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In-vivo evaluation of Antidiabetic Properties of Plant Extracts in Streptozotocin Induced Diabetes

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ABSTRACT

The primary objective of this study was to evaluate the hypoglycemic effects of alcoholic extracts obtained from Annona squamosa stem bark in rats. Additionally, the research investigated the antidiabetic potential of both alcoholic and aqueous extracts of Annona squamosa stem bark in rats with Streptozotocin-induced diabetes. Preliminary phytochemical analysis revealed the presence of glycosides, fixed oils, tannins, phytosterols, and phenolic compounds in the alcoholic extracts. However, significant hypoglycemic effects were observed only at higher doses.

In Streptozotocin-induced diabetic rats, the alcoholic extract of Annona squamosa stem bark demonstrated marked antidiabetic activity at elevated doses, exceeding the efficacy of the standard drug Glibenclamide. Morphological assessments, including water intake, food consumption and body weight measurements, showed comparable effects between the alcoholic extracts and Glibenclamide at higher dosages. Histopathological analysis further revealed substantial restoration of damaged islet cells in the pancreas, surpassing the restorative effects observed with Glibenclamide.

These findings suggest that the alcoholic extract of Annona squamosa stem bark exhibits significant antidiabetic properties, validating its traditional use in diabetes management.

Keywords: *Annona squamosa, Hypoglycemic, Antidiabetic, Streptozotocin, Glibenclamide*

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1. INTRODUCTION

Diabetes mellitus, often referred to as the "silent invader," is increasingly prevalent with advancing age in both genders. However, its incidence is notably higher in males than females between the ages of 25 and 50. This condition is marked by persistent high blood sugar levels (hyperglycaemia), abnormal lipid profiles (hyperlipidaemia), oxidative stress, excessive urination (polyuria), increased appetite (polyphagia), excessive thirst (polydipsia), ketosis, kidney damage (nephropathy), nerve disorders (neuropathy), and cardiovascular complications.

Globally, nearly 40 million individuals are affected by diabetes mellitus, making it a significant public health concern. It is a major contributor to mortality, impacting both developed and developing countries. In the United States, it ranks as the fourth leading cause of death, while in India, it affects roughly 1-2% of the population.

A recent report highlights the alarming global surge in diabetes cases. Currently, an estimated 1.7% of the global population is affected by diabetes mellitus, with projections indicating this figure may rise to 4% by 2030. In countries like the United States, the prevalence is expected to double by 2040, while alternative estimates suggest that the number of new cases could triple. In the near future, it is anticipated that 851,000 individuals with diabetes will require treatment for end-stage kidney disease.

Despite advancements in medical science, an effective and satisfactory cure for diabetes mellitus remains elusive. Insulin therapy is commonly used for disease management, but it has limitations, including insulin resistance, anorexia nervosa, brain shrinkage, and fatty liver. Prolonged use of medications like sulfonylureas and biguanides is also associated with adverse effects. Furthermore, issues such as the need for drug refrigeration, the requirement for skilled healthcare professionals, and high treatment costs pose significant barriers, particularly for low-income populations.

As we know, modern medicine has yet to provide a reliable solution for achieving strict glycemic control without undesirable side effects. Since ancient times, herbal remedies have been a cornerstone in the treatment of diabetes mellitus and various other health conditions, both in India and worldwide. This preference arises from the relatively lower risk of adverse effects associated with herbal treatments compared to synthetic drugs. Herbal medications are generally non-toxic or exhibit minimal toxicity, leading to fewer side effects compared to synthetic alternatives. As a result, there continues to be significant global interest in exploring alternative therapeutic systems such as Ayurveda, Unani, Homeopathy, Siddha, and others, valued for their perceived effectiveness, safety, and affordability.

