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ANTIHYPERGLYCEMIC ACTIVITY OF *Alphonsea Sclerocarpa Thw*. IN STZ-INDUCED DIABETIC RATS

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ABSTRACT

Back ground and objectives: *Alphonsea sclerocarpa* a well-known traditional herb famous for its immense use in folklore medicine. Since diabetes is the most ubiquitous health issue in the century, the drive towards antidiabetic drug discovery is continued for decades. In this regard, the current work is engrossed in determining the antihyperglycemic potential of *A. sclerocarpa* leaves.

Methods: Antihyperglycemic through the Streptozotocin-induced diabetic rat model was performed and Glibenclamide was taken as standard, the flavonoid-rich fraction of *A. sclerocarpa* was given to the animals in two doses (200 and 400 mg/kg body weight), and blood glucose and lipid levels were analyzed along with histological studies. Phytochemical screening, total phenolic, flavonoid, and tannin contents were determined to support this investigation. The antioxidant activity studies by DPPH assay, NO free radical scavenging assay, and total antioxidant assay were also screened using standard protocols.

Results: Preliminary phytochemical screening of the leaf extract exposed various secondary metabolites. The results show that the plant is rich in polyphenolic contents and regularized blood glucose levels at 200 mg/kg and 400mg/kg dose in 15 days. The biochemical studies disclosed that the plant significantly improved the abnormal blood glucose and lipid profile. The histopathological study sustenance the protective effect of the plant against diabetes.

Conclusion: To conclude, all the biochemical data professed that the flavonoid-rich fraction of *A. sclerocarpa* is effective among all the extracts to give protection against oxidative stress and subsequent improvement of the blood sugar levels, serum lipid parameters, and β-cell recovery. The antioxidants of the phytochemicals embedded in the extract may be responsible for the stated activity.

Key words: *Alphonsea sclerocarpa;* antioxidant; anti-hyperglycemic; acute toxicity; lipid profile

INTRODUCTION

Diabetes is one of the progressive metabolic turmoil that became a global concern in the health care system. The chronic cumulative disorders are the culprits that turn diabetes into a serious health hurdle by cramming other metabolic functions. Plant-based medicine is gaining importance nowadays that led to the exploration of massive plant-derived compounds to use various ailments [1,2]. India is a hotspot for medicinal plants, and the utilization of natural medicine is well documented in traditional literature such as Ayurveda, Unani, and Sidha. The advancement in plant research catalyzed the drug discovery from natural products. Ample literature evidenced the application of many plants and plant extracts to handle diabetes and associated complications [3,4].

Alphonsea sclerocarpa belongs to the family Annonaceae, a rarely distributed tree in tropical Asian countries [5]. Traditionally, *Alphonsea* plants are famous for treating infections, urinary problems, kidney stones, etc. [6]. Phytochemical investigation of *A. sclerocarpa* leaves reported the presence of crotsparine sparsiflorine, laurotetanine, isoboldine, liriodenine and petalinemethine. The bark contains liriodenine, anonaine, norushinsunine, ushinsunine, stepharine, stepholidine, candicine, phenethyltrimethylonium and magnoflorine [7]. The plant also possesses polyphenolic components such as tannins and flavonoids. Pharmacologically *A. sclerocarpa* is proved to exhibit antioxidant [8], antibacterial, antifungal [9], anticancer [10], antiurolithiatic activity [11] in various experimental models.

Oxidative stress is causation for many chronic diseases, and foraging free radicals at the cellular level can help to contrive the stress-related physiological challenges [12] such as aging, Alzheimer's disease, Parkinsonism, diabetes, liver, kidney, and heart diseases [13]. Atypical food and food habits, exposure to radiation, chemicals & toxic pollutants can increase the oxidative burden on the cell and disturb the normal physiology of the body. [14,15] Polyphenols are the metabolic end products of the plant and are considered to possess various health-promoting properties [16] and led to the extensive utilization of flavonoid and phenolic-rich food in nutrient diet regime [17].

