Ind. J. Pharm. Edu. Res., 2026; 60(4):1720-1729.
https://www.ijper.org

Original Article

Nephroprotective effect of *Dichrostachys cinerea* Bark Extract against Streptozotocin Induced Diabetic Nephropathy Via Alteration of Oxidative Stress and Inflammation

Kavitha Kandasamy[1], Zain Alabden Ghanim Ahmed Ahmed[2], Zead Helmi Abudayeh[3], Samer Hasan Hussein-Al-Ali[4], Shilpa Perumal Narasimhamoorthi[5], Palanisamy Arulselvan[6], Karunakaran Saravanan[7], Subbarayan Sarathbabu[8,*]

[1]Department of Biochemistry, Vivekanandha College of Arts and Sciences for Women, Tiruchengode, Namakkal, Tamil Nadu, INDIA.
[2]College of Pharmacy, Al-Kitab University, Kirkuk, IRAQ.
[3]Basic Pharmaceutical Sciences Department, Faculty of Pharmacy, Isra University, Amman, JORDAN.
[4]Department of Chemistry, Faculty of Sciences, Isra University, Amman, JORDAN.
[5]Centre for Applied Research, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, INDIA.
[6]Department of Biochemistry, Karpagam Academy of Higher Education (Deemed to be University), Coimbatore, Tamil Nadu, INDIA.
[7]Department of Chemical Engineering, KPR Institute of Engineering and Technology, Avinashi Road, Arasur, Coimbatore, Tamil Nadu, INDIA.
[8]Muthayammal Centre for Advanced Research, Muthayammal College of Arts and Science, Rasipuram, Namakkal, Tamil Nadu, INDIA.

ABSTRACT

Introduction: The primary trigger of End-Stage Renal Disease (ESRD) in several affluent nations, including the US, is diabetic nephropathy. **Objectives:** Diabetic nephropathy is a microvascular consequence that can occur in patients with Type 1 Diabetes (T1D) or Type 2 Diabetes (T2D). Because of its anti-inflammatory and antioxidant effects, *Dichrostachys cinerea* (L.) Wight and Arn. (DC) treats many inflammatory diseases, especially rheumatoid arthritis. **Materials and Methods:** The experimental rats were given an intraperitoneal dose of 60 mg/kg of STZ to cause diabetes. Following diabetic induction, fasting blood glucose samples were taken on the 0thand 30th day. Following the study, we evaluated the activity of pro-inflammatory indicators, renal biochemical markers, and levels of oxidative and antioxidant stress following DCBE treatment. **Results:** Glibenclamide is an anti-diabetic medication that was utilized in the study to compare treatment with DCBE. When DCBE was administered to STZ-induced diabetic rats, the levels of ROS, plasma AGEs, BUN, Serum Creatinine, uric acid, NAG, and U-mAlb were found to decrease. TNF-α, IL-1β, and IL-6 activities were all markedly downregulated. Following DCBE treatment, SOD, GSH, and GPx levels increased. **Conclusion:** According to the current investigation, DCBE treatment alleviated STZ-induced diabetes in a manner comparable to that of glibenclamide treatment. It might therefore be utilized as a possible treatment for diabetic nephropathy in the future.

Keywords: *Dichrostachys Cinerea* Bark Extract (DCBE), Glibenclamide, Streptozotocin (STZ), Diabetic Nephropathy (DN), Oxidative stress, Pro-inflammatory biomarkers, Renal biochemical markers.

Correspondence:
Dr. Subbarayan Sarathbabu
Muthayammal Centre for Advanced Research, Muthayammal College of Arts and Science, Rasipuram, Namakkal-637408, Tamil Nadu, INDIA.
Email: sarath07bio@gmail.com

Received: 06-04-2026;
Revised: 21-05-2026;
Accepted: 14-07-2026.

