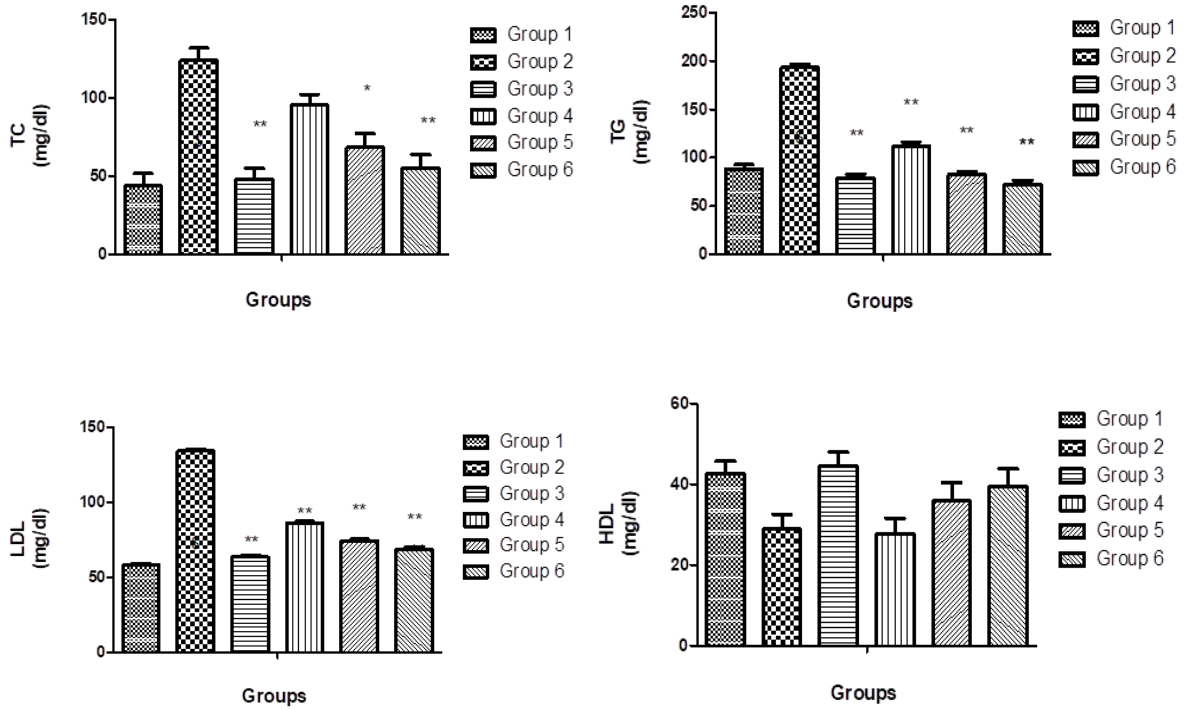


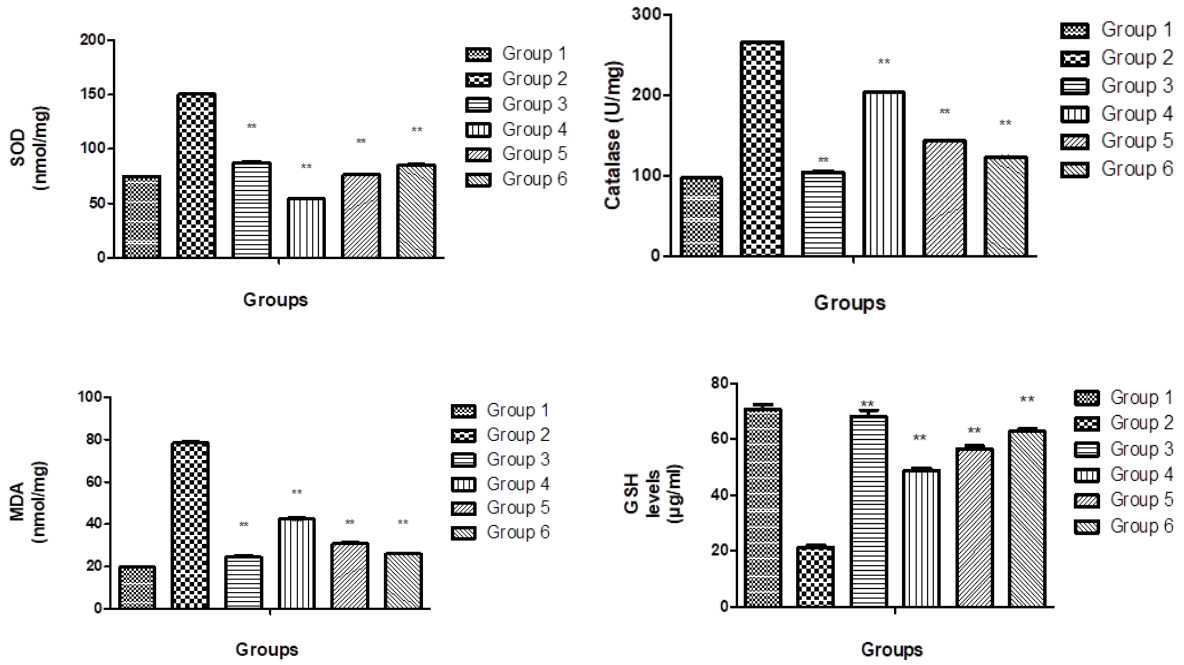
Table 4: Effect of ethanolic extract of leaves of *S. arboricola* on oxidative stress parameters

