RESEARCH PAPER

Unravelling The Renoprotective Potential Of *Eleutherococcus Senticosus*-Derived Eleutherosides In Experimental Diabetic Nephropathy

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Abstract

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Introduction

Diabetic nephropathy (DN) is a major microvascular complication of diabetes mellitus and represents a progressive cause of renal dysfunction. The disease is characterized by structural and functional alterations in the kidney, including glomerular injury, mesangial expansion, albuminuria, and progressive deterioration of renal function. Persistent hyperglycemia plays a central role in the development of DN by promoting oxidative stress, inflammatory responses, cellular injury, and apoptosis within renal tissues.[1–3]

Excessive generation of reactive oxygen species (ROS) under hyperglycemic conditions can impair endogenous antioxidant defenses and promote lipid peroxidation, protein oxidation, and cellular damage.[4–6] In parallel, activation of inflammatory pathways and increased production of pro-inflammatory cytokines contribute to the progression of renal injury and tissue remodeling.[7–10] The interaction between oxidative stress and inflammation is therefore considered an important component of diabetic renal damage.

Despite advances in the management of diabetes and its renal complications, the progressive nature of diabetic kidney injury continues to support the investigation of additional pharmacological approaches. Medicinal plants and their bioactive constituents have received considerable attention because of their potential antioxidant, anti-inflammatory, and metabolic effects.[11] *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., commonly known as Siberian ginseng, is a medicinal plant traditionally recognized for its pharmacological and adaptogenic properties. Its roots contain several bioactive constituents, particularly eleutherosides, which have been investigated for their diverse biological activities.[12–17]

Previous experimental studies have reported beneficial metabolic effects of *E. senticosus* and its constituent syringin in experimental diabetes, including improvements in glucose metabolism and insulin-related responses.[18,19] However, the protective potential of the root extract against diabetes-associated renal injury requires further experimental investigation. Therefore, the present study was designed to evaluate the nephroprotective activity of hydroalcoholic root extract of *E. senticosus* in streptozotocin-induced diabetic nephropathy, with particular emphasis on biochemical alterations, oxidative stress, inflammatory responses, and renal histopathological changes.

2. Materials and Methods

2.1 Preparation of Hydroalcoholic Root Extract

The dried roots of *Eleutherococcus senticosus* were coarsely powdered and passed through a 40-mesh sieve. Approximately 100 g of the powdered material was extracted with 800 mL of 70% ethanol using a Soxhlet apparatus for 6–8 h, until the siphoning solvent became nearly colorless. The resulting extract was cooled and filtered through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator maintained at 40–45 °C. The concentrated extract was further dried in a vacuum desiccator to obtain a semisolid hydroalcoholic extract. The percentage yield was calculated with respect to the initial weight of the dried root material. The final extract was transferred into airtight amber-colored containers and stored at 4 °C until further use.[20]

2.2 Preliminary Phytochemical Screening

A preliminary qualitative phytochemical investigation was performed on the hydroalcoholic root extract of *Eleutherococcus senticosus*. Standard chemical tests were employed to identify different classes of phytoconstituents. Alkaloids were evaluated using Hager's, Wagner's, and Dragendorff's tests, whereas flavonoids were examined using Shinoda, alkaline reagent, and lead acetate reactions. Phenolic compounds and tannins were assessed using ferric chloride, gelatin, lead acetate, and potassium dichromate tests. Carbohydrates were investigated using Molisch's, Benedict's, and Fehling's tests, while proteins were detected using Biuret, Xanthoproteic, and Millon's reactions. Saponins were evaluated by the froth test, steroids by Salkowski and Liebermann–Burchard reactions, and fixed oils and fats by the stain test. The observations were recorded according to the characteristic reactions obtained.[21,22]

2.3 Isolation of Eleutherosides

The hydroalcoholic root extract of *Eleutherococcus senticosus* was subjected to successive solvent fractionation using n-hexane, chloroform, and ethyl acetate. The individual fractions were concentrated separately and further subjected to silica gel column chromatography using a chloroform–methanol gradient system. The collected fractions were monitored by thin-layer chromatography (TLC), and fractions exhibiting similar chromatographic profiles were pooled. The separated constituents were examined under ultraviolet light and, where necessary, visualized by spraying with 10% sulfuric acid followed by gentle heating. The isolated compounds were characterized using chromatographic and spectroscopic techniques, including UV, IR, ^{1}H-NMR, and ^{13}C-NMR analyses. Based on comparison of the obtained spectral characteristics with previously reported data, the isolated constituents were assigned as Eleutheroside B and Eleutheroside E.[14–17,23,24]

2.4 Experimental Design

Male BALB/c mice weighing 20–22 g and aged 4–6 weeks were maintained under controlled laboratory conditions and acclimatized for 7 days before initiation of the experiment. The animals were housed at 24 ± 1 °C with a relative humidity of 60 ± 2% under a 12 h light/12 h dark cycle and provided free access to standard feed and drinking water. Following acclimatization, the animals were randomly divided into six experimental groups, with six animals in each group (n = 6). The experimental design was based on established streptozotocin-induced diabetic animal models.[25–27]

Group 1: Normal control
Group 2: Diabetic control – STZ 50 mg/kg, i.p.
Group 3: Diabetic + metformin 250 mg/kg, p.o.
Group 4: Diabetic + *E. senticosus* extract 100 mg/kg, p.o.
Group 5: Diabetic + *E. senticosus* extract 300 mg/kg, p.o.
Group 6: Diabetic + combination treatment of *E. senticosus* extract and metformin.