INTRODUCTION

The diagnosis of Diabetic Nephropathy (DN), often referred to as Diabetic Kidney Disease (DKD), has historically been made based on the presence of Microalbuminuria (MA). DN is a debilitating consequence that can occur in patients with both Type 1 and Type 2 Diabetes mellitus (T1D and T2D).[1] Both genetic and environmental variables contribute to the genesis of diabetic nephropathy, making genetics a key element in its development. Diabetic nephropathy is more likely to develop in people with a family history of diabetes or kidney disease.[2]

The most commonly administered oral hypoglycemic medication is glibenclamide. Chemically speaking, 5-chloro-N- [2-[4-cyclohexyl carbamoyl sulfamoyl) phenyl] ethyl]-2-methoxy benzamide is a sulfonylurea molecule that increases insulin release from beta cells by acting either as an extra-pancreatic

DOI: 10.5530/ijper.20264263

or pancreatic mechanism. In hyperglycemic circumstances, glibenclamide works by promoting the generation of insulin from the pancreatic beta cells already present. It exhibits other pancreatic effects in addition to this direct action. Therefore, in clinical diabetes, the single medication glibenclamide decreases hepatic glucose synthesis and increases insulin secretion.[3]

Dichrostachys cinerea is also referred to as the Kalahari Christmas tree, Chinese lantern tree, sickle bush, and bell mimosa. Different ethnic groups in Nigeria have different names for it. For centuries, it has been used as a diuretic and laxative, and to cure worms, syphilis, boils, headaches, gonorrhea, elephantiasis, and dysentery. Postpartum discomfort and diarrhoea are treated with dried bark. *Dichrostachys cinerea* leaf, root, and bark extracts have been shown to have antibacterial and anticandidal properties; they are effective towards *Candida albicans, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli*, and *Staphylococcus aureus*.[4-7] *Dichrostachys cinerea* extract was shown to have a dose-dependent relaxing effect on guinea pig trachea preparations. Research has demonstrated that extracts from *Dichrostachys cinerea* have strong antimalarial, antidiarrheal, analgesic, and anti-inflammatory properties, and anticancer properties on liver carcinoma and brain tumour cell lines.[8]

Renal impairment is indicated by elevated blood levels of creatinine, a metabolic byproduct. One blood test that provides information on kidney function is BUN. An elevated BUN concentration may indicate renal injury and malfunction when the kidneys are unable to properly eliminate this product, which builds up in the blood. According to Campion *et al.*[9] The BUN-to-creatinine proportion is typically a more accurate indicator of kidney activity and its potential underlying cause than the creatinine level alone. The renal tubules are the primary location for the lysosomal enzyme N-Acetyl-β-d-Glucosaminidase (NAG). For many tubulointerstitial disorders, urinary NAG activity is elevated. It is higher in diabetic populations than in controls, even in patients with normoalbuminuria.[10]

Antioxidants are a defense system in the human body that balances the effects of oxidants. Antioxidants can be classified as either enzymatic or nonenzymatic. Superoxide Dismutase (SOD), catalase, Glutathione Peroxidase (GPx), Hemo Oxygenase-1 (HO-1), and thioredoxin are the main enzymatic antioxidants.[11] Glutathione (GSH), Vitamins C and E, and β-carotene are the main nonenzymatic antioxidants.

Oxidative stress plays an important role in the occurrence of diabetic issues, including diabetic nephropathy, in addition to being a key contributor to the progression of type 1 and type 2 diabetes.[12] The actual source of oxidative stress is uncontrolled hyperglycemia, which damages different tissues by generating free radicals, particularly Reactive Oxygen Species (ROS).[3] Advanced Glycation End Products (AGEs) are produced in tissue and plasma by hyperglycemia. There are two known ways that AGEs might cause kidney problems. One is by staying permanently attached to tissue proteins, like matrix proteins (like type IV collagen and laminin), and preventing matrix metalloproteinases from breaking them down. This leads to fibrosis by causing an excessive buildup of extracellular matrix proteins. Second, by engaging with the kidney's endothelium, mesangial, and podocyte-expressed Receptor for AGE (RAGE).[13]

TNF-α has been found to cause proliferation and ECM accumulation in capillary and cylindrical areas, which alters glomerular filtering, tubular permeability, and reabsorption, in addition to playing a significant role in generating inflammation.[14] When glucose levels are high, macrophages, tubular cells of the kidney, and glomerular mesangial cells all generate the crucial cytokine TNF-α.[15] Fibronectin is a high molecular weight (~440 kDa) plasma glycoprotein primarily synthesized by fibroblasts and endothelial cells, and its production is elevated in diabetic patients, where increased plasma levels have been correlated with Microalbuminuria (MA) and Diabetic Retinopathy.[6] As TGF-β1 stimulates the buildup of Extracellular Matrix (ECM) and promotes renal cell hypertrophy, two characteristics of diabetic renal disease, it plays a significant role in the development of DN.[17,18]

MATERIALS AND METHODS

Materials

Streptozotocin (STZ), DCBE and glibenclamide have been bought from Sigma-Aldrich in the US. Every additional chemical and reagent that was required and obtained was of a higher diagnostic group.