Parameters → **Groups ↓**	**SOD (nmol/mg)**	**MDA (nmol/mg)**	**Catalase (U/mg)**	**GSH (µg/ml)**
1. (*Normal group*)	74.81±0.30	19.63±0.30	96.75±0.01	70.73±1.54
2. (*Diabetic control group*)	150.37±0.56	78.39±0.56	266.31±0.03	21.33±0.76
3. (*Diabetic group Standard drug*)	87.31±0.62**	24.37±0.62**	104.73±0.09**	68.16±2.20**
4. (*Test group low dose*)	54.33±0.48**	42.41±0.48**	203.32±0.04**	48.67±1.04**
5. (*Test group intermediate dose*)	76.18±0.58**	30.73±0.58**	143.68±0.09**	56.48±1.10**
6. (*Test group high dose*)	85.31±0.37**	25.98±0.37**	122.64±0.05**	62.68±0.86**

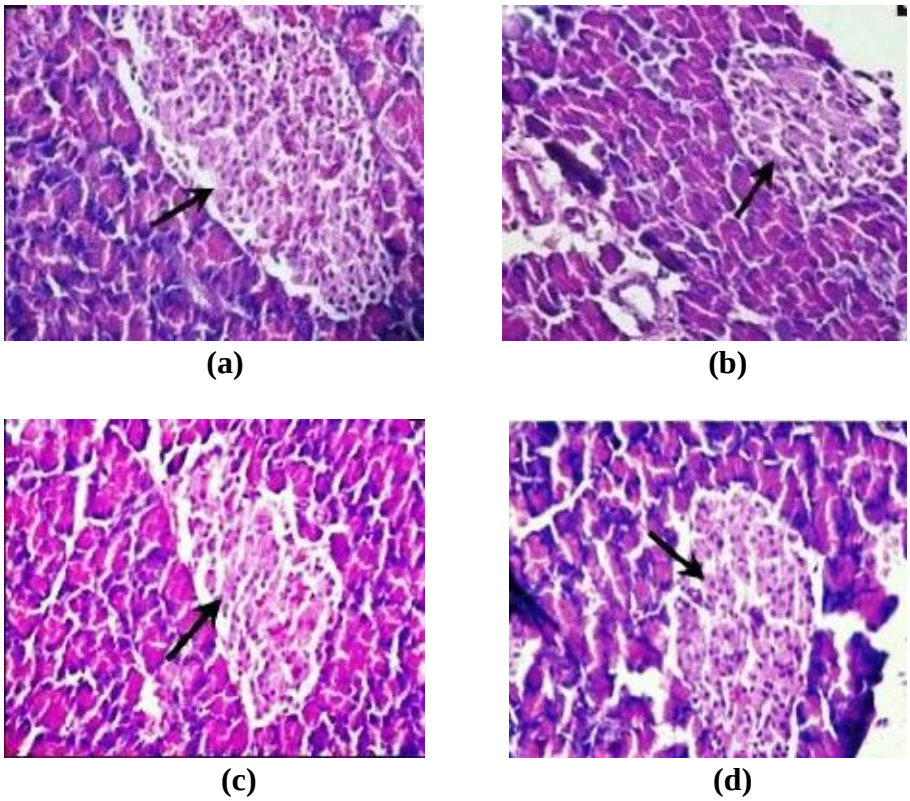
n=6, data is represented as mean±SEM, *p<0.05, **p<0.01, ***p<0.001

Diabetic control group animals had a significantly elevated level of MDA, catalaze and SOD while decreased levels of glutathione. Treatment with plant extracts significantly reduced lipid peroxidation and increased glutathione and reduces catalase levels, as presented in figure2a, 2b, 2c and 2d. The effect of extracts on the antioxidant parameters of different groups is depicted in figure 2.

