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Original Article

In vivo Investigation to Validate the Traditional Usage of *Sorbaria tomentosa* (Lindley) Reh. Root Extract against Alloxan-induced Diabetic Rats

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ABSTRACT

Background: DM is a complicated condition that impacts the handling of sugar, fat, and protein metabolism in our bodies. Medicinal herbs play a significant role in diabetes care. As a result, we focused on the plant components traditionally employed by regional medical professionals. **Objectives:** The current research was carried out to check if *Sorbaria tomentosa* (Lindley) Reh. (ST) the ethanolic root extracts anti-diabetic potential. ST is a member of the Rosaceae family commonly known by the common name "false spirae." The root extract of ST is used by the locals in the Kinnaur district of Himachal Pradesh to effectively treat diabetes. **Materials and Methods:** Plant ethanolic extracts were evaluated for their potential on alloxan-induced diabetic rats for 14 days. The animals were put into one of four treatment groups at random: a normal control group, not treated diabetic control group (150 mg/kg bw), a drug control group that got glibenclamide (5 mg/kg bw), and the last treatment group medicated with ST root extract (50 mg/kg BW). Liver, pancreas, and kidney histopathology were analyzed to correlate biochemical findings with histological changes. **Results:** Our results showed that the extract and medication-treated diabetic groups gained statistically significant weight ($p \leq 0.001$) and that their BGL went back to normal. Along with more common biological markers such as SGOT, SGPT, ALP, TC, TG, HDL, LDL, VLDL, creatinine level, uric acid, urea, and uric acid were measured. After the extract treated hyperglycaemic rats, all biochemical markers came under the normal range ($p \leq 0.001$). Improvement of the damaged structure of the liver, pancreas, and kidneys was observed after treatment with standard medication and root extracts of ST. **Conclusion:** Based on the data gathered from the tribal research area, to the best of our capacity, we have uncovered for the very first time the anti-diabetic efficacy of the plant parts. The empirical knowledge of previous generations is typically the basis for the traditional utilization of medicinal plants for controlling diabetes. Nevertheless, *in vivo*, research has assisted to discover the anti-diabetic potential of ST's ethanolic root extract and provides scientific proof to back up these claims. The plant component may also be used to create natural antidiabetic drugs that are effective.

Keywords: Diabetes mellitus, *Sorbaria tomentosa*, Traditional knowledge, Alloxan, Wistar rats, Kinnaur.

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INTRODUCTION

Since that DM is a long-term metabolic condition, it has a considerable effect on physical wellness, the standard of living, and patients' life expectancy as well as the healthcare structure. The major characteristic of DM is the inability to control blood glucose levels as a result of dysfunction in the metabolism of carbohydrates, fats, and proteins coupled with inefficient insulin release or activity.[1]

The pervasiveness of type 2 diabetes is increasing globally due to sedentary lifestyles, inactivity, poor diet, junk food consumption, and environmental contaminants. Managing long-term diabetic consequences such as nephropathy, retinopathy, and vascular disorders is challenging. According to previous statistics reports, by 2040, there will be 622 million more people with diabetes in comparison to current reports i.e., 422 million.[2] Maintaining BGL near normal will aid in the prevention, delay, or lessening of these complications.

The utilization of medicinal plants as a natural diabetic treatment has received a lot of interest in recent years. Numerous ancient healthcare systems, including Ayurveda, Unani, and Traditional Chinese Medicine, have depended on medicinal plants to manage diabetes. It is undeniable that plants are used in healthcare by more than eighty percent of the global population. Currently, there is

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interest in finding natural alternatives to synthetic molecules that can be used in diet or medications.[3] The primary justification for such approval is that medicinal plants are non-toxic, have fewer side effects than synthetic medications, and have a long history of safety testing.

ST is commonly referred to as false spirae of the Rosaceae family. It is a sizable woody shrub with creamy white blooms that grow in the temperate Himalayas, eastern Afghanistan, and Central Asia highlands (1400-3800m) on rocky soil, along streams and rivers. The sole Sorbaria species reported in India is tomentosa. The Plant parts were collected from several regions in the Kinnaur area. Kinnaur is a district in Himachal Pradesh's alpine and sub-alpine Himalaya, tribal communities, sometimes known as the "Kinner Community," prevail. Because of the scarcity of medical facilities in these areas, as well as the difficulty in obtaining them, the communities rely only on traditional herbal treatments to cure many ailments and health conditions that exist there. They have been using these plant roots for a very long time. Locally called as Kusht, ST is also said to have hypoglycaemic properties, and it is said that taking a decoction or infusion made from fresh or shade-dried roots was taken orally twice a day to treat diabetes. It is considered that traditional knowledge is produced through many years of observation and via the trial-and-error technique. According to the information gathered, they initially employed the leaf section for the treatment but didn't see any real improvements. Then they conducted an experiment using a root decoction to treat diabetes, with amazing results. The root portions are still the essential component for effective treatments for diabetes. Therefore, the current plant species were chosen based on their regional availability and historical use as folk remedies for diabetes. The current study looked at the hypoglycaemic activity of ethanolic root extracts of ST on alloxan-induced diabetic rat models. To determine the antidiabetic potential, changes in body weight and blood glucose levels, lipid profile (TC, TG, HDL, LDL, VLDL), liver parameters (SGOT, SGPT, ALP), and renal parameters (urea, uric acid, and creatinine level) were evaluated. The liver, pancreas, and kidneys were also histopathologically examined.

