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Cardio- and hepato-protective potential of methanolic extract of *Syzygium cumini* (L.) Skeels seeds: A diabetic rat model study

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ABSTRACT

Objective: To evaluate the effect of methanolic extract of *Syzygium cumini* (L.) Skeels (*S. cumini*) seeds on the major organs in an animal model of diabetes through biochemical and histopathological studies.

Methods: The methanolic extracts of *S. cumini* seeds (100 and 200 mg/kg body weight) were administered to alloxan-induced diabetic rats daily, with fasting blood glucose levels being measured by glucometry at one-day interval for a duration of two weeks. Biochemical assays to evaluate changes in the functions of the heart, liver, pancreas and kidney were carried out. Histopathological changes in the diabetic rat organs (pancreas, liver, heart, kidney and spleen) were also observed after the 14 days of treatment with the extracts.

Results: Oral administration of methanolic extracts of *S. cumini* seeds (100 and 200 mg/kg body weight), with gliclazide as a positive control (25 mg/kg), showed beneficial effects including lowering blood glucose levels ($P < 0.001$), improved heart and liver functions, and hyperlipidemia due to diabetes. At 200 mg/kg, the extracts reversed cardiac and liver damage caused by alloxan.

Conclusions: In addition to the anti-hyperglycemic activity of methanolic extracts of *S. cumini* seeds, the extracts demonstrates potential to minimize cardiac and hepatic complications.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and glucosuria resulting from the lack of insulin secretion from the beta cells of the pancreas and desensitization

of insulin receptors. The global prevalence of diabetes mellitus in 2010 was 6.4%, affecting 285 million adults aged between 20 and 79 years and this figure is set to rise to a prevalence of 7.7% with 439 million diabetic adults by the year 2030 [1,2]. As a result of their metabolic disorder, people with diabetes mellitus are at a high risk of developing a host of serious conditions. Diabetes-specific microvascular disease is a leading cause of blindness, renal failure and nerve damage. In addition, diabetes-accelerated atherosclerosis leads to increased risk of myocardial infarction, stroke and limb amputation.

The goal of diabetes mellitus treatment is primarily to secure a quality of life and extend lifespan comparable to those of healthy people [3]. The use of drugs in the management of diabetes mellitus and its complications, however, is limited by the fact that many of the synthetic medications have adverse effects and some with high secondary failure rates [4]. Alternative medicines, particularly herbal medicines, are available for the treatment of diabetes and its complications. Advantages of

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herbal medicines are perceived to be safety, affordability and acceptability [5]. The efficacies of herbal medicines are often a point of controversy. A number of medicinal plants and their products have been used in the Indian traditional system of medicine (Ayurveda) for diabetes and have been reported as showing experimental and/or clinical antidiabetic activity [6,7]. Furthermore, the World Health Organization has also recommended the evaluation of traditional plant treatments for diabetes [8].

Among the thousands of medicinal plants, *Syzygium cumini* (L.) Skeels (*S. cumini*; synonyms: *Eugenia jambolana*, *Syzygium jambolanum*), is deserving of special attention due to its antidiabetes potential [9]. *S. cumini* is colloquially named as ‘jamun’ in India, ‘black plum’ in Europe, ‘jambolan’ in Spanish-speaking countries, and it is an evergreen tropical tree belonging to the family Myrtaceae [10]. All parts of the tree can be used medicinally and it has a long history of use in traditional medicine [11]. Various traditional practitioners in the Indian subcontinent use the different parts of this tree in the treatment of a wide range of conditions, including diabetes, blisters in the mouth, cancer, colic, diarrhea, digestive complaints, dysentery, piles, pimples and stomachache [12]. In Unani medicine various parts of *S. cumini* are used and said to act as a liver tonic, enrich blood, strengthen teeth and gums and be able to remove ringworm infections of the head in a lotion form [13]. The use of *S. cumini* was introduced to Western medicine in the mid-nineteenth century, when the first report on the investigations of its antidiabetic properties were published [14]. Pharmacological studies have reported hypoglycemic properties of this medicinal species [15–18]. Other experimental studies using different extracts of the plant reported that *S. cumini* possesses antioxidant activity [19], antihypertensive and anti-atherosclerotic potentials [20], cardio-protective effect [21–23] and hepato-protective effect [24].

