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RESEARCH ARTICLE

Evaluation of the Antioxidant, Antihyperglycemic and Hypolipidemic Potential of *Alstonia scholaris* Leaves Extracts in Streptozotocin-Induced Diabetic Rats

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Abstract

The evergreen tree *Alstonia scholaris* (L) R. Br. (Family: Apocynaceae) is native to Australasia, southern China and the tropics of Asia. Despite its importance as a medicinal plant, little is known about its potential role in complementing standard methods of treating diabetes and its associated consequences. Therefore, the present study scientifically investigated extracts from the leaves of *A. scholaris* for their antioxidant (*in vitro*), anti-diabetic, and hypolipidemic effects in rats with type 2 diabetes mellitus. Male Wistar rats were administered streptozotocin (45 mg/kg, i.p.) and fed a high-fat diet to induce type 2 diabetes mellitus. They were treated with 400 mg/kg of an ethyl acetate (EAEAS) and ethanolic (EAAS) extract of *A. scholaris* leaves after complications persisted. Typical drugs were metformin (200 mg/kg) and canagliflozin (10 mg/kg). In the end, blood was drawn to determine various biochemical parameters such as fasting blood sugar, lipid profile and markers of heart, liver and kidney damage. In addition, the rat's weight, urinary glucose concentration, urine volume, blood pressure, Electrocardiogram (ECG), and antioxidant potential of EEAS were measured. The pancreas, heart, kidneys, and liver were all subjected to histopathological analysis. A wide range of biochemical and physiological markers, including blood and urine glucose, lipid profile, markers of heart, kidney and liver damage, antioxidant levels and blood pressure, showed significant improvement in response to EEAS. Histopathology illustrates the reverse modulation in heart, kidney, and liver tissue compared to disease control. Based on the data obtained, the EAEAS achieved is far inferior to that required to treat diabetes mellitus. In summary, this present study demonstrates that EEAS (400 mg/kg) can lower blood sugar levels, fight free radicals, and lower bad cholesterol levels in rats with diabetes and complications. Further investigations can be undertaken to explore its mechanism of action at the molecular level.

Keywords: *Alstonia scholaris*, Anti-hyperglycemia, Antioxidant, Hypolipidemia, Streptozotocin

1. Introduction

Insulin resistance and persistent hyperglycemia are the hallmarks of diabetes mellitus[1]. According to the International Diabetes Federation (IDF) report, 10th edition 2021, 537 million adults aged 20 to 75 are living with diabetes and approximately 6.7 million deaths are attributed worldwide[2]. Organ dysfunction in the liver, heart, kidneys, blood vessels, urogenital tract system, and eyes is a common complication of type 2 diabetes mellitus[3]. Chronic hyperglycemia is associated with a decrease in antioxidant status, an increase in reactive oxygen species formation, and dyslipidemia[4]. The rate of diabetes and its complications evolve most directly with oxidative stress. Although several scientific and pharmacological arsenals are available, including

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many potent hypoglycemic drugs, approximately half of the patients receive the glycolic treatment that is recommended based on our expertise. Side effects of currently available anti-hyperglycemic drugs include hypoglycemia, haematological problems, obesity, osteoporosis, hepatotoxicity, and lactic acidosis. These assessments of diabetics are the reason for the transition from symptomatic and allopathic therapy to natural therapy[5]. Therefore, there is a need to identify effective drugs for diabetes with fewer side effects, and traditional medicinal plants are preferred for this purpose. Globally, Herbal medicines are gaining popularity as a viable option for treating diabetes due to their low cost and low risk of side effects[6,7]. Many medicinal plants are currently available that also reduce the risk of cardiovascular disease due to hyperlipidemia in diabetics[8,9]. *Alstonia scholaris R.Br.* (Apocynaceae) is the medicinal plant described in Ayurveda with its therapeutic importance. Sapta means seven, and Parna means leaves; hence, another name for this plant is Saptaparna. It is widely distributed in India, Thailand, the Philippines, Africa, China, and Australia[10]. Plant-based products possess rich sources of antioxidant activity[11]. Tissue oxidative stress is managed by antioxidants by indirectly enhancing the cell's natural defence mechanisms and directly scavenging oxidative free radicals[12]. Many experimental studies indicate that antioxidants reduce oxidative stress caused by diabetes and manage its complications[13]. Therefore, medicinal plants are gradually gaining attention for the treatment of diabetes with complication management. Antibacterial, anxiolytic, antiulcer, antimicrobial, antioxidant, and antidiarrheal are just a few of its pharmacological effects[14]. Traditionally, the plant has been used to treat whooping cough, chronic bronchitis, asthma and other respiratory infections[15]. Phyto components such as alkaloids, steroids, reducing sugars, and flavonoids are present in the plant. Previous studies only reported the hypoglycemic effect of *A. scholaris* leaves in Wistar rats[16]. However, the anti-hyperglycemic and antioxidant activities of the various active extracts of *A. scholaris* have not yet been recognized, nor has the mechanism involved in its action. Herbal remedies cannot be used therapeutically in the modern era without supporting scientific data. Therefore, the effects of ethyl acetate and ethanolic extract of *A. scholaris* leaves on antioxidant activity, anti-hyperglycemic activity, and lipid-lowering STZ-induced diabetic rats were examined.

2. Materials and Methods

2.1 Plant Authentication and Collection

In September 2020, we collected the leaves of *Alstonia scholaris* from Ahmedabad, Gujarat, India. The botanical identity of the plant material was verified by a taxonomist from Gujarat University (GU), Ahmedabad. The AS-1 specimen has been deposited in the GU herbarium in Ahmedabad for further reference.