MATERIALS AND METHODS

Plant material

Roots of ST were gathered in June of 2018 during their blooming season in the village of Mebar, District Kinnaur, Himachal Pradesh (India), at an elevation of 30,390 m above sea level. Herbarium of Botanical Survey of India, Northwest Circle, Dehradun was consulted in order to authenticate the collected plant materials and use diagnostic keys and floras to identify them (BSI) and submitted under the accession number 149.

Plant-extract preparation

The ST roots were collected, cleaned with running water, and then dried in the shade for few days. At first, secateurs were used to chop the dried roots into manageable chunks. Afterward, they spent another 7-8 hr drying in the oven before being pounded into a fine powder in grinding equipment. The fine material weighed 30 grams, and the extraction process took 24 hr in a shaker containing 60 mL of 90% ethanol. To remove impurities, we filtered the extract through Whatman filter paper before evaporating the concentrate at ambient temperature. We extracted the plant material from the leftover material and preserved it at 4°C for later use.

Chemicals Used

All chemicals, including Sigma-alloxan Aldrich's monohydrate, GK enterprises' (Chandigarh, India) glucose solution, sodium chloride, and chloroform, and calculated by private limited diagnostics' (Singapore) commercial kits (for lipid, renal, and liver parameters), were procured.

Animal testing

Twenty-four male Wistar rats considering body weight between 230 and 280 g were employed in this study, with six rats in each of the four groups. All trial protocols were authorized by the University's Central Animal Ethics Committee. The rats were kept in sanitary polypropylene cages at standard conditions (12 hr of light/dark cycle, 23±2°C).

Assay for toxicity

Following OECD recommendation 420, an acute oral toxicity test was performed. Extracts were given orally via gavage feeding at varying doses of 5, 50, 300, and 2,000 mg/Kg. An elevated mortality rate, anxiety, hostility, convulsions, diarrhea, touch sensitivity, and disturbed sleep were all noted in the subsequent 72 hr.

Diabetes induction

A single dosage of 150 mg/Kg Alloxan dissolved in 154 mM saline was administered intraperitoneally. Those with hypoglycemic shock responded positively to 24 hr of having 5% glucose added to their drinking water. After 72 hr of therapy, a Code free glucometer placed into the tail vein of the rats revealed diabetic rats with blood sugar levels > 200 mg/dL.

Experimental layout

During the course of two weeks, the rats were segregated into four groups of six and given various treatments. The medicine and plant extract in ethanol were administered via oral gavage. The experimental study was conducted using standard methods, with only minor adjustments.[4] The rats were divided in four different treatment groups:

1) Normal control group.

2) Diabetic control group.

3) Diabetic rats given glibenclamide (5 mg/kg of body weight).

4) Diabetic rats were given an ethanolic root extract of ST (50mg/kg BW).

Blood sugar and body weight were checked on the rats on days 1, 7, and 14. On the final day of study, the rats were killed via dislocation of cervical spine after a 12 hr fast. Blood samples are being collected in Eppendorf tube for biochemical assays. Histopathology was performed on the removed organs. Histopathology was performed on the kidneys, liver, and pancreas.

Biochemical testing

Blood was centrifuged at 4°C and 4000 revolutions every minute for 10 min to isolate the serum. Reckon private limited diagnostic's commercial kits were used to detect lipid profile, liver, and renal parameters.

Histopathological examinations

Following the sacrificial procedure, the kidneys, liver, and pancreas were removed. Histopathological analysis typically involves dehydrating and blocking a material before cutting it into 5 μM sections, haematoxylin, and eosin using to stain and examining them under an electron microscope.

Statistical Examination

The statistical analysis was performed in SPSS16. The standard error of the mean of 6 replicas is applied. The graph was made using Origin Pro 2023 software. The outcomes were assessed by applying one-way analysis of variance and the Turkey post hoc test to figure out the least significant difference. The result is expected significant if the probability value is lower than 0.05.