Alphonsea is a source for various plant secondary metabolites, and few researchers investigated multiple plants in the genus and reported few categories of biomolecules with an established structure. The literature shows that the genus is rich in polyphenolic compounds like tannins, flavonoids, chalcones, dihydrochalcones, lignans, terpenes, steroids, saponins, alkaloids,

proteins, and lipids. The present work aims to evaluate the antihyperglycemic potential of the flavonoid-rich fraction of *Alphonsea sclerocarpa* in the STZ-induced diabetes rat model.

MATERIALS AND METHODS

Plant material

Alphonsea sclerocarpa was collected from the forest areas of Tirupati in February and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, and voucher specimen was (Pt 0332) preserved in the herbarium.

Extraction and Fractionation

Five kilograms of the fresh leaves were shade dried at temperatures 25-30 ºC for 7 days. The dried leaves were powdered in a grinder. The methanolic extract of the leaves was prepared by subjecting to exhaustive extraction using the Soxhlet apparatus for 72 hours with methanol. Then each of the extracts was filtered using cotton plugs followed by Whatman No. 1 filter paper. The filtrates were then concentrated, dried under reduced pressure in the rotary evaporator, and lyophilized to get in powder form. The percentage of yield was calculated.

The methanolic extract was suspended in distilled water and then partitioned between various solvents such as n-hexane, Ethyl acetate, Chloroform, n-butanol, methanol, and water successively to get respective fractions [18].

Experimental animals

In this study, male Wistar rats were selected and were randomly grouped in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2˚C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed free access to water and fed with a standard commercial pelleted rat diet [19]. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional animal ethics committee (IAEC) before experimental studies (1292/ac/09/CPCSEA).

Phytochemical Screening

The preliminary phytochemical screening of all the leaf extracts of *A. sclerocarpa* was performed according to the standard procedures **[20]**.

Total Phenolic content estimation

Preparation of Standard Gallic Acid for Calibration Curve:

Concentrations ranging from 25-100 µg/mL of standard gallic acid solution was prepared by dissolving pure gallic acid in methanol; 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7% Sodium carbonate were also added to make a final volume of 10 mL followed by the 30 minutes incubation at 40°C. The coloured solution

thus obtained was measured at 760 nm using a UV-visible spectrophotometer and a calibration curve was plotted for the average values of the results obtained in triplicates.

Estimation of total Phenolic content:

The total phenolic content of all the extracts of *A. sclerocarpa* was estimated by the Folin Ciocalteu method described by Singleton *et al*., (1965) with slight modifications. Various extracts ranging from 25-100 µg/mL were prepared, and the total phenolic content was estimated as described above. The total phenolic content of the extractswas expressed as mg of gallic acid equivalents (GAE) per gram of sample indry weight (mg/g) **[21]**.

Total Flavonoid content estimation

The flavonoids content for *A. sclerocarpa* was determined by the $AlCl_3$ method using quercetin as standard. 125µL of the extract solution was added to 75 µL of a 5% $NaNO_2$ solution. After 6 min, 150 µL of 10% $AlCl_3$ was added, followed by 5 min of incubation. 750 µL of 1M NaOH solution was added to this mixture, diluted the final volume to 2500 µL with distilled water, and incubated for 15 min to get the pink colour. The absorbance was measured at 510 nm. The total flavonoid content was expressed as µg of quercetin equivalents per mg dry matter (µg QE/ mg dry weight) using thecalibration curve. All the experiments were run in triplicate. The mean values and standard deviations were calculated **[22]**.

Total Tannin content estimation

The tannin content for *A. sclerocarpa* was determined by Broadhurst et al. (1978) method with slight modification, taking tannic acid as a standard. 400µL of the extract solution is added to 3 mL of vanillin (4% in methanol) solution and 1.5 mL of concentrated hydrochloric acid followed by 15 min of incubation. The absorbance was read at 500 nm, and the total condensed tannin content was expressed as µg of tannic acid equivalents per mg dry matter (µg TAE/ mg dry weight). All the experiments were run in triplicate. The mean values and standard deviations were calculated **[23]**.