2.2 Chemicals

Streptozotocin (STZ) was procured from ANJ biomedical, USA. The standard drugs are metformin from Apollo, Knoll Pharmaceuticals Ltd., and canagliflozin from INVOKANA, Johnson & Johnson. The chemicals and solvents used in the experiments were the entire analytical grade.

2.3 Preparation of Various Extracts

The leaves of *A. scholaris* were thoroughly washed with water and shaded at room temperature for seven days. Subsequently, they were reduced to a powder. The leaves of A. scholars were extracted using a Soxhlet apparatus with Petroleum Ether (PE), Chloroform (CH), Ethyl Acetate (EA), and Ethanol (Et) as solvents. The pulverized leaves of *A. scholaris* were packed in a Soxhlet apparatus and extracted with these four solvents by successive methods for 48 hours. A rotary evaporator was then used to concentrate the extracts. The percentage yields of PE, CH, EA, and Et were 4.2 ± 0.33 %, 8.2 ± 0.54 %, 13 ± 0.36 %, and 9.3 ± 0.71 % (w/w), respectively. For future research purposes, the powdered extracts were stored in a cool and dark place.

2.4 Preliminary Phytochemical Screening

The photochemical approach was used to perform a qualitative screening for bioactive components in four different *A. scholaris* preparations[17].

2.5 Animal

Male albino Wistar rats (230-250 g) were considered for this present experiment. All animals were maintained at 22°C temperature, 12–12 hour light-dark cycle, and 55% relative humidity. The rats were allowed to consume water ad libitum and SPD (Standard Pellet Diet) with 3% fat, 55% carbohydrates and 20% protein with 310 kJ/kg total calories. High-fat content with 15% butter, 10% egg yolk, 2% coconut oil, and 73% standard feed provides 412 kJ/kg calories for animals[18]. The study protocol with reference number: LMCP/Cology/17/09 was sanctioned by the Institutional Animal Ethics Committee (IAEC) according to Committee for Control and Supervision of Experiment on Animals (CPCSEA) guidelines, Animal Welfare Division, India[19].

2.6 Assessment of the Anti-Diabetic Effect of the EAEAS and EEAS

2.6.1 Induced Experimental T2DM

Animals were freely fed a high-fat diet for four weeks except for the vehicle control group. A single dose of STZ (45 mg/kg/i.p.) in chilled 0.1 M sodium citrate buffer adjusted to pH 4.3 was administered[20]. Drinking water was replaced with a 5% sucrose solution before and after the administration of STZ to overcome drug-induced hypoglycemia[21]. Moderately diabetic range rats (250–400 mg/dl blood glucose level) were included in the study. Then, the diabetic rats were kept for four weeks for the induction of complications.

2.6.2 Experimental Design and Treatment Protocol

Thirty-six rats were randomized into six different groups (G). Each group carried six animals.

G I: Vehicle Control (VC) treated with 0.9% saline

G II: Diabetes Control (DC) treated with STZ (45 mg/kg, i.p)

G III: EAEAS (400 mg/kg, PO bw)

G IV: EEAS (400 mg/kg, PO bw)

G V: MET (200 mg/kg, PO bw)

G VI: CANA (10 mg/kg, PO bw)

The dose of both extracts was selected based on the previously performed OGTT analysis. Both extracts and standard drugs were dissolved in 1% sodium Carboxymethylcellulose (CMC).

2.7 Bodyweight, Food, and Water Intake

The weight, water, and food intake of the rats were measured every week during the experiment.

2.8 Fasting Blood Glucose (FBG)

Throughout the experiment, FBG readings were recorded weekly. They were tested by pricking the tail veins of the rats with a glucometer (Accu Check, Germany). Estimates of fasting serum glucose levels were made on the last day of the experiment using a diagnostic kit and spectrometry (Span Diagnostic Ltd., Surat, and Gujarat, India).

2.9 Urine Output and Glucose Concentration

A metabolic cage was used to collect urine from animals. The urinary output was measured in ml using a glass cylinder. Glucose concentrations in urine were determined using a urine glucose assay kit (Oxidase method, India).

2.10 Analysis of Biochemical Parameters

In the end, the biochemical parameters were determined by collecting blood from the retro-orbital of Wistar rats. They were kept at room temperature for 30 min before centrifugation. They were centrifuged at 2500 rpm for 15 min at 4°C to isolate the serum. The appropriate spectrometric diagnostic kit was used to examine all biochemical variables.

2.10.1 Lipid Profile

Serum Total Cholesterol (T-CH), Triglyceride (TG), and High-Density Lipoprotein Cholesterol (HDL-CH) were determined with diagnostic kits (ERBA Diagnostics, Gujarat, India). Friedewald's formula[22] was used to calculate Low-Density Lipoprotein Cholesterol (LDL-CH) and very–low-density lipoprotein cholesterol (VLDL-CH).

$$\text{VLDL-CH} = \text{TG } 5 \text{ LDL-CH} = \text{T-CH} - (\text{HDL-CH} + \text{VLDL-CH})$$

2.10.2 Cardiac Function Parameters

Standard diagnostic kit instructions were followed to measure cardiac injury markers Lactate Dehydrogenase (LDH) and Creatine Phosphokinase-MB (CK-MB) (Crest Biosystems, Goa, India). An automated plate reader was used to analyze the microplates (Lab System Multi-scan 51118220, Thermo Bioanalysis, Helsinki, Finland).

