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Research

Evaluation of anti-diabetic potential of *Sceletium tortuosum* aerial parts against streptozotocin-induced diabetes on wistar rats

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Abstract

` Diabetes Mellitus, the metabolic syndrome where the body either fails to produce or effectively utilize insulin, is associated with chronic morbidity. While a definitive cure for the disease is lacking, with the modern medicine offering mainly the means to control the extent of the disease, Complementary and Alternative Medicine (CAMs) offers additional/alternate means to tackle the disease. On the other hand, the lack of evidenced medical practices is a lacuna in most of the traditional medical applications. *Sceletium tortuosum* (Aizoaceae family), a perennial shrub found in the tropics, has been known for its numerous pharmacological properties and is found as a constituent in many Ayurvedic and Siddha drugs. Any evidence based evaluations have not been conducted on the anti-hyperglycemic effect of the plant. In the current study, the ethanolic extract of *S.tortuosum*, was scientifically assessed for its effect on *In-vitro* α-amylase inhibition assay, *In-vitro* α-glucosidase inhibition assay, In vitro anti-glycation assay and *In-vivo* anti-diabetic activity by streptozotocin induced diabetes in Wistar albino rats. The diabetic rats were divided into 5 groups of 6 animals each. For testing the efficacy of extracts, two groups were intra-orally provided with dosages of 100 mg/Kg and 200 mg/ Kg of body weight of animals, respectively, ofEthanolic extract of *S.tortuosum*. Control groups were maintained for evaluation, which included vehicle control as well as with Glibenclamide, a standard anti-diabetic drug. The extracts at a dose of 400 mg/Kg body weight was found to be associated with significant amelioration of many of the diabetes induced conditions, suggesting that the plant extract could be a strong potential CAM candidate for therapeutic management of diabetes.

Keywords: Complementary and alternative medicine; *Sceletium tortuosum*; diabetes; streptozotocin

INTRODUCTION

Diabetes mellitus (simply referred diabetes), a metabolic disorder in which the body fails either to produce or to respond to insulin and thus resulting in faulty glucose metabolism, is a chronic disease condition with a prevalence of 8.5% of the world adult population [1]. Diabetes type II is the most common, responsible for 90% of diabetes, arises due to dysfunctional pancreatic beta cells or impairment to insulin response by the body

cells. The disorder is associated with morbidities and reduction in the quality of life and in addition, also could shorten the lifespan due to complications such as cardiovascular diseases [2]. There are no curative therapies yet identified for diabetes, while management of the blood glucose levels by restriction of sugar and calorie intake (especially low glycemic index foods), regular monitoring of blood sugar parameters, and more importantly by adoption of healthy lifestyle [3], in an optimal manner have been shown to improve the quality of life near normal. In chronic cases, the effective management of type 2 diabetes involves insulin therapy. However, the administration of the same is fraught with difficulties such as the requirement of a trained person for administration, pain and relative cost. Other medicines taken orally including sulfonylureas and biguanides have limited utility and are also fraught with side effects.

Sceletium tortuosum, a succulent plant in the Mesembryanthemaceae (*Aizoaceae*) family, is native to South Africa. It is particularly renowned among the indigenous people, especially in Namaqualand, for its frequent use due to its medicinal and psychoactive properties. Four basic ring systems, differentiated by the shape and oxidation of the carbon ring, have been identified in *S. tortuosum*. The mesembrine alkaloids are the most active of these subgroups. Other alkaloids include mesembrone, mesembrenol, Hordenine, (-)-Tortuosamine, Mesembranol, Mesembrenol, D7-Mesembrenone, and Mesembrenone. Mesembrine is notable for its effects on the central nervous system and functions as a serotonin-uptake inhibitor. In specified doses, these compounds act as antidepressants, minor tranquilizers, and anxiolytics, making them useful in treating mild to moderate depression, psychological and psychiatric disorders involving anxiety, major depressive episodes, alcohol and drug dependence, bulimia nervosa, and obsessive-compulsive disorders (U.S. Patent 6,288,104). [4,5] Another study has given a comprehensive review of data on Sceletium accumulated over 300 years, documenting traditional methods for preparing Kougoed and reporting its psychoactive properties based on the experiences of several test subjects. The aim of the present study is to investigate anti-diabetic potential of *Sceletium tortuosum* aerial parts against streptozotocin-induced diabetes on wistar rats.

