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Evaluation of the Anti-Diabetic Activity of Ethanolic Extract of *Momordica Charantia* in Streptazocin induced Diabetic Rats in Male Sprague Dawley Rats

Mekala Shirisha [1], Madhurima Yadav [2*], Dr I Veena Rani [3]

[1] Student, Department of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Rangareddy District, Telangana, India

[2-3] Department of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Gandipet, Rangareddy District, Telangana, India

* Corresponding Author: **Madhurima Yadav**

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Abstract

Background: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and oxidative stress, often leading to complications such as dyslipidemia and organ damage. This study aimed to evaluate the antidiabetic, antioxidant, and nephroprotective effects of an ethanolic plant extract in streptozotocin-induced diabetic rats.

Methods: Experimental rats were divided into five groups: normal control, diabetic control, diabetic + Glibenclamide (2.5 mg/kg), and diabetic rats treated with ethanolic extract at 250 mg/kg and 500 mg/kg. Oral glucose tolerance test (OGTT), biochemical profiling (glucose, lipid profile, serum creatinine, blood urea, total protein), antioxidant enzyme assays (SOD, catalase, MDA), and free radical scavenging assays (DPPH, nitric oxide, hydrogen peroxide, hydroxyl radical) were conducted. SEM analysis was used to observe morphological changes in liver and kidney tissues.

Results: Diabetic rats showed elevated glucose, lipid abnormalities, oxidative stress, and kidney dysfunction. Treatment with the ethanolic extract, especially at 500 mg/kg, significantly improved glucose tolerance, normalized biochemical parameters, enhanced antioxidant enzyme levels, and reduced oxidative damage. SEM analysis revealed restoration of normal tissue architecture in extract-treated groups, comparable to the standard drug Glibenclamide.

Conclusion: The ethanolic extract exhibited significant antidiabetic, antioxidant, hypolipidemic, and nephroprotective effects in diabetic rats. These findings suggest its potential as a natural therapeutic agent for managing diabetes and its complications. Further studies are recommended to isolate active constituents and evaluate clinical safety.

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Keywords: Diabetes, SEM Analysis, Lipid Abnormalities, Glucose Tolerance

1. Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by alteration in the metabolism of carbohydrates, lipids and proteins. DM is probably one of the most terrible oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago [1]. It is well known that diabetes and hypertension are chief risk factors in the development of nephropathy, retinopathy, and cardiomyopathy which progress to myocardial infarction [2].

Types of Diabetes

During long standing hyperglycemic state in diabetes mellitus, glucose forms covalent adducts with the plasma proteins through a non-enzymatic process known as glycation. Protein glycation and formation of advanced glycation end products (AGEs) play an important role in the pathogenesis of diabetic complications like retinopathy, nephropathy, neuropathy, cardiomyopathy along with some other diseases such as rheumatoid arthritis, osteoporosis and aging. Glycation of proteins interferes with normal functions by disrupting molecular conformation, altering enzymatic activity, and interfering with receptor functioning. AGEs form intra and extracellular cross linking not only with proteins, but with some other endogenous key molecules including like lipids and nucleic acids to contribute in the development of diabetic complications [3]. Classification of diabetes mellitus is based on its etiology and clinical presentation. As such, there are four types or classes of diabetes mellitus; they are,

1. Type-1 (Insulin dependent diabetes mellitus)
2. Type-2 (non-insulin dependent diabetes mellitus)
3. Gestational diabetes mellitus
4. Other specific types

The origin and etiology of DM (Sicree *et al*., 2006) can fluctuate greatly but constantly include defects in either insulin secretion or response or in both at some point in the course of disease. Mostly patients with diabetes mellitus have either type 1 diabetes (which is immune-mediated or idiopathic) or type 2 DM (formerly known as non-insulin dependent DM) which is the most universal form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [4].

Type 1 diabetes

Type 1 Diabetes Mellitus (DM) is a catabolic disorder in which circulating insulin is very low or absent, plasma glucagon is elevated, and the pancreatic beta cells stop working to respond to all insulin secretory stimuli. The pancreas shows lymphocytic infiltration and destruction of insulin secreting cells of the islets of Langerhans finally leads to insulin deficiency. Patients need exogenous insulin to overturn this catabolic condition, prevent ketosis, decrease hyperglucagonemia, and normalize lipid and protein metabolism [5]. Type 1 diabetes leads to inability to discharge insulin that results in low down rates of glucose uptake into muscles and adipose tissue [6].

The usual rapid onset of the insulin dependent diabetes mellitus is due to the autoimmune attack on the pancreatic cells. Though the disease persists for several years the immune system slowly destroys the pancreatic cells. When >80% of the pancreatic cells have been damaged it leads to classic symptoms of diabetic disease [7].

Materials and Methods

Collection and authentication of Plant material

The flowers of *Elephantopus scaber*, was selected for investigation and were procured from the nearest area of Hyderabad Ranga Reddy District. The plant material was taxonomically identified and authenticated by Dr. Madhava Chetty, Head of Department, Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh.

Preparation of plant extract

The fresh flower was air dried in shade and extracted with 1:4 ratio ethanol extract, using a Soxhlet extractor for 8 hrs at 55-60^{0}C. The supernatant was filtered through Whatman filter paper No.1 and concentrated under reduced pressure using vacuum at 44±10^{0}C in a rotavapor (IKA ® RB 10 Rota Evaporator, India) The extract was stored at 22^{0}C in a seeded air tight container.

The percentage of extract yield was calculated by using the formula

$$\% \text{ of extract yield} = \frac{\text{weight in gm of extract obtained}}{\text{weight in gm of plant material taken}} \times 100$$

***In-vitro* antioxidant activities**

Assay of superoxide radical scavenging activity

The assay was focused on the ability of different specimens to prevent formazan development by scavenging the superoxide radicals produced in the framework of riboflavin–light–NBT [8]. There were 50 mM sodium phosphate buffer (pH 7.6), 12 mM EDTA, 20 µg riboflavin, 0.1 mg NBT and 100 µl test medium in each 3 ml response combination. The response began by illuminating the sample extract response combination for 90 seconds. The absorbance was evaluated at 590 nm immediately after illumination. In a cabinet covered with aluminium foil, the full response installation was contained. Identical response blend pipes held as blanks in the dusk. Superoxide anion production inhibition proportion was calculated as:

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where,

A0 is the absorbance of the control, and

A1 is the absorbance of the sample extract/standard.