Diabetes was induced by a single intraperitoneal administration of streptozotocin (STZ) prepared freshly in citrate buffer (pH 4.5). Fasting blood glucose was assessed 72 h after STZ administration, and animals showing blood glucose concentrations above 250 mg/dL were considered diabetic. The respective treatments were administered orally once daily for 28 consecutive days. STZ-induced diabetic models are widely used for experimental investigation of diabetic renal injury.[25–28]

All procedures involving experimental animals were performed in accordance with the guidelines approved by the Institutional Animal Ethics Committee (IAEC), under IAEC registration/approval number 2331/PO/Re/S/2025/CCSEA.

2.5 Physiological and General Health Assessment

General health and observable behavioral changes were monitored throughout the 28-day treatment period. Body weight was recorded at weekly intervals, and the percentage change in body weight was calculated using the following equation:

% Change in body weight = [(Final body weight − Initial body weight) / Initial body weight] × 100

At the end of the treatment period, urine samples were collected using the experimental urine-collection procedure followed in the study. The samples were centrifuged at 3000 × g for 20 min, and the resulting samples were stored at −80 °C until biochemical analysis.

General external appearance was also monitored during the experimental period. Fur condition was assessed according to observable characteristics such as cleanliness, density, shininess, color uniformity, and presence or absence of visible lesions.

2.6 Assessment of Renal Hypertrophy

At the end of the 28-day treatment period, the animals were sacrificed, and both kidneys were carefully removed. Adhering tissues were removed, and the kidneys were blotted to eliminate excess moisture before individual weighing. Renal hypertrophy was expressed as the kidney-to-body weight ratio using the following equation:

Kidney-to-body weight ratio (%) = [Kidney weight / Body weight at sacrifice] × 100

Changes in kidney-to-body weight ratio were used to assess diabetes-associated alterations in renal size.[27,28]

2.7 Biochemical Analysis

Following completion of the treatment period, blood samples were collected by retro-orbital puncture under light anesthesia. The samples were centrifuged at 3000 × g for 15 min to separate serum, which was aliquoted and stored at −80 °C until biochemical analysis. Serum glucose, creatinine, albumin, and insulin concentrations were determined using appropriate commercially available diagnostic kits according to the manufacturers' instructions.

For urinary biochemical assessment, animals were placed individually in metabolic cages, and urine was collected over a 4–6 h period. The collected urine samples were centrifuged at 3000 rpm for 10 min, and the resulting supernatants were used for estimation of urinary albumin using an enzyme-linked immunosorbent assay (ELISA). Urinary albumin concentration was normalized against urinary creatinine, and the albumin-to-creatinine ratio (ACR) was expressed as µg/mg creatinine. ACR is an established measure used for the assessment of albuminuria and diabetic kidney injury.[29,30]

2.8 Oxidative Stress and Antioxidant Assays

Kidneys were collected following sacrifice and immediately rinsed with ice-cold saline. The tissues were homogenized at a concentration of 10% (w/v) in 50 mM phosphate buffer (pH 7.4) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatants were collected for biochemical analysis.

Renal lipid peroxidation was evaluated by estimating malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) method.[31] Reduced glutathione (GSH) content was determined according to the method of Ellman.[32] Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were determined using established spectrophotometric methods.[33–35]

The free-radical-scavenging capacity of the extract was additionally evaluated in vitro using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[36] Where applicable, the biochemical results were normalized to the protein concentration of the respective tissue homogenates using the Bradford protein assay.[37]

2.9 Inflammatory and Histopathological Evaluation

Inflammatory responses were evaluated by measuring tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) concentrations in serum and/or kidney tissue samples using commercially available ELISA kits according to the respective manufacturers' instructions. Increased inflammatory signaling and cytokine production are recognized components of diabetic renal injury.[38,39]

For histopathological examination, kidney tissues were collected and immediately fixed in 10% neutral buffered formalin. The fixed tissues were processed using standard histological procedures, embedded in paraffin wax, and sectioned at a thickness of approximately 5 µm. The sections were stained with haematoxylin and eosin (H&E) and examined microscopically. Standard histological procedures were followed for tissue processing and microscopic evaluation.[40]

2.10 Statistical Analysis

The experimental results were expressed as mean ± standard error of the mean (SEM), with n = 6 animals per group. Statistical comparisons among the experimental groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test. Statistical analysis was performed using GraphPad Prism software. A p value of <0.05 was considered statistically significant. The corresponding significance levels were indicated in the respective tables and figures.