The reported constituents include flavonoids (quercetin, rutin and 3,5,7,4-tetrahydroxy flavanone) [23,25], phenolic acids (caffeic acid, ellagic acid, ferulic acid and gallic acid), tannins (corilagin, 3,6-hexahydroxydiphenoyl glucose, 4,6-hexahydroxydiphenoyl glucose, 1-galloyl glucose and 3-galloyl glucose) [23], terpenes (α -terpineol, β -pinene, β -terpinene and betulinic acid) [25,26]. A reportedly antidiabetic compound, mycaminose, was isolated from *S. cumini* seeds extract [27].

So, taking into account the traditional use of this plant in diabetes treatment, we designed biochemical and histopathological studies to evaluate the protective action of the methanolic extract of the seeds of *S. cumini* over the major organs of alloxan-induced diabetic rats.

2. Materials and methods

2.1. Collection of plant material

The fresh *S. cumini* fruits were collected from a locally growing *S. cumini* plant at Chittagong, Bangladesh in the month of April, 2015. The fresh seeds were isolated and dried. The plant was identified by using standard taxonomical methods, supervised by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh. The collected and identified plant material was deposited as a voucher specimen (Accession No. S246 CTGUH) in the herbarium of the University of Chittagong.

2.2. Preparation of the plant extract

After washed in water, the seeds were dried under shade and powdered to coarse particles. A total of 1890 g powder was then macerated as three 630 g samples each in 1500 mL methanol (99.9%), with 10 min shaking every 24 h over a period of 7 days. Extracts were then filtered through Whatman No. 1 paper, concentrated under reduced pressure at a temperature of 40–45 °C (rotary evaporator), then dried under vacuum and kept in a refrigerator at 4 °C for experimental usage. The yield of the methanolic extract of *S. cumini* seeds was 15.32% (w/w).

2.3. Animals

Wistar albino rats of either sex, aged 8–10 weeks, weighing 230–250 g each, obtained from the animal house of Jahangirnagar University, Savar, Bangladesh were used for the experiment. The procedures in this study for animal handling were performed in accordance with the Animal Resources Branch of the International Centre for Diarrheal Disease and Research, Bangladesh. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments. The animals were kept in clean dry polypropylene cages at (25 ± 2)°C and 45%–55% relative humidity in the animal house of University of Science and Technology Chittagong and were provided with standard laboratory food and tap water *ad libitum* and maintained in a natural day–night cycle. All animal experiments were carried out according to the guidelines of the Institutional Animals Ethics Committee and study protocols were approved by the University of Science and Technology Medical Ethics, Biosafety and Biosecurity Committee (Reference No. USTMEBBC/15/03/201) at the Basic Medical and Pharmaceutical Sciences Faculty, University of Science and Technology Chittagong, Bangladesh. Moreover, the experiments were conducted in an isolated and noise free environment.

2.4. Chemicals and kits

All reagents used in the study were of analytical grade. Alloxan monohydrate (98.82%) (Sigma–Aldrich, Darmstadt, Germany), formalin (10%) (Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh), xylene, ethanol (95%) (Merck, Mumbai, India), eosin (WELL's Health Care, Spain), hematoxylin, and normal saline solution (0.9% NaCl) (Popular Pharmaceuticals Ltd., Dhaka, Bangladesh) were used.

Total cholesterol, serum high-density lipoprotein (HDL), serum creatinine, serum urea, serum alkaline phosphatase (ALP), alanine transaminase (ALT), serum aspartate transaminase (AST) and triglyceride standard kits were obtained from Erba diagnostics Mannheim GmbH, Germany. Blood glucose level was measured using an Optium Xceed glucose meter (Abbott Laboratories, MediSense Products, Illinois, USA).

2.5. Diabetes induction

The Wistar albino rats were fasted for 12 h prior to the induction of diabetes. Alloxan monohydrate freshly prepared in 0.2 mL saline was administered intraperitoneally at a single dose of 150 mg/kg. Development of diabetes was confirmed by

measuring blood glucose concentration 10 days after the administration of alloxan monohydrate. Rats with blood glucose levels of above 140 mg/dL were considered to be diabetic and used for the studies.

2.6. Experimental design

Overnight-fasted rats were divided into five groups, each containing six animals, and treated every alternate day for 14 days as follows: Group I: Normal healthy control rats given only vehicle (saline, 10 mL/kg, *p.o.*); Group II: Diabetic control rats given vehicle (saline, 10 mL/kg, *p.o.*); Group III: Diabetic rats given methanolic extract of *S. cumini* seeds (100 mg/kg, *p.o.*); Group IV: Diabetic rats given methanolic extract of *S. cumini* seeds (200 mg/kg, *p.o.*); Group V: Diabetic rats given gliclazide (25 mg/kg, *p.o.*).