2.10.3 Liver Function Parameters

At the end of the study, Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were estimated with standard diagnostic kits (Crest Biosystems, Goa, India). Microplates were stored for further analysis using a plate reader.

2.10.4 Kidney Function Parameters

The levels of urea and creatinine were measured using standard test kits (Span Diagnostics, India).

2.11 *In Vitro* Liver Antioxidant Activity

2.11.1 Goat Liver Slices Preparation

Goat liver was chosen as the mammalian tissue to analyze the antioxidant effects of EEAS[23]. Fresh livers were collected from the local slaughterhouse immediately after the animal was sacrificed. The tissue was quickly immersed in a cold sterile (4°C) Phosphate Buffer Saline (PBS) solution. Liver tissue (1 mm) was cut with a sterile scalpel. 250 mg tissue was stored in sterile PBS (1.0 ml). Both H_2O_2 and EEAS were mixed to incubate for 1 hour at 37°C with gentle shaking. Suitable Normal Control (NC) groups were established and H_2O_2 (2 ml/kg tissue) was used as the standard oxidant. After the incubation, a homogenizer was used to homogenize liver tissue with PBS buffer and centrifuged to collect the supernatant. It was used to estimate various parameters for evaluating the antioxidant potential.

2.11.2 Assessment of Enzymatic Antioxidants

Homogenized tissue (goat liver) was used for enzymatic antioxidant analysis, including Superoxide Dismutase (SOD) was estimated by Misra, *et al*.,[24] methods, reduced Glutathione (GSH) activity was estimated by the method of Moron, *et al*.,[25] and Catalase (CAT) was measured according to the procedure of Jhansyrani, *et al*[26].

2.12 Blood Pressure and Electrocardiogram (ECG)

Rats were given urethane i.p. at a dose of 1.3–1.5 g/kg in a solution of 1.5 g/5 ml. They were cannulated carotid arteries for monitoring systemic blood pressure and heart rate using the BIOPAC MP-150 data acquisition system (Biopac Systems Inc. Camino Goleta, CA, USA). ECG signals were also recorded using the appropriate three-needle electrode method for the limb lead at position II[27]. Three-needle electrodes were attached to theleft arm, right arm, and left hind leg. A negative electrode was placed under the skin of the front paw of the right arm and the positive electrode was placed on the left hind paw. Analysis and documentation of changes in the P wave, QT interval, and QRS complex in ECG were performed.

2.13 Histopathology

In the end, animals were sacrificed from which the heart, pancreas, kidney, and liver were isolated for histopathological examination. Formalin (10% v/v) was used to preserve all tissues of these organs after they had been irrigated with normal saline. Hematoxylin and eosin were used to stain each part of the organ (H and E). Digital microscope photos (MLX-B plus, Magnus microscope, China) were captured at 40x or 10x magnification to record histological details.

2.14 Statistical Analysis of Data

All data were presented in mean±SEM (Standard Error Mean) form. All data information was obtained from a one-way ANOVA following Tukey's multiple comparisons with Graph pad Prism 8.01 software. Compared to the respective controls, $P<0.05$ (p-value) was identified as statistically significant.

3. Results

3.1 Qualitative Phytochemical Screening

The phytochemical screening of EAEAS and EEAS reveals the presence of flavonoids, alkaloids, and steroids as the main chemical constituents, followed by a standard protocol[28].

3.2 Bodyweight Water and Food Intake

Table 1 summarizes the weight, water intake, and food intake data of male Wistar rats during the experiments. DC participants lost more weight compared to those in the vehicle control group. After four weeks of treatment, the body weights of the rats in the EEAS group improved significantly compared to those in the DC group. The diabetic group consumed significantly ($p<0.05$) more water than the VC group. There was no discernible difference in water consumption between the EEAS-treated group and the diabetic rat group. Comparing the DC to the VC, we find that the DC significantly $p<0.05$ increases its food consumption. At the end of the treatment period, the EEAS groups showed a statistical ($p<0.05$) reduction in food intake compared to the DC group. The water intake of the DC was significantly ($p<0.05$) increased compared with the VC. As a final result, the EEAS treatment group showed no significant difference in water intake compared with diabetic rats. A significant ($p<0.05$) increase in food intake was observed in the DC compared to the VC. The EEAS groups showed a significant ($p<0.05$) reduction in food intake compared to the DC group on the 28th day of the treatment period.

3.3 Fasting Blood Glucose

Figure 1 summarizes the data on the FBG levels of the various groups. A reduction in fasting blood glucose levels was observed after treatment with EAEAS and EEAS compared to the DC group. EAEAS and EEAS, reduced fasting blood glucose by 70.94% and 56.14% in diabetic rats. Canagliflozin and metformin, two common diabetes drugs, have been shown to reduce fasting blood glucose levels by 45.25% and 53.07%, respectively.