Assay of nitric oxide scavenging activity

The procedure is focused on the technique in which sodium nitroprusside accidentally produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitrite ions that can be measured using Greiss reagent. Nitric oxide scavengers struggle with oxygen resulting in decreased nitrite ion manufacturing. For the experiment, sodium nitroprusside (10 mM) was mixed with 100 µl sample solution of various extracts in phosphate buffered saline (0.2 M, pH 7.4) and incubated for 150 min at room temperature. After the incubation era, 0.5 ml of Griess reagent (1% sulphanilamide, 2% H3PO4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was introduced to the same response blend without the test. The chromophore absorbance created at 546 nm was reported [9].

Hydrogen Peroxide Scavenging Activity

Ruch *et al*. (1989) technique determined the capacity of the seeds to scavenge hydrogen peroxide. In phosphorus base (0.2 M, pH 7.4) a batch of hydrogen peroxide (2 mmol / l) was provided. The level of hydrogen peroxide was determined by absorption at 230 nm spectrophotometrically with molar absorbability of 81 (mol / l) -1 /cm. Extracts (10 µl) and oxygen peroxide solution (0.6 ml) were introduced to 3.4 ml of phosphorus buffer. Hydrogen peroxide absorbance at 230 nm was determined after 10 min against a blank

solution without hydrogen peroxide comprising phosphate buffer.

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where,
A0 is the absorbance of the control, and
A1 is the absorbance of the sample extract/standard.

Hydroxyl radical scavenging activity
Chloroform, acetone, methanol, and warm water samples from H scavenging operation. herbacea and N. Hydroxyl radical alata was evaluated using Klein *et al.* (1991) technique. Various levels (20, 40, 60 and 80 µg) of substances have been combined with 1.0 ml of iron-EDTA fluid (0.13 per cent ferrous ammonium chloride and 0.26 per cent EDTA), 0.5 ml of EDTA fluid (0.018 per cent) and 1.0 ml of dimethyl sulphoxide (DMSO) (0.85 per cent v / v in 0.1 M nitrate buffer, pH 7.4). The response was launched by supplying 0.5 ml (0.22 cents) of ascorbic acid and incubated in a fluid tub at 80–90oC for 15 min. The response was terminated after incubation by adding 1.0 ml of icecold TCA (17.5% w / v). Three milliliters of Nash reagent (75.0 g ammonium acetate, 3.0 ml bacterial acetic acid, and 2 ml acetyl acetone were blended and elevated to 1L with distilled H2O) were added and kept at bed heat for 15 minutes. The sample-free response combination has been used as command. The strength of the dye created was evaluated against reagent void spectroscopically at 412 nm. By the previous formula, the percentage of hydroxyl radical scavenging activity (HRSA) calculated as:

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where,
A0 is the absorbance of the control, and
A1 is the absorbance of the sample extract/standard.

4.4.5. Free radical scavenging activity on DPPH
The antioxidant behavior of the samples was determined using the stable radical DPPH according to the **Blios (1958)** technique[12] in cases of hydrogen donation or radical scavenging capacity. Sample samples were drawn at different levels and methanol adapted the quantity to 100 µl. Added and strongly stirred 5 ml of a 0.1 mM DPPH methanolic fluid. The tubes sit at 27ºC for 20 minutes. The sample absorption was evaluated at 517 nm. The sample displayed radical scavenging exercise as the proportion of free radical inhibition and was calculated using the equation:

$$\% \text{ of inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where,
A0 is the absorbance of the control, and
A1 is the absorbance of the sample extract/standard.

Pharmacological evaluation
Animals and Management
For this research, Wistar albino rats of both sexes measuring between 150 and 200 gm were used. The animals were picked up from the residence of the pets, from our institute. The pets were randomly put upon entry and assigned as bedding in polypropylene tanks with paddy husk to treatment groups. Animals were housed at a temperature of 24±2ºC and relative humidity of 30 to 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat. The Institutional Animal Ethics Committee (_____ CCSEA) studied all the laboratory processes and protocols used in this research and complied with the institutional ethical standards.

Acute Toxicity Study [13]
The acute toxicity study is use to establish the therapeutic index, i.e. the ratio between the pharmacologically effective dose and lethal dose on the same strain and species (LD50/ED50). Greater is the index; safer is the compound and vice versa. The acute toxicity study was done according to OECD (Organization of Economic Co-operation and Development) guidelines 425- Fixed Dose Procedure (FDP).

Procedure:
The animals were divided into two groups and each group consisted of five rat. The defined or fixed dose level of aqueous and ethanolic extracts (2000 mg/kg) were given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death.
Hence in our studies we selected 1/4th and 1/8th of the highest dose.

Preparation of Doses
Doses equivalent to 500 mg and 250 mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v tween 80 solutions for the experiment.

Oral Glucose Tolerance Test [14]
It is the test for the diagnosis of diabetes. It can be made on the basis of individual's response to the oral glucose load, commonly referred to as oral glucose tolerance test (OGTT). The response of standard oral test dose of glucose was determined. For this study normal rats were selected.

Procedure: Animals were divided into six groups and each group consisted of six rats. Overnight fasted rats were used for study.
Group I: Normal control rats administered saline (0.9% w/v);
Group II: Rats administered standard drug Glibenclamide (2.5 mg / kg) daily
Group III: Rats administered hydroalcoholic extract of *Elephantopus scaber* (EEES) (250 mg/kg);
Group IV: Rats administered hydroalcoholic extract of *Elephantopus scaber* (EEES) (500 mg/kg);
Hydroalcoholic extracts was administered orally to all overnight fasted animals. After 30 min. extract administration, the glucose (4gm/kg) was administered orally to all groups. The blood samples were collected from the orbital plexus of each animal at 0 min, 30 min, 60 min and 120 min after glucose loading. The fasting blood glucose level was determined using a glucose oxidase-peroxidase reactive strips and a glucometer.

Induction of Diabetes:
Diabetes was induced by a single intraperitoneal injection of 60 mg/kg Streptozotocin (Sigma Aldrich, Germany)

followed by (Ranbaxy Chemicals Ltd, Mumbai, India) 120 mg/kg, i.p, 15 min afterwards. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >126 mg/dl. Only those rats that were found to have permanent NIDDM were used for the study.

The diabetic rats after confirmation of stable hyperglycemia, were divided into 5 different groups of 6 rats each. That day was considered as the 0th day. Drug and doses were administered as mentioned.

Group I: Normal control rats administered saline (0.9% w/v);
Group II: Diabetic control rats administered saline (0.9% w/v);
Group III: Diabetic rats administered standard drug Glibenclamide (2.5 mg / kg) daily
Group IV: Diabetic rats administered Ethanolic extract (250 mg/kg);
Group V: Diabetic rats administered Ethanolic extract (500 mg/kg);

Preparation of 0.1 M citrate buffer solution pH 4.5 [16]: 14.9 gm of trisodium citrate was dissolved in sufficient distilled water to produce 1000 ml and the necessary pH (4.5) was adjusted with Concentrated HCl.