2.7. Biochemical parameters

The blood glucose levels were measured on Days 0, 1, 3, 5, 7, 9, 11 and 14 using an Optium Xceed glucose meter (Abbott Laboratories, MediSense Products, Illinois, USA) after daily oral administration of the extract. After blood glucose estimation on Day 14, whole blood was collected by cardiac puncture under

various treated groups. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Hypoglycemic activity

Administration of a single dose of alloxan monohydrate (150 mg/kg, *i.p.*) to the animals led to increased blood glucose levels that were maintained over a period of 14 days in the diabetic control animals. Daily treatment with the different doses of methanolic extract of *S. cumini* seeds led to a decrease in blood glucose levels.

In the case of Group III, serum glucose levels were reduced significantly ($P < 0.05$) when treated with 100 mg/kg body weight of the extract from Day 9 [(15.8 ± 0.8) mmol/L] to 14 [(10.5 ± 0.4) mmol/L] compared to those of the diabetic control (Group II) with serum glucose values ranging from (18.8 ± 0.8) mmol/L to (20.3 ± 0.4) mmol/L in the same time period (Table 1).

The Group IV animals treated with 200 mg/kg body weight of the extract showed statistically highly significant (to $P < 0.01$) reduction of serum glucose from Day 7 onwards and having the highest depletion power from (22.5 ± 0.7) mmol/L to (6.0 ± 0.5) mmol/L.

Table 1

Effect of the methanolic extract of *S. cumini* seeds on the blood glucose and serum insulin in diabetic rats.

Group	Treatment	Blood glucose level (mmol/L)					Serum insulin (IU/dL)
		Initial day	Day 7	Day 9	Day 11	Day 14	
I	Normal + vehicle	4.1 ± 0.3	5.0 ± 0.15	4.6 ± 0.2	4.5 ± 1.1	4.9 ± 0.4	4.5 ± 0.2
II	Diabetic + vehicle	22.7 ± 0.5	21.2 ± 1.2	20.3 ± 0.4	19.1 ± 0.6	18.8 ± 0.8	1.4 ± 1.2
III	Diabetic + the extract (100 mg/kg)	21.1 ± 0.1	18.9 ± 0.6	15.8 ± 0.8*	14.8 ± 0.7*	10.5 ± 0.4	2.3 ± 0.7
IV	Diabetic + the extract (200 mg/kg)	22.5 ± 0.7	12.8 ± 0.8*	8.2 ± 0.9**	7.1 ± 0.4**	6.0 ± 0.5**	3.0 ± 2.5*
V	Diabetic + gliclazide (25 mg/kg)	20.9 ± 0.5	8.1 ± 0.6**	6.1 ± 0.7***	4.4 ± 0.5***	4.1 ± 0.3***	3.6 ± 1.5**

Data are represented as mean ± SEM ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ when compared with diabetic control (Group II).

mild ether anesthesia of rats. Serum insulin levels were determined by using ELISA. Total cholesterol, triglycerides, creatinine, urea, ALP, low density lipoprotein (LDL), HDL and total proteins levels were also evaluated in normal and alloxan-induced diabetic rats as reported by Monday and Uzoma [28]. Serum ALT and serum AST were measured by autoanalyser (Erba Chem 7, Mannheim, Germany) using Erba diagnostic kits [29,30].

2.8. Histopathology of major organs

After sacrificing the rats, the major organs, including pancreas, heart, liver, kidney and spleen were removed and immediately washed with ice cold normal saline. Portions of organ tissues were fixed in 10% neutral formalin for histopathological studies. After fixation, tissues were embedded in paraffin, solid sections were cut (5 µm) followed by section staining with hematoxylin and eosin.

2.9. Statistical analysis

All values of blood glucose, body weight and biochemical parameters were presented as mean ± SEM. One-way ANOVA followed by Dunnett's multiple comparison *post hoc* test was used for statistical comparison between control and

At the end of the experiment (14th day) blood glucose levels were (10.5 ± 0.4) mmol/L and (6.0 ± 0.5) mmol/L in the groups treated with the extract at doses of 100 and 200 mg/kg body weight, respectively in comparison with the gliclazide-treated animals as standard [(4.1 ± 0.3) mmol/L] (Table 1). In addition, serum insulin levels also significantly improved ($P < 0.05$) for animals treated with 200 mg/kg body weight of the extract in comparison to diabetic control.