In traditional medicine, many plants are believed to have therapeutic properties for managing diabetes mellitus. As such, they hold promise as a source of potential antidiabetic treatments. However, this idea has yet to gain widespread acceptance within the scientific community. Several factors contribute to this hesitation, including skepticism among conventional medical practitioners toward alternative treatments, the lack of clear definitions for alternative medical practices, and concerns over fraudulent claims of miraculous cures. Additionally, natural remedies can vary significantly in their composition, quality, and safety, further complicating their acceptance.

The use of this plant extract in the treatment of diabetes mellitus is widely recognized in traditional medicine. However, scientific studies supporting its efficacy remain scarce. Therefore, research has been undertaken to evaluate its potential hypoglycemic and antidiabetic properties through experiments involving an animal model.

2. MATERIALS AND METHODS

Plant Material Collection:
The stem bark of *Annona squamosa* was sourced from the hilly areas of the district and authenticated by specialists from the Department of Botany and the Botanical Survey of India. The collected bark was then shade-dried, ground into a fine powder, and subjected to extraction using Pet Ether, Ethanol, Methanol, and Aqueous solvents with the aid of a Soxhlet apparatus.

Chemicals
Streptozotocin was procured from Sigma Chemical Company, while all other chemicals utilized were of the highest purity grade.

Experimental animals
Albino rats of both sex weighing between 150 and 200 grams, were supplied by the central animal house for experimental studies. The animals were acclimated to the laboratory environment for a period of 7 days. They were fed a standard diet available commercially and provided with unrestricted access to water under hygienic conditions. All animal experiments were conducted in accordance with guidelines set by the Institutional Animal Ethical Committee (IAEC) and CCSEA regulations.

Acute toxicity study
The acute toxicity of the extracts was evaluated using albino mice of both sex weighing between 20 and 25 grams, maintained under controlled conditions. Before the experiment, the animals were subjected to an overnight fasting period. The extracts were administered orally following a modified up-and-down procedure in compliance with OECD guideline 425. The LD_{50} value was determined, and doses corresponding to $1/10^{th}$, $1/20^{th}$ and $1/5^{th}$ of the LD_{50} were selected as low, medium, and high dose levels for the extract, respectively.

Assessment of Antidiabetic Activity
Hypoglycaemic activity:
“Albino rodents of both sex weighing between 150 and 200 grams, were allocated into four groups, each consisting of six animals.”

Group A: Normal control (Saline solution)
Group B: Standard (Glyburide)
Group C: Ethanolic extract of the stem bark of *A. squamosa* (100 mg/kg)
Group D: Ethanolic extract of the stem bark of *A. squamosa* (200 mg/kg)

Antidiabetic action:
Rodents of both sex weighing between 150 and 200 grams, were divided into five groups, each containing six animals.
Group A: Normal group
Group B: Diabetic group (STZ treated)
Group C: Standard (STZ + Glyburide treated)
Group D: STZ + Extract of the stem bark of *A. squamosa* (100 mg/kg)

Group E: STZ + Extract of the stem bark of *A. squamosa* (200 mg/kg)

3. PHARMACOLOGICAL STUDY

Hypoglycemic activity
To evaluate hypoglycaemic activity healthy animals were utilized. This approach was consistently applied to all extracts analysed in this study.

Here, we outline the standard protocol used to evaluate the hypoglycaemic potential of extracts, which is uniformly applicable to all other extracts studied. Animals in each group underwent a fasting period of 16-18 hours before the experiment, which continued until the study's conclusion. However, the animals had unrestricted access to water throughout the experiment. A consistent 12-hour light/dark cycle was maintained, with relative humidity levels kept between 45-55% and ambient temperature regulated for all animals during the study period.

Before administering the vehicle, glyburide, or extract, blood samples were collected from overnight-fasted animals to determine baseline glucose levels. Subsequently, the animals in each group were treated with the respective vehicle, glyburide, or extract. Blood samples were then collected at various time intervals and analysed for glucose concentration using the GOD/POD method.