Preparation of solution: solution was freshly prepared by dissolving 480 mg of in 8 ml of 0.9 % NaCl solution, and the volume of the solution was made upto 10 ml with the same solution.

Preparation of Streptozotocin-solution: A solution of Streptozotocin was prepared by dissolving the weighed quantity of Streptozotocin in 0.1 M freshly prepared ice-cold citrate buffer (pH 4.5) solution.

Blood Sample Collection

Blood was removed at 0, 4, 7 and 14 days of drug administration from the tail vein and glucose concentrations are evaluated using a glucometer. The rats were fasted overnight at the end of the experimental period, anesthetized with Pentobarbitone sodium (60mg / kg, i.p) anaesthesia, and the blood was collected in non-heparinized tubes by a retro-orbital puncture.

Blood samples were put at room temperature for 30 minutes to achieve serum and centrifuged at 3000 X g for 10 minutes and the supernatant was drawn to determine lipid characteristics, liver activity (AST, ALT, ALP) and kidney function test.

Homogenate preparation and Biochemical profiling

The animals were sacrificed with excess pentobarbitone sodium, removing the liver from the animal and keeping it in the phosphate buffer of 0.2 M, pH 6.6. The liver was shattered and the homogeneous result was centrifuged and centrifuged for 10 min at 3000 rpm. The supernatant acquired was used for enzymatic and non-enzymatic testing. The determination of the decreased glutathione (GSH) non-enzymatic antioxidant estimate was evaluated. using the technique outlined in Beutler *et al*., [17] 1986. Determination of enzymatic anti-oxidant studies such as catalase (CAT) [18], superoxide dismutase (SOD), glutathione reductase (GSSH), peroxides (Px) effect [19], glutathione peroxidase (GPx) effect was explored using standard conventional procedures. Estimation of lipid hydroperoxides and evaluation of malondialdehyde (MDA) lipid peroxidation evaluation as demonstrated by the creation of thiobarbituric acid explosive materials (TBARS) and hydroperoxides (HP) was assessed using the Nieshus and Samuelsson (1986) methods.

Analysis of serum Lipid Profile and Total Protein

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein, cholesterol (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein cholesterol (VLDL) blood levels were evaluated according to conventional techniques. Total protein in the homogeneous tissue was measured by Lowry *et al*. (1951) technique [20] using bovine serum albumin as a standard. Similarly, blood urea assessment [21] and serum creatinine analysis were done by standard technique and were reported.

Total cholesterol

Total cholesterol in serum was determined by a colorimetric method. The assay principle is based on enzymatic hydrolysis and oxidation of cholesterol and the indicator compound, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The reagents consisted of 4-aminoantipyrine (0.03 mmol/l), phenol (6 mmol/l), peroxidase (≥0.5 U/ml), cholesterol esterase (> 0.15 U/ml), cholesterol oxidase (> 0.1 U/ml) and pipes buffer (80 mmol/L pH 6.8). The serum sample (10 µl) was mixed with 1 ml of reagent, incubated at 37oC for 5 min, and absorbance measured at 500 nm against the reagent blank [117]. The cholesterol standard was 5.17mmol/l (200 mg/dl). The concentration of total cholesterol in the sample was calculated by

$$\text{Total cholesterol} = \frac{\Delta \text{A sample}}{\Delta \text{ A standard}} \times \text{concentration of standard}$$

Triglycerols

Serum triacylglycerol's (TG) were determined by a colorimetric method. The assay principle is based on the enzymatic hydrolysis of TG with lipases and the indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic activity of peroxidase. The enzyme reagent consisted of 4-aminophenazone (0.5 mmol/l), ATP (1.0 m.mol/l), lipases (≥150 U/ml), glycerol-kinase (≥0.4 U/ml), glycerol-3-phosphate oxidase (≥1.5 U/ml), peroxidase (≥0.5 u/ml). The serum sample (10 µl) was mixed with 1000 µl of enzyme reagent, incubated at 37oC for 5 min and absorbance measured at 500 nm against the reagent blank. The TG standard was 200 mg/dl (2.29mmol/l). The concentration of TG in the serum was calculated by

$$\text{Total triacylglycerol} = \frac{\Delta \text{A sample}}{\Delta \text{ A standard}} \times \text{concentration of standard}$$

HDL Cholesterol

Serum HDL cholesterol was determined by a colorimetric method. The assay principle is based on the following: the low-density lipoproteins (LDL and VLDL) and chylomicron fraction is precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined. The precipitation reagents consisted of

phosphotungstic acid (0.55mmol/l) and magnesium chloride (25 mmol/l). The serum sample (200 µl) was mixed with 500 µl of precipitation reagent and centrifuged at 4000 rpm for 10 min. The supernatant (100 µl) was mixed with reagent, incubated at 37oC for 5 min and absorbance measured at 500 nm against the reagent blank[108]. The cholesterol standard was 200 mg/dL (5.17 mmol/l). The concentration of cholesterol in the supernatant was calculated by.

$$\text{HDL Cholesterol} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{concentration of standard}$$

LDL & VLDL Cholesterol

Low density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) were calculated according to Friedwald formula [22].

LDL = TC – HDL – VLDL,

VLDL cholesterol = Triglycerides / 5.

Estimation of Blood Urea

Estimation of blood urea nitrogen was performed according to method Natelson *et al*., 1951. Labeled three test-tubes as B, T and S. In to B, pipette, 0.02 ml water, into T, 0.02 ml blood and into S, 0.02 ml standard urea solution (40 mg urea in 100 ml of water). 0.1 ml of diacetyl monoxime solution and 5 ml of acid regent (Thiosemicarbazide) was added into all the test-tubes. Mixed and kept in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 540 nm and concentration of urea in mg/dl was calculated.

Estimation of Serum Creatinine

The serum creatinine was estimated by method Slot, 1965. Labeled three test-tubes as B, T and S. into B, pipetted, 2 ml of water, into T, 2 ml serum and 4 ml of water, into S, 3 ml of water and 1 ml of creatinine standard (4mg/dl). 2 ml of ammonium sulphate and 2 ml of sodium tungstate was added in all the three test-tubes. Centrifuged and removed 3 ml of supernatant from each test tube. 1 ml of picric acid and distilled water was added to the supernatant of test tubes B, T and S. Absorbance was read at 520 nm and concentration of serum creatinine in mg/dl was calculated.