Table 1. Effect of EAEAS and EEAS on body weight, food and water intake in STZ-induced diabetic

Experimental groups	Body weight (gm)		Food intake (gm/rat/day)		Water intake (ml/rat/day)	
	Initial	Final	Initial	Final	Initial	Final
VC	240.7±3.81	309.7±13.65	20.9±0.6	24.68±0.51	24.01±1.683	38.49±1.831
DC	246.8±2.27	196.3±8.25***	22.45±0.35	30.7±1.1*	23.8±0.19	63.15±2.15**
EAEAS	248.16±5.24	197±6.97***	22.88±0.28	28±0.5	21.9±0.6	56.2±2.2**
EEAS	246±6.40	228.7±13.29$^{\#\#}$	22.83±0.16	25.78±0.61$^{\#}$	22.31±1.01	63.65±0.64***
CANA	244.67±1.88	240.7±1.88$^{\#\#\#}$	24.4±0.4	25.56±0.76$^{\#}$	25.47±0.13	69.11±1.11***
MET	208.5±3.56	257.7±4.49$^{\#\#}$	23.63±0.03	21.58±1.03$^{\#\#}$	25.31±0.98	48.2±3.2$^{\#}$

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ as compared to VC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

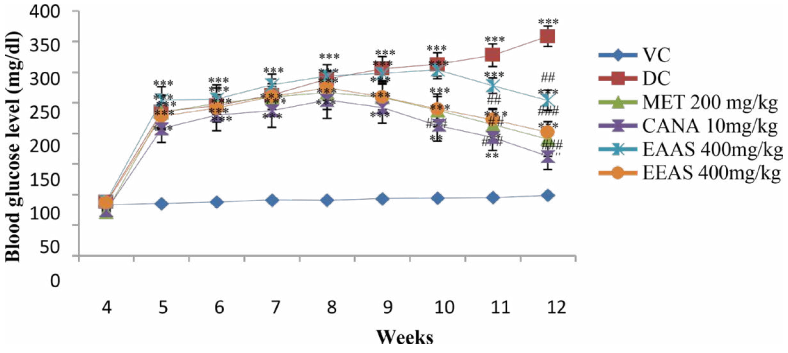

Figure 1. Effects of EAEAS and EEAS on blood glucose levels in STZ-induced diabetic rats. The results are expressed in Mean ± SEM. (n = 6). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ as compared to VC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

3.4 Urine Output and Glucose Concentration

There was a statistically significant ($p<0.001$) increase in urination in diabetic rats compared to VC (Table 2). In diabetic rats, urine output was significantly ($p<0.001$) increased compared to VC. Compared to DC, EEAS treatment showed no significant effect on urinary output. Based on the results, the data of the EEAS-treated group showed no significant difference in urinary glucose concentration compared to diabetic rats.

3.5 Lipid Profile

Table 3 compiles the lipid profile changes caused by EAEAS and EEAS. The effects of EAEAS and EEAS on lipid profile. The STZ-induced diabetic rats reflected a significant increase in TG, T-CH, LDL-CH, and VLDL-CH while HDL-CH decreased compared to VC. EAEAS and EEAS (400 mg/kg) showed a significant decrease in lipid profile, while both treated groups significantly improved HDL-CH levels.

3.6 Cardiac, Liver, and Kidney Parameters

The results of the heart, liver, and kidney parameters are summarized in Table 3. The EEAS treatment group significantly ($p<0.001$) improved CK-MB and LDH levels compared to diabetic rats. Treatment with EEAS significantly ($p<0.001$) reduced ALT and AST levels in diabetic rats. Oral administration of EEAS lowers serum urea and creatinine levels. Standard drugs significantly improved markers of cardiac, renal, and hepatic damage in diabetic rats.

Table 2. Effect of EAEAS and EEAS in urine profile in STZ-induced diabetic rats

Parameters	VC	DC	EAEAS	EEAS	CANA	MET
Urine output (ml/rat/day)	16.38± 0.36	37.52±0.69***	31.34±1.41***	39.35±1.08***	43.78±0.57***	25.24±1.20##
Urine glucose level (mg/dl)	6.76±0.70	465.23±16.5***	407.63±11.1#	484±10.2	550.46±10.13###	374.29±10###

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6*p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; #p < 0.05, ##p < 0.01 and ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison

Table 3. Effect of EAEAS and EEAS on biochemical parameters in STZ-induced diabetic rats

Parameters		VC	DC	EAEAS	EEAS	CANA	MET
Lipid profile	CH (mg/dl)	129.90±3.183	257.00±5.55***	220.00±1.06###	173.40±2.78###	174.20±3.23###	196.00±3.68###
	TG (mg/dl)	80.04±1.23	130.00±0.49***	108.3±4.11###	98.00±3.46###	82.77±3.29###	98.23±3.52###
	HDL (mg/dl)	85.82±2.09	56.95±3.9***	50.55±2.15#	85.07±2.74###	94.47±3.06###	87.93±4.22###
	LDL (mg/dl)	30.45.±2.24	174.00±1.81***	148.30±3.93##	68.40±3.48###	62.86±1.79###	88.54±4.48###
	VLDL (mg/dl)	16.01±0.24	26.14±0.49***	21.67±0.82###	19.60±0.69###	16.55±0.65###	19.65±0.65###
Liver function test	ALT (IU/L)	37.48±2.87	76.96±2.51***	70.20±1.90	57.83±2.93#	52.00±5.02##	58.30±0.95#
	AST (IU/L)	33.95±2.10	63.82±2.22***	60.75±0.95	50.03±4.71#	44.73±4.43##	49.33±1.81#
Kidney function test	Creatinine (mg/dl)	0.58±0.01	0.88±0.01***	0.89±0.02	0.73±0.03#	0.63±0.03###	0.73±0.06#
	UREA (mg/dl)	20.00±0.86	33.75±1.49***	30.19±2.19	20.57±1.01###	17.17±0.46###	21.54±1.70###
Cardiac function test	CK-MB (IU/L)	14.47±0.48	30.96±2.06***	25.45±1.75	19.03±0.93###	18.23±0.93###	18.40±0.58###
	LDH (IU/L)	136.80±3.06	240.00±19.10***	196.50±14.50	149.00±10.97###	138.70±3.75###	143.00±5.85###

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; #p < 0.05, ##p < 0.01 and ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey multiple tests for comparison.