RESULTS

Toxicity test results

In order to determine whether or not the plant extract was hazardous, toxicity experiments were conducted using rats given doses of up to 2,000 mg/kg BW of the extract, but none of the animals displayed any abnormal behavior. The toxicity of plant extracts was not observed up to a concentration of 2,000 mg/kg, and there were no signs detected, aberrations from the normal behavior of the animals, fatalities, or other undesirable consequences. It was decided to use the plant as the subject of this inquiry.

Effects of a plant extract on change in body weight

The diabetic rats in the second group (alloxan-induced150 mg/kg) showed a reduction of body weight (group 2). Significant weight gain ($p \leq 0.001$) was seen in both group 3 (glibenclamide 5 mg/Kg) and group 4 (ST ethanolic root extracts 50 mg/kg) in contrast to the diabetic control group. The result is displayed in Table 1.

Effect of a plant extract on change in BGL

Increased BGL was seen in group 4 after fourteen days testing with an ethanolic root extract of ST (blood glucose level). While gp 2's BGL remained rather stable at around 596-595 mg/kg, group 4's (the extract-treated group) BGL decreased significantly ($p \leq 0.001$) from the first day (384.6 mg/dL) to the 14th day (98.5 mg/dL). In group 1 (normal control), BGL remained consistent (115-114 mg/dL), however in group 3 (Glibenclamide treatment), BGL decreased from 587 to 132 mg/dL. The outcomes demonstrated that both the treated and untreated groups saw measurable shifts in BGL. The ST ethanolic root extract (50 mg/Kg) or with standard medication Glibenclamide (5mg/Kg) had significantly ($p \leq 0.001$) lowered BGL in comparison with group 2. The difference in change of BGL in different treatment groups is shown in Table 2.

Effect on lipid profile

An investigation was conducted into an alloxan-induced diabetic group that received no treatment (group 2) and found that there was an escalation in TC and LDL, while the level of good cholesterol, also known as HDL, fell. While in the case of the normal control (group 1), diabetic rats treated with the standard drug glibenclamide (group 3), and diabetic rats treated with ethanolic root extracts of ST (group 4), there was a significant lowering ($p \leq 0.001$) in TC, LDL, and TG after 14 days of treatment, along with a significant improvement in HDL levels, as shown in Figure 1.

Table 1: Effect of root extract of *Sorbaria tomentosa* on the body weight of diabetic rats (*n*=6).

Treatment groups	Body weight (g)			% change in body weight
	Initial day	7th day	14th day	
Normal control	234 ± 2.00^{a}	240 ± 3.71	265 ± 3.54^{a}	-22.23%
Diabetic control (150 mg/kg alloxan)	276 ±2.10^{x}	248 ±2.13	226 ± 2.53^{x}	13.38%
Alloxan + SD glibenclamide (5mg/kg)	273 ± 4.94^{x}	289 ± 3^{x,}	304±3.83^{x,a}	11.15%
Alloxan + roots extract of ST (50 mg/kg)	231± 3.07^{y,a}	242 ± 2.5^{y,a}	257±4.03^{y}	9.957%

All the values presented as SEM, *n*=6.x= $p \leq 0.001$ when compared with group 1, the normal control group, y= $p \leq 0.05$ (When compared with group 1).a = $p \leq 0.001$ when compared with group 2, the diabetic control group.

Table 2: Effect of root extract of *Sorbaria tomentosa* on the blood glucose level on diabetic rats (*n*=6).

Treatment groups	Blood glucose level mg/dL			% change in blood glucose level
	Initial day	7th day	14th day	
Normal control	115.2 ± 2.03[a]	113.7 ± 2.64[a]	144.7 ± 1.40[a]	4.85
Diabetic control (150 mg/kg alloxan)	596.3 ± 2.40[x]	591.83 ± 3.43[x]	595.3 ± 3.12[x]	1.75
Alloxan + SD glibenclamide (5 mg/kg of bw)	587 ± 3.92[a]	273.3 ± 3.04[x,a]	132 ± 3.11[a]	-181.6
Alloxan + roots extract of *Sorbaria tomentosa* (50 mg/kg of bw)	384.6 ± 1.89[y,a]	204 ± 3.42[a]	98.5 ± 4.77[a]	-59.53

All the values presented as SEM, *n*=6x= $p \leq 0.001$ when compared with group 1, the normal control group, y= $p \leq 0.05$ (When compared with group 1). a = $p \leq 0.001$ when compared with group 2, the diabetic control group.