3.2. Effect on body weight of rats

Continuous reduction in body weight was observed in the case of the diabetic rats as shown in Table 2. Significant increments (to $P < 0.01$) of body weight were observed for the animals treated with 200 mg/kg body weight of extract compared with that of the diabetic control. This weight gain was comparable with that of the standard antidiabetic drug, gliclazide, administered at a dose of 25 mg/kg.

3.3. Effect on lipid profile

In the diabetic rats, there was a significant increase of serum total cholesterol, LDL cholesterol and triglycerides, accompanied by a significant decrease in HDL cholesterol in comparison to those of the normal control animals. The standard

Table 2Effect of the methanolic extract of *S. cumini* seeds on the body weight in diabetic rats (g).

Group	Treatment	Body weight				
		Initial day	Day 7	Day 9	Day 11	Day 14
I	Normal + vehicle	272.22 ± 2.44	275.91 ± 2.11	276.14 ± 2.02	277.70 ± 1.88	279.13 ± 2.01
II	Diabetic + vehicle	268.44 ± 1.88	263.35 ± 1.55	261.66 ± 1.70	258.81 ± 3.01	253.55 ± 1.78
III	Diabetic + the extract (100 mg/kg)	270.20 ± 2.01	268.52 ± 2.13	268.35 ± 2.01	268.11 ± 1.77	267.90 ± 2.10
IV	Diabetic + the extract (200 mg/kg)	269.52 ± 2.33	272.40 ± 1.88*	272.90 ± 1.44*	273.25 ± 1.98*	275.13 ± 2.55**
V	Diabetic + gliclazide (25 mg/kg)	270.90 ± 2.19	273.12 ± 1.90*	273.88 ± 1.56*	274.73 ± 2.22*	276.88 ± 2.34**

Data are represented as mean ± SEM (n = 6). *: P < 0.05, **: P < 0.01 when compared with diabetic control (Group II).

drug, gliclazide (25 mg/kg) and the extract (200 mg/kg) significantly reduced total cholesterol and LDL cholesterol. Most importantly, the methanolic seed extract reduced serum triglyceride concentration and increased serum HDL concentration to values comparable with those of gliclazide (25 mg/kg) significantly (P < 0.01) after 14 days of treatment (Table 3). Based on the lipid profiles in the animals treated with the extract, the results demonstrated the potential cardio-protective effect of the methanolic seed extract of *S. cumini*.

3.5. Effect on kidney function

The effect of the extract on kidney function was evaluated by measuring the markers serum creatinine and serum urea. It was observed that these were elevated significantly in the alloxan-induced diabetic rats when compared to those of normal rats. However, the extract treatment did not show any statistically significant reduction in either of the level (Table 5).

Table 3Effect of the methanolic extract of *S. cumini* seeds on lipid profile in diabetic rats 14 days after the treatment (mg/dL).

Group	Treatment	Total cholesterol	LDL cholesterol	Triglycerides	HDL cholesterol
I	Normal + vehicle	72.00 ± 2.25	78.45 ± 3.13	88.35 ± 3.22	48.00 ± 3.22
II	Diabetic + vehicle	135.61 ± 2.33	125.88 ± 5.16	240.11 ± 4.77	29.66 ± 2.77
III	Diabetic + the extract (100 mg/kg)	105.65 ± 5.80	101.34 ± 4.12	110.72 ± 3.55	35.33 ± 3.02
IV	Diabetic + the extract (200 mg/kg)	99.00 ± 3.11*	92.13 ± 3.55*	95.12 ± 4.01**	42.88 ± 3.66**
V	Diabetic + gliclazide (25 mg/kg)	95.77 ± 4.11*	85.23 ± 4.94**	91.88 ± 2.88**	43.78 ± 3.85**

Data are represented as mean ± SEM (n = 6). *: P < 0.05, **: P < 0.01 when Groups III, IV and V were compared with diabetic control (Group II).

3.4. Effect on liver function

The hepato-protective effect of the methanolic seed extract is clearly shown in Table 4. The total protein level was decreased significantly in diabetic rats, but after 14 days of treatment with 200 mg/kg dose of the extract, the protein concentration increased significantly (P < 0.01), even more than that of the rats treated with the gliclazide (25 mg/kg).