3. RESULTS

3.1. Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the *Eleutherococcus senticosus* root extract indicated the presence of several classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, and carbohydrates. Positive reactions were observed in the Shinoda test for flavonoids and in ferric chloride and lead acetate tests for phenolic constituents. The observed phytochemical profile indicates the presence of diverse bioactive constituents in the hydroalcoholic root extract. However, these qualitative tests provide only preliminary evidence of the respective phytochemical classes and do not specifically establish the presence of individual eleutherosides. The subsequent isolation and spectroscopic characterization were therefore used for the identification of Eleutheroside B and Eleutheroside E.

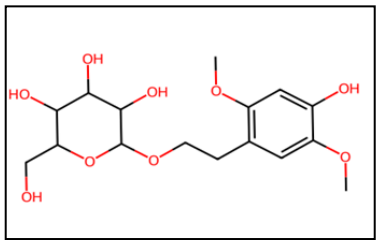


Fig.1. Structure of Eleutheroside B

3.2. FT-IR Analysis

The FT-IR spectra of the isolated Eleutheroside B and Eleutheroside E fractions showed characteristic absorption bands associated with their major functional groups. A broad absorption band observed around 3400 cm^{-1} was attributed to O–H stretching, indicating the presence of hydroxyl groups. Absorption bands in the region of approximately 1660–1665 cm^{-1} were associated with carbonyl-related vibrations, while bands observed within the 1610–1520 cm^{-1} region were attributed to aromatic C=C stretching. The observed spectral features were consistent with the presence of hydroxylated aromatic structures and glycosidic components. These FT-IR characteristics, together with the other spectroscopic findings, supported the assignment of the isolated compounds as Eleutheroside B and Eleutheroside E.

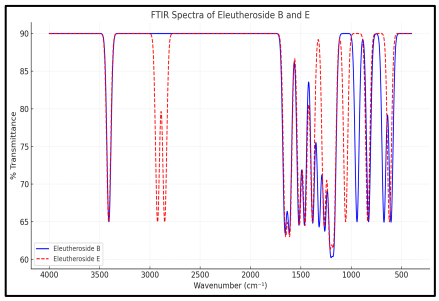


Fig.2. FT-IR spectrum of Eleutheroside B and Eleutheroside E

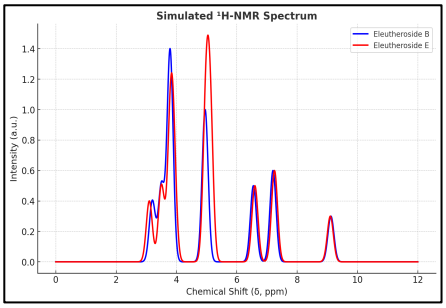


Fig.3.^{1}H NMR spectrum of Eleutheroside B and Eleutheroside E

3.3. Nuclear Magnetic Resonance (NMR) Analysis

The ^{1}H-NMR spectrum of the isolated Eleutheroside B fraction showed characteristic signals in the aromatic proton region, together with resonances in the δ 3.2–5.2 ppm range attributable mainly to oxygenated aliphatic and sugar-related protons. Signals observed in the aromatic region were consistent with the presence of a substituted aromatic moiety. The ^{13}C-NMR spectrum showed resonances within the aromatic and oxygenated carbon regions, including signals in the δ 101–103 ppm range that were attributable to anomeric carbon environments. The overall spectral pattern was consistent with a glycosylated aromatic phenylpropanoid structure.

The ^{1}H-NMR spectrum of the isolated Eleutheroside E fraction exhibited characteristic aromatic proton signals in the δ 6.42–7.62 ppm region, together with signals in the δ 3.2–5.2 ppm range corresponding to oxygenated and sugar-associated protons. The ^{13}C-NMR spectrum displayed resonances distributed across the δ 62–165 ppm range, corresponding to oxygenated aliphatic, aromatic, and glycosidic carbon environments. The combined ^{1}H-NMR and ^{13}C-NMR spectral characteristics, together with the UV and FT-IR findings, supported the assignment of the isolated fractions as Eleutheroside B and Eleutheroside E.

3.4. Body Weight

A progressive reduction in body weight was observed in the diabetic control group during the experimental period, which was associated with STZ-induced diabetic alterations. Treatment with *Eleutherococcus senticosus* root extract at 100 mg/kg produced a partial improvement in body weight compared with the diabetic control group. The 300 mg/kg dose produced a greater restoration of body weight, with values approaching those observed in the normal control group. The metformin-treated group also showed improvement in body weight compared with the diabetic control animals. Overall, the findings

demonstrated a dose-related attenuation of STZ-associated body-weight loss following treatment with *E. senticosus* root extract.

Group	Initial Body Weight (g)	Final Body weight (g)	% Change in Body Weight
Normal Control	21.0 ± 0.5	23.5 ± 0.6	+11.90%
Diabetic **Control**	21.2 ± 0.4**	18.0 ± 0.5**	–15.09%
Diabetic + Low Dose (ELD)	21.1 ± 0.6**	20.00020± 0.5**	–5.21%
Diabetic + High Dose (EHD)	21.3 ± 0.5***	21.8 ± 0.6***	+2.35%
Diabetic + Standard Drug (STD)	21.2 ± 0.4***	22.5 ± 0.5***	+6.13%

Table 1 Effect of Eleutherococcus senticosus Root Extract on Body Weight in STZ-Induced Diabetic Nephropathy Mice.