Housing and feeding practices for animals

The Institutional Animal Ethical Committee approved the purchase of mature and competent Wistar rats weighing 180-200 g by the Animal Facility. For one week, the ambient temperature was held at 25ºC, the relative humidity was maintained at 55ºC, and a cycle of light and dark for 12 hr in a completely hygienic laboratory. After adapting, rats were given a normal rat diet and were always given access to fresh water. Only every three days was the rat cage updated, but the bedding was changed every day. The rats were treated with the highest care, and all experimental methods used in this work were authorized by the ethics committee. The experimental protocol entitled "Investigating the potential of *D. cinerea* bark extract and its derived fractions on diabetes and diabetes-related complications in a chemically induced diabetic animal model" was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Muthayammal Centre for Advanced Research, Muthayammal College of Arts and Science, Rasipuram, Namakkal, Tamil Nadu, India (Approval No: MCAS/IAEC/03/05, dated 04.04.2024). All procedures were conducted in accordance with the guidelines of the Committee

for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Diabetes Induction

The present study was conducted during the year 2024-2025. Experimental diabetes was induced in rats using Streptozotocin (STZ). A single intraperitoneal injection of STZ (60 mg/kg body weight), dissolved in 0.1 M citrate buffer (pH 4.4), was administered to the animals. After seven days of STZ administration, fasting blood glucose levels were measured to confirm the induction of diabetes. Rats with blood glucose levels greater than 11 mmol/L were considered diabetic and included in the study.[19,20]

Design and Sampling of Experiments

The study employed five groups, each consisting of six rats. The groups had been divided at random. Initially, the rats in the control group were given an intraperitoneal injection of approximately 0.5% DMSO. With rats given an intraperitoneal injection of the drug STZ at a dosage of 60 mg/kg, the diabetic-triggered group came in second. The third group was administered STZ followed by DCBE (25 mg/kg bwt). The fourth group was administered STZ followed by DCBE (50 mg/kg bwt). After receiving a diabetic induction treatment, the fifth group was administered the anti-diabetic medicine Glibenclamide (600 µg/kg bwt). DCBE and Glibenclamide were consumed orally once a day in the morning for 30 days after being dissolved in 0.5% DMSO. The rats were euthanized at the end of the treatment, and their kidney, liver, and pancreatic tissues were taken for histopathological examination, as well as their blood for biochemical analysis.[21]

Calculating the animals' body weight and fasting blood glucose levels

The body weight (BW) of the experimental animals was measured on the first and last days of the procedure and analysed for each group in the experiment. Blood samples were collected during fasting on the 0^{th} and 30^{th} days after the drug was administered. The rats were fasted for the whole night before the Fasting Blood Glucose (FBG) was measured. Glucose levels were estimated using the glucose testing kit on carefully obtained orbital sinus blood samples.[22]

Evaluation of STZ-induced animals' food intake, HOMA-IR, kidney weight, insulin levels, and water intake

Throughout the experiment, frequent food and water intake measurements were made for every group of rats. After 30 days of fasting, all of the rats in the experiment were put to death by anesthesia (24 mg/kg bwt. of ketamine intramuscularly). Both anticoagulants and non-anticoagulants were used for extracting blood into collection tubes. The kidney and liver were weighed as soon as they were separated, and any blood was removed by thoroughly cleaning them in ice-cold saline. The 10% homogenate was then produced using 0.1 M Tris-HCl buffer and centrifuged for 10 min at 1000 g. Biochemical parameter tests were performed with the separated supernatants. The insulin levels in the test animals were measured using a commercially available ELISA kit. The ultimate absorbance of the standard and sample at 450 nm was measured using a microplate ELISA reader shortly after the stop solution was introduced. The experimental animals' plasma insulin resistance level was ascertained using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Using the fasting insulin and glucose readings, HOMA-IR was calculated using the following formula.

HOMA-IR=Fasting Glucose (mmol/L) * Fasting Insulin (uIU/mL) / 22.5.