ALT, AST, ALP and bilirubin levels were significantly elevated in the alloxan-induced diabetic animals. The rats treated with methanolic seed extract of *S. cumini*, particularly at 200 mg/kg dose, showed significant (P < 0.01) reduction in the elevated levels of liver enzymes. In the case of bilirubin, the serum concentration also decreased significantly (P < 0.05) for the extract treated (200 mg/kg) diabetic rats.

Table 5Effect of the methanolic extract of *S. cumini* seeds on kidney profile in diabetic rats (mg/dL).

Group	Treatment	Serum urea	Serum creatinine
I	Normal + vehicle	32.23 ± 1.33	0.69 ± 0.57
II	Diabetic + vehicle	66.22 ± 1.14	1.12 ± 1.44
III	Diabetic + the extract (100 mg/kg)	60.12 ± 1.34	0.90 ± 1.66
IV	Diabetic + the extract (200 mg/kg)	57.55 ± 0.92	0.85 ± 0.55
V	Diabetic + gliclazide (25 mg/kg)	35.77 ± 0.50**	0.64 ± 0.34**

Data are represented as mean ± SEM (n = 6). **: P < 0.01 when Groups III, IV and V were compared with diabetic control (Group II).

Table 4Effect of the methanolic extract of *S. cumini* seeds on liver parameters in diabetic rats 14 days after the treatment.

Group	Treatment	Total protein (g/dL)	Bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I	Normal + vehicle	7.88 ± 1.55	0.55 ± 2.02	47.24 ± 2.34	62.11 ± 2.13	130.44 ± 3.12
II	Diabetic + vehicle	4.83 ± 2.13	1.01 ± 1.94	110.26 ± 3.58	115.33 ± 1.03	200.45 ± 4.13
III	Diabetic + the extract (100 mg/kg)	6.29 ± 1.01	0.88 ± 1.26	89.15 ± 4.12	87.33 ± 2.99	181.67 ± 1.66
IV	Diabetic + the extract (200 mg/kg)	7.32 ± 0.76**	0.61 ± 3.33*	55.32 ± 2.54**	59.62 ± 3.22**	127.35 ± 2.67**
V	Diabetic + gliclazide (25 mg/kg)	7.69 ± 1.83*	0.49 ± 3.12*	52.12 ± 1.55**	60.88 ± 2.77*	133.67 ± 3.77**

Data are represented as mean ± SEM (n = 6). *: P < 0.05, **: P < 0.01 when Groups III, IV and V were compared with diabetic control (Group II).

3.6. Histopathological study

3.6.1. Microscopic findings in the pancreas

The histopathology of the pancreas (Figure 1) showed normal acini and normal islets of Langerhans, with abundant β -cells in the normal control group (Figure 1A). Pancreatic sections of the diabetic control rats showed atrophy of β -cells and degenerative changes in the islets (Figure 1B). The pancreatic section of a diabetic rat treated with the extract at 200 mg/kg revealed the preservation of compact islets and β -cells, along with mild β -cell hyperplasia (Figure 1C). The pancreatic section of a diabetic rat treated with gliclazide (25 mg/kg) showed the recovery of islets and β -cells to almost normal (Figure 1D).

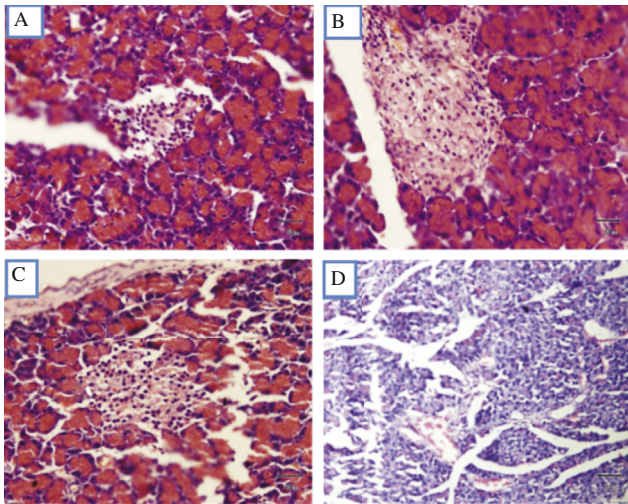


Figure 1. Effect of the methanolic extract of *S. cumini* seeds (200 mg/kg) on rat pancreases (400 \times).
A: Normal rat; B: Diabetic rat; C: The extract (200 mg/kg) treated rat; D: Gliclazide (25 mg/kg) treated rat.