MATERIALS & METHOD

Collection, authentication, and preparation of *Sceletium tortuosum* extract

The plant was obtained and subjected to authentication by Dr. S. S. Hameed, Scientist 'F' & Head of Office (I/C), Ministry of Environment, Forest & Climate change, Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2024-25/Tech-350). The aerial portions were gathered, subjected to air drying within the temperature range of 40°C to 50°C, and subsequently ground into powder form. A quantity of 100 grams from the resulting powder was subjected to soxhlet extraction utilizing 500ml of ethanol, over a period of 48 hours. Following extraction, the ethanol extract underwent concentration at 50°C and then dried. Finally, the percentage yield of the extract was determined and the extract was labelled as STEE (*Sceletium tortuosum* ethanol extract) [6].

Acute toxicity study

Following an overnight fast with access only to water, an acute toxicity test of plant extracts was conducted on rats. Prior to extract administration, each rat's weight was recorded. Random allocation placed the rats into control and treatment groups, each consisting of 1 rat. Over the initial four hours post-administration, the animals were closely observed for overt toxicities and behavioral changes such as restlessness, tremors, diarrhea, sluggishness, weight loss, and paralysis at regular intervals. Subsequently, they were monitored daily for two weeks to detect any alterations in general behavior or physical activity. Food access was restored four hours after extract administration (OECD 423) [7].

***In-vitro* α-amylase inhibition assay**

The α-amylase inhibition activity of the ethanol extracts was assessed using the following procedure: Porcine pancreatic α-amylase solution (1U/mL, 100 µL) was combined with 100 µL of STEE (dissolved in ethanol, concentrations ranging from 50 to 300 µg/mL) and pre-incubated at 37°C for 30 minutes. Following this, 100 µL of 1% starch solution was added to the mixture, and the reaction was allowed to proceed at 37°C for an additional 20 minutes. Subsequently, 200 µL of dinitro salicylic acid was introduced, and the reaction tubes were subjected to boiling water for 5 minutes. After cooling to room temperature, the reaction mixture was diluted with 1.5 mL of distilled water, and its absorbance was measured at 540 nm using a UV-visible spectrophotometer. The percentage of α-amylase inhibition activity was determined relative to a solvent control, with acarbose serving as the positive control for comparison [8].

$$\% \text{ of Inhibition} = A_{control} - A_{sample} / A_{control} \text{ X } 100$$

$A_{control}$ - control absorbance; A_{sample} - sample absorbance

***In-vitro* α-glucosidase inhibition assay**

To determine the α-glucosidase inhibition activity of the extracts, we followed the protocol outlined by Lordan *et al*., 2013 with slight modifications. Initially, 10µL of STEE, dissolved in ethanol at concentrations ranging from 5 to 300 µg/mL, was mixed with 120µL of 0.1 M phosphate buffer (pH 6.9) and 20µL of enzyme solution. This mixture was allowed to pre-incubate at 37°C for 15 minutes. Following pre-incubation, 20µL of 5mM p-nitro-α-D-glucopyranoside was added to initiate the enzymatic reaction, and the mixture was further incubated at 37°C for 15 minutes. To halt the reaction, 80µL of 0.2 M sodium carbonate was added. The absorbance of the resulting solution was then measured at 409 nm. The percentage inhibition of α-glucosidase activity was calculated accordingly. Acarbose served as the positive control in this assay [9].

% of Inhibition = $A_{control} - A_{sample} / A_{control}$ X 100

$A_{control}$ - control absorbance; A_{sample} - sample absorbance

In vitro anti-glycation assay

The procedure involved testing the impact of ethanol extract on the formation of AGEs (advanced glycation end products). STEE samples, ranging from 50 to 300 µg/mL dissolved in DMSO, were prepared. These samples were then mixed with a reaction solution composed of 40 mg/mL bovine serum albumin (BSA) in 100mM sodium phosphate buffer (pH 7.4), along with 0.02% sodium azide in 500mM glucose. The mixture was then incubated for 21 days at 37°C, with measurements taken at 24-hour intervals. After the incubation period, the fluorescence intensity was measured using excitation and emission wavelengths of 335 nm and 385 nm, respectively. The percentage inhibition of AGE formation was calculated relative to a control sample treated with the solvent alone. As a reference, ascorbic acid was included as a positive control according to previous studies [10].