In vitro antioxidant activity

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay

In DPPH assay, 1ml of different concentrations (25-200 µg/ml) of *A. sclerocarpa* extracts were added to the reference solution (0.004% in methanol) in test tubes. The tubes were incubated in the dark for 30min at room temperature; absorbance of the reaction mixture was measured in UV Visible spectrophotometer (Labindia 3000+) at 517nm. Ascorbic acid was usedas standard. Methanol replacing the extract/ascorbic acid served as control (i.e., 1ml of methanol + 3ml of DPPH radical solution). Inhibition of DPPH radicals (%) was calculated and IC_{50}

was determined **[24]**.

% Scavenging activity =

$[(A_{control} - A_{test})/A_{control}] \times 100$

NO free radical scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of Griess Ilosvoy rection. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of *A. sclerocarpa* extracts at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture, 0.5ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5min. Finally, 1.0 ml naphthethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min **[25]**. Standard Ascorbic acid was taken for the comparison and the absorbance at 540nm was measured with a spectrophotometer, and percentage inhibition was calculated as

% NO radical scavenging activity =
$[(A_{control} - A\ test)/A_{control}] \times 100$

Total antioxidant activity

Total antioxidant activity for *A. sclerocarpa* was determined by the phosphomolybdenum method. 0.1 ml of various concentrations of plant extracts (25-200µg/ml) were added to 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). After an incubation of 90 minutes at 95°C, samples were cooled to room temperature, and theabsorbance of the mixture was measured at 695nm using a UV Visible spectrophotometer (Labindia 3000+). Ascorbic acid was used as standard. 0.1ml of methanol was used as blank **[26]**.

Acute toxicity studies

Acute oral toxicity study of the flavonoid-rich fraction of *A. sclerocarpa* was carried according to OECD-425 guidelines. A dose of 2000 mg/kg, body weight was administered orally to the first rat, and this rat was observed for mortality and clinical signs like aggressiveness, restlessness, sedation tremor, ataxia, paralysis, convulsion, prostration, unusual locomotion, etc. for the first hour, then hourly for 3 h and, finally periodically until 48 h (short term toxicity). The animal was survived, and then four additional rats were orally administered at dose 2000 mg/kg, sequentially at 48-h intervals. All the experimental animals were maintained under close observation for 14 days (long-term outcomes), and the number of ratsthat died within the study period was noted. The 50% lethal dose (LD_{50}) valuewas calculated **[27]**.

Antihyperglycemic activity in albino Wistar rats

STZ- induced diabetic model was used to assess the antihyperglycemic property of the flavonoid-rich fraction of *A. sclerocarpa* **[28]**. Animals were kept for fasting 24 hrs before the induction of diabetes. Streptozotocin (60mg/kg body weight, I.P.) was administered as a single dose in a normal saline solution to induce diabetes in rats. Animals with diabetes were divided into five groups (I, II, III, IV, and V), having five rats in each group **[29]**. Group-I & II served as Normal and diabetic control, respectively. Group-III and group-IV (test groups) received 200mg/kg and 400mg/kg of the flavonoid-rich fraction of *A. sclerocarpa* for 15 days, and group-V (standard group) received glibenclamide (5mg/kg p.o.). Serum was collected and analyzed for glucose, cholesterol, triglycerides, LDL, VLDL, and HDL using the Automatic Biochemistry Analyzer.

Histopathological study

The pancreas was separated from the rats on the 15th day, retained in the formalin solution. Hematoxylin and eosin were used to stain the incised sections of pancreatic tissue (4 microns) and subjected to histological examination.

RESULTS Phytochemical Screening

Preliminary phytochemical screening of all the extracts revealed that the plant is rich in various phytochemicals such as alkaloids, glycosides, terpenoids, steroids, tannins, flavonoids, saponins, carbohydrates, and proteins.

Total Phenolic content estimation

A calibration curve was plotted by the absorbance and concentrations (mg/mL) using prepared dilutions. The regression analysis was performed, and the resulting equation was Abs= y = 6.827x + 0.2228. The coefficient of determination for standard curves was greater than 0.99 (R^2 = 0.9918). Thus, the calculated straight line could explain more than 99% of the experimental data. The total phenolic content of the extracts was calculated and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Where y is the absorbance at 760 nm and x is the total phenolic content in the extracts of *A. sclerocarpa*. The values are varied from 52.3±1.35 mg GAE/g for the n-hexane extract to 115.18±1.87 mg GAE/g for the methanol extract **(Table 1)** which are lower and highest content values.