3.7 Liver Oxidative Stress of Enzymatic and Non-Enzymatic Biomarkers

Figure 2 illustrates SOD, CAT, and GSH antioxidant activity performed on goat liver tissue reflecting a significant ($p<0.001$) declination in the toxic (H_2O_2 exposed) group compared to the untreated-Normal Control (NC). The EEAS treatment group has significantly ($p<0.001$) increased SOD, GSH, and CAT levels compared with the Toxic Group (TG).

3.8 Effect of EEAS on Blood Pressure and ECG

At the end of the study, Systolic (SBP) and Diastolic Blood Pressure (DBP) were elevated in the DC group (Table 4). Compared to vehicle control, EEAS significantly ($p>0.05$) reduces SBP but does not affect DBP. STZ-induced diabetic rats had a lower heart rate while they improved in the EEAS-treated group. Table 4 shows the relationship between PR interval, QT interval, and QRS duration. Both the PR interval and the QRS duration were significantly longer in the STZ-induced diabetic rats. Diabetic rats treated with EEAS observed a reduction in prolonged intervals.

3.9 Histology Study of Liver, Pancreas, Kidney, and Heart

Figure 3 shows a histological section of the liver. No obvious microscopic or morphological changes were found in the tissue sample of the VC group. Hepatocytes with significant vacuolation in their cytoplasm, a disrupted portal connection, disorganized hepatocytes, and a central vein were seen in the DC group. The microvasculature of hepatocytes was found to be moderately enhanced in the CANA group. Hepatocyte,

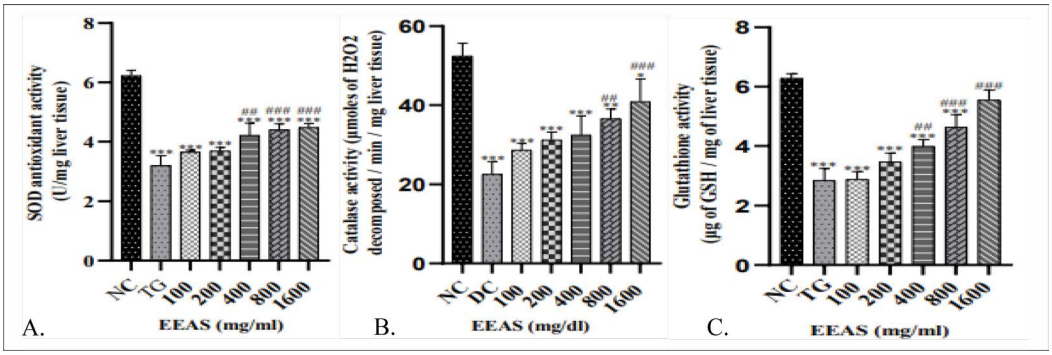

Figure 2. Antioxidant enzyme activity of EEAS on goat liver homogenate. **(A)** SOD. **(B)** CAT and **(C)** GSH. NC, untreated normal control; TG, Toxic group; EEAS, ethanolic extract of *A. scholaris* leaves. The results are expressed in Mean ± SEM. Each group comprised triplicates. *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to NC; #p < 0.05, ##p <0.01 and ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

Table 4. Effect of EAEAS and EEAS on blood pressure and ECG on STZ-induced diabetic rats

Parameters	VC	DC	EAEAS	EEAS	CANA	MET
Systolic BP (mmhg)	122.00± 1.0	150.00±2.0***	144.50± 1.50	130.50± 1.50##	126.50± 1.5###	122.70± 0.85##
Diastolic BP (mmhg)	102.60± 5.9	140.50±18.69***	137.50± 5.56	122.90± 4.45##	109.40± 3.0###	116.40± 2.96##
Heart rate (beats/min)	325.50± 0.5	430.00±2.0***	400.0± 6.0#	344.50±7.5###	332.50± 4.50###	357.00± 4.0###
PR interval (ms)	50.50±1.53	59.00±1.0**	58.00±1.0	55.50±0.50	53.00±1.0#	55.00±1.0
QT interval (ms)	71.00±1.0	88.00±1.0*	80.00±2.0#	74.50±0.50##	72.00±1.00##	75.00±1.0##
QRS interval (ms)	19.50±0.50	25.50±0.5**	24.50±0.5	21.50±0.50#	20.00±1.0##	22.50±1.50#

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; #p < 0.05, ##p < 0.01 and ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