Anti-diabetic activity
To evaluate antidiabetic activity, diabetic animals with blood glucose levels exceeding 250 mg/dl (induced by STZ treatment) were used. Before the experiment, all diabetic animals (glucose levels > 250 mg/dl) in each group underwent a 16-18 hour fasting period, which was maintained throughout the experimental duration. Treatment began on the same day, except for the control groups, and continued for a period of 28 days.

During this period, all groups of animals had free access to a standard diet and water. Parameters including body weight, food consumption, and water intake were monitored from day 1 to day 28 of treatment. At the end of the 28-day treatment period, blood samples were collected from overnight-fasted rats via the tail vein at various intervals. These samples were analyzed to measure blood glucose levels.

At the conclusion of the study, all animals were euthanized using ether, and their pancreases were carefully extracted and preserved in a 10% formalin solution for histopathological analysis.

Measurement of Fasting Blood Glucose Level
Transfer 1 ml of glucose oxidizing reagent into labeled test tubes and add 10 µl of serum or plasma to each tube. Incubate the tubes for 15 minutes, then measure the absorbance readings.

Calculations:

$$\% \text{ BGL (mg/dl)} = \frac{\text{Initial reading (at '0' time)} - \text{Test reading (at regular intervals of time)}}{\text{Initial reading (at '0'time)}} \times 100$$

Morphological studies

a. Body weight
All processes for body weight assessment were conducted alongside the antidiabetic study. The average body weight of each experimental group was monitored throughout the study, from day 1 to day 24, to identify any changes over time. At the beginning of the experiment, all animals were carefully weighed on the first day before receiving any treatment. After 24 hours, the body weights of all animals in each group were recorded again, and this process was repeated daily for the entire 24-day duration. Changes in the average body weight for each group were analyzed and compared with the standard group.

b. Water intake
All procedures related to water consumption assessment were carried out alongside the antidiabetic study. The average water intake for each experimental group was measured daily throughout the 24-day study period to identify variations in water consumption among the rats. The standard water intake for rats was set at 10-12 ml per day per rat. To conduct the assessment, precisely 200 ml of water was dispensed into labeled feeding bottles for each group, consisting of 6 animals, and the start time was recorded. After 24 hours, the remaining water was measured and subtracted from the initial 200 ml. The resulting value was then divided by 6 to determine the mean water intake per rat for that group. This process was repeated daily for all groups over the entire 24-day experimental period.

c. **Food consumption**

All procedures for assessing food consumption were conducted alongside the antidiabetic study. The focus was on determining the average food intake of each experimental group throughout the 24-day study period to observe any variations in mean food consumption. Given that the standard food intake for rats ranges between 20-40 grams per day per rat, a precisely measured 300 grams of food was provided to each group, consisting of 6 animals, with the time recorded. After 24 hours, the leftover food was collected, measured, and subtracted from the initial 300 grams. The resulting amount was then divided by 6 to calculate the mean food intake for that group. This process was repeated daily for all experimental groups over the 24-day period.

Statistical analysis:

The values were expressed as mean ± SEM. The statistical analysis was carried out by One-way analysis of variance (ANOVA) followed by Dunnet‟s t‟- test. p values <0.05 were considered significant.

4. RESULTS

Effect of the alcoholic extract of *A. squamosa* stem bark on fasting blood glucose levels in both normal and diabetic rats.

The alcoholic extract of *A. squamosa* proved a significant, dose-dependent decrease in blood glucose levels following a single-dose administration. Remarkably, the hypoglycemic effect of the extract at a dose of 200 mg/kg was nearly comparable to that of the reference standard, Glibenclamide.