Total Flavonoid content estimation:

The total flavonoid content profile of the plant extracts was established through the colorimetric method using $AlCl_3$. A calibration curve (y = 7.6262x + 0.0831, R^2 = 0.9953) was plotted using various concentrations of standard quercetin (0–100 µg/mL) and expressed in quercetin equivalents (QE) per gram. The total flavonoid content of the extracts was calculated and expressed

as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). Where y is the absorbance at 760 nm and x is the total flavonoid content in the extracts of *A. sclerocarpa* were depicted in **Table 1**. The results show that methanolic fraction is found to possess high flavonoid content 28.37±2.05 mg QE/mg dry weight followed by the n-butanol extract.

Total condensed tannin content

The total tannin content of the plant extracts was determined using the Vanillin-HCl colorimetric method. A calibration curve ($y = 6.9973x + 0.1014$, $R^2 = 0.9953$) was plotted using various concentrations of standard Tannic acid (0–100 µg/mL) and expressed in Tannic equivalents (TAE) per gram. The total tannin content of the extracts was calculated and expressed as mg Tannic acid equivalents (TAE) per gram of sample in dry weight (mg/g). Where y is the absorbance at 760 nm and x is the total tannin content in the extracts of *A. sclerocarpa* in µg TAE per mg dry weight of the extract. Results disclosed that the methanolic fraction is lavished with high tannin content 198.65±1.87 mg TAE/mg dry weight followed by water extract 124.57±1.05 mg TAE/mg dry weight **(Table 1)**.

In vitro antioxidant activity DPPH assay

Antioxidant potential of all the extracts of A. *sclerocarpa* leaves was evaluated using DPPH assay and observed in a dose-the dependent manner in comparison with standard Ascorbic acid. A calibration curve ($y = 6.7859x + 0.2233$, $R^2 = 0.9928$) was plotted using various concentrations of standard Ascorbic acid (0–75 µg/mL) and expressed in percentage. When compared to the standard Ascorbic acid (88.27±2.76), Methanol (79.32±1.88) and n-butanol (56.36±1.42) fractions were found comparatively better inhibitors than other fractions **(Table 2)**. IC_{50} values 30.45 and 55.32 µg/mL of methanol and n-butanol fractions were also comparable with the standard (22.67 µg/mL).

NO free radical assay

All the leaf fractions of *A. sclerocarpa* were evaluated for nitrite free radical scavenging property. Percentage free radical scavenging was plotted against the concentration of the extracts. The plants exhibited antioxidant activity by competing with oxygen to scavenge for the nitrite radical generated from sodium nitroprusside at physiological pH in an aqueous environment. The antioxidant activity increased with an increased polarity up to methanol in a dose-dependent manner. The maximum free radical scavenging activity was interpolated to give results, as shown in Table 3. *A. sclerocarpa* methanol fraction (77.34%) inhibited the nitrite radical efficiently compared to the other fractions compared with standard Ascorbic acid (81.73%). *A. sclerocarpa* had maximal scavenging activity with a potent IC50 value 75 µg/mL followed by the n-butanol fraction (61.04 µg/mL) ascorbic acid is having 28.52 µg/mL.

Total antioxidant activity

Phosphomolybdenum assay was used to determine the total antioxidant activity of the *A. sclerocarpa* leaf fractions. Ascorbic acid being as a reference standard for comparison (83.12±1.54 mg TE/g), methanolic fraction showed comparatively better total antioxidant activity than other fractions; methanolic leaf fraction of *A. sclerocarpa* (78.38±1.77) mg TE/g), exhibited higher antioxidant activity followed by methanolic leaf fraction of *A. sclerocarpa* **(Table 4)**. IC_{50} values of the fractions were comparable with the standard 29.78 µg/ml and calculated as 38.39 & 59.22 µg/ml respectively.