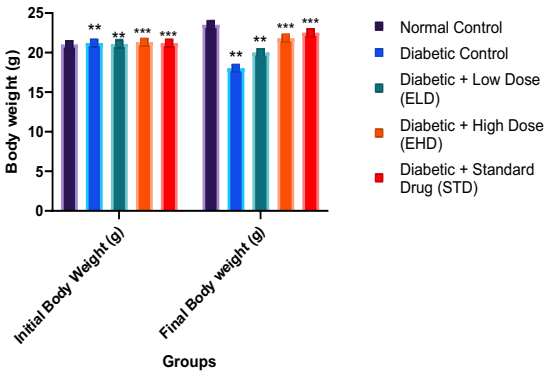


Fig. 4 Initial and Final Body weight

3.5. Urine Volume (Polyuria)

Urine output was markedly increased in the diabetic control group compared with the normal control group, indicating the development of polyuria following STZ administration. Treatment with *Eleutherococcus senticosus* root extract at 100 mg/kg produced a partial reduction in urine volume. A greater reduction was observed in animals receiving the 300 mg/kg dose, with urine output showing a trend toward the values observed in the normal control group. The standard drug-treated group also exhibited a reduction in urine volume compared with the diabetic control group. Overall, the findings suggest a dose-related attenuation of STZ-associated polyuria following treatment with *E. senticosus* root extract.

Group	**Urine Volume (mL/4–6 h)**
Normal Control	0.35 ± 0.05
Diabetic Control	1.20 ± 0.08**
Diabetic + Low Dose (ELD)	0.85 ± 0.07**
Diabetic + High Dose (EHD)	0.50 ± 0.06***
Diabetic + Standard Drug (STD)	0.45 ± 0.05***

Table 2 Effect of Eleutherococcus senticosus Root Extract on Urine volume in STZ-Induced Diabetic Nephropathy Mice

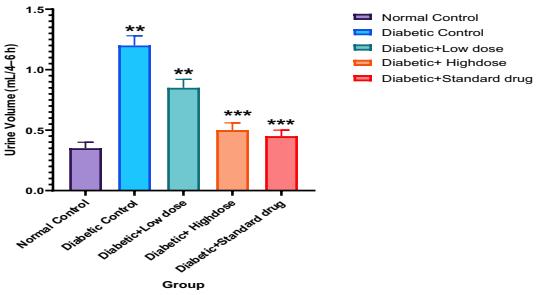


Fig 5. Urine volume

3.6. Fur Condition

The diabetic control group exhibited observable changes in fur appearance, including reduced smoothness, dullness, and patchy distribution, compared with the normal control animals. Treatment with *Eleutherococcus senticosus* root extract produced an improvement in the general appearance of the fur. The improvement was more evident at the 300 mg/kg dose, where the fur characteristics were closer to those observed in the standard drug-treated group. These observations indicate an improvement in general external appearance during treatment; however, fur condition was considered a general health observation rather than a specific measure of the pharmacological mechanism of the extract.

3.7. Renal Hypertrophy

The kidney-to-body weight ratio was increased in the diabetic control group compared with the normal control group, indicating an alteration in renal size associated with experimental diabetes. Administration of *Eleutherococcus senticosus* root extract resulted in a reduction in the kidney-to-body

weight ratio compared with the diabetic control group. The decrease was more pronounced in animals treated with the 300 mg/kg dose, with values approaching those observed in the standard drug-treated group. These findings demonstrate an attenuation of the diabetes-associated increase in kidney-to-body weight ratio following treatment with *E. senticosus* root extract.

3.8. Biochemical Parameters

The effects of *Eleutherococcus senticosus* root extract on serum biochemical parameters are presented in Table 3. The diabetic control group showed a marked increase in serum glucose (320 ± 10 mg/dL) and creatinine (0.85 ± 0.05 mg/dL) compared with the normal control group, which recorded values of 95 ± 5 mg/dL and 0.35 ± 0.02 mg/dL, respectively. In contrast, serum albumin and insulin concentrations were reduced in diabetic animals, with values of 2.8 ± 0.1 g/dL and 5 ± 0.5 µIU/mL, respectively, compared with 4.2 ± 0.1 g/dL and 12 ± 1 µIU/mL in the normal control group.

Treatment with *E. senticosus* root extract produced dose-related improvements in these parameters. The 100 mg/kg treatment reduced serum glucose and creatinine to 220 ± 8 mg/dL and 0.65 ± 0.04 mg/dL, respectively, while serum albumin and insulin increased to 3.5 ± 0.1 g/dL and 7 ± 0.5 µIU/mL. A greater improvement was observed with the 300 mg/kg dose, which reduced glucose and creatinine levels to 140 ± 7 mg/dL and 0.45 ± 0.03 mg/dL, respectively, and increased albumin and insulin concentrations to 4.0 ± 0.1 g/dL and 10 ± 0.8 µIU/mL.