Fig. 2: a) SOD levels b) MDA levels c) catalase level d) GSH levels in all groups.

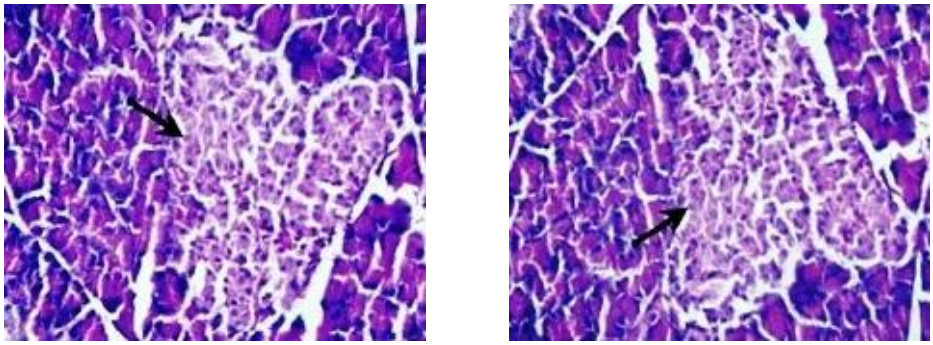


Histopathological examination: Histopathology of pancreas was done after twenty eight days of treatment with different concentrations of ethanolic extract of *S. arboricola* leaves at 100, 200, and 400 mg/kg doses. The tissue slices were stained, images were taken, and the findings from this stage were examined fig. 3.



(a) **(b)**

(c) **(d)**



(e) **(f)**

fig. 3: Histopathology of pancreas from (a) Normal group (b) Diabetic control group (c) Diabetic rat treated with standard drug. (d) Diabetic rats treated with 100 mg/kg extract. (e) Diabetic rats treated with 200 mg/kg extract. (f) Diabetic rat treated with 400 mg/kg extract.

DISCUSSION

Diabetes mellitus is a long term ailment that primarily affect glucose metabolism[20]. These changes are brought on by long term insulin deficiency or some sort of acquired resistance against the action of insulin. Together these factors causes an abnormal glucose homeostasis [21, 22]. Diabetes causes vast numbers of quietus globally[23]. It was estimated by WHO, that in 2030 the number of diabetics will be around 18 millions across the world[24]. Long term fluctuation in plasma glucose concentration causes numerous complications like retinopathy, neuropathy and cardiomyopathy [25]. At present pharmacotherapy can control hyperglycemia, but no medication persist which can cure diabetes[26].

Plants have been the primary source of medication due to the relatively lesser side effects[27] and availability at lower cost[28]. Manny herbal preparations known as rasayana have been utilized in Indian traditional health care system against DM, since hundreds of years[29]. Several herbal preparations are under study to counteract multifactorial diseases. Hence the current study focused on evaluation of glucose reducing potential of *S. arboricola.*

Alloxan monohydrate causes cessation of pancreatic β- cells and a decrement, in the amount of insulin secreted by them[30]. Which is responsible of altered glucose homeostasis. When alcoholic extract of *S. arboricola* was given to such animal, it regenerate β – cells of pancreatic islets and improve the production of insulin.

Extract obtained from the leaves of this plant reduces plasma glucose level, when administered to the alloxan induced diabetic rats. This may be due to the presence of terpenoids[31,32]. The Terpenoids discern from vegetation, express antitumour, antimicrobial and antidiabetic potential[33]. A diterpenoids, shows insulino-mimetic activity in diabetic animals.[34]

Severe hyperlipidemia was also observed in alloxan induced diabetic rats which may be due to expansion of cholesterol pool[35]. *S arboricola* extract also shows anti-hypercholesteromic effect but the mechanism of this action is not clear.

Over production of ROS results, an escalation in oxidative stress, which shrink antioxidant defence system[36]. This phenomenon is commonly encountered in diabetes mellitus. Administration of plant extract (*S. arboricola*) at different doses have a restorative effect on different antioxidant parameters[37,38].

CONCLUSION

Due to its traditional uses, *S. arboricola* leaves were chosen for the current anti-diabetic studies. The Wistar albino rats were chosen for assessing the effect of ethanolic extract against alloxan-induced diabetes. At varying concentrations of *S. arboricola* ethanolic extract, it showed marked anti-diabetic, anti-cholesteromic and antioxidant restorative efficacy. According to our research, the herbal plant extract of *S. arboricola* has anti-hypoglycemic properties that may be useful in halting the course of diabetes. Further studies are required to determine the precise underlying mechanism of action, especially the underlying biomolecules of the plant extract responsible for the noted pharmacologic actions.

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