Acute toxicity studies

From the results, it is evident that the administration of flavonoid-rich fraction of *A. sclerocarpa* leaf was safe up to a dose of 2000 mg/kg. No aforementioned toxic symptoms or mortality were observed at this dose. Hence, the present study selected 1/10th and 1/5th of 2000 mg/kg, i.e., 200 mg/kg and 400 mg/kg as working doses.

Anti-hyperglycemic activity

In the current study, the flavonoid-rich fraction of *A. sclerocarpa* was investigated for its antihyperglycemic activity in STZ-induced diabetic rats. After the induction of diabetes, the rats with diabetes were treated with the extract for 15 days. **Table 5** designates the fluctuations in body weight in normal and diabetic rats. STZ significantly reduced body weight in the diabetic group compared to the normal group during the study. Diabetic control rats sustained to drop weight till the end of the study while *A. sclerocarpa* treated rats at a dose (200 & 400 mg/kg body weight) showed significant improvement in body weight (126.62 & 129.32gm) compared to the diabetic control group (118gm) after 5 days.

Results of the effect of *A. sclerocarpa* on STZ-induced diabetic rats are presented in **Table 6**. From the 5^{th} day, a substantial reduction in blood glucose levels was noticed. The antihyperglycemic effect was extreme on the 15^{th} day, at a dose of 400 mg/kg body weight of *A. sclerocarpa* (163.8mg/dL), the results were comparable with that of glibenclamide (134.8mg/dL). The outcomes of the current investigation evidently showed that the flavonoid-rich fraction of *A. sclerocarpa* exhibited significant antihyperglycemic activity (Figure 1).

Effect of *A. sclerocarpa* on the lipid profile of the animals was also compared with the controls. The results show that the streptozotocin disturbed the animals' lipid metabolism, and the successive treatment with test and standard groups restored the lipid profile **(Table 7)**. After 15 days of the treatment of the diabetic group with the extracts, the serum cholesterol, triglyceride levels significantly declined ($p<0.001$) and

HDL level was improved similar to the standardglibenclamide.

Histopathological study

Glibenclamide successfully restored the aberrant anatomical features of the Langerhans, such as ruptured pancreatic cells, dilated pancreatic canal, and enlargement of epithelial tissue caused by streptozotocin treatment. The flavonoid-rich fraction of *A. sclerocarpa* revived the β-cells with improved anatomical architecture at the given doses proportional. After 15 days of treatment, the extract exhibited marked enhancement in the β-cells and invigorated morphological contours, as shown in **Figure 2**.

Table 1: Phytochemical profile of *A. sclerocarpa* extracts

Extracts	Total Phenolic content (mg GAE/mg dry weight)	Total flavonoid content (mg QE/mg dry weight)	Total condensed tannin content (mg TAE/mg dry weight)
***A. sclerocarpa* n-hexane extract (ASH)**	**52.3±1.35**	**2.14±1.04**	**1.02±2.31**
***A. sclerocarpa* Ethyl acetate extract (ASE)**	**74.52±0.65**	**10.07±1.25**	**24.24±2.09**
***A. sclerocarpa* Chloroform extract (ASC)**	**81.79±2.34**	**18.61±1.67**	**31.02±0.38**
***A. sclerocarpa* n-butanol extract (ASB)**	**113.56±1.95**	**24.13±1.63**	**35.43±1.64**
***A. sclerocarpa* methanol extract (ASM)**	**115.18±1.87**	**28.37±2.05**	**198.65±1.87**
***A. sclerocarpa* water extract (ASW)**	**58.35±2.04**	**9.94±2.07**	**124.57±1.05**

Values are expressed as mean ± SD, done in triplicates

Table 2: DPPH Scavenging activity of *A. sclerocarpa* extracts with IC_{50} values