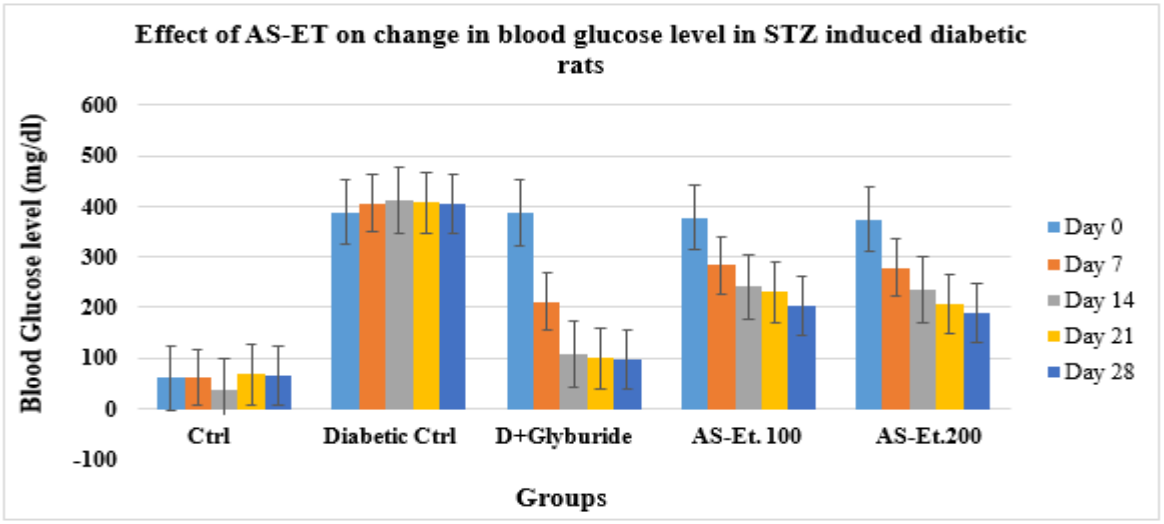


Chart 1: Blood Glucose Level

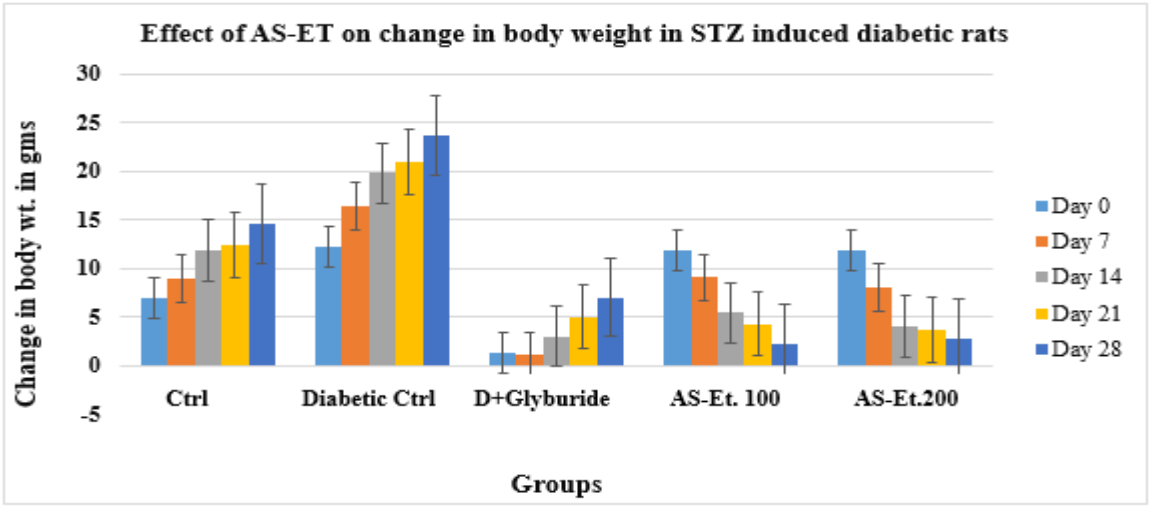


Chart 2: Change in body weight

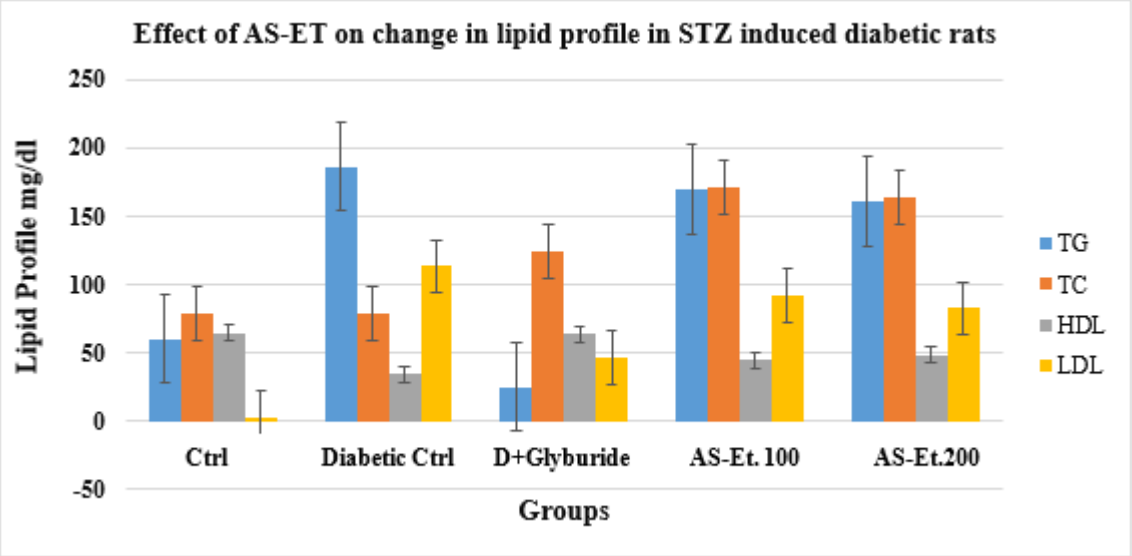


Chart 3: Lipid Profile

Table no 01: Effect of *A. squamosa* stem bark extracts on Blood Glucose Levels and Body Weight in Diabetic Rats