The standard drug-treated group showed serum glucose, creatinine, albumin, and insulin values of 130 ± 6 mg/dL, 0.40 ± 0.02 mg/dL, 4.1 ± 0.1 g/dL, and 11 ± 0.7 µIU/mL, respectively. Thus, the 300 mg/kg extract treatment produced biochemical responses approaching those observed with the standard treatment.

Plasma catalase activity was markedly reduced in the diabetic control group (20 ± 2 U/mL) compared with the normal control group (45 ± 3 U/mL). Treatment with the extract increased catalase activity to 30 ± 2 U/mL at 100 mg/kg and 40 ± 3 U/mL at 300 mg/kg. The standard drug-treated group showed a catalase activity of 42 ± 2 U/mL. These findings indicate that *E. senticosus* treatment was associated with improvement in the measured metabolic, renal, and antioxidant parameters, with the higher extract dose producing a greater response.

Table 3 Effect of Eleutherococcus senticosus Root Extract on Biochemical Analysis

Group	Serum Glucose (mg/dL)	Serum Creatinine (mg/dL)	Serum Albumin (g/dL)	Serum Insulin (µIU/mL)	Plasma Catalase (CAT) Activity (U/mL)
Normal Control	95 ± 5	0.35 ± 0.02	4.2 ± 0.1	12 ± 1	45 ± 3
Diabetic Control	320 ± 10***	0.85 ±0.05***	2.8 ± 0.1***	5 ± 0.5***	20 ± 2***
Diabetic + Low Dose (ELD)	220 ± 8***	0.65 ±0.04***	3.5 ± 0.1***	7 ± 0.5***	30 ± 2***
Diabetic + High Dose (EHD)	140 ± 7###	0.45 ± 0.03***###	4.0 ± 0.1***###	10 ± 0.8***###	40 ± 3***###
Diabetic + Standard Drug (STD)	130 ± 6###	0.40 ± 0.02***###	4.1 ± 0.1***###	11 ± 0.7***###	42 ± 2***###