Evaluation of STZ-induced animals' Blood Urea Nitrogen (BUN), Serum Creatinine (SCr), uric acid, NAG, and U-mAlb levels

Serum was centrifuged for 10 min at 3600 g to assess renal function. BUN and Scr levels were tested on a programmed biochemical scanner. Using any appropriate commercially available assays, the levels of NAG, uric acid, and U-mAlb have been determined through the urine samples that were obtained.[23]

Evaluation of oxidative stress and antioxidant biomarker values in animals exposed to STZ

The calorimetric estimation of the amounts of the antioxidant biomarkers, such as SOD, GSH, and GPx, was done using commercially available kits and by following the manufacturer's instructions. To measure the amounts of plasma AGEs, ELISA kits were used in accordance with the manufacturer's instructions. Oxidation of the nonfluorescent label 2,7-Dichloro fluorescein Diacetate (DCFDA) to the fluorescent compounds Dichlorofluorescein (DCF) allowed for the fluorometric measurement of intracellular ROS generation in rat kidney homogenate. A summary is that 30 µL of kidney homogenate in PBS was mixed with 5 µM DCF-DA and allowed to sit at room temperature for 30 min. Then, at the emission wavelength of 535 nm and the excitation wavelength of 485 nm, the mean fluorescence intensity was measured directly.[24]

Evaluation of pro-inflammatory biomarkers in animals induced by STZ

Utilizing a commercial ELISA kit purchased from the market, the pro-inflammatory marker levels such as Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), and Interleukin-1 (IL-1β) were assessed in each group. Following the manufacturer's directions, the experiment was conducted. To find the absorbance at 450 nm, the experiment was conducted in triplicate. Calculating the final results was done using the standard curve with set standard concentrations.[15]

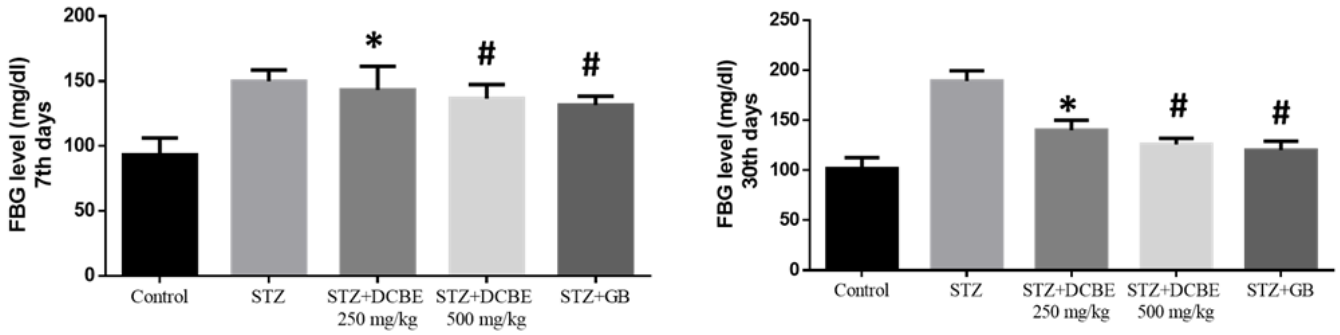

Figure 1: Effect of DCBE on the Fasting blood glucose (FBG) level in the experimental rats. The data were provided as the Mean±SD of six distinct values. Tukey's *post hoc* test and one-way ANOVA were used to statistically evaluate all of the results. "*" Significantly different compared with the control group ($p < 0.05$); "#" significantly different compared with DN-induced rats ($p < 0.01$).

Estimation of fibronectin and TGF-β1 levels in STZ-provoked animals

Fibronectin and TGF-β1 levels in the renal homogenate were measured using ELISA kits. The renal cortex was immediately homogenized in ice-cold phosphate buffer saline with 0.05% Tween 20 and then centrifuged for 20 min at 4°C at a speed of 9000 g rpm. Then, using commercially available ELISA kits, the quantities of TGF-β1 and fibronectin were estimated from the supernatants.[25,26]

Histopathological studies

Histopathological analysis was performed on the renal tissues of all five study groups: control, STZ-induced diabetic rats, STZ-induced diabetic rats treated with DCBE (25 mg/kg and 50 mg/kg), and STZ-induced diabetic rats treated with glibenclamide. The tissues were first treated for 24 hr with 10% formalin. After that, the tissue was cut using a rotary microtome into segments that were 3-5 µm thick, embedded in paraffin wax, and treated with Hematoxylin and Eosin (H&E). An Olympus light microscope was then used for photomicroscopy inspection of the stained slides.[27]