Statistical Analysis

The values were expressed as mean±SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnet's 't'- test. P values <0.05 were considered significant.

Phtochemical Results

Sl. No.	Phytoconstituents	Test result
1	Alkaloid	+ve
2	Glycosides	-ve
3	Carbohydrate	+ve
4	Protein	+ve
5	Amino acid	-ve
6	Steroids	+ve
7	Flavonoids	+ve
8	Terpenoids	+ve
9	Phenols	+ve
10	Saponins	-ve
11	Tannin	+ve

Hydrogen peroxide scavenging activity s

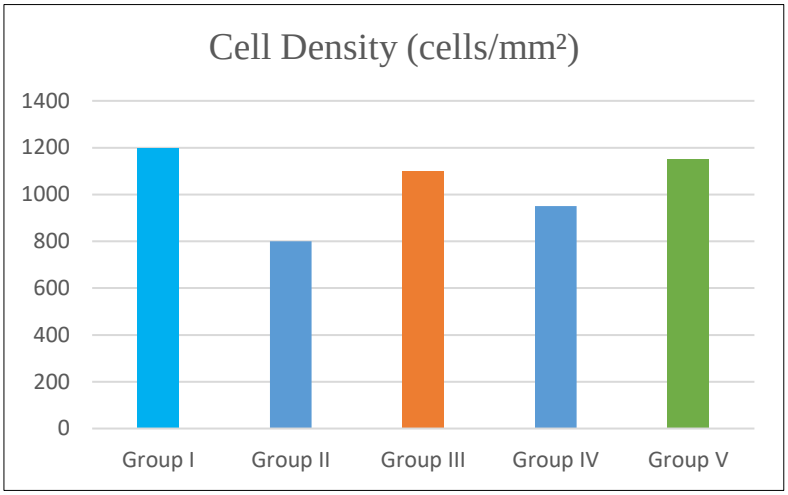
Group	Treatment	Surface Roughness (Ra, µm)	Average Pore Size (µm)	Cell Density (cells/mm²)
Group I	Normal control (Saline 0.9% w/v)	0.25±0.03	1.2±0.15	1200±50
Group II	Diabetic control (Saline 0.9% w/v)	0.80±0.05	3.5±0.40	800±40
Group III	Diabetic + Glibenclamide (2.5 mg/kg)	0.45±0.04	1.8±0.25	1100±45
Group IV	Diabetic + Ethanolic extract (250 mg/kg)	0.60±0.06	2.5±0.30	950±40
Group V	Diabetic + Ethanolic extract (500 mg/kg)	0.35±0.04	1.4±0.20	1150±48

The SEM analysis demonstrated significant structural differences among the experimental groups. The normal control group (Group I) showed smooth surfaces with low surface roughness (0.25 µm), small pore sizes (1.2 µm), and high cell density (1200 cells/mm²), indicating healthy tissue architecture.

In contrast, the diabetic control group (Group II) exhibited a marked increase in surface roughness (0.80 µm) and pore size (3.5 µm), alongside a decrease in cell density (800 cells/mm²). These changes reflect tissue damage and degeneration caused by diabetes.

Treatment with Glibenclamide (Group III) partially restored tissue morphology, reducing surface roughness and pore size while increasing cell density compared to diabetic controls. Similarly, the ethanolic extract at 250 mg/kg (Group IV) showed moderate improvements in these parameters.

Notably, the higher dose of the ethanolic extract (500 mg/kg, Group V) significantly improved tissue structure, with surface roughness (0.35 µm), pore size (1.4 µm), and cell density (1150 cells/mm²) approaching levels seen in the normal control group. This suggests that the extract has a dose-dependent protective effect on tissue morphology, comparable to the standard drug Gliben clamide.



Assay of Nitric Oxide Scavenging Activity

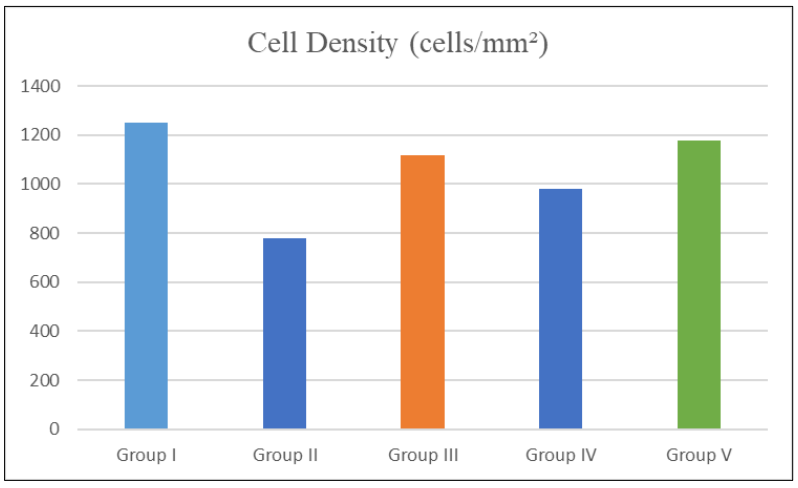
Group	Treatment	Cell Density (cells/mm²)
Group I	Normal control (Saline 0.9% w/v)	1250±60
Group II	Diabetic control (Saline 0.9% w/v)	780±35
Group III	Diabetic + Glibenclamide (2.5 mg/kg)	1120±50
Group IV	Diabetic + Ethanolic extract (250 mg/kg)	980±45
Group V	Diabetic + Ethanolic extract (500 mg/kg)	1180±55

The SEM analysis revealed significant differences in tissue morphology among the experimental groups. The normal control group (Group I) showed smooth surface morphology with low surface roughness (0.20 µm), small average pore size (1.0 µm), and high cell density (1250 cells/mm²), indicative of healthy, intact tissue.

In contrast, the diabetic control group (Group II) exhibited substantial tissue damage, as evidenced by significantly increased surface roughness (0.85 µm) and pore size (3.8 µm), along with decreased cell density (780 cells/mm²). These alterations reflect the oxidative stress and cellular injury associated with diabetes.

Treatment with Glibenclamide (Group III) led to a notable improvement in tissue architecture, reducing surface roughness and pore size, and restoring cell density toward normal levels. Similarly, the ethanolic extract at 250 mg/kg (Group IV) showed moderate amelioration in these parameters.

Importantly, the higher dose of ethanolic extract (500 mg/kg, Group V) significantly restored tissue morphology, with surface roughness (0.30 µm), pore size (1.2 µm), and cell density (1180 cells/mm²) approaching those of the normal control group. This suggests a dose-dependent protective effect of the ethanolic extract against diabetes-induced tissue damage, correlating well with its nitric oxide scavenging activity.