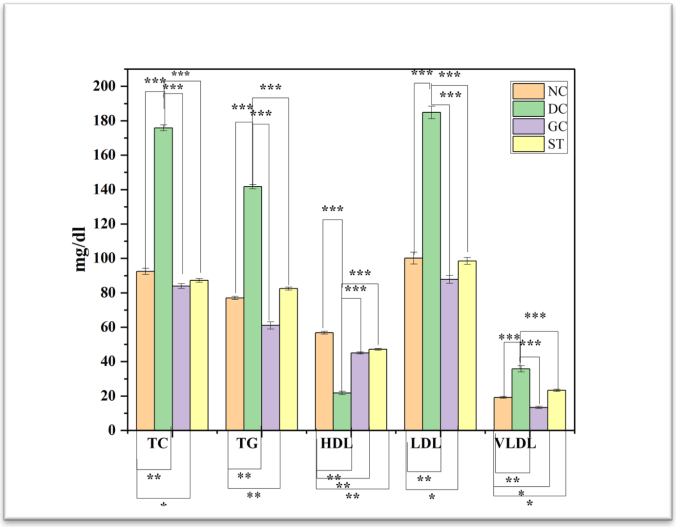

Figure 1: Demonstrate evaluation of lipid profile level on alloxan induced diabetic rats treated with ethanolic extract of ST.

All the values presented as SEM, *n*=6. ** = $p \leq 0.001$ when compared with normal control group; * = $p \leq 0.05$ (when compared with normal control group (group 1); *** = $p \leq 0.001$ when compared with the diabetic control group (group 2).

Effects on the liver's ability to function

The beneficial effects of ST ethanolic root extracts on liver functions are shown graphically in the displayed Figure 2. The SGOT and SGPT, as well as ALP levels, were significantly elevated in the untreated diabetic rats (group 2). The levels of liver marker enzymes were found to be within the normal range in the normal control group. however, the liver parameters were found to be significantly decreased ($p \leq 0.001$) in groups that had received treatment with the common medication glibenclamide (group 3) and ethanolic root extracts of ST (group 4).

Effect on Kidney Functions

When compared to normal rats, the levels of renal function markers like urea, uric acid, and creatinine in alloxan-induced diabetic rats (group 2) were significantly elevated ($p \leq 0.001$) in comparison to the normal control group (group 1). After 14 days of treatment, diabetic rats given the standard medication glibenclamide as well as ethanolic root extracts of (group 3, group 4) displayed a significant restoration ($p \leq 0.001$) to the normal range of renal marker enzymes. A comprehensive description of the studied data is provided in Figure 3.

Histology of pancreas

In the normal control group, the histology of the pancreas showed that the acini cells and the location of the islets of Langerhans were normal (group 1). Abnormalities such as distortion of the islet of Langerhans and lymphocyte infiltration were observed in the diabetic control group (group 2). Whereas groups treated with the standard drug glibenclamide and with ethanolic root extracts of ST (group 3, group 4) showed normal acinar cells and restoration of the normal shape of the islet of Langerhans as shown in Figure 4.

Histology of liver

Examining a liver segment from a normal control group (group 1) reveals a liver with a typical histological appearance. Hepatic parenchyma and portal vein inflammation was observed in the diabetic control group (group 2). Figure 5 shows that after treatment with ST root extracts and the standard medication, both groups (group 3 and group 4) returned to their original, healthy shapes.

Histology of kidney

Section of the kidney from the normal control group (group1) was examined histologically, and they showed no signs of inflammation in the glomerulus, which was encompassed by Bowman's capsule, or in the proximal and distal convoluted tubules. Diabetic rats given the standard medication and ethanolic root extract did not exhibit any of the detrimental alterations observed in the groups (group 3 and group 4). Sectional histology of the kidneys from Wistar rats, as shown in Figure 6.

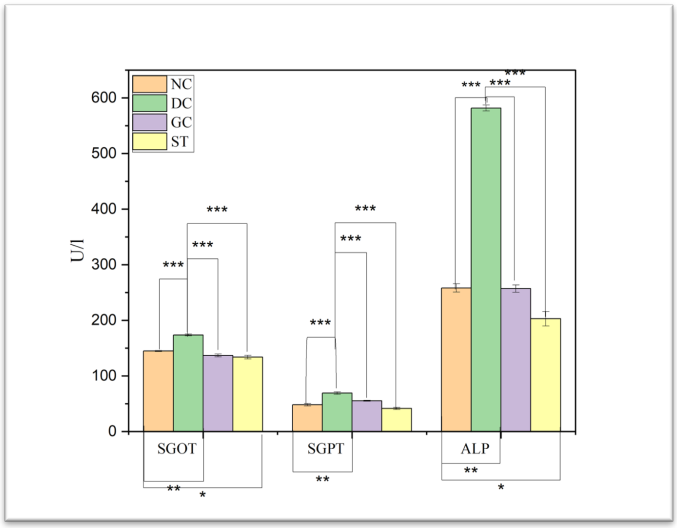

Figure 2: Presents estimation of liver parameters on alloxan induced diabetic rats treated with ethanolic extract of ST.