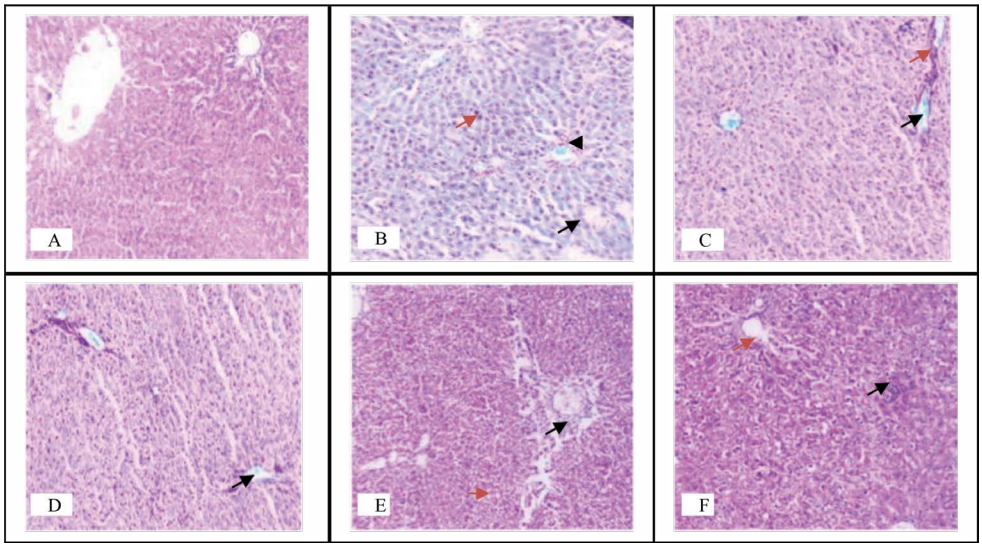

Figure 3. Histological sections of rat liver from different groups (H and E x10). **(A).** VC group showing normal hepatic cords and portal triad; **(B).** DC group representing increased cytoplasmic vacuolation of hepatocytes (black arrowhead), mononuclear cell infiltration of liver tissue (black arrow) and activation of Kupffer cells (red arrow); **(C).** CANA group shows areas of leukocyte aggregations (red arrow) and an area of the portal tract with dilated obstructed portal vein (black arrow); **(D).** MET group shows congestion of some central veins (black arrow); **(E).** EAEAS group showing hepatocytes associated with mononuclear cell infiltration (black arrow), and cytoplasmic vacuolation of hepatocytes (red arrow) and portal vein arrays; **(F).** EEAS treated group showing improved portal vein triad, central vein, mild hydropic degeneration (black arrow) and mild hepatic sinusoid congestion (red arrow).

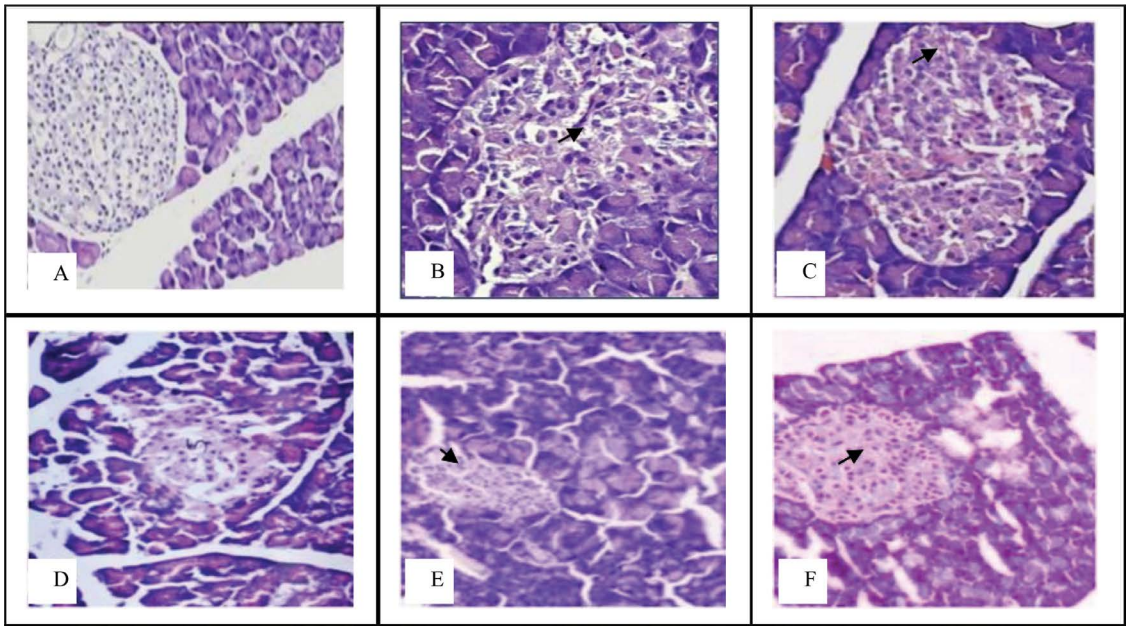

Figure 4. Histological sections of rat pancreas from different groups (H and E x40). **(A).** VC group representing normal islet of Langerhans; **(B).** DC group showing extensive degranulation, destruction of cells, presence of necrotic cells and inflammatory cells (black arrow); **(C).** CANA group showing improved pancreatic islets of Langerhans with beta cell enhancement (black arrow); D: MET group showing recovery of normal Langerhans islet cell population; **(E).** EAEAS group representing abnormal islets of Langerhans; **(F).** EEAS group showing improvement in the islet of Langerhans morphology with an increasing number of beta cells (black arrow).