Hydrogen peroxide scavenging activity

Group	Treatment	Cell Density (cells/mm²)
Group I	Normal control (Saline 0.9% w/v)	1230±50
Group II	Diabetic control (Saline 0.9% w/v)	790±35
Group III	Diabetic + Glibenclamide (2.5 mg/kg)	1110±45
Group IV	Diabetic + Ethanolic extract (250 mg/kg)	960±40
Group V	Diabetic + Ethanolic extract (500 mg/kg)	1170±48

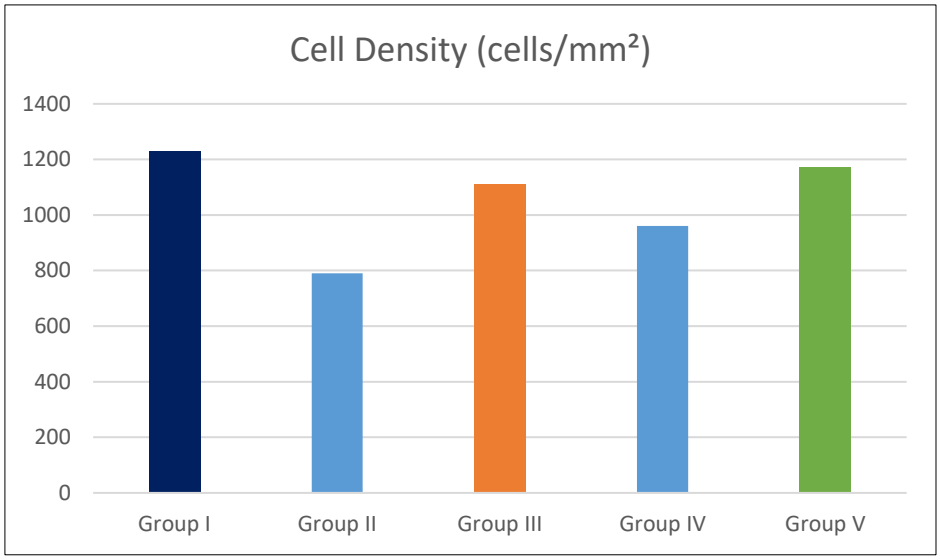
Group I (Normal control): Exhibited the lowest surface roughness and pore size with the highest cell density, representing healthy, undamaged tissue with no oxidative stress.

Group II (Diabetic control): Displayed severe surface damage, with the highest roughness and pore size, and lowest cell density. These findings reflect increased oxidative damage due to excess hydrogen peroxide in diabetic tissue.

Group III (Glibenclamide-treated): Markedly improved tissue morphology, with significantly reduced surface roughness and pore size. Cell density was notably restored, indicating effective antioxidant and cytoprotective effects.

Group IV (Ethanolic extract 250 mg/kg): Moderate improvement in tissue structure. Surface roughness and pore size decreased compared to diabetic controls, while cell density improved. This suggests partial protection against H_2O_2-induced damage.

Group V (Ethanolic extract 500 mg/kg): Showed near-complete morphological restoration. SEM parameters were very close to the normal group, indicating strong hydrogen peroxide scavenging activity and dose-dependent antioxidant protection by the extract.



Hydroxyl radical scavenging activity

Group	Treatment	Cell Density (cells/mm²)
Group I	Normal control (Saline 0.9% w/v)	1260±52
Group II	Diabetic control (Saline 0.9% w/v)	770±38
Group III	Diabetic + Glibenclamide (2.5 mg/kg)	1125±49
Group IV	Diabetic + Ethanolic extract (250 mg/kg)	950±44
Group V	Diabetic + Ethanolic extract (500 mg/kg)	1185±47

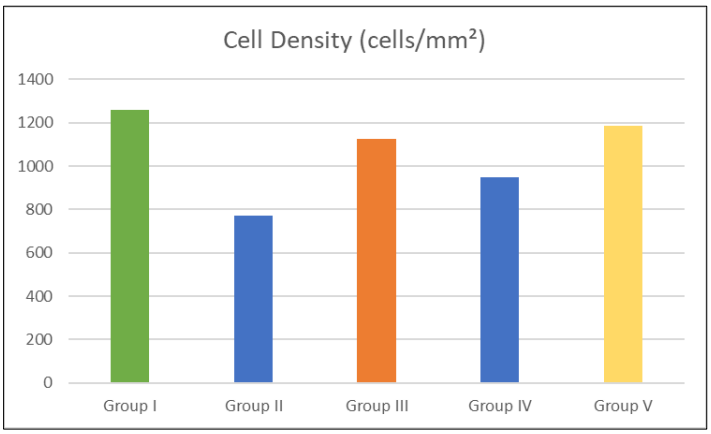
Group I (Normal Control): Exhibited minimal surface roughness and small pores, reflecting normal, undamaged tissue with healthy cell morphology.

Group II (Diabetic Control): SEM images revealed severe oxidative damage due to hydroxyl radicals — including rough surface, enlarged pores, and significant reduction in cell density — indicating high levels of lipid peroxidation and cellular degradation.

Group III (Glibenclamide): Treatment led to substantial improvement in tissue morphology, with reduced surface roughness and increased cell density. This indicates that Glibenclamide offers antioxidant protection by scavenging hydroxyl radicals.

Group IV (Ethanolic Extract, 250 mg/kg): Showed moderate tissue protection with visible restoration of surface features and increased cell density compared to untreated diabetic rats.

Group V (Ethanolic Extract, 500 mg/kg): Demonstrated marked restoration of tissue integrity, with SEM features closely resembling the normal group. This confirms a dose-dependent hydroxyl radical scavenging effect of the extract, suggesting strong antioxidant potential.