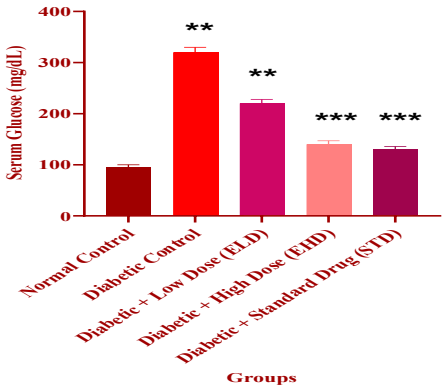


Fig. 6. Serum glucose (p<0.0001*)(p<0.001**)**

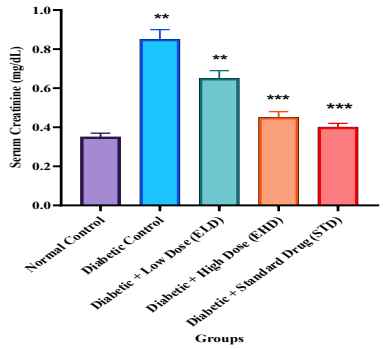


Fig. 7. Serum Creatinine (p<0.0001*)(p<0.001**)**

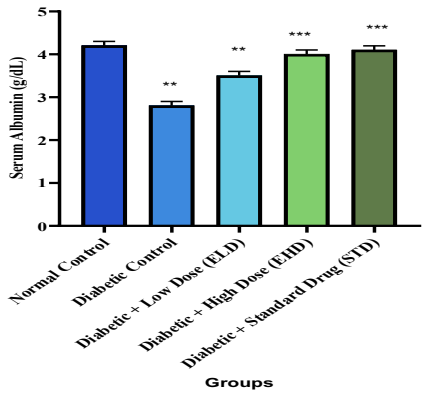


Fig. 8. Serum Albumin (p<0.0001*)(p<0.001**)**

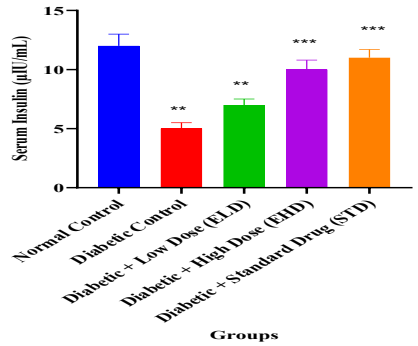


Fig. 9. Serum Insulin (p<0.0001*)(p<0.001**)**

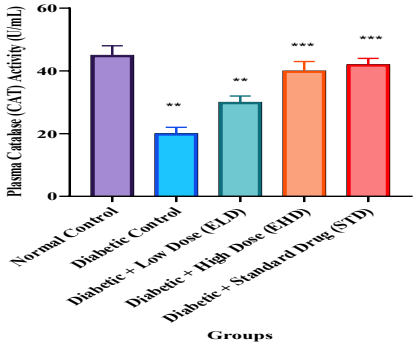


Fig .10. Serum Insulin (p<0.0001*)(p<0.001**)**

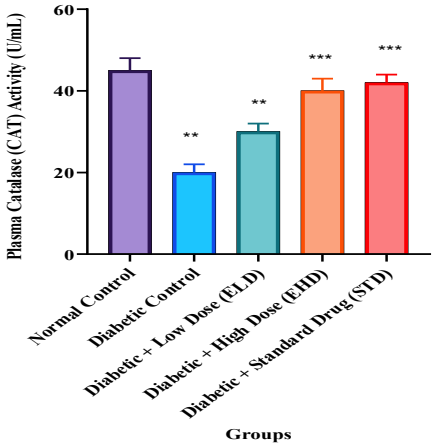


Fig. 11. Plasma Catalase Activity

3.9. Estimation of Urinary Albumin and Albumin-to-Creatinine Ratio

Urinary albumin excretion and albumin-to-creatinine ratio (ACR) were increased in the diabetic control group compared with the normal control group, indicating increased albuminuria associated with experimental diabetes. Treatment with *Eleutherococcus senticosus* root extract resulted in a reduction in urinary albumin excretion and ACR compared with the diabetic control group. The reduction was more evident at the 300 mg/kg dose, with ACR values approaching those observed in the normal and standard drug-treated groups. The findings indicate that *E. senticosus* treatment attenuated diabetes-associated albuminuria, with a greater response observed at the higher dose.

Table 4 .Effect of Eleutherococcus senticosus Root Extract on urine albumin in STZ-Induced Diabetic Nephropathy Mice

Group	Urinary Albumin (µg/mL)	Urinary Creatinine (mg/mL)	ACR (µg/mg)
Normal Control	25 ± 3	1.8 ± 0.2	13.9 ± 2.1
Diabetic Control	120 ± 8	1.2 ± 0.1	100.0 ± 5.6**

Diabetic + Low Dose (ELD)	85 ± 6	1.4 ± 0.1	60.7 ± 4.8**
Diabetic + High Dose (EHD)	60 ± 5	1.6 ± 0.2	37.5 ± 3.9***
Diabetic + Standard Drug (STD)	55 ± 4	1.7 ± 0.2	32.4 ± 3.1***

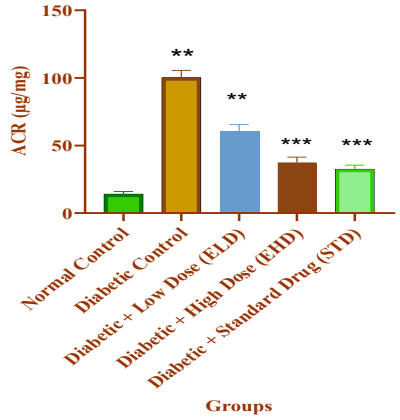


Fig. 12 ACR

3.10. Oxidative Stress Markers

The effects of *Eleutherococcus senticosus* root extract on renal oxidative stress and antioxidant status are presented in Table 4. The diabetic control group exhibited a marked elevation in MDA level (6.5 ± 0.30 nmol/mg protein) compared with the normal control group (2.8 ± 0.15 nmol/mg protein), indicating increased lipid peroxidation. In parallel, the levels of GSH and the activities of SOD, CAT, and GPx were substantially reduced in diabetic animals, with values of 3.2 ± 0.20 µmol/mg protein, 5.4 ± 0.25 U/mg protein, 30.2 ± 1.5 µmol H_2O_2/min/mg protein, and 4.6 ± 0.20 nmol NADPH/min/mg protein, respectively.

Administration of *E. senticosus* root extract resulted in a dose-related improvement in the measured oxidative stress parameters. At 100 mg/kg, MDA was reduced to 4.8 ± 0.25 nmol/mg protein, accompanied by increases in GSH, SOD, CAT, and GPx to 5.6 ± 0.30 µmol/mg protein, 8.0 ± 0.40 U/mg protein, 45.8 ± 2.1 µmol H_2O_2/min/mg protein, and 7.2 ± 0.30 nmol NADPH/min/mg protein, respectively.

The 300 mg/kg treatment produced a greater response, reducing MDA to 3.6 ± 0.20 nmol/mg protein and increasing GSH, SOD, CAT, and GPx to 7.2 ± 0.35 µmol/mg protein, 10.5 ± 0.50 U/mg protein, 56.4 ± 2.5 µmol H_2O_2/min/mg protein, and 9.1 ± 0.40 nmol NADPH/min/mg protein, respectively. The standard drug-treated group showed values of 3.2 ± 0.18 nmol/mg protein for MDA, 7.8 ± 0.40 µmol/mg protein for GSH, 11.5 ± 0.55 U/mg protein for SOD, 60.8 ± 2.8 µmol H_2O_2/min/mg protein for CAT, and 9.8 ± 0.45 nmol NADPH/min/mg protein for GPx.

The DPPH radical-scavenging activity was also reduced in the diabetic control group (41.5 ± 2.0%) compared with the normal control group (82.0 ± 2.5%). Treatment with the extract increased DPPH inhibition to 59.0 ± 2.2% and 71.2 ± 2.4% at 100 and 300 mg/kg, respectively, while the standard treatment produced 75.5 ± 2.6% inhibition. Overall, the findings demonstrate that *E. senticosus* root extract was associated with reduced lipid peroxidation and improved antioxidant status, with the higher dose producing a greater response.