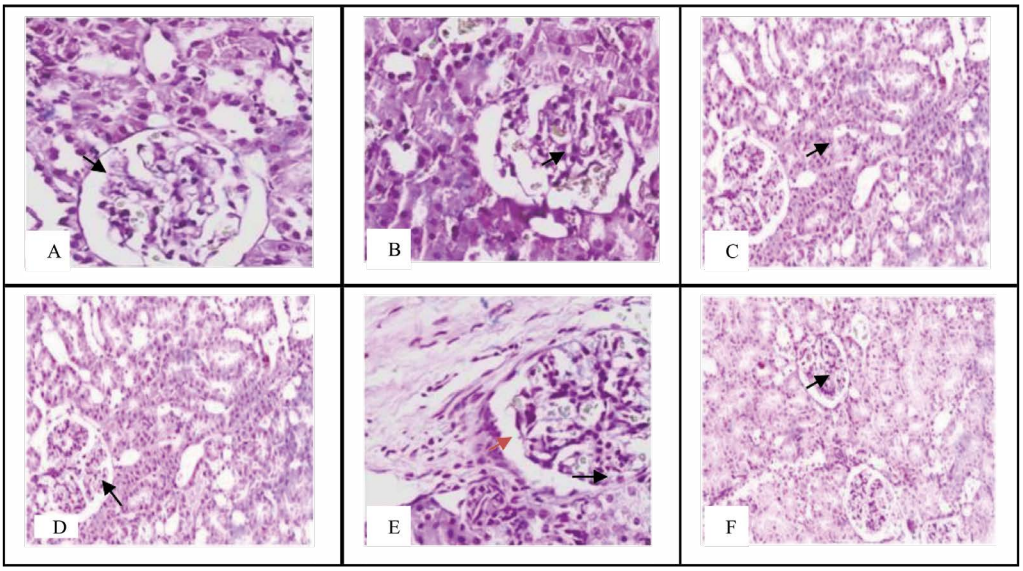

Figure 5. Histological sections of rat kidneys from different groups (H and E x40). **(A).** VC group showing normal glomeruli with normal baseline and tubules (black arrow); **(B).** DC group representing disrupted glomeruli with baseline fat deposition along with infiltration of lymphocytes (black arrow); **(C).** CANA with improved vascular system (black arrow) and less intestinal infiltration; **(D).** MET group with improved glomeruli with normal baseline and tubules (black arrow); **(E).** EAEAS group with glomerular lymphocytic infiltration (black arrow) thickening of basement membrane (red arrow); **(F).** EEAS group showing improved vasculature and glomeruli (black arrow).

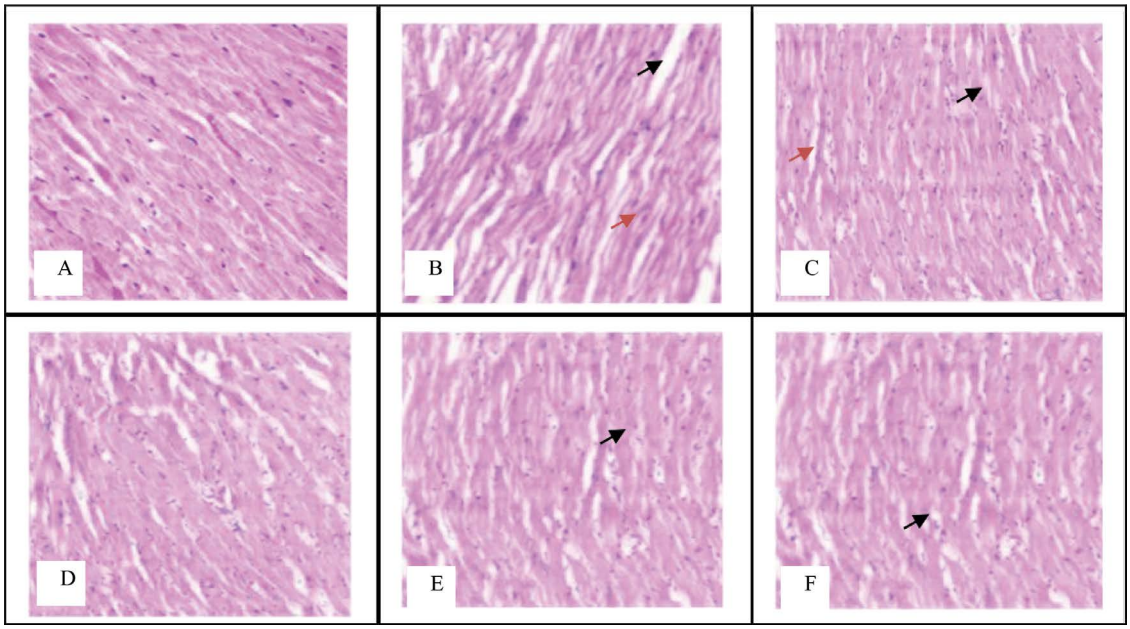

Figure 6. Histological sections of rat hearts from different groups (H and E x40). **(A).** VC group showing well-arranged cardiomyocytes and normal streaks; **(B).** DC group depicting disordered cardiac myocytes with disrupted streaks, enlarged interstitial space (red arrow) and distorted intercalated disc (black arrow); **(C).** CANA group showing normal heart tissue with enhanced features of myocytes, less visualization of the interstitial space (red arrow) and distortion of the intercalated disc (black arrow); **(D).** MET group with some distorted intercalated discs; **(E).** EAEAS group showing stronger evidence of interstitial space (black arrow); **(F).** EEAS group showing the mild imaging of the interstitial space (black arrow) and distorting the intercalated disc.

portal vein, and central vein damage in STZ-induced diabetic rats were alleviated in the EEAS therapy group. Figure 4 depicts a microscopic histological segment of the pancreas. Massive degranulation, cell death, and the presence of necrotic and inflammatory cells were all seen in the DC group. EEAS restores the damage, increases B cells, and improves islets of Langerhans morphology. A histological kidney section is shown in Figure 5. Damaged glomeruli and early lipid deposition, lymphocyte infiltration, and renal deformation from inflammation were all observed in the diabetic rat. EEAS treatment improved the vasculature and glomeruli. Figure 6 shows a heart section under a microscope. Disorganized cardiomyocytes were observed in diabetic rats, characterized by a lack of striations, a large interstitial space, and a deformed intercalated disc. The EEAS group exhibits slight displacement of the gap and few numbers of the degenerated cell.