Statistical analysis

The statistical analysis was carried out using GraphPad Prism version 6.01. The Mean±SD is used to report the results. The Tukey *post hoc* test and ANOVA have been utilized to evaluate how much the groups differed in any way. We considered mean differences to be statistically significant if $p<0.05$.[28]

RESULTS

Impact of DCBE on the Fasting blood glucose levels in the experimental Rats

The impact of DCBE on the experimental animals' fasting blood glucose levels on days 7 and 30 is shown in Figure 1. All experimental groups, except for the control group, had nearly identical blood glucose levels on the seventh day. On the 30th day, all groups' blood glucose levels were lower than those of the STZ-treated control rats (group II). However, animals treated with DCBE in a dose-based manner (groups III and IV) showed a considerable drop in blood glucose levels on the 30th day. The fasting blood glucose levels of the animals treated with DCBE and those treated with glibenclamide did not significantly differ at the end of the research.

Impact of DCBE on the body weight of experimental Rats

The impact of DCBE on the body weight of rats treated with STZ is seen in Figure 2. The experimental animals in each group had the same body weight on the 0th day. Rats in group II (STZ-treated) had a dose-dependent decrease in body weight when compared to STZ-induced rats treated with DCBE extract (group III and group IV). In Groups III and IV, the animal body weight was nearly maintained with DCBE extract.

Impact of DCBE on food intake, HOMA-IR, Kidney weight, Water intake, and Insulin level in experimental Rats

The impact of DCBE medicine on a variety of indicators, such as insulin levels, food intake, kidney weight, HOMA-IR, and water intake, was examined in the DN animals. In comparison to the control animals, the STZ-induced DN animals exhibited higher levels of water consumption, kidney weight, HOMA-IR, and average meal intake. Treatment with DCBE significantly reduced food intake, HOMA-IR, kidney weight, and water intake in rats induced by STZ. Furthermore, the material increased the insulin level in animals treated with DCBE as opposed to rats exposed to STZ (Figure 3). Glibenclamide therapy had results that were similar to DCBE.

Impact of DCBE on the renal biochemical markers level in experimental Rats

The impact of DCBE on the serum levels of Blood Urea Nitrogen, Serum Creatinine, uric acid, NAG, and Reactive Oxygen

Species in rats treated with STZ is shown in Figures 4a-4e, respectively. The results showed that the rats developed diabetic nephrotoxicity 24 hr after taking STZ, as shown by a significant increase in Blood Urea Nitrogen, Serum Creatinine, uric acid and NAG levels. Blood Urea Nitrogen, Serum Creatinine, NAG and uric acid levels were significantly lower in rats with STZ-induced nephrotoxicity treated with DCBE in a dose-dependent manner (group III and group IV) than in the animals treated with STZ (group II). Glibenclamide therapy had results that were similar to DCBE.

Impact of DCBE on the oxidative stress and antioxidant biomarkers in rats exposed to STZ

It was assessed how DCBE treatment affected the amounts of several antioxidants in both treated and untreated rats. When compared to control rats, STZ-stimulated animals' renal tissues exhibited significantly lower levels of antioxidants, such as SOD, GSH, and GPx (Figure 5). It is important to note that the STZ-induced rats' SOD, GSH, and GPx levels were significantly elevated after receiving DCBE treatment. The animals that were exposed to STZ had higher levels of ROS and plasma AGE than the control rats. Plasma AGEs and ROS levels decreased after DCBE