Group	**DA (nmol/mg protein)**	GSH(µmol/mg protein)	SOD (U/mg protein)	CAT (µmol H_2O_2/min/mg protein)	GPx (nmol NADPH/min/mg protein)	DPPH % Inhibition
Normal Control (NC)	2.8 ± 0.15	8.5 ± 0.40	12.2 ± 0.60	65.5 ± 3.2	10.8 ± 0.50	82.0 ± 2.5
Diabetic Control	6.5 ± 0.30**	3.2 ± 0.20**	5.4 ± 0.25**	30.2 ± 1.5**	4.6 ± 0.20**	41.5 ± 2.0**
Diabetic + Low Dose (ELD)	4.8 ± 0.25**	5.6 ± 0.30**	8.0 ± 0.40**	45.8 ± 2.1**	7.2 ± 0.30**	59.0 ± 2.2**
Diabetic + High Dose (EHD)	3.6 ± 0.20***	7.2 ± 0.35***	10.5 ± 0.50**	56.4 ± 2.5**	9.1 ± 0.40**	71.2 ± 2.4***

Diabetic + Standard Drug (STD)	3.2 ± 0.18***	7.8 ± 0.40***	11.5 ± 0.55***	60.8 ± 2.8***	9.8 ± 0.45***	75.5 ± 2.6***

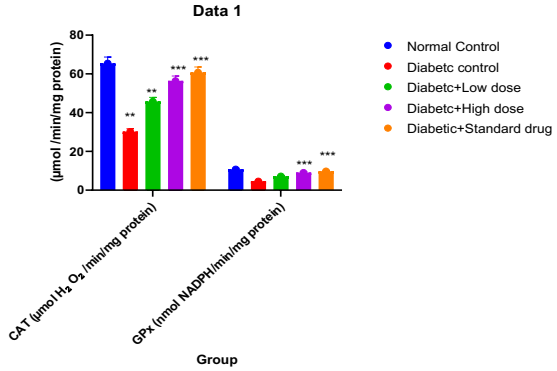


Figure 13. CAT and GPx

3.11. Assessment of Inflammatory Markers

Group	Serum TNF-α (pg/mL)	Serum IL-1β (pg/mL)	Serum IL-6 (pg/mL)
Normal Control	18.5 ± 1.2	12.2 ± 0.8	15.8 ± 1.0
Diabetic Control	45.6 ± 2.5**	32.4 ± 1.8**	40.2 ± 2.0**
Diabetic + Low Dose (ELD)	33.2 ± 1.8**	24.5 ± 1.3**	30.4 ± 1.6**
Diabetic + High Dose (EHD)	25.8 ± 1.5***	18.6 ± 1.1***	22.8 ± 1.4***
Diabetic + Standard Drug (STD)	25.8 ± 1.5***	18.6 ± 1.1***	22.8 ± 1.4***

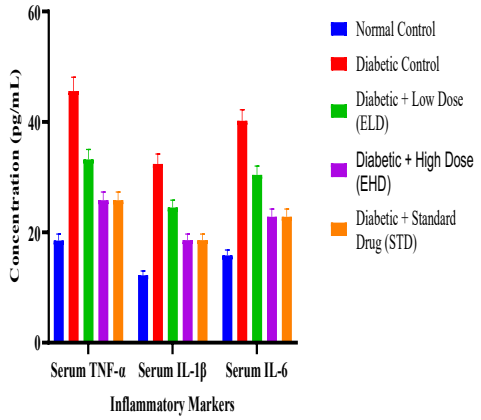


Figure. 14. Inflammatory markers (Serum)

Group	KidneyTNF-α (pg/mL)	KidneyIL-1β (pg/mL)	Kidney IL-6 (pg/mL)
Normal Control	**22.5 ± 1.4**	**15.2 ± 1.0**	**18.6 ± 1.2**
Diabetic Control	**52.8 ± 2.6****	**38.4 ± 1.9****	**46.5 ± 2.1****
Diabetic + Low Dose (ELD)	**38.6 ± 1.9****	**28.2 ± 1.5****	**32.8 ± 1.6****
Diabetic + High Dose (EHD)	**38.6 ± 1.9****	**21.6 ± 1.2*****	**25.5 ± 1.4*****
Diabetic	**29.4 ±**	**19.5 ±**	**23.2 ±**

Table 5. Effect of Eleutherococcus senticosus Root Extract on oxidative stress markers in STZ-Induced Diabetic Nephropathy Mice

The diabetic control group showed elevated concentrations of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 compared with the normal control group. Treatment with *Eleutherococcus senticosus* root extract resulted in a reduction in the measured cytokine levels compared with the diabetic control group. The decrease was more pronounced in animals receiving the 300 mg/kg dose, with cytokine concentrations approaching those observed in the standard drug-treated group. A similar trend was observed in the corresponding renal tissue measurements. Overall, treatment with *E. senticosus* was associated with attenuation of the diabetes-related increase in inflammatory cytokines, with a greater response observed at the higher dose.