***In-vivo* anti-diabetic activity**

The study was performed as per the protocol approved by the ethics committee (SSMCOP/IAEC/M.Pharm/02/04/2024). Diabetes was induced in rats by intraperitoneal injection of Streptozotocin (STZ) which was dissolved in citrate buffer (0.01 mol/L, pH 4.5). After one week of STZ administration, blood glucose levels were measured by a one-touch glucometer to confirm diabetes. Blood samples were drawn by picking the rat's tail. After one week of STZ injection, the animals were divided into groups (with 6 animals each) for the anti-diabetic study of the potent extract. Treatment started one week later. Group I served as the control and received normal saline; group II was treated with STZ 50 mg/kg (negative control), and group III was treated with the standard drug (Glibenclamide). Group IV and group V were treated with STEE 100 mg/kg and STEE 200 mg/kg. Blood glucose levels were measured with the help of a one-touch glucometer at one week (assumed as 0 hrs.), after 3 hrs., on the 5th day, 10th day, 15th day, and 21st day of the experiment [11].

Evaluation of biochemical parameters

Random blood glucose level, Glycated Hemoglobin (%HbA1c), Lipid Profile, aspartate amino transferase (AST), serum alanine amino transferase (ALT), serum creatinine. Method of Blood Withdrawal for the biochemical parameters from the tail vein of treated male Wistar rats and the withdrawal volume is 1 ml.

Histopathological analysis

The liver, kidney, and pancreas of the rats were extracted and stained with *Hematoxylin and evaluated.*

STATSTICAL ANALYSIS

All statistical analyses were performed using the graphpad prism 8.3.0. To assess the significance of differences, two-way ANOVA was performed. Post hoc tests using the dunnet test were conducted to identify specific group differences in case of a significant main effect.

RESULT & DISCUSSION

Acute toxicity study

During the initial four hours post-administration, the rats were closely monitored for any signs of toxicity and behavioral changes, including restlessness, tremors, diarrhea, sluggishness, weight loss, and paralysis. Following this intensive monitoring period, the rats were observed daily for two weeks for any further behavioral or physical activity changes. Food was reintroduced four hours after extract administration. The absence of observable toxic effects or behavioral changes in rats administered *Sceletium tortuosum* extract at doses of 1000 mg/kg and 2000 mg/kg suggests that the extract is well tolerated.

***In-vitro* α-amylase inhibition assay**

The results of the α-amylase inhibition assay indicate that both *Sceletium tortuosum* extract ethanol extract (STEE) and the standard acarbose exhibit dose-dependent inhibition of α-amylase activity. As the concentration of STEE and acarbose increased, there was a corresponding increase in the inhibition of α-amylase activity, as demonstrated by the decreasing trend in IC50 values. Specifically, the IC50 of STEE was found to be 169.594 µg/mL, while the IC50 of acarbose was determined to be 115.001 µg/mL. In this context, the correlation between the mechanism of the α-amylase inhibition assay and the obtained results underscores the potential of both STEE and acarbose as inhibitors of α-amylase activity, highlighting their relevance in managing postprandial hyperglycemia in diabetes. The α-amylase inhibition assay is crucial in evaluating the potential of natural extracts or compounds in managing hyperglycemia by inhibiting the breakdown of complex carbohydrates into glucose. In this study, both STEE and acarbose demonstrated significant inhibition of α-amylase activity, suggesting their potential as antidiabetic agents. The dose-response relationship observed in this study supports the notion that higher concentrations of STEE and acarbose lead to increased inhibition of α-amylase activity. This suggests that higher doses of these compounds may be more effective in managing postprandial hyperglycemia by slowing down carbohydrate digestion and reducing the rate of glucose absorption. Overall, the results of this study highlight the potential of STEE as a natural alternative to synthetic α-amylase inhibitors like acarbose in managing diabetes. Further studies exploring the mechanism of action and potential synergistic effects of STEE with other antidiabetic agents are warranted to validate its therapeutic efficacy and safety. [Fig 1]