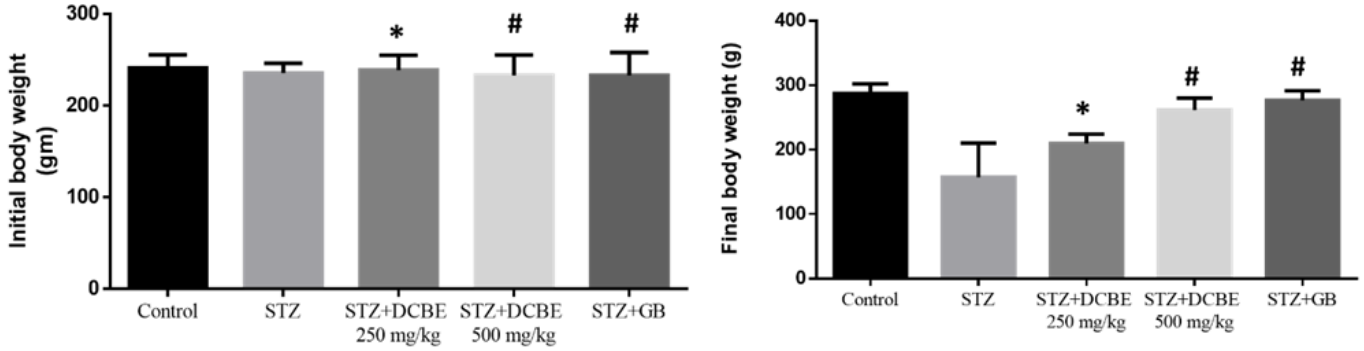

Figure 2: Effect of DCBE on the initial and final body weight of experimental rats. The data were provided as the Mean±SD of Six distinct values. Tukey's *post hoc* test and one-way ANOVA were used to statistically evaluate all of the results. "*"Significantly different compared with the control group ($p < 0.05$); "#" significantly different compared with DN-induced rats ($p < 0.01$).

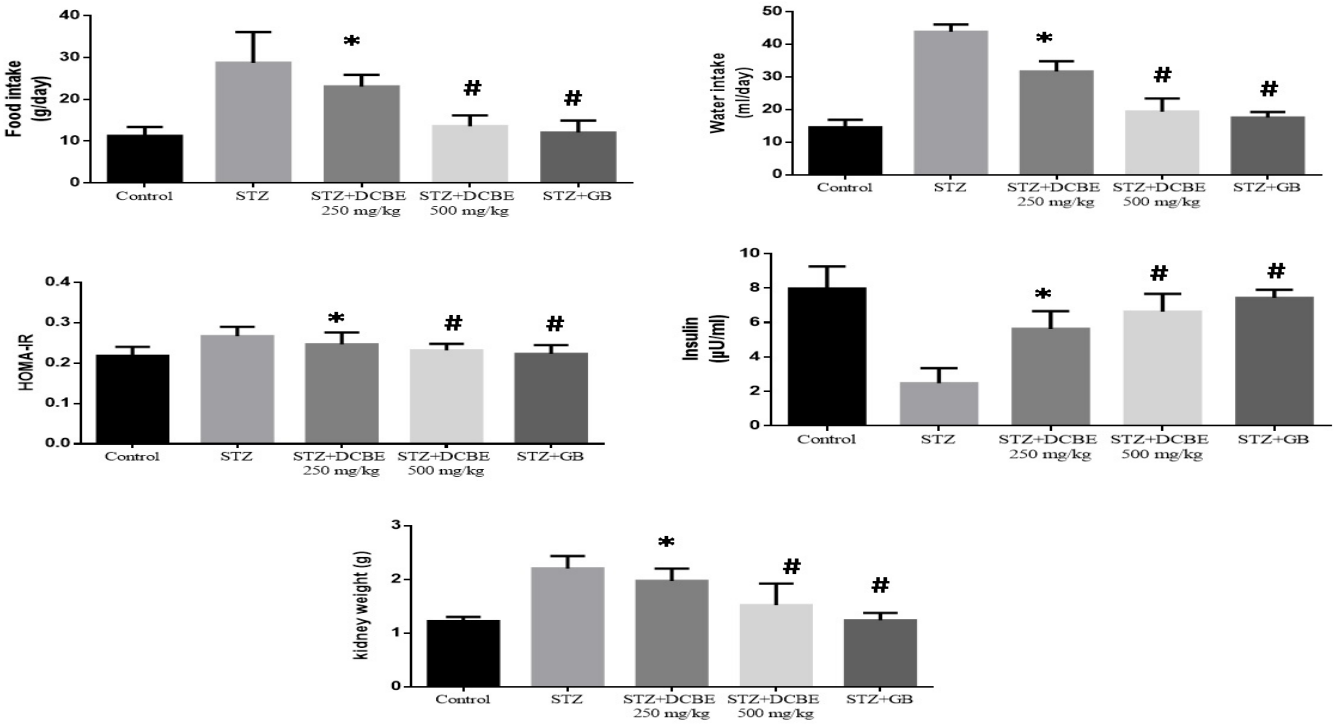

Figure 3: Effect of DCBE on hyperglycemia in diabetic rats. Food intake, Water intake, HOMA-IR, Insulin and Kidney weight. Tukey's *post hoc* test and one-way ANOVA were used to statistically evaluate all of the results. Data are expressed as Mean ± SD (n=6)."*"Significantly different compared with the control group ($p < 0.05$); "#" significantly different compared with DN-induced rats ($p < 0.01$)..