4. Discussion

The World Health Organization (WHO) ranked type 2 diabetes mellitus (T2DM) as the ninth leading cause of death worldwide in 2019[29]. Despite the widespread availability of various anti-diabetic drugs, natural products have always had an advantage in the management of diabetes. In the present study, the fasting blood glucose level of the EEAS group (400 mg/kg) in diabetic rats was significantly lower after four weeks of treatment in this study. Both EAEAS (400 mg/kg) and EEAS (400 mg/kg) significantly reduced FBG levels in normoglycemic rats, as reported in our previous Oral Glucose Tolerance Test (OGTT) results. The anti-diabetic effects of the ethanolic extract of *A. scholaris* were more pronounced. Diabetic rats lose weight steadily due to an increase in blood sugar levels, loss of tissue proteins (gluconeogenesis), and increased muscle wasting[30]. EEAS improved weight loss in diabetic rats because it offers opportunities to restore protein metabolism. High protein catabolism and hyperosmolarity from high blood glucose levels promote polyphagia and polyuria in diabetic rats. The result of the present study indicates that increased glucose consumption is associated with decreased food and water intake. Metformin improves treatment by increasing hepatic glucose metabolism, while canagliflozin causes excess glucose excretion in the urine. To investigate the potential mechanism underlying the anti-hyperglycemic effect, we analyzed several biochemical markers and urinalysis results. EEAS (400 mg/kg) increases both urine production and urine glucose concentration, leading to a decrease in high blood glucose levels. Consequently, the anti-hyperglycemic effect of EEAS can be attributed to increased urinary glucose excretion. Irregularities in lipid metabolism lead to atherosclerosis, which is more common in people with diabetes mellitus[31]. Oral administration of EEAS improved lipid profiles and HDL-CH levels. EEAS's ability to lower blood cholesterol levels reflects its effect in preventing cardiovascular problems that can arise from diabetes. Long-term use of certain herbal plants can be harmful to the liver and kidneys. Therefore, we evaluated EEAS activity in diabetic rats to examine its long-term impact on kidney and liver function. Alanine aminotransferase and aspartate aminotransferase are two reliable liver indicators. This STZ induces liver toxicity in diabetic control rats due to leakage of these protein enzymes from the liver cytosol into the blood flow region[32]. EEAS restores levels of these enzymes that have been damaged by STZ. Creatinine and urea are the marker functions of kidney damage reduced by EEAS treatment in diabetic rats. Reactive oxygen species are unstable chemicals that have negative effects on many biological components, including lipids, proteins, and DNA, through their oxidative action. In our study (*in vitro*), EEAS showed significant SOD, CAT, and GSH activity, suggesting that it also has antioxidant potential. Antioxidants present in the body provide resistance to oxidative stress by scavenging free radicals and suppressing both oxidation and lipid peroxidation to prevent disease[33]. Diabetic rats showed morphological abnormalities and microscopic changes in the liver, pancreas, kidneys, and heart as compared to VC animals. Extensive vacuolation in the cytoplasm of the hepatocytes, a damaged portal specimen, disorganized hepatocytes, and a central vein were observed in a histological segment of the liver. The rate of morphological damage in the EEAS treatment group decreased to levels similar to the VC group. Microscopy of a pancreatic tissue segment of diabetic rats showing severe degranulation, cell death, and the

presence of necrotic and inflammatory cells. The shape of the islets of Langerhans and the number of beta cells improved during EEAS treatment. Histological analysis of a kidney tissue sample reveals that glomeruli have been damaged, with fat depositing at baseline and lymphocytes infiltrating the area. Diabetic rats induced by Streptozotocin (STZ) show severe damage and morphological abnormalities, although EEAS therapy successfully mitigates these effects. The cardiac tissue of diabetic rats showed significant interstitial space and myocardial cell dysfunction with disrupted streaks upon histological examination. Minor interstitial space movement, intercalated disc distortion, and a few deteriorated cells were seen in the EEAS therapy group. For any interpretation of the pharmacological action, it is necessary to evaluate the phytochemical compositions of various extracts of each herbal drug. Qualitative phytochemical screening identified the primary bioactive components of EEAS were identified as alkaloids, flavonoids, and phenols. Bioactive components such as alkaloids, flavonoids, and phenols are discussed for their role in the treatment of diabetes[34]. Fortunately, all of these bioactive components are found in *A. scholaris* in moderate concentrations. Therefore, phytochemical components of *A. scholaris* leaves can be considered a significant approach to enhancing the antioxidant, hypolipidemic, and anti-hyperglycemic potential for treating type two diabetes mellitus. Future developments in experimental design are intended to provide its mechanism of action, which will make an innovative contribution to research communities.

5. Conclusion

This research leads to a valid conclusion and supports the ethanolic extract of *A. scholaris* at a dose of 400 mg/kg for the reduction of common symptoms of type 2 diabetes mellitus including polydipsia, polyphagia, and hyperlipidemia in diabetic rats. It significantly reduces markers of heart, kidney and liver damage. It also reflects the improvement in blood pressure, ECG, antioxidant levels, and reverse modulation in the histopathology of various organs. Consequently, it can exert anti-hyperglycemic, antioxidant and hypolipidemic effects in diabetes with its attendant complications. In the future, studying its effect on intestinal absorption at the molecular level and *in vitro* research could shed light on the primary mechanism of its anti-diabetic efficacy.

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