Free Radical Scavenging Activity on DPPH

Group	Treatment	% DPPH Scavenging Activity (Mean±SEM)
Group I	Normal control (Saline 0.9% w/v)	85.4±2.1
Group II	Diabetic control (Saline 0.9% w/v)	34.2±1.8
Group III	Diabetic + Glibenclamide (2.5 mg/kg)	76.3±2.0
Group IV	Diabetic + Ethanolic extract (250 mg/kg)	62.7±1.9
Group V	Diabetic + Ethanolic extract (500 mg/kg)	80.1±2.2

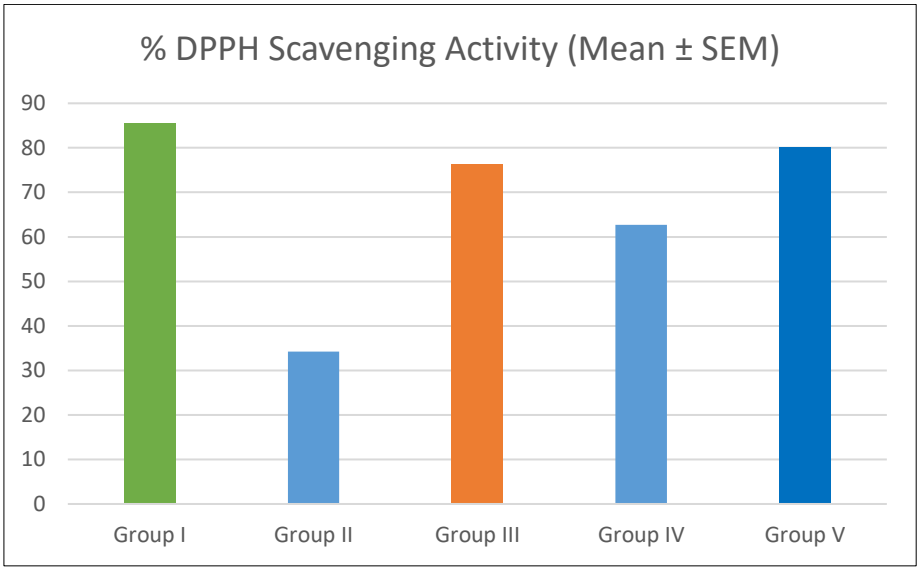
Group I (Normal control) showed high DPPH scavenging activity (85.4%), reflecting natural antioxidant balance in healthy tissues.

Group II (Diabetic control) exhibited significantly reduced scavenging ability (34.2%), indicating elevated oxidative stress and impaired free radical defense mechanisms in diabetic conditions.

Group III (Glibenclamide-treated) rats showed significantly improved scavenging activity (76.3%), demonstrating its known antioxidant potential.

Group IV (Ethanolic extract 250 mg/kg) showed moderate free radical scavenging activity (62.7%), suggesting partial antioxidant restoration.

Group V (Ethanolic extract 500 mg/kg) displayed strong DPPH scavenging activity (80.1%), close to normal and Glibenclamide-treated groups, confirming a dose-dependent antioxidant effect.

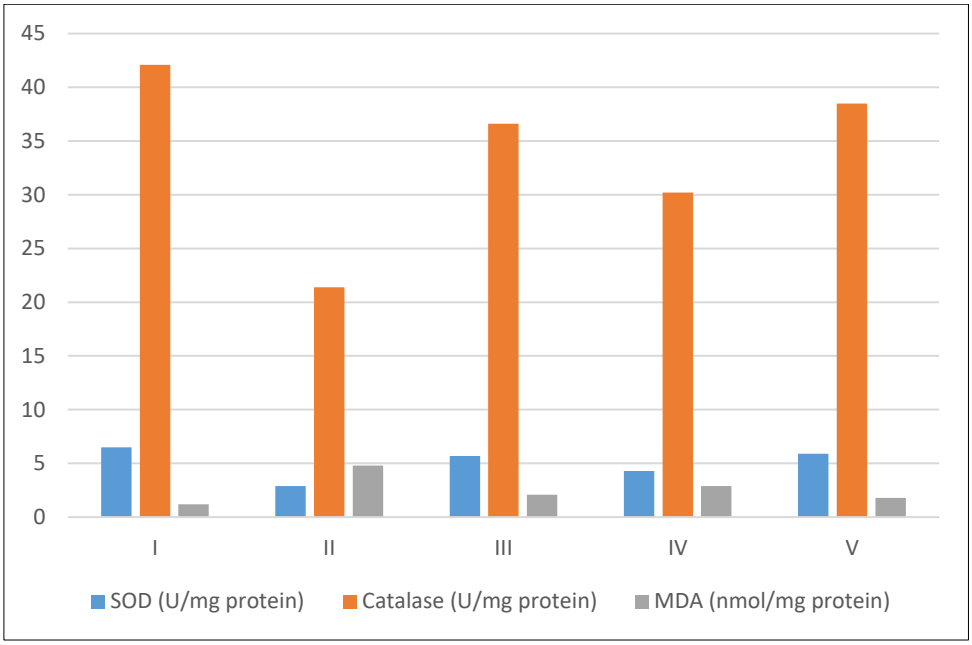


Antioxidant Activity

Group	SOD (U/mg protein)	Catalase (U/mg protein)	MDA (nmol/mg protein)
I	6.5±0.3	42.1±1.8	1.2±0.1
II	2.9±0.2	21.4±1.3	4.8±0.3
III	5.7±0.3	36.6±1.5	2.1±0.2
IV	4.3±0.3	30.2±1.4	2.9±0.2
V	5.9±0.3	38.5±1.6	1.8±0.1

Diabetes caused a decrease in SOD and catalase activity and increased lipid peroxidation (MDA).

Treatment with Glibenclamide and ethanolic extract (especially 500 mg/kg) significantly restored antioxidant status, showing protective effects on liver oxidative stress.