	Percentage of inhibition						
Conc µg/ml	**AA**	**ASH**	**ASE**	**ASC**	**ASB**	**ASM**	**ASW**
10	**44.18±1.45**	**8.21±1.34**	**12.87±2.58**	**6.74±0.47**	**32.75±1.11**	**36.79±1.29**	**15.11±1.07**
15	**57.3±0.13**	**9.98±1.87**	**18.13±1.46**	**8.02±2.47**	**35.67±2.74**	**54.43±1.07**	**21.17±0.37**
25	**65.47±1.14**	**14.03±1.91**	**22.57±4.31**	**9.22±0.42**	**40.12±1.61**	**57.33±1.64**	**24.39±1.54**
50	**71.38±1.22**	**16.14±0.57**	**29.64±1.74**	**11.71±1.48**	**46.27±1.49**	**65.71±1.39**	**32.74±1.39**
75	**88.27±2.76**	**18.11±1.65**	**39.41±1.43**	**15.73±0.62**	**56.36±1.42**	**79.32±1.88**	**47.21±1.22**
IC_{50} µg/ml	**22.67**	**220.14**	**130.09**	**174.57**	**55.32**	**30.45**	**78.12**

Values are expressed as mean ± SD, done in triplicates

Table 3: NO free radical Scavenging activity of *A. sclerocarpa* extracts

Conc µg/ml	AA	ASH	ASE	ASC	ASB	ASM	ASW
10	34.28±0.91	7.12±±1.26	8.2±0.55	7.74±1.43	31.3±0.49	27.17±0.33	14.38±1.27
15	48.42±0.25	8.21±1.83	9.32±1.08	8.89±1.28	31.78±0.45	42.97±1.38	16.47±1.58
25	44.48±1.87	10.39±1.22	10.67±1.07	10.07±1.17	38.76±1.27	38.34±1.26	19.94±1.53
50	62.32±1.24	12.01±1.51	16.74±0.44	14.35±1.53	41.87±1.39	54.04±1.67	27.47±1.35
75	81.73±1.03	15.12±1.33	21.17±0.89	19.15±1.59	57.23±1.46	77.34±1.28	36.23±1.49
IC_{50}	28.52	378.15	215.91	254.93	61.04	38.01	117.17

Values are expressed as mean ± SD, done in triplicates

Table 4: Total antioxidant activity of *A. sclerocarpa* extracts

Conc µg/ml	AA	ASH	ASE	ASC	ASB	ASM	ASW
10	36.17±0.84	8.22±1.34	9.91±1.28	8.47±1.63	21.37±1.96	28.84±1.04	16.44±1.08
15	39.39±1.22	10.18±1.39	10.87±1.39	10.08±1.27	21.48±1.73	32.14±0.54	17.89±0.23
25	45.87±1.36	13.08±1.26	11.98±1.27	12.09±1.83	34.12±1.65	41.17±1.63	21.33±0.74
50	64.38±1.27	14.27±1.38	19.07±1.23	15.28±1.97	43.17±1.85	56.74±1.32	28.14±1.39
75	83.12±1.54	16.54±1.32	23.74±1.65	21.08±2.36	59.36±1.72	78.38±1.77	38.12±1.07
IC_{50} µg/ml	29.78	364.89	193.13	235.66	59.22	38.39	113.3

Values are expressed as mean ± SD, done in triplicates

Table 5: Effect of flavonoid-rich fraction of *A. sclerocarpa* on body weight in STZ-induced diabetic rats

Treatment	Body weight in grams			
	Day 0	Day 5	Day 10	Day 15
Group-I (Normal control)	126.04±2.44	126.4±2.57	133.18±4.9	135.36±2.5
Group-II (Diabetic control)	126.4±2.57	118±1.56*	116.24±1.89*	112.84±4.25*
Group-III (200mg/kg of extract)	129.78±2.76	126.62±2.08#	124.22±2.09#	121.22±0.9#
Group-IV (400mg/kg of extract)	129.86±5.96	129.32±2.19#	128.52±2.6#	125.12±4.3#
Group-V (5mg/kg of glibenclamide)	129.36±6.09	131.36±5.86#	130.76±6.3#	126.16±3.91#

Values are given as mean ± SEM for groups of five animals each #P < 0.05 (graph pad prism) using one way analysis followed by Tukey's post hoc test. * Groups were compared with the normal control and # Groups were compared with the diabetic control

Table 6: Effect of flavonoid-rich fraction of *A. sclerocarpa* on Blood Glucose levels in STZ-induced diabetic rats