3.6.2. Microscopic findings in the heart

Cardiac tissue sections (Figure 2) illustrated the non-diabetic control group displaying normal histological features (Figure 2A). The diabetic group section showed mild

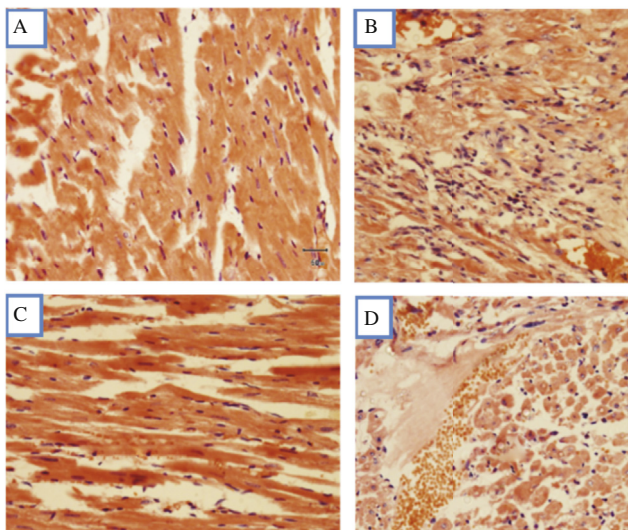


Figure 2. Effect of the methanolic extract of *S. cumini* seeds (200 mg/kg) on rat hearts (400 \times).
A: Normal rat; B: Diabetic rat; C: The extract (200 mg/kg) treated rat; D: Gliclazide (25 mg/kg) treated rat.

edematous changes in the cardiac muscle fiber and infiltration by a few mixed inflammatory cells (Figure 2B). The cardiac section of a diabetic rat treated with the extract at 200 mg/kg (Figure 2C) showed marked improvement and recovery from damage compared to both the diabetic control and gliclazide-treated animals, which was a clear indication of the cardio-protective effect of the extract. The gliclazide-treated (25 mg/kg) rat tissue (Figure 2D) retained similar pathological features to that of the diabetic animal.

3.6.3. Microscopic findings in the liver

The histopathology of liver (Figure 3) showed the diabetic control group with marked morphological changes, including necrosis in the hepatic muscle fiber and infiltration by mixed inflammatory cells (Figure 3B). A liver section of the extract treated (200 mg/kg) diabetic rat revealed normal hepatic tissue compared to that of the diabetic control (Figure 3C). The liver section of gliclazide-treated (25 mg/kg) diabetic rat revealed partial recovery of damage (Figure 3D). It was evident that the methanolic extract of *S. cumini* seeds treatment supported recovery of liver damage even better than the treatment with gliclazide.

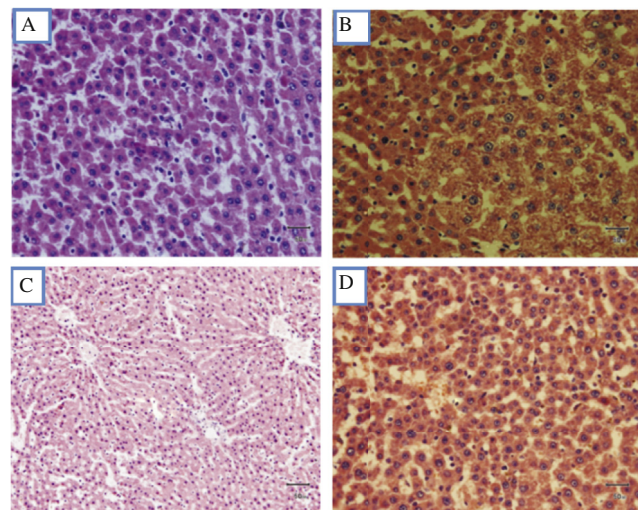


Figure 3. Effect of the methanolic extract of *S. cumini* seeds (200 mg/kg) on rat livers (400 \times).
A: Normal rat; B: Diabetic rat; C: The extract (200 mg/kg) treated rat; D: Gliclazide (25 mg/kg) treated rat.