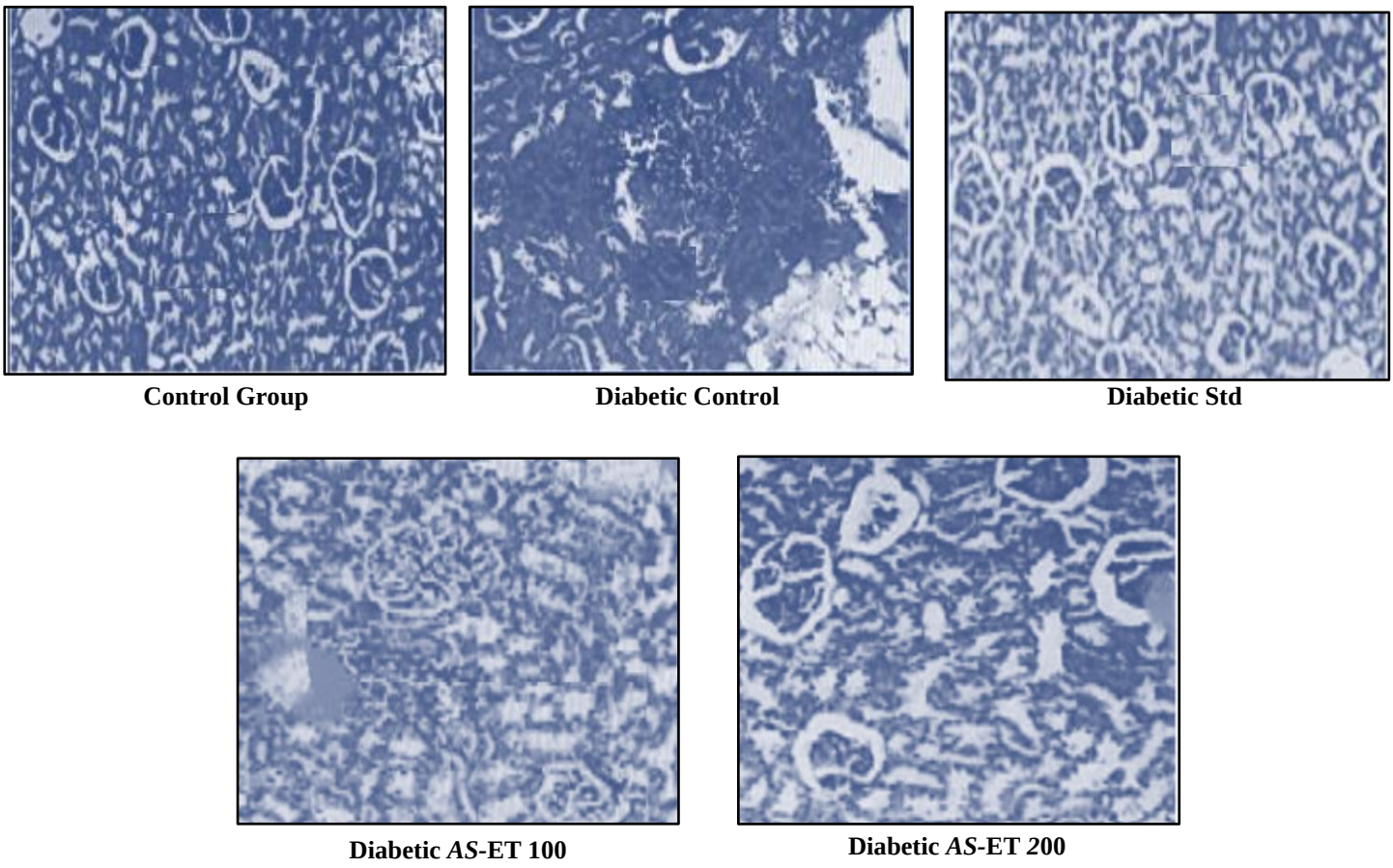
Groups	Blood Glucose Level (mg/dl)					Body weight (gm)				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Ctrl	59.90 ± 3.85	60.03 ± 2.56	61.59 ± 1.12	66.56 ± 1.09	63.14 ± 1.47	5.97 ± 0.45	8.03 ± 0.35	10.88 ± 0.30	11.46 ± 0.77	12.64 ± 0.74
Diabetic Ctrl	386.61 ± 3.52	404.41 ± 5.48	409.83 ± 4.75	406.10± 4.74	402.15 ± 4.02	11.21 ± 0.32	15.41 ± 0.74	17.83 ± 0.44	20.03 ± 0.44	21.65 ± 0.44
D+ Glyburide (10mg/kg)	385.20 ± 7.52	209.29 ± 3.67**	105.81 ± 2.56**	99.23± 1.83**	95.43 ± 2.57**	1.25 ± 0.33**	1.04 ± 0.18**	2.04 ± 0.31**	4.05 ± 0.41**	6.04 ± 0.31**
AS-Et. 100 mg/kg	375.36 ± 3.48	281.57 ± 1.47**	239.24 ± 1.09**	228.24± 1.09**	201.4± 2.92**	10.91 ± 0.67	8.09 ± 0.33**	4.44 ± 0.35**	3.33 ± 0.84**	1.99 ± 0.80**
AS-Et. 200 mg/kg	371.66 ± 3.63	275.63 ± 1.92**	232.14 ± 2.19**	205.35± 2.56**	185.8± 3.29**	10.18 ± 0.23	7.01 ± 0.35**	3.07 ± 0.30**	2.60 ± 0.40**	1.96 ± 0.42**

Values are expressed as Mean±SEM. (n=6), ANOVA followed by Tukey test. *p<0.05 significant difference, **p<0.00 highly significant difference when compared with Diabetic-control. #p>0.05 non-significant difference when compared with standard

Table no 02: Effect of *A. squamosa* stem bark extracts on Lipid profile, Liver Function and Kidney Parameter in diabetic rats