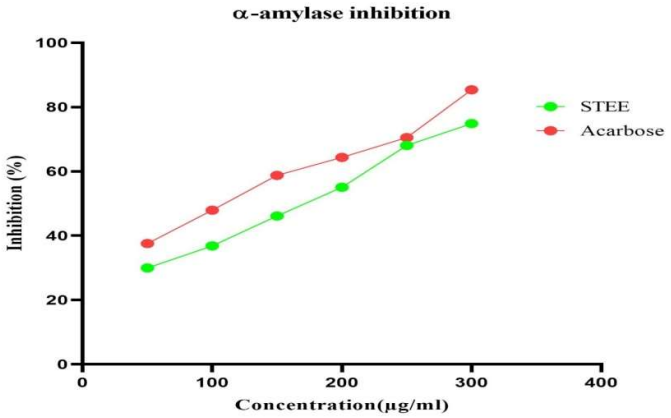


Fig 1: *In-vitro* α-amylase inhibition assay of STEE

***In-vitro* α-glucosidase inhibition assay**

The α-glucosidase inhibition assay was conducted to evaluate the inhibitory activity of *Sceletium tortuosum* extract ethanol extract (STEE) in comparison with the standard drug, acarbose. The results revealed concentration-dependent inhibition for both STEE and acarbose. As the concentration of both substances increased, the inhibition percentage also increased. The half-maximal inhibitory concentration (IC50) values were determined to be 234.698 µg/mL for STEE and 68.070 µg/mL for acarbose. This indicates that acarbose exhibited a stronger inhibitory effect on α-glucosidase compared to STEE, as evidenced by its lower IC50 value. [Table 1]

Table 1: *In-vitro* α-glucosidase inhibition assay

Concentration (µg/ml)	Inhibition percentage	
	STEE	Acarbose
50	41.26 ± 1.33	47.23 ± 0.10
100	44.49 ± 0.06	55.19 ± 0.99
150	44.70 ± 0.51	64.14 ± 0.22
200	49.03 ± 0.67	70.25 ± 0.04
250	49.15 ± 0.83	77.14 ± 0.79
300	55.48 ± 0.03	81.36 ± 0.77

Values are expressed as mean ± SEM (n=3)

***In-vitro* anti-glycation assay**

The results of the anti-glycation assay indicate that both *Sceletium tortuosum* ethanol extract (STEE) and acarbose exhibit significant inhibitory effects on protein glycation. Acarbose, a known standard inhibitor, showed a strong inhibitory effect across all tested concentrations, with a maximum inhibition of 97.2% at 300 µg/mL. The IC50 value for acarbose was 94.513 µg/mL, demonstrating its high potency in inhibiting glycation. [Fig 2]

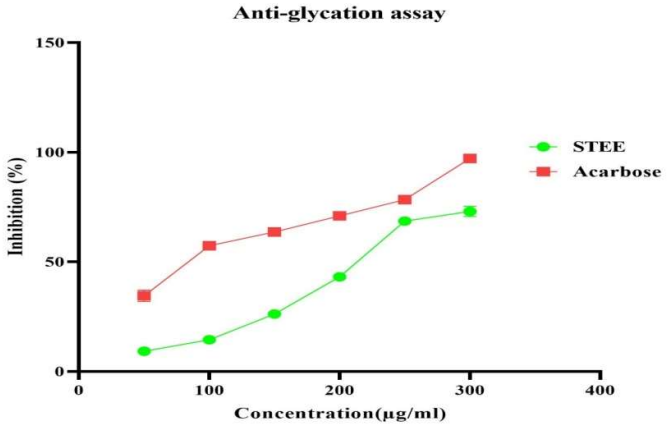


Fig 2: *In-vitro* anti-glycation assay of STEE

STEE also demonstrated a dose-dependent inhibition of glycation, with inhibition percentages increasing from 9.28% at 50 µg/mL to 73.02% at 300 µg/mL. Although the inhibition percentages were generally lower than those of acarbose, STEE still showed considerable anti-glycation activity, with an IC50 value of 208.41 µg/mL. This suggests that STEE, while less potent than acarbose, has potential as a natural anti-glycation agent.

***In-vivo* anti-diabetic activity**

STZ is a well-known diabetogenic agent that selectively destroys insulin-producing beta cells in the pancreas, leading to hyperglycemia. The successful induction of diabetes in this study was confirmed by measuring elevated blood glucose levels one week post-STZ administration. This confirms that the model was effectively established for evaluating the anti-diabetic potential of the tested extract. The experimental design included a control group (normal saline), a negative control group (STZ only), a standard treatment group (Glibenclamide), and two treatment groups receiving STEE at doses of 100 mg/kg and 200 mg/kg. This setup allowed for a comprehensive comparison of the extract's efficacy against a known pharmaceutical agent (Glibenclamide) and untreated diabetic rats. Blood glucose levels were measured at several time points: baseline (0 hours), after 3 hours, and on days 5, 10, 15, and 21. This time-course assessment provided insights into the immediate and long-term effects of the treatments. The use of a one-touch glucometer ensured consistency and reliability in the measurements. [Table 2]