3.6.4. Microscopic findings in the kidney

The histopathology of kidney (Figure 4) showed the tissue from the non-diabetic control group with normal histological features (Figure 4A). The diabetic control group showed glomerular lesions with capillary basement membrane thickening and moderated mixed inflammatory infiltration along with global tubular degeneration and necrosis (Figure 4B). The treated groups, both the extract (200 mg/kg) and gliclazide (25 mg/kg) did not show any significant recovery in the diabetic kidney (Figure 4C, D).

3.6.5. Microscopic findings in the spleen

The histopathology of spleen (Figure 5) showed that samples from the spleen tissues of normal control (Figure 5A), diabetic control (Figure 5B), the extract (200 mg/kg) treated (Figure 5C) and gliclazide (25 mg/kg) treated (Figure 5D)

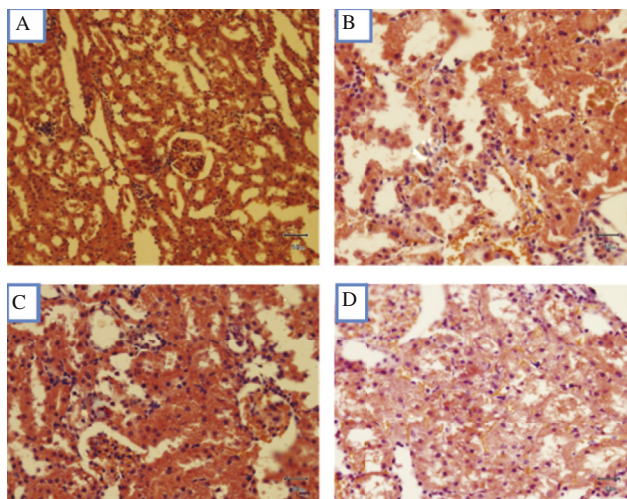


Figure 4. Effect of the methanolic extract of *S. cumini* seeds (200 mg/kg) on rat kidneys (400×).

A: Normal rat; B: Diabetic rat; C: The extract (200 mg/kg) treated rat; D: Gliclazide (25 mg/kg) treated rat.

groups revealed normal histological features, which may indicate that there might be no damage to the spleen by alloxan (150 mg/kg) or by the diabetic condition over the period of the experiment.

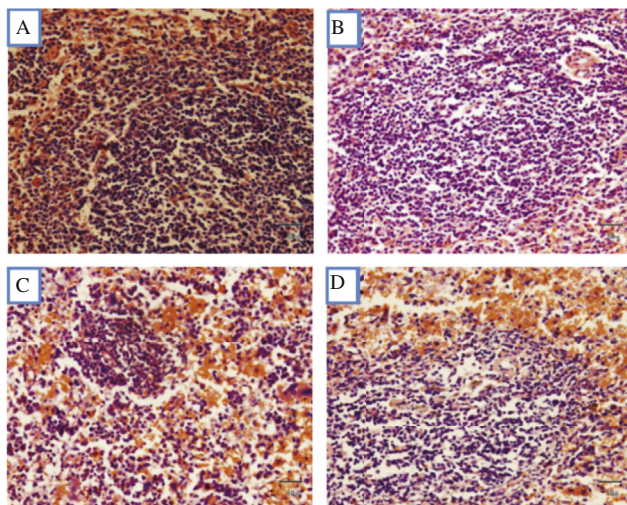


Figure 5. Effect of the methanolic extract of *S. cumini* seeds (200 mg/kg) on rat spleens (400×).

A: Normal rat; B: Diabetic rat; C: The extract (200 mg/kg) treated rat; D: Gliclazide (25 mg/kg) treated rat.

4. Discussion

Diabetes mellitus is a chronic metabolic disorder that leads to impaired carbohydrate, fat and protein metabolism. It affects many millions of people worldwide. Therefore, it is of vital public importance because of the physiological complications associated with it. These include, multi-organ dysfunction especially, but not limited to the pancreas, heart, liver and kidney [31,32].

Although a large variety of synthetic compounds have been found to be effective in reducing diabetic complications as well as organ protective action, there are relatively few compounds that are clinically successful, especially in regard of the organ

protective function. This is due to undesirable side effects and poor pharmacokinetic profiles [33]. Thus there is a great need to search for new molecules from nature, with plants remaining the most promising source due to the extraordinary biodiversity which yields an impressive variety of phyto-constituents.