Groups	Lipid profile (mg/dl)				Liver Function (IU/lit)		Kidney Parameter		
	TG	TC	HDL	LDL	SGPT	SGOT	CRT (mg/dl)	ALB (g/dl)	TP (g/dl)
Ctrl	59.02±0.73	76.91±0.36	63.75±3.83	02.10±0.15	35.80±1.82	36.05±1.47	0.721±0.11	4.734±0.21	5.884±0.14
Diabetic Ctrl	185.61±3.64	77.91±0.36	33.61±3.56	110.3±0.73	97.66±1.12	98.83±1.49	2.115±0.18	1.745±0.18	2.250±0.18
D+ Glyburide (10mg/kg)	22.90±3.22**	122.9±3.42**	63.53±3.51**	45.44±8.09**	50.85±0.37**	49.83±0.37**	1.214±0.10**	3.551±0.19**	3.155±0.18
AS-Et 100 mg/kg	166.7±3.89	170.03±3.76	44.59±3.52	91.49±5.11	71.03±1.47**	77.83±1.11*	1.568±0.30	2.055±0.18	3.437±0.17
AS-Et 200 mg/kg	159.2±3.18**	162.33±3.52*	48.43±3.22**	81.66±0.43*	70.83±1.48**	72.33±1.12**	1.630±0.22	2.258±0.18	3.471±0.19

Values are expressed as Mean±SEM. (n=6), ANOVA followed by Tukey test. *p<0.05 significant difference, **p<0.00 highly significant difference when compared with Diabetic-control. #p>0.05 non-significant difference when compared with standard