Table 2: *In-vivo* anti-diabetic study

Treatment	Fasting blood glucose (mg/dL)					
	0 hrs	3 hrs	5th day	10th day	15th day	21st day
Group 1 (normal control)	76.4 ± 2.9	76.8 ± 4.2	78.2 ± 3.7	79.3 ± 2.1	78.2 ± 1.9	77.5 ± 3.1
Group 2 (negative control)	268.3 ± 4.3	261.8 ± 4.7	264.7 ± 3.2	254.1 ± 2.8	251.3 ± 5.3	250.2 ± 3.3
Group 3 glibenclamide	260.6 ± 1.2	253.7 ± 4.4	201.4 ± 5.5*	173.5 ± 2.2*	137.4 ± 5.4**	97.1 ± 3.5***
Group 4 STEE 100 mg/kg	261.2 ± 4.3	259.4 ± 7.1	258.3 ± 2.8	217.2 ± 4.1*	173.5 ± 2.7**	141.6 ± 7.5**
Group 5 STEE 200 mg/kg	264.3 ± 3.6	260.5 ± 4.7	221.7 ± 2.7*	184.3 ± 3.5**	145.1 ± 4.5**	101.6 ± 5.8***

*Values are expressed as mean ± SEM (n=6) for estimation of fasting blood glucose. Significance was analyzed using a two-way ANOVA followed by a dunnet test. Comparisons between groups 2 to 5 with the negative control group. p-value * represents p<0.05; ** represents p<0.01; *** represents p< 0.001.*

At the beginning of the study (0 hrs), there were no significant differences in fasting blood glucose levels between Group III (glibenclamide), Group IV (STEE 100 mg/kg), and Group V (STEE 200 mg/kg) compared to

the negative control group (Group 2). The values were 260.6 ± 1.2 mg/dL, 261.2 ± 4.3 mg/dL, and 264.3 ± 3.6 mg/dL, respectively, while the negative control group had a value of 268.3 ± 4.3 mg/dL. This indicates that the immediate effect of STEE, whether at 100 mg/kg or 200 mg/kg, and glibenclamide was not substantial within the first few hours of administration. After 3 hours, the fasting blood glucose levels slightly decreased in Group III (253.7 ± 4.4 mg/dL), Group IV (259.4 ± 7.1 mg/dL), and Group V (260.5 ± 4.7 mg/dL) compared to the negative control group (261.8 ± 4.7 mg/dL). However, these changes were not statistically significant. [12-15]

Short-term effects

By the 5th day, a significant reduction in fasting blood glucose was observed in Group III (201.4 ± 5.5 mg/dL, $p < 0.05$) and Group V (221.7 ± 2.7 mg/dL, $p < 0.05$) compared to the negative control group (264.7 ± 3.2 mg/dL). Group IV showed a slight reduction (258.3 ± 2.8 mg/dL), but it was not statistically significant. STEE likely contains bioactive compounds that stimulate insulin secretion or enhance insulin sensitivity. Phytochemicals such as flavonoids, alkaloids, and glycosides, commonly found in plant extracts, can improve β-cell function and promote insulin release, similar to the action of sulfonylureas like glibenclamide.

Mid-term effects

By the 10th day, both Group III (173.5 ± 2.2 mg/dL, $p < 0.05$) and Group V (184.3 ± 3.5 mg/dL, $p < 0.01$) showed a significant reduction in fasting blood glucose levels compared to the negative control group (254.1 ± 2.8 mg/dL). Group IV also showed significant improvement (217.2 ± 4.1 mg/dL, $p < 0.05$). On the 15th day, the reductions became more pronounced in Group III (137.4 ± 5.4 mg/dL, $p < 0.01$), Group IV (173.5 ± 2.7 mg/dL, $p < 0.01$), and Group V (145.1 ± 4.5 mg/dL, $p < 0.01$) compared to the negative control group (251.3 ± 5.3 mg/dL). The pharmacodynamic profile of STEE suggests that its active compounds may be accumulating or progressively interacting with targets involved in glucose metabolism. Flavonoids and polyphenols in STEE could be enhancing glucose uptake by peripheral tissues, improving insulin receptor signaling, or exhibiting antioxidant properties that protect pancreatic β-cells from oxidative stress-induced damage.