All the values presented as SEM, *n*=6. ** = $p \leq 0.001$ when compared with normal control group; * = $p \leq 0.05$ (when compared with group 1); *** = $p \leq 0.001$ when compared with diabetic control group.

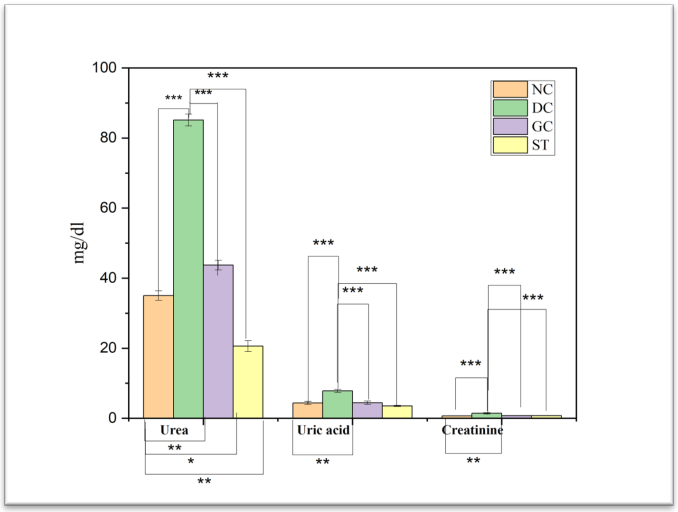

Figure 3: Presents estimation of renal parameters on alloxan induced diabetic rats treated with ethanolic extract of ST.

All the values presented as SEM, *n*=6. ** = $p \leq 0.001$ when compared with normal control group; * = $p \leq 0.01$ (when compared with normal control group); *** = $p \leq 0.001$ when compared with diabetic control group.

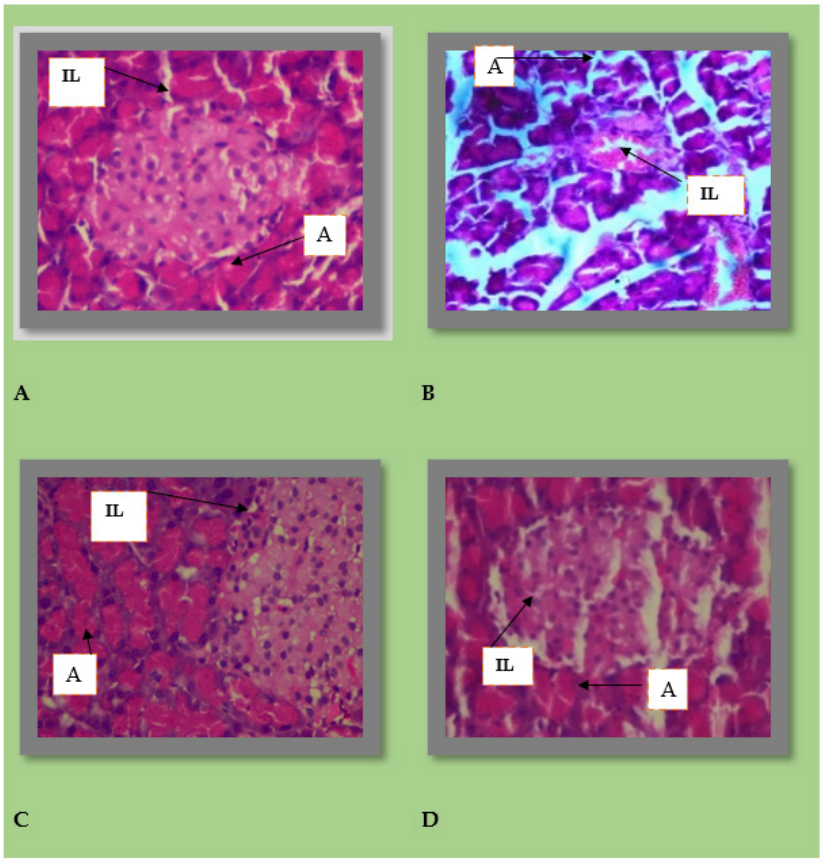

Figure 4: Histological study of the pancreas of alloxan-induced diabetic rats treated with ethanolic extract of ST and standard drug glibenclamide.