Control Group **Diabetic Control** **Diabetic Std**

Diabetic *AS*-ET 100 **Diabetic *AS*-ET 200**

Fig 1. Histopathology of Kidney

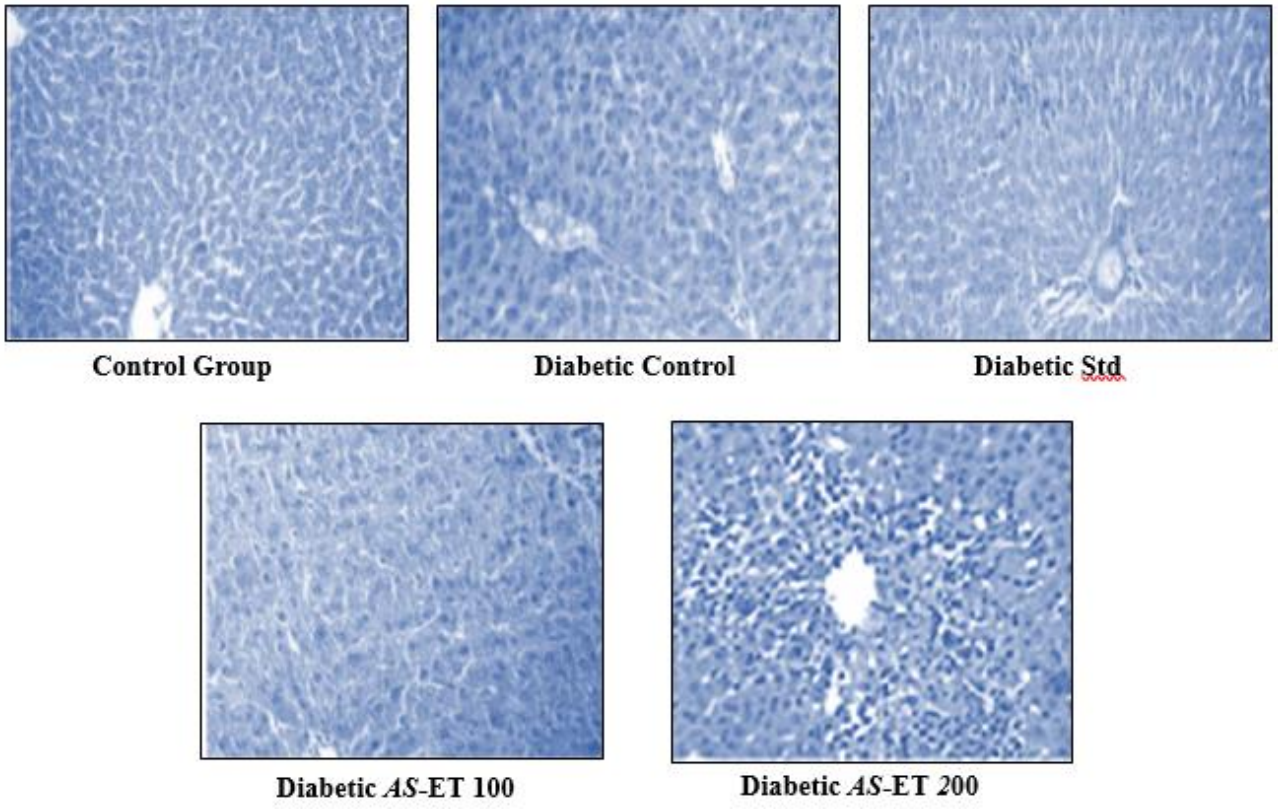


Fig 2. Histopathology of Liver

5. DISCUSSION

In the present study, we investigated the potential antidiabetic properties of *A. squamosa* stem bark extracts using a Streptozotocin (STZ)-induced diabetes model. STZ is commonly utilized in animal research to induce diabetes due to its ability to selectively target and destroy pancreatic beta cells, leading to insulin deficiency and elevated blood glucose levels, mimicking type 1 diabetes in humans.

The findings of this study suggest that the stem bark of *A. squamosa* possesses significant antidiabetic properties. The group treated with the stem bark extract demonstrated a substantial reduction in blood glucose levels compared to the untreated diabetic control group. This decrease in blood glucose levels indicates that the extract may enhance glucose utilization or promote insulin secretion.

Additionally, the improvement in glycemic control was supported by the increased insulin levels observed in the treated group. This suggests that the *A. squamosa* stem bark extract may positively impact pancreatic beta-cell function or insulin production, highlighting its potential therapeutic value in managing diabetes mellitus.

In addition to its antidiabetic effects, the stem bark extract demonstrated potential antioxidant properties, as evidenced by a decrease in malondialdehyde (MDA) levels. Since oxidative stress is a key factor in the progression of diabetes, the extract's ability to neutralize free radicals and reduce oxidative damage may contribute to its antidiabetic effectiveness.