Treatment	Blood Glucose levels (mg/dL)			
	Day 0	Day 5	Day 10	Day 15
Group-I (Normal control)	92.6±1.14	93.4±1.14	72.9±1.38	73.92±0.94
Group-II (Diabetic control)	278.8±1.3*	359.4±2.7*	386.08±5.24*	408.6±4.15*
Group-III (200mg/kg of extract)	276.8±1.64	350.6±5.45	272.6±2.07#	213.6±2.07#
Group-IV (400mg/kg of extract)	279.2±4.2	321.6±1.51#	211.2±3.96#	163.8±1.48##
Group-V (5mg/kg of glibenclamide)	255.4±6.5#	284.6±4.9##	186.2±2.58##	134.8±2.38##

Values are given as mean ± SEM for groups of five animals each #P < 0.05 ## P < 0.001 (graph pad prism) using one way analysis followed by Tukey's post hoc test. *Groups were compared with the normal control and # Groups were compared with the diabetic control.

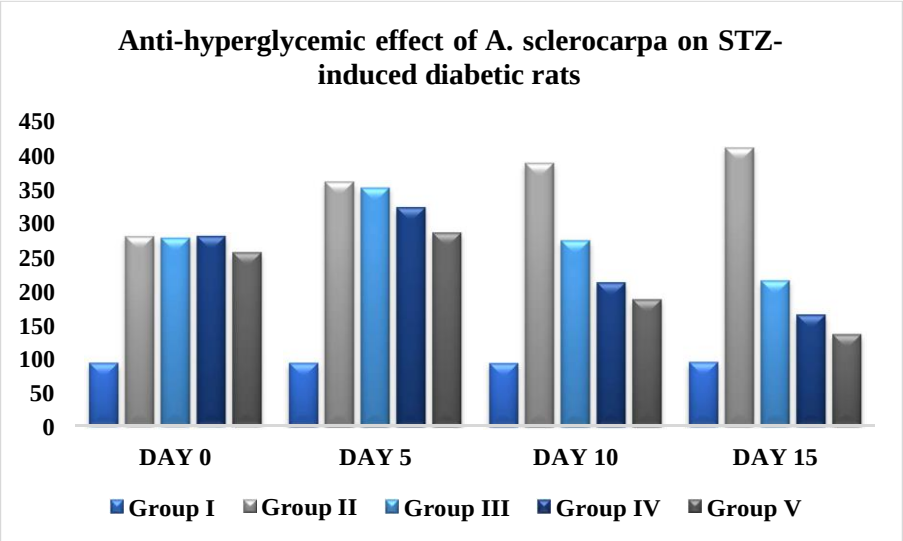

Figure 1: Effect of flavonoid-rich fraction of *A. sclerocarpa* on blood glucose levels in STZ-induced diabetic rats

Table 7: Effect of flavonoid-rich fraction of *A. sclerocarpa* on lipid profile in STZ-induced diabetic rats

Treatment	Effect of *A. sclerocarpa* on lipid profile in STZ-induced diabetic rats on 15th day				
	Cholesterol levels	Triglyceride levels	LDL levels	VLDL levels	HDL levels
Group-I (Normal control)	75.02±1.14	77.23±2.13	30.71±1.03	16.15±0.87	30.29±1.34
Group-II (Diabetic control)	226.14±2.16*	211.03±2.95*	171.08±1.27*	42.27±1.54*	12.26±0.6*
Group-III (200mg/kg of extract)	152.04±1.34#	128.11±2.54#	92.11±2.06#	26.34±1.07#	29.73±0.62#
Group-IV (400mg/kg of extract)	145.32±1.34#	114.16±1.32#	74.33±2.98#	21.27±0.6#	39.74±1.95#
Group-V (5mg/kg of glibenclamide)	126.5± 1.66#	89.14±1.33#	40.14±3.72#	19.13±3.15#	42.35±1.65#

Values are given as mean ± SEM for groups of five animals each # P < 0.001 (graph pad prism) using one way analysis followed by Tukey's post hoc test. * Groups were compared with the normal control and # Groups were compared with the diabetic control