Therefore, the present study was designed to investigate the organ protective effect of a methanolic extract of the seeds of *S. cumini* through histopathological and biochemical studies along with an examination of its reputed hypoglycemic action. For the evaluation of the antidiabetic potential of plants, apart from streptozotocin, alloxan-induced hyperglycemia in rats is considered to be an effective preliminary screening model and is widely used [34]. Alloxan is a beta-cell cytotoxin that has been extensively used to induce diabetes mellitus in a wide variety of animals. It effectively destroys the beta-cells in the islets of Langerhans in the pancreas, causing a reduction of endogenous insulin secretion which paves the way for the decreased utilization of blood glucose by tissues [35]. Therefore, it results in the elevation of blood glucose levels, reduces protein content and elevates total cholesterol, LDL cholesterol and triglycerides [36].

Single dose (*i.p.*) treatment of rats with alloxan monohydrate (150 mg/kg) significantly ($P < 0.05$) increased blood glucose as shown in Table 1. Treatment with methanolic extract of *S. cumini* seeds and gliclazide were found to reduce the elevated glucose levels markedly in alloxan-induced diabetes test animals. The *S. cumini* extract may act on islet functions by increasing the number of pancreatic islets (Figure 1). Hypertriglyceridemia and hypercholesterolemia are the most common lipid abnormalities in diabetes [37]. Daily administration of the methanolic extract of *S. cumini* seeds for 14 days significantly ($P < 0.01$) decreased LDL cholesterol, triglyceride and total cholesterol, while the HDL cholesterol level was increased.

Flavonoids found in *S. cumini* are expected to account for this activity, since it has been established that this class of compounds increases the expression of cAMP-dependent phosphokinase, the enzyme responsible for 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibition [38]. However, such reduced lipid levels might also be due to reduced intestinal absorption of cholesterol as well as increased free fatty acid and triacylglycerol clearances subsequent to insulin action improvement [39–41].

Histopathological studies of organ tissues (heart, liver, pancreas, kidney and spleen) did not indicate any toxic effects of the extract. The methanolic extract of *S. cumini* seeds did not show any protective or recovery effects on the kidney or spleen in comparison to the diabetic control animals (Figures 4 and 5). However, the histopathological study on the heart and liver of the methanolic extract of *S. cumini* seeds-treated groups shows very promising results.

The histopathological observations on the heart show that the cardiac tissue from the diabetic group displays marked morphological changes like mild edema in the cardiac muscle fiber and infiltration by mixed inflammatory cells. These are in stark contrast to the tissue sections of the normal control animals. The cardiac tissue of methanolic extract of *S. cumini* seeds extract-treated (200 mg/kg) group shows normal morphological features compared to both the diabetic control and the gliclazide (25 mg/kg) treated animals (Figure 1), which clearly reveals that the extract has protective and recovery ability on cardiac tissue. This is due to its ability to reduce myocardial necrosis biomarkers, specifically aspartate aminotransferase, alanine aminotransferase, uric acid, creatine phosphokinase, and lactate dehydrogenase as seen our study and also reported by Mastan *et al.* [22].

Myricetin, quercetin, rutin, ellagic acid and gallic acid have been reported to act on distinct pathways of cardio-metabolic disorders [23], so the presence of these compounds in the *S. cumini* seed extract could be the reason for the cardio-protective ability of the extract. On the other hand, the histopathological study on liver tissues reveals that the diabetic control group has marked morphological changes including necrosis in the hepatic muscle fiber and infiltration by mixed inflammatory cells, whereas the methanolic extract of *S. cumini* seeds extract (200 mg/kg) treated group shows significant recovery from damage in comparison to the diabetic control and gliclazide (25 mg/kg) groups (Figure 2). This correlates with ability of the extract to reduce serum AST, ALT, total protein and bilirubin levels. Furthermore, the liver protective activity of the extract may be attributed to saponins, tannins and flavonoids present in the seeds of the plant. Our results indicate a significant positive effect of the methanolic extract of *S. cumini* seeds on diabetic heart and liver.

The present biochemical and histopathological experiments on diabetic rats confirmed that the methanolic extract of *S. cumini* seeds has not only anti-hyperglycemic and anti-hyperlipidemic activities, but more importantly, it can lead to recovery from cardiac and liver damage. Accordingly, our results support the traditional notions that the plant extract has significant effects on the control or cessation of diabetes-related complications such as cardiovascular disease and liver damage.

Conflict of interest statement

We declare that we have no conflict of interest.

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