A – Acini; IL – Islet of Langerhans.
Histopathological examination of Hematoxylin and eosin-stained pancreas from various treatment groups.
A- In the untreated normal control group, the regular architecture of islets of Langerhans and acinar cells was seen. B- IL rupture and an inflammatory reaction were observed in diabetic control group of untreated groups. C- Diabetes rats treated with glibenclamide (standard treatment) had improved architecture of IL, and the inflammatory regions were also repaired. D- Diabetic rats given an ethanolic root extracts of ST had significantly improved histological architecture compared to a normal control group.

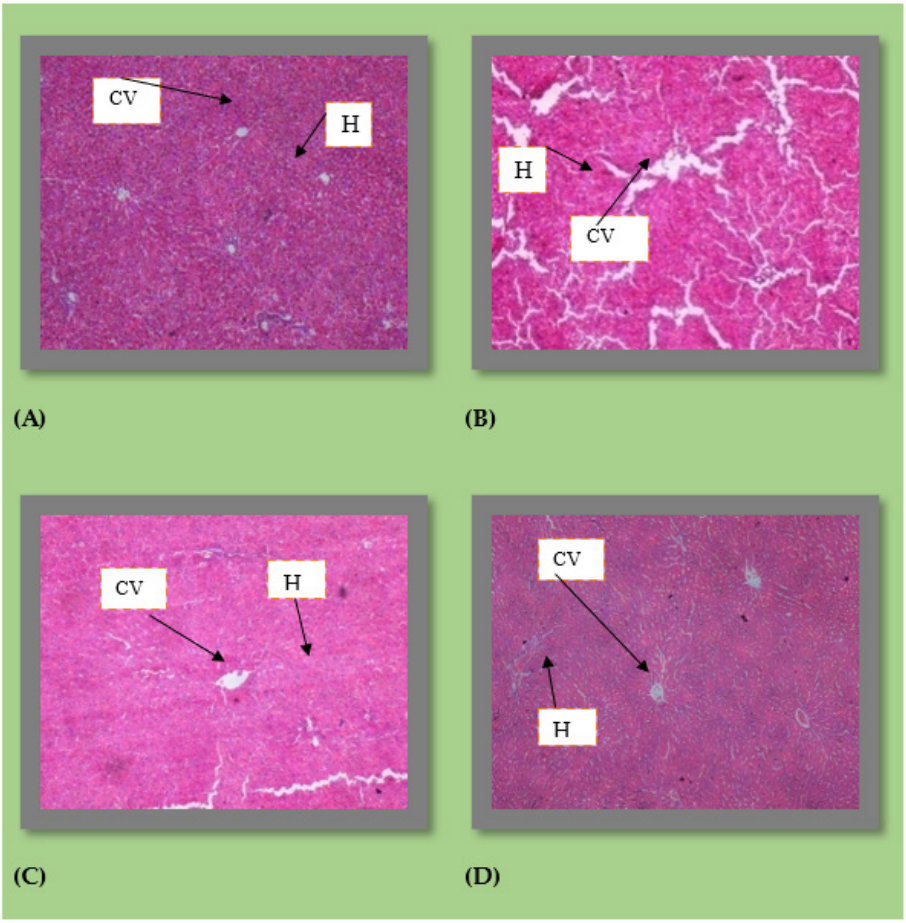

Figure 5: Histological study of the liver of alloxan-induced diabetic rats treated with ethanolic extract of ST and standard drug glibenclamide.

CV – Central Vein; H – Hepatocytes.
Histopathological examination of Hematoxylin and eosin-stained liver tissue from various treatment groups.
A- Normal hepatocytes and central veins were found in an untreated normal control group. B- The untreated alloxan-induced diabetes control group demonstrated hepatocyte and central vein architectural impairment. C – Diabetic rats treated with the reference drug glibenclamide showed improvement in the destructive structure of hepatocytes and central veins. D – The structure of hepatocytes and central veins in diabetic rats treated with ethanolic extract improved dramatically. The extract-treated groups outperform the drug-treated ones.

DISCUSSION

In traditional medicine across the world, medicinal plants have been used for centuries. Increased vigilance of the value of medicinal plants as a significant source for discovering drugs for a wide range of human maladies has been sparked by factors such as a rising population, a lack of traditional drugs, the high expense of treating illness, the unfavorable consequences of numerous orthodox medicines, and the emergence of drug tolerance to some diseases treated with presently available drugs.[5]

People in impoverished nations frequently use medicinal plants as a kind of alternative medicine. Approximately, a thousand plant species have been utilized in India to treat diabetes mellitus traditionally.[6,7] Regrettably, only a handful of these medicinal plants have been scientifically studied.