Long-term effects

By the 21st day, Group III (97.1 ± 3.5 mg/dL, $p < 0.001$), Group IV (141.6 ± 7.5 mg/dL, $p < 0.01$), and Group V (101.6 ± 5.8 mg/dL, $p < 0.001$) demonstrated a highly significant reduction in fasting blood glucose levels compared to the negative control group (250.2 ± 3.3 mg/dL) indicating a robust anti-diabetic effect. The significant reduction in Group IV (STEE 100 mg/kg) also confirms the efficacy of STEE, although the higher dose consistently showed greater efficacy. These findings suggest that STEE might be modulating several pathways involved in glucose homeostasis, such as enhancing insulin secretion, improving insulin sensitivity, inhibiting hepatic gluconeogenesis, and increasing glucose uptake in muscles and adipose tissues.

The clinical relevance of these results lies in the potential use of STEE as an anti-diabetic agent. Glibenclamide acts by stimulating insulin release from pancreatic β-cells through ATP-sensitive potassium channels. Similarly, STEE might contain compounds that have insulinotropic effects, enhancing insulin secretion or possibly improving insulin sensitivity. Bioactive components in STEE, such as flavonoids, saponins, and polyphenols, are known to exhibit multiple anti-diabetic mechanisms. [15-18] [Figure 3]

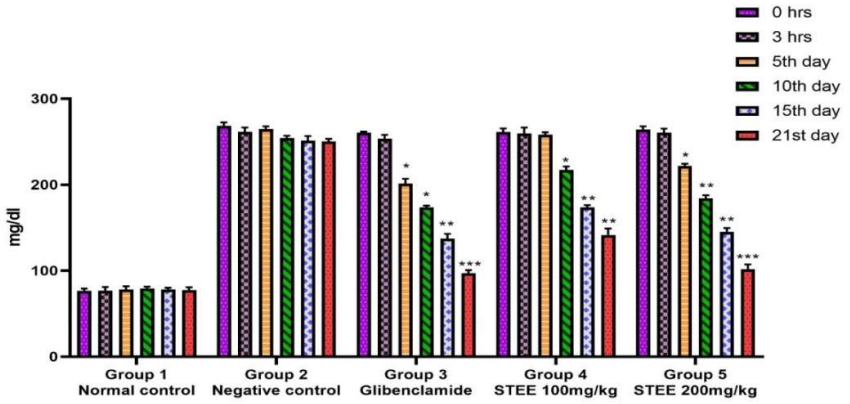


Fig 3: Fasting blood glucose levels of experimental rats

Values are expressed as mean ± SEM (n=6) for estimation of fasting blood glucose. Significance was analyzed using a two-way ANOVA followed by a dunnet test. Comparisons between groups 2 to 5 with the negative control group. p-value * represents $p<0.05$; ** represents $p<0.01$; *** represents $p< 0.001$.

Estimation of biochemcial parameters

The study examined the effects of glibenclamide and STEE (at 100 mg/kg and 200 mg/kg) on various biochemical parameters in diabetic rats. The parameters measured were Hba1c (%), SGOT (AST) (IU/L), SGPT (ALT) (IU/L), creatinine serum (mg/dL), and random blood glucose (mg/dL). The results for each parameter were compared to the negative control group to evaluate the significance of the treatments. **):** The Hba1c level further decreased to 6.9 ± 0.41 ($p<0.001$), comparable to the reduction seen with glibenclamide, indicating that the higher dose of STEE is highly effective in managing blood glucose levels over the long term. The reduction in Hba1c in all treatment groups compared to the negative control highlights the potential of both glibenclamide and STEE, particularly at higher doses, in achieving sustained glycemic control. The SGPT level significantly reduced to 36.42 ± 1.85 IU/L ($p<0.05$), indicating a beneficial effect on liver function at the higher dose. The reduction in SGOT and SGPT levels with STEE treatment, particularly at 200 mg/kg, suggests that STEE has a protective effect on the liver, reducing the enzyme levels that indicate liver stress and damage. The creatinine level was 0.41 ± 0.07 mg/dL, indicating no significant impact on kidney function. The similar creatinine levels across all groups suggest that neither glibenclamide nor STEE, at the doses tested, adversely affect kidney function. [Table 3]