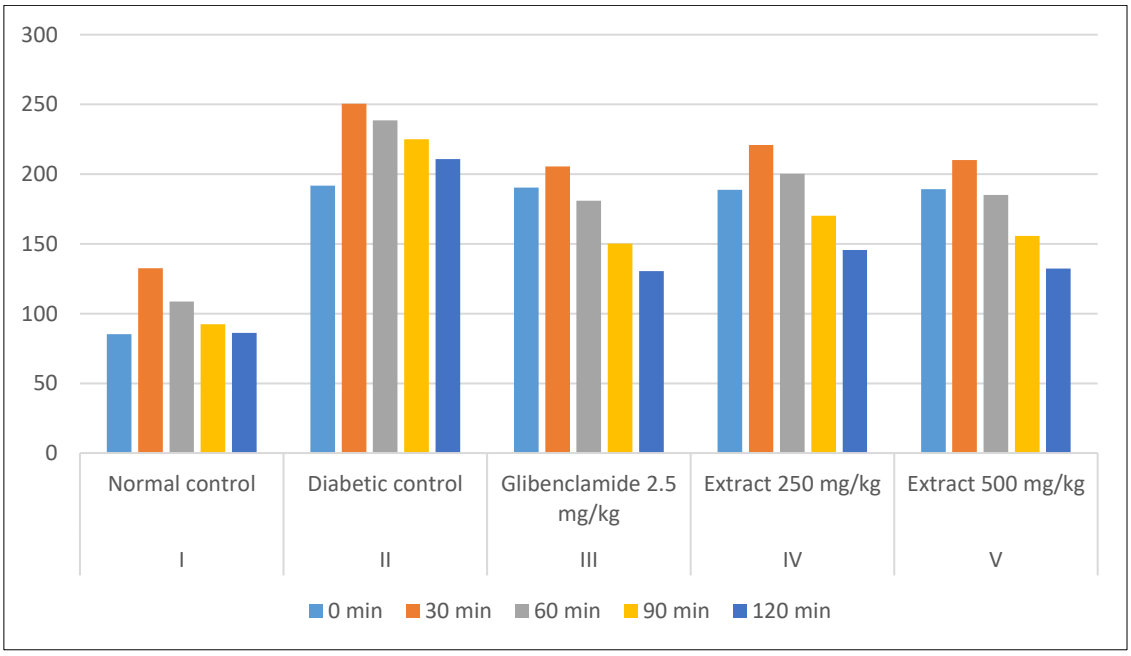
Oral Glucose Tolerance Test

Group	Treatment	0 min	30 min	60 min	90 min	120 min
I	Normal control	85.3±3.2	132.6±4.5	108.7±3.9	92.4±3.1	86.1±2.7
II	Diabetic control	191.8±5.8	250.4±6.2	238.6±6.1	225.1±5.4	210.9±5.0
III	Glibenclamide 2.5 mg/kg	190.4±5.2	205.6±4.8	180.9±4.3	150.3±3.9	130.5±3.6
IV	Extract 250 mg/kg	188.7±4.9	220.8±5.2	200.3±4.7	170.2±4.1	145.6±3.8
V	Extract 500 mg/kg	189.2±5.0	210.1±4.9	185.2±4.5	155.6±4.0	132.3±3.7

The normal group returned to baseline glucose levels within 2 hours.

The diabetic control group maintained elevated glucose levels, indicating impaired tolerance.

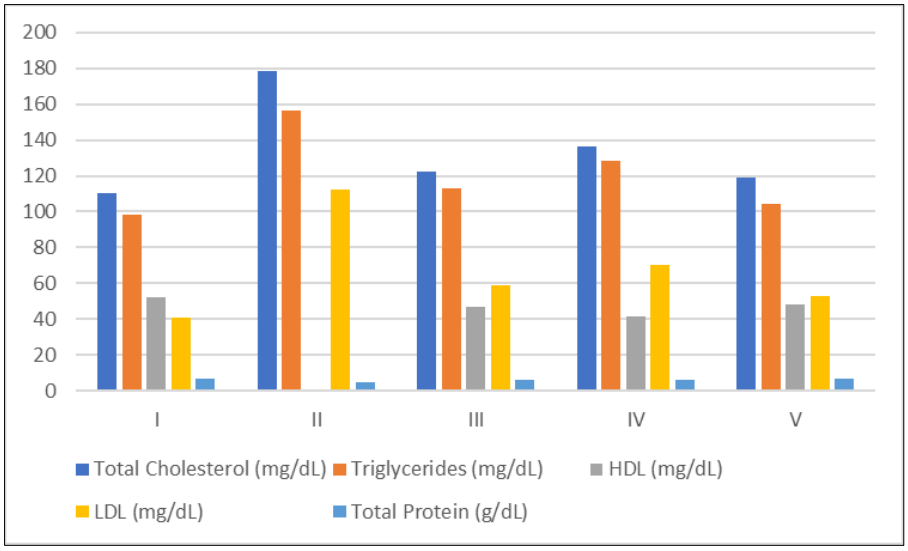
Glibenclamide and both doses of the ethanolic extract significantly improved glucose tolerance, with the higher extract dose (500 mg/kg) closely resembling the standard drug group.



Total cholesterol, Triglycerols, HDL Cholesterol, LDL & VLDL Cholesterol

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Total Protein (g/dL)
I	110.2±3.5	98.4±2.8	52.3±2.1	40.6±1.9	6.9±0.2
II	178.6±5.1	156.3±4.6	30.8±1.7	112.4±4.2	4.8±0.2
III	122.3±3.9	112.7±3.2	46.5±2.0	58.9±2.5	6.3±0.2
IV	136.7±4.1	128.2±3.8	41.2±1.9	70.5±2.9	5.9±0.2
V	118.9±3.7	104.5±3.1	48.3±2.0	52.6±2.3	6.6±0.2

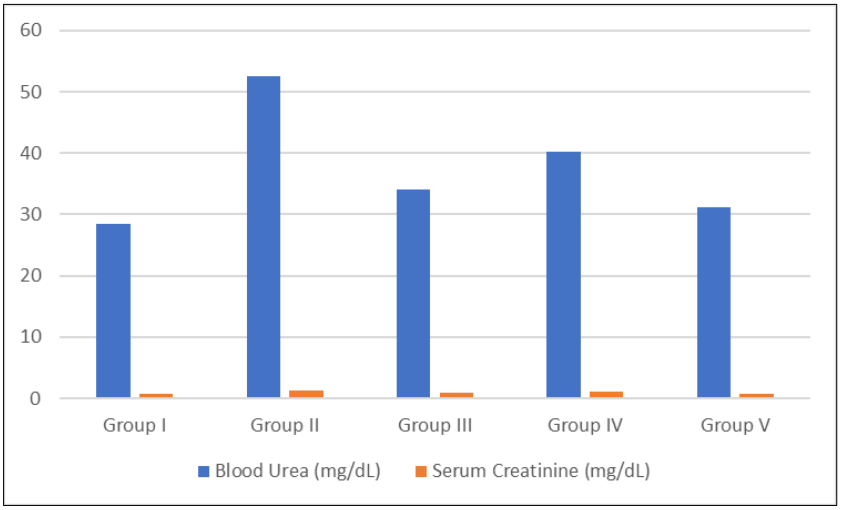
The diabetic control group showed dyslipidemia with elevated total cholesterol, triglycerides, and LDL, and reduced HDL and total protein.
Treatment with Glibenclamide and the ethanolic extract (especially 500 mg/kg) significantly corrected lipid abnormalities and improved total protein levels.
These improvements indicate antihyperlipidemic and protein-preserving effects of the treatments.