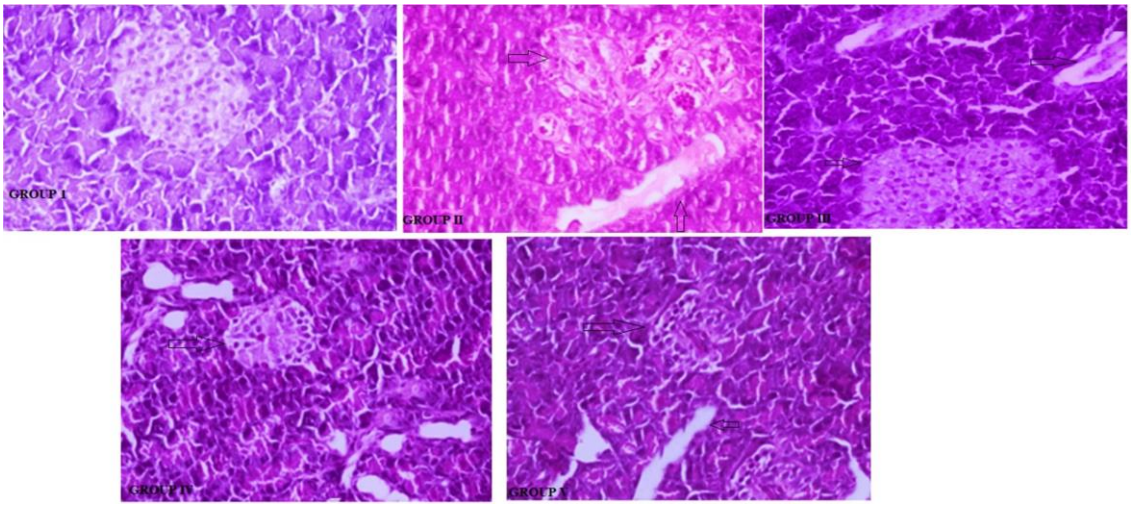

Figure 2: Histopathological study of pancreas

DISCUSSION

Flavonoids and tannins are the major polyphenolic compounds produced as metabolic end products of plants that help the detoxification process. They are considered as powerful agents to neutralize the free radicals due to their ability to react with them and are regarded as antioxidants, supporting the scavenging mechanism of the human body. Ample literature is available to support the protective effect of these polyphenolics to address various chronic and lifestyle disorders associated with stress. Since synthetic agents are associated with fewer side effects, naturally occurring antioxidants are preferred in nutraceuticals for promoting health **[32]**.

It is essential to determine the antioxidant potential of the plants to understand their efficiency in protecting against cellular stress. Numerous procedures were adopted to screen the antioxidant (*In vitro*& *In vivo*) such as DPPH assay, nitric oxide free radical assay, etc **[33]**.

A. sclerocarpa methanolic leaf extract demonstrated the highest antioxidant activity of other extracts. The antioxidant activity was assessed through DPPH assay, NO free radical scavenging assay and total antioxidant assay. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, terpenoids, sterols, etc. Anti-hyperglycemic activity of the flavonoid-rich fraction of *A. sclerocarpa* was established *In vivo* using the STZ-induced rat model by assessing biochemical parameters of the blood. The extracts also improved the lipid profile of the diabetic animals in a dose-dependent manner. The histological studies substantiate the restoration of the β-cell framework with improved function. The mechanism of activity may be due to the protective effect of the extract on the islets of Langerhans or regeneration of the STZ-damaged β cells. Since the induction of diabetes in group-II is by destructing the β cells, a regular dose of the extract (200mg/kg and 400mg/kg per body weight) might have increased the regeneration of the β-cells in group III and IV, which might be the reason for the recovery from the hyperglycemia after 15 days. The presence of various phytochemicals like flavonoids, lignans, tannins, and saponins may contribute to the protection against diabetes.

CONCLUSION

The flavonoid-rich fraction of *A. sclerocarpa* holds substantial anti-hyperglycemic potential in adult Wistar rats in a dose-dependent manner to curb the present study. The polyphenolic compounds present in the fraction such as flavonoids may have a direct relationship with the reported anti-hyperglycemic activity. From the current investigation, it is apparent to report the anti-hyperglycemic activity of

sclerocarpa leaves and further investigation is under progress to comprehend the exact mechanism intricate in the antihyperglycemic action of this plant.

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