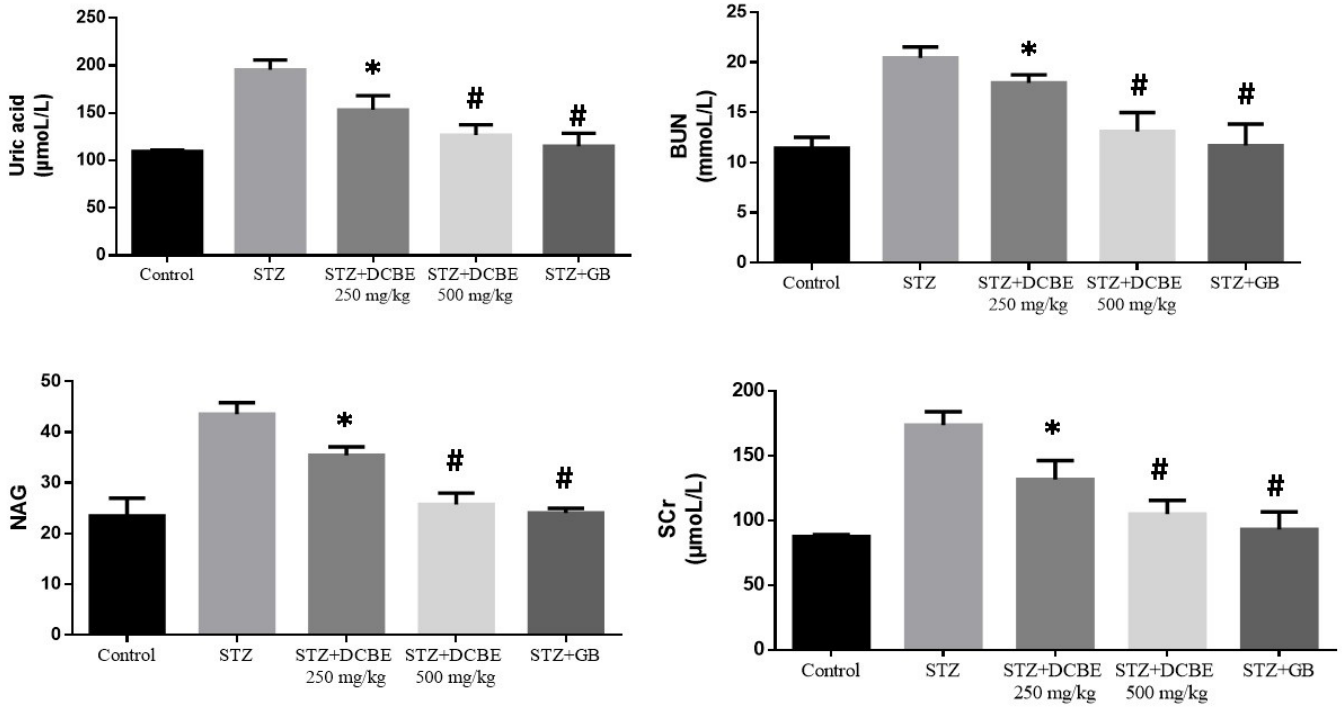

Figure 4: Effect of DCBE on the renal biochemical markers level in the experimental rats. (a) BUN (mmoL/L), (b) SCr (µmoL/L), (c) Uric acid (µmoL/L), (d) NAG. Tukey's *post hoc* test and one-way ANOVA were used to statistically evaluate all of the results. Data are expressed as Mean ± SD (*n*=6)."*"Significantly different compared with the control group ($p < 0.05$); "#" significantly different compared with DN-induced rats ($p < 0.01$).

therapy. When DCBE was administered to the STZ-induced DN rats, their antioxidant status improved.

Impact of DCBE on the pro-inflammatory biomarkers in rats induced by STZ

The renal tissues of both treated and untreated rats were examined for levels of IL-6, TNF-α, and IL-1β, which are displayed in Figure 6. When compared to the control group, the STZ-stimulated rats exhibited significantly higher levels of IL-6, TNF-α, and IL-1β in their renal tissues. However, the STZ rats' IL-6, TNF-α, and IL-1β production dramatically dropped when DCBE was given as a supplement (Figure 6). Glibenclamide therapy also reduced the synthesis of these markers in the renal tissue of rats that were stimulated with STZ.