The current study aims to scientifically confirm a traditionally utilized ST ethanolic root extract on alloxan-induced diabetic male Wistar rats. To the greatest extent of our information, this is the first *in vivo* investigation on the root extract of ST. Therefore, to ascertain the antihyperglycemic properties of root extract of selected plant species the current investigation was thus executed.

By following the protocols from previous research work, the ethanolic root extract of ST (50 mg/Kg body weight) was employed for an anti-diabetic investigation.[4] Alloxan induces diabetes by killing pancreatic beta cells, which leads to hyperglycemia since the wounded pancreas is either entirely or partially unable to release insulin.[8] Plants have therapeutic action owing to the presence of several phytoconstituents. Previous investigations have shown that plant extracts have an anti-hyperglycemic impact by preserving β-cells from degeneration, encouraging their regeneration, and limiting glucose load by encouraging limitless endogenous insulin activity. Plant extracts may potentially have an antihyperglycemic impact by stimulating β-cells to make insulin or by activating insulin receptors to absorb blood sugar and speed up peripheral glucose consumption.[9] The antidiabetic action of ethanolic root extract might be attributed to its capacity

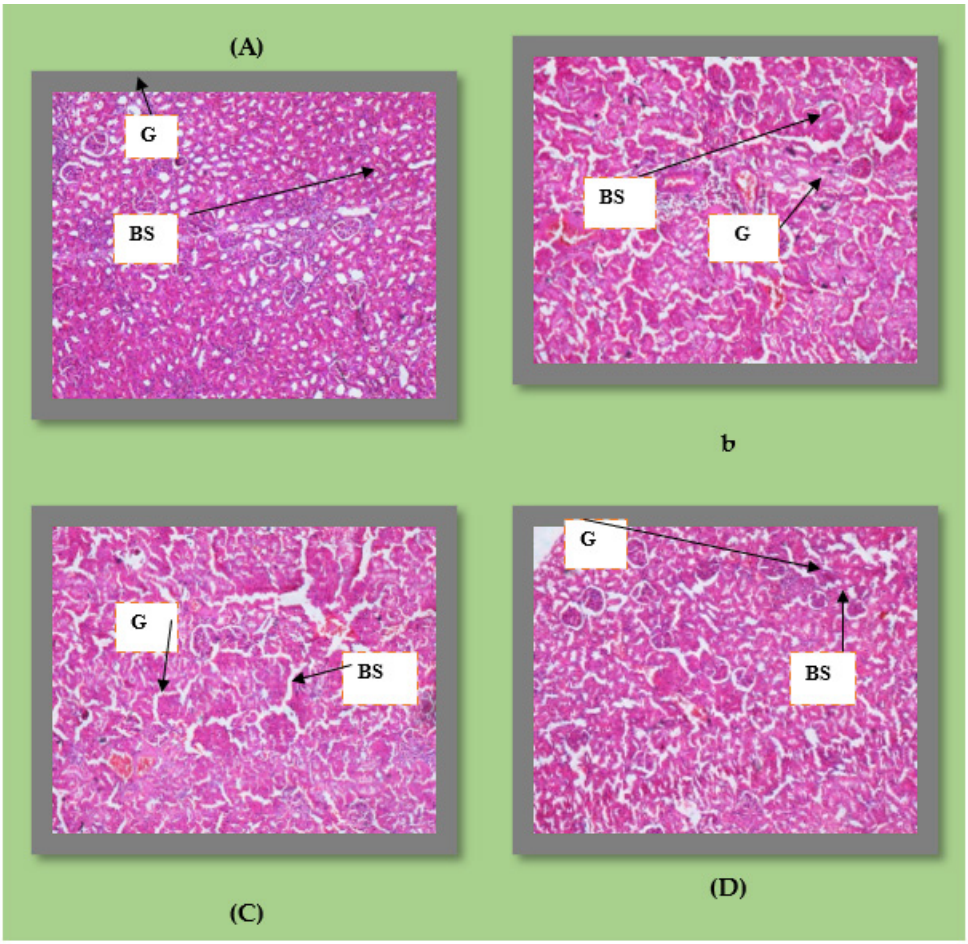

Figure 6: Histological study of the kidneys of alloxan-induced diabetic rats treated with ethanolic extract of ST and standard drug glibenclamide.