Table 6. Effect of Eleutherococcus senticosus Root Extract on inflammatory markers of serum in STZ-Induced Diabetic Nephropathy Mice.

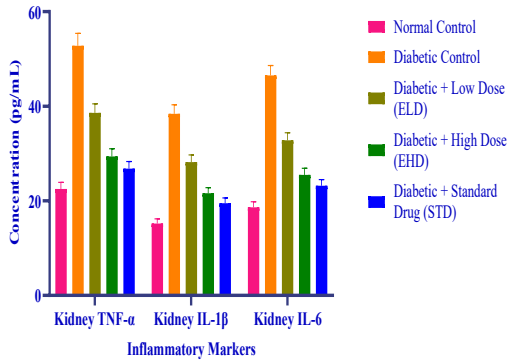


Figure. 15. Inflammatory markers (kidney)

+ Standard Drug (STD)	**1.6*** **	**1.1*** **	**1.3*** **

Table 7. Effect of Eleutherococcus senticosus Root Extract on inflammatory markers of kidney in STZ-Induced Diabetic Nephropathy Mice

3.12. Histopathological Evaluation

Histopathological examination of kidney sections from the normal control group showed preserved glomerular and tubular architecture. In contrast, the diabetic control group exhibited evident renal structural alterations, including glomerular degeneration, tubular dilation, and inflammatory cell infiltration. Treatment with *Eleutherococcus senticosus* root extract resulted in progressive improvement in the renal architecture. The protective effect was more pronounced at the 300 mg/kg dose, with relatively preserved glomerular and tubular structures comparable to those observed in the standard drug-treated group. These findings indicate that *E. senticosus* root extract attenuated the histopathological changes associated with STZ-induced diabetic renal injury.

Group	Glomerular Sclerosis	Tubular Degeneration	Interstitial Inflammation	Basement Membrane Thickening
Normal Control	Normal	Normal	Normal	Normal
Diabetic Control	Severe	Severe	Severe	Severe
Diabetic + Low Dose (ELD)	Moderate	Moderate	Moderate	Moderate
Diabetic + High Dose (EHD)	Mild	Mild	Mild	Mild
Diabetic + Standard Drug (STD)	Minimal	Minimal	Minimal	Minimal

Table 8 . Effect of Eleutherococcus senticosus Root Extract on Histopathological Changes in Kidney of STZ-Induced Diabetic Nephropathy Mice

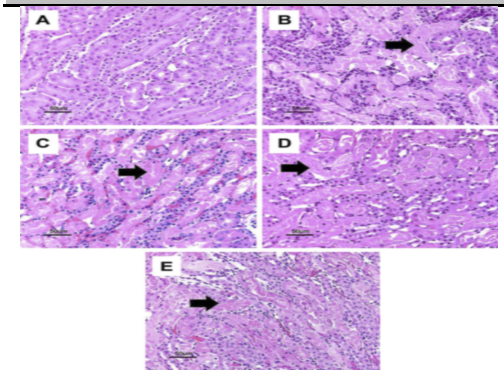


Fig. 12. Histopathological changes in Kidney tissue (H&E staining at 40 X magnification) A) normal control group B) diabetic nephropathy group C)Low dose treatment group; D) High dose treatment group E) Metformin (Std dose)

4. DISCUSSION

The present study demonstrated that *Eleutherococcus senticosus* root extract attenuated several alterations associated with STZ-induced diabetic nephropathy. Diabetic control animals showed body-weight loss, polyuria, renal hypertrophy, hyperglycemia, increased serum creatinine, albuminuria, and elevated ACR. Treatment with the extract, particularly at 300 mg/kg, improved these metabolic and renal parameters.

STZ-induced diabetes was also associated with increased MDA and reduced GSH, SOD, CAT, and

GPx levels, indicating enhanced oxidative stress. *E. senticosus* treatment reduced lipid peroxidation and improved antioxidant status. The extract additionally reduced TNF-α, IL-1β, and IL-6 levels in serum and kidney tissue, suggesting attenuation of the inflammatory response associated with diabetic renal injury.

Histopathological examination supported these biochemical findings, with reduced glomerular and tubular abnormalities in extract-treated animals. The overall protective effect may be related to the combined antioxidant and anti-inflammatory properties of constituents present in the root extract, including the isolated eleutherosides. However, the individual contribution of Eleutheroside B and Eleutheroside E requires further investigation using purified compounds.

5. CONCLUSION

The present study demonstrated that hydroalcoholic root extract of *Eleutherococcus senticosus* exerted a protective effect against STZ-induced diabetic renal injury in mice. The extract improved metabolic and renal parameters, reduced oxidative stress and inflammatory cytokines, and preserved renal histological architecture. The 300 mg/kg dose produced a greater protective response than the 100 mg/kg dose. These findings support the potential of *E. senticosus* root extract as a source of nephroprotective phytoconstituents, while further studies are required to establish the specific contribution and molecular mechanisms of its individual eleutherosides.

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