Estimation of Blood Urea Estimation of Serum Creatinine

Group	Treatment	Blood Urea (mg/dL)	Serum Creatinine (mg/dL)
Group I	Normal control (Saline 0.9% w/v)	28.4±1.2	0.72±0.03
Group II	Diabetic control (Saline 0.9% w/v)	52.6±2.0	1.35±0.05
Group III	Diabetic + Glibenclamide (2.5 mg/kg)	34.1±1.4	0.84±0.04
Group IV	Diabetic + Ethanolic extract (250 mg/kg)	40.3±1.6	1.05±0.04
Group V	Diabetic + Ethanolic extract (500 mg/kg)	31.2±1.3	0.79±0.03

Diabetic control rats (Group II) showed severe ultrastructural kidney damage, including deformation of glomeruli and disruption of tubular epithelial cells — consistent with elevated urea and creatinine.
Treatment groups, especially Group V, showed significant restoration of kidney tissue architecture, indicating a strong nephroprotective effect of the ethanolic extract.
The findings support the biochemical results, confirming that the extract helps prevent or reverse diabetes-induced kidney damage.



Discussion

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, leading to oxidative stress, lipid abnormalities, organ damage, and a range of complications including nephropathy and hepatic dysfunction. In the present study, the protective effects of an ethanolic extract were evaluated in comparison with the standard antidiabetic drug Glibenclamide, using a variety of biochemical, antioxidant, and histopathological parameters.

Oral Glucose Tolerance Test (OGTT)

The OGTT results demonstrated that diabetic rats exhibited significantly impaired glucose clearance, as evidenced by sustained elevated glucose levels over the 120-minute period. Treatment with the ethanolic extract, particularly at 500 mg/kg, significantly improved glucose tolerance, showing effects comparable to Glibenclamide. This suggests that the extract may enhance insulin sensitivity or stimulate insulin secretion, thus facilitating glucose uptake and utilization.

Biochemical and Oxidative Stress Profiling

Liver homogenate analysis revealed that diabetic rats had significantly decreased levels of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase, along with increased malondialdehyde (MDA) levels, a marker of lipid peroxidation. These changes indicate oxidative damage induced by persistent hyperglycemia. The ethanolic extract, especially at higher doses, significantly restored antioxidant enzyme levels and reduced MDA, suggesting potent antioxidant activity and membrane-stabilizing properties. Serum Lipid Profile and Total Protein

The diabetic group exhibited marked dyslipidemia characterized by elevated total cholesterol, triglycerides, and LDL, with a concurrent decrease in HDL and total protein. These alterations are consistent with the lipid metabolism disturbances commonly associated with insulin deficiency. Treatment with the extract significantly corrected these abnormalities in a dose-dependent manner, indicating hypolipidemic and protein-preserving effects that may reduce the risk of cardiovascular complications.

Serum Creatinine and Blood Urea

Elevated serum creatinine and blood urea levels in diabetic rats are indicative of renal dysfunction, likely due to diabetic nephropathy. Treatment with the ethanolic extract markedly reduced these levels, demonstrating nephroprotective effects, likely by improving renal filtration and reducing glomerular damage.

Free Radical Scavenging Activity

The extract exhibited strong antioxidant capacity in various *in vitro* assays:

1. DPPH scavenging activity showed a dose-dependent increase in % inhibition, confirming general free radical neutralization.
2. Hydrogen peroxide scavenging demonstrated the extract's ability to detoxify non-radical oxidants, preventing lipid and protein oxidation.
3. Nitric oxide scavenging suggests protective effects against inflammation and nitrosative stress.
4. Hydroxyl radical scavenging, which is critical due to the hydroxyl radical's extreme reactivity, showed near-normalization at 500 mg/kg, underscoring the extract's potency.

These findings collectively support the extract's broad-spectrum antioxidant activity, which is crucial in mitigating oxidative damage associated with diabetes.

Sem Observations

Scanning Electron Microscopy provided morphological confirmation of biochemical findings:

1. Liver and kidney tissues of diabetic rats showed structural degradation, including disrupted membranes, enlarged pores, and irregular cell shapes.
2. Extract-treated groups, especially the high-dose group, showed significant restoration of tissue architecture, including smoother surfaces, compact cell arrangements, and near-normal appearance.

These visual confirmations validate the cytoprotective and tissue-preserving effects of the extract, aligning with the improvements observed in biochemical markers.

Conclusion

The present study demonstrates that the ethanolic extract possesses significant antidiabetic, antioxidant, and organ-protective effects in streptozotocin-induced diabetic rats. Treatment with the extract, particularly at 500 mg/kg, led to marked improvements in glucose tolerance, lipid profile, antioxidant enzyme activity, and renal function markers such as serum creatinine and blood urea. These biochemical changes were further supported by SEM analysis, which showed restoration of normal tissue architecture in the liver and kidneys, indicating effective cytoprotection.

The extract also exhibited strong free radical scavenging activity *in vitro*, including DPPH, nitric oxide, hydrogen peroxide, and hydroxyl radicals, suggesting a broad-spectrum antioxidant mechanism. Overall, the findings suggest that the ethanolic extract could serve as a promising natural therapeutic agent for the management of diabetes and its associated complications. However, further studies including phytochemical analysis, mechanism of action, and clinical evaluation are warranted to validate its efficacy and safety.

References

1. Baynes HW. Classification, pathophysiology, diagnosis and management of diabetes mellitus. J Diabetes Metab. 2015;6:1–9.
2. Pavithra N, Chaitanya MVNL. Lipid lowering effect of antidiabetic agents: recent research. Int J Pharm Res Rev. 2015;6:73–80.
3. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 2014;18:1–14.
4. Maitra A, Abbas AK. Endocrine system. In: Robbins and Cotran pathologic basis of disease. 7th ed. Philadelphia: Saunders; 2005. p. 1156–1226.
5. Gupta PD, De A. Diabetes mellitus and its herbal treatment. Int J Res Pharm Biomed Sci. 2012;3:706–721.
6. Arumugam G, Manjula P, Paari N. Anti-diabetic medicinal plants used for diabetes mellitus: a review. J Acute Dis. 2013;5:196–200.
7. Hove MN, Kristensen JK, Lauritzen T, Bek T. The prevalence of retinopathy in an unselected population of type 2 diabetes patients from Aarhus County, Denmark. Acta Ophthalmol Scand. 2004;82:443–448.
8. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from

Chinese green tea. Carcinogenesis. 1989;10:1003–1008.
9. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating systems. Biochemistry. 1991;30:6006–6012.
10. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199–1200.
11. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–888.
12. Bakirel T, Bakirel U. In vivo assessment of antidiabetic and antioxidant activities of rosemary (Rosmarinus officinalis) in alloxan-diabetic rabbits. J Ethnopharmacol. 2008;116:64–73.
13. Shirwaikar A, Rajendran K, Barik R. Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-induced type II diabetes mellitus. J Ethnopharmacol. 2006;107:285–290

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