G – Glomerulus; BS – Bowman's Space.
Histopathological examination of Hematoxylin and eosin-stained kidney tissue from various treatment groups.
A- Normal control group with glomeruli enclosing in Bowman's space. B- Diabetic control group showing the destructive glomeruli and vasculature. C- Minor improvements were noted in diabetic rats receiving medication, but inflammation persisted. D- Group 4 treated with root extract showed inflammation, but demonstrated superior improvement in histological structure than the drug-treated group.

to release insulin or to its potential to quench reactive oxygen species produced by autooxidation in hyperglycaemic conditions.

ST ethanolic root extract was orally administered to rats in the acute oral toxicity investigation at a dosage of up to 2000 mg/kg BW. The extract was either non-toxic or safe after 14 days since there were no indicators of toxicity, death, or diagnostic abnormal symptoms. The OECD guideline number 420 was followed in conducting the acute oral toxicity test.[10]

Significant ($p \leq 0.05$) increases in bw and decreases in BGL were seen in the treatment groups following dosage of the ethanolic root extract of ST (50 mg/kg of Bw) and the common medication glibenclamide 5 mg/kg of bw) as shown in Tables 1 and 2. When the disease progresses in diabetic rats, their body weight decreases.[11]

According to a previous study, alterations in serum lipids and diabetes may be related. As per previous studies, coronary heart disease is associated with elevated levels of TG, LDL, TC, and HDL level dropped.[12] Similar results were seen in our studies after treatment with root extract and with reference drug, lipid profile levels were found to be normal.

To evaluate the health of the plasma membrane and endoplasmic reticulum, experts frequently employ the liver marker enzyme ALP. Increased activity of these enzymes in the serum is a sign of liver structural integrity loss and is likely due to leakage from changed cell membrane structure.[13] A previous study suggested that high levels of AST, ALT, and ALP in plasma are indications of liver function and high levels of these enzymes are indicative of liver toxicity, hyperglycemia in mice results in liver damage.[14] The presence of hepatic steatosis, liver cholestasis, and fibrosis was not altered throughout testing with *B. vulgaris*, although polymorphonuclear infiltration and glycogen deposition did. They noticed that individuals who received therapy from this plant had lower glucose, SGOT, SGPT, and ALP levels.[15] SGOT, SGPT, and ALP levels significantly ($p \leq 0.01$) decreased to normal levels following therapy with ethanolic root extract of ST as compared to earlier study work.

Proteinuria, an increase in uric acid, and urea nitrogen accumulation all contribute to the development of renal failure with glomerulosclerosis in diabetic nephropathy.[16] However, after treatment with ethanolic root extract of ST (50 mg/kg bw), The levels of urea, uric acid, and creatinine were considerably lowered in diabetic rats (Figure 3). Although histological assessment revealed improvement toward normal histoarchitecture with no evident nephritis symptoms. According to our investigation findings, which align with the studies mentioned above, ST root extract has a therapeutic impact on alloxan-induced diabetic rats. Although allopathic treatment lowered blood glucose levels and improved biological markers, it had a deleterious influence on heart, kidney, liver, and neurological function. The benefit of employing our plant extract is that it has no additional detrimental effects on our bodily function.

CONCLUSION

The study revealed that ST's ethanolic root extract had an outstanding hypoglycaemic and hypolipidemic effect. Ethanolic extract from the roots of the ST has significantly ($p \leq 0.001$) reduced BGL in alloxan-induced diabetic rats while also improving bw, lipid profile, liver parameters, and kidney function. Hence, supporting the efficacy of using this extract in traditional medicine to treat diabetes and some of its consequences. Phytoconstituents are prevalent in plants. Phytochemicals have a high antioxidant capacity and are of significant interest because of their positive influence on human health, and they provide a variety of health advantages. To date, no study has been done on the root component of this plant, analysis of numerous phytochemicals and isolation of significant bioactive compounds will need to be done in the future. As a result, they contribute to the development of natural anti-diabetic drugs. Also, Clinical research is still required to fill in the knowledge gaps and completely understand the therapeutic impact of the medicinal plant.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ST: *Sorbaria tomentosa* (Lindley) Reh; **DM:** Diabetes mellitus; **OECD:** Organisation for Economic Co-operation and Development; **BGL:** Blood glucose level; **bw:** Body weight; **NC:** Normal control; **DC:** Diabetic control; **GC:** Glibenclamide control; **SGOT:** Serum glutamic-oxaloacetic transaminase; **SGPT:** Serum glutamic pyruvsic transaminase; **ALP:** Alkaline phosphatase; **TC:** Total cholesterol; **TG:** Triglycerides; **HDL:** High density lipoprotein, **LDL:** Low density lipoprotein.

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