Table 3: Estimation of biochemcial parameters in experimental rats

Treatment	Hba1c (%)	SGOT (AST) (IU/L)	SGPT (ALT) (IU/L)	Creatinine serum (mg/dL)	Blood glucose random (mg/dL)
Group 1 (normal control)	5.9 ± 1.4	102.20 ± 3.15	36.40 ± 2.01	0.35 ± 0.21	99.5 ± 3.2
Group 2 (negative control)	11.1 ± 1.3	129.20 ± 1.28	45.26 ± 1.9	0.41 ± 0.3	259.7 ± 2.9
Group 3 glibenclamide	6.4 ± 1.0***	107.42±3.12*	46.10 ± 1.5	0.38 ± 0.02	107.5 ± 3.17***
Group 4 STEE 100 mg/kg	8.5 ± 0.93**	113.26±2.17*	44.70 ± 2.74	0.39 ± 0.21	143.6 ± 1.39***
Group 5 STEE 200 mg/kg	6.9±0.41***	95.4±2.55**	36.42±1.85*	0.41 ± 0.07	119.3 ± 2.43***

*Values are expressed as mean ± SEM (n=6) for estimation of fasting blood glucose. Significance was analyzed using a two-way ANOVA followed by a dunnet test. Comparisons between groups 2 to 5 with the negative control group. p-value * represents p<0.05; ** represents p<0.01; *** represents p< 0.001.*

Lipid profile

The observed improvements in lipid profiles following treatment with glibenclamide and STEE are closely intertwined with their anti-diabetic activity. Diabetes mellitus and dyslipidemia frequently coexist and share common pathophysiological mechanisms, including insulin resistance and inflammation. Glibenclamide's efficacy in lowering fasting blood glucose levels aligns with its favorable effects on lipid parameters, indicating a comprehensive approach to metabolic health. Similarly, the lipid-lowering effects of STEE corroborate its anti-diabetic activity, suggesting a multifaceted mechanism of action. The interplay between glucose and lipid metabolism underscores the potential of glibenclamide and STEE as dual-action therapies for managing metabolic disorders. [19-21] [Fig 4]

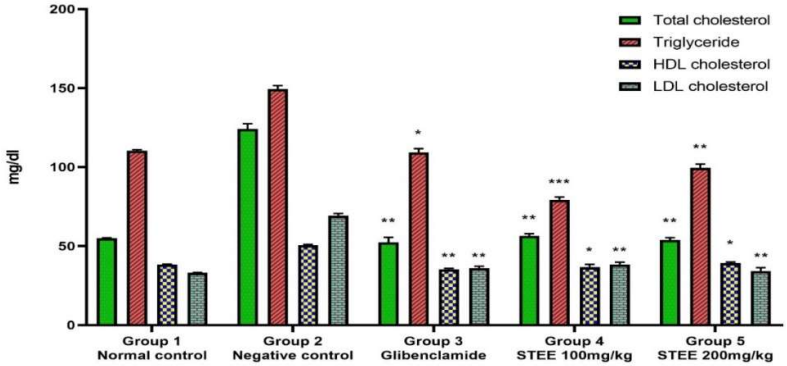


Fig 4 : Lipid profile of the experimental rats

*Values are expressed as mean ± SEM (n=6) for estimation of fasting blood glucose. Significance was analyzed using a two-way ANOVA followed by a dunnet test. Comparisons between groups 2 to 5 with the negative control group. p-value * represents p<0.05; ** represents p<0.01; *** represents p< 0.001.*

Histopathological study of Pancreas

Histopathological examination revealed that the normal control group exhibited healthy tissue architecture with no pathological changes, while the negative control group showed significant tissue damage and inflammation in all organs studied. The positive control group treated with Glibenclamide demonstrated reduced damage and inflammation. Notably, the STEE 100 mg/kg group showed moderate improvement in tissue architecture, with some reduction in damage and inflammation, while the STEE 200 mg/kg group exhibited significant protection, with tissue conditions approaching those of the normal control group. These findings indicate that STEE exerts a dose-dependent protective effect against diabetes-induced organ damage, with the 200 mg/kg dose providing the most substantial benefits. [Fig 5]