Furthermore, the treated group showed a significant improvement in body weight compared to the untreated diabetic group. Diabetes often causes weight loss due to increased fat and protein breakdown. The observed weight gain suggests that the stem bark extract may help protect against tissue wasting, potentially by enhancing nutrient utilization.

The study also assessed the safety profile of the stem bark extract, with no adverse effects or toxic reactions observed at the administered doses. This information is crucial when considering the potential therapeutic application of the extract for individuals with diabetes.

In conclusion, the findings of this study support the traditional use of *A. squamosa* stem bark in managing diabetes mellitus. However, further research is needed to elucidate the exact mechanisms responsible for its antidiabetic properties.

Additionally, long-term studies on animal models and clinical trials with human participants are crucial to confirm its efficacy and safety for diabetic patients.

6. CONCLUSION

This study provides promising evidence of the antidiabetic potential of *A. squamosa* stem bark extract in a Streptozotocin-induced diabetes mellitus model. Its ability to lower blood glucose levels, enhance insulin secretion, and exhibit antioxidant properties highlights its potential as a candidate for developing complementary and alternative therapies for diabetes management. However, comprehensive research is necessary to validate these results and explore its feasibility as a therapeutic option for diabetic patients.

Ethical approval

The study protocol received approval from the institutional animal ethics committee under reference number SNIOP/CCSEA/IAEC/729/12-12-2023. All ethical oversight and experimental procedures strictly complied with the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was officially registered with the identification number 729/PO/Re/S/11/CPCSEA.

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