Effect of DCBE on Fibronectin (FN) and TGF-β1 Levels

The levels of fibronectin (FN) and transforming growth factor-beta 1 (TGF-β1), two key markers associated with renal fibrosis and extracellular matrix accumulation, were significantly elevated in the diabetic nephropathy (DN) group compared with the control group. Treatment with Dichrostachys cinerea bark extract (DCBE) markedly reduced the expression levels of both FN and TGF-β1 in a dose-dependent manner. The higher dose of DCBE exhibited a more pronounced effect, bringing these biomarkers closer to normal levels. Similarly, glibenclamide treatment significantly attenuated the elevated FN and TGF-β1 levels observed in STZ-induced diabetic rats. These findings suggest that DCBE may exert nephroprotective effects by suppressing renal fibrotic pathways and inhibiting the overproduction of extracellular matrix components associated with diabetic nephropathy (Figure 7).

DISCUSSION

In the present study, we analyzed pro-inflammatory biomarkers, antioxidant and oxidative stress markers, and renal biochemical markers in diabetic nephropathy.

In many industrialized nations, including the US, diabetic nephropathy is the leading trigger of End-Stage Renal Disease (ESRD). Diabetic nephropathy is a microvascular consequence that can occur in patients with both Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). Prolonged albuminuria and a steadily declining Glomerular Filtration Rate (GFR) are the condition's hallmarks.[2]

Type 2 diabetes is becoming more and more common at a startlingly rapid rate. End-stage renal disease, especially diabetic nephropathy (post-type 2 diabetes), is among the main causes of diabetes. Deregulation of the local metabolic environment brought on by inflammation and oxidative stress, followed by tissue remodelling, is the primary cause of renal failure, even though nephropathy in diabetic conditions is the best indicator of patient death.[29] However, no one has fully recovered from

this noncommunicable disease, and the majority of diabetics experience treatment-related problems.[3]

Cells that are involved in the synthesis and secretion of insulin can be specifically destroyed by Streptozotocin (STZ). According to Singh *et al.* (2015), STZ can directly kill pancreatic B cells in the peripheral system (such as intraperitoneal injection) and result in insulin resistance, which can lead to diabetes.[30] Furthermore, rats can exhibit altered emotional behaviour in response to intraventricular injections of varying dosages of STZ. As a result, different STZ injection routes may result in various behavioural and blood glucose modifications, including adjustments to emotions and spatial memory.[31] Glibenclamide is a useful medication that helps the beta cells release insulin. Long-term use of this sulfonylurea molecule also increases the risk of hypoglycemia and has other pancreatic effects. This oral hypoglycemic medication is used to treat type 2 diabetes.

The levels of important indicators, including creatinine, urea, uric acid, and certain electrolytes, make it simple to evaluate the functioning of the kidneys. According to the authors, rats given STZ injections showed elevated plasma levels of urea, uric acid, and creatinine, which may be impairing renal function.[32] The liver produces urea as a waste product during the metabolism of proteins. After being released into the bloodstream, urea gets processed by properly functioning kidneys and discharged as urine. Typically, the blood contains a modest but steady level of urea nitrogen. Urine microalbuminuria, particularly in diabetic patients, is a precursor to increased gross proteinuria or raised creatinine levels in the serum in patients vulnerable to kidney dysfunction.[32]

A 2019 study by Rani *et al.*, found that oxidative stress is more prevalent in diabetes patients and that it is much greater in DN cases compared to controls.[33] In the pathophysiology of DN, oxidative stress brought on by hyperglycemia is a significant factor. According to a number of studies, oxidative stress is linked to both the overproduction of free radicals in experimental diabetes and the depletion of free radical scavengers. In experimental rats, STZ, a medication administered intraperitoneally at a certain dosage, can successfully cause hyperglycemia.[34] The observable symptoms of this medication, including excessive eating, excessive thirst, and frequent urination, along with decreased weight following administration, guarantee its diabetogenic potential. According to the study, STZ hinders the production of insulin through molecular mechanisms because it produces free radicals, which can damage DNA and induce beta cell degeneration in rats.[35]

If the treatment approaches are effectively focused, the outcome of diabetic kidney disease will undoubtedly be better. Even with diabetes medication, DN can still occur, even if optimal glycemic control may be the best way to prevent it. The understanding of potent antioxidants can be used to infer such tailored approaches. Inhibitors of inflammation and oxidative stress thought to be