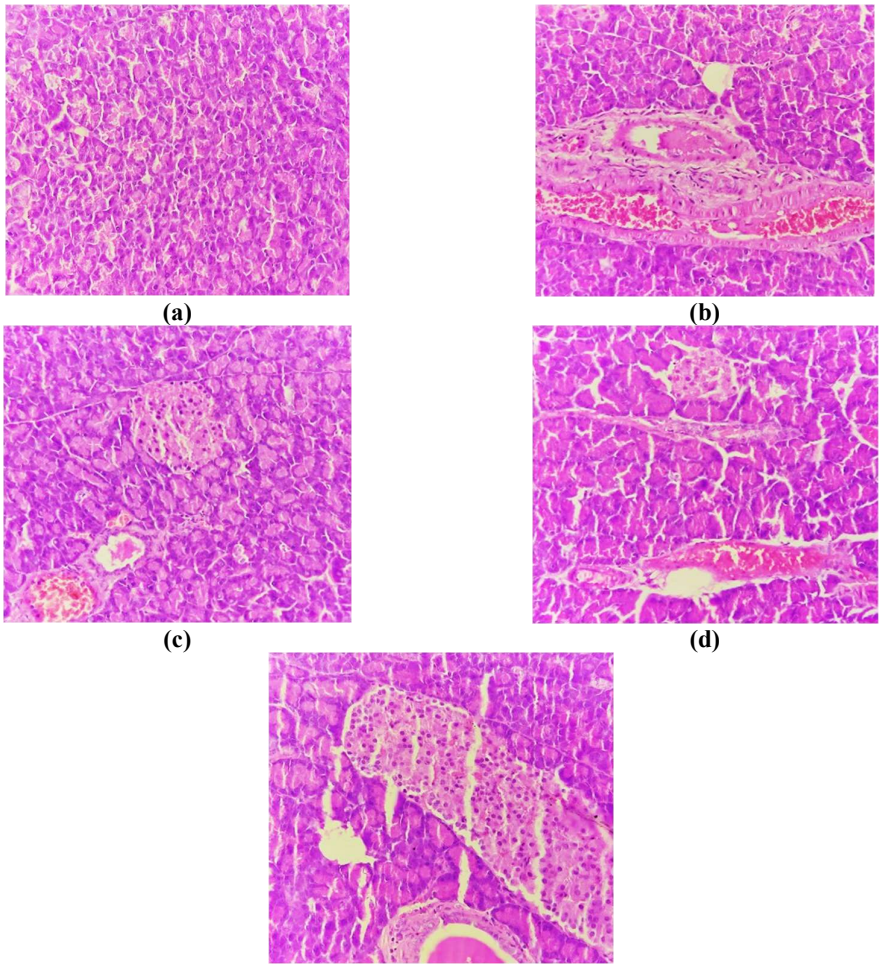


Fig 5: Histopathology of pancreas

CONCLUSION

The *Sceletium tortuosum* ethanol extract (STEE) demonstrates significant bioactive properties, including anti-diabetic effects, and inhibition of α-amylase, α-glucosidase, and protein glycation. The extract effectively reduces fasting blood glucose levels, suggesting its potential in managing diabetes through mechanisms such as enhancing insulin sensitivity, inhibiting hepatic gluconeogenesis, and increasing glucose uptake in muscles and adipose tissues. Although STEE shows lower potency compared to the standard drug acarbose in enzyme inhibition assays, its considerable biological activities highlight its potential as a therapeutic agent, warranting further investigation. Both doses of STEE reduced total cholesterol, triglycerides, and LDL cholesterol levels while also improving HDL cholesterol levels. *Sceletium tortuosum* ethanol extract (STEE) at both 100 mg/kg and 200 mg/kg doses improved metabolic parameters, particularly HbA1c and random blood glucose levels. The 200 mg/kg dose showed greater improvements, approaching the efficacy of glibenclamide and significantly better than the negative control. Both doses improved SGOT and SGPT levels, with the higher dose showing significant reductions. STEE, especially at higher doses, shows potential as an effective anti-diabetic treatment with positive

effects on liver function and glucose metabolism. The histopathological analysis of liver, kidney, and pancreas tissues in diabetic Wistar rats demonstrated that *Sceletium tortuosum* ethanol extract (STEE) exhibits a dose-dependent protective effect. The administration of STEE at both 100 mg/kg and 200 mg/kg doses resulted in reduced tissue damage and inflammation compared to the negative control group. The 200 mg/kg dose of STEE showed the most significant improvement across all tissues, closely approaching the conditions observed in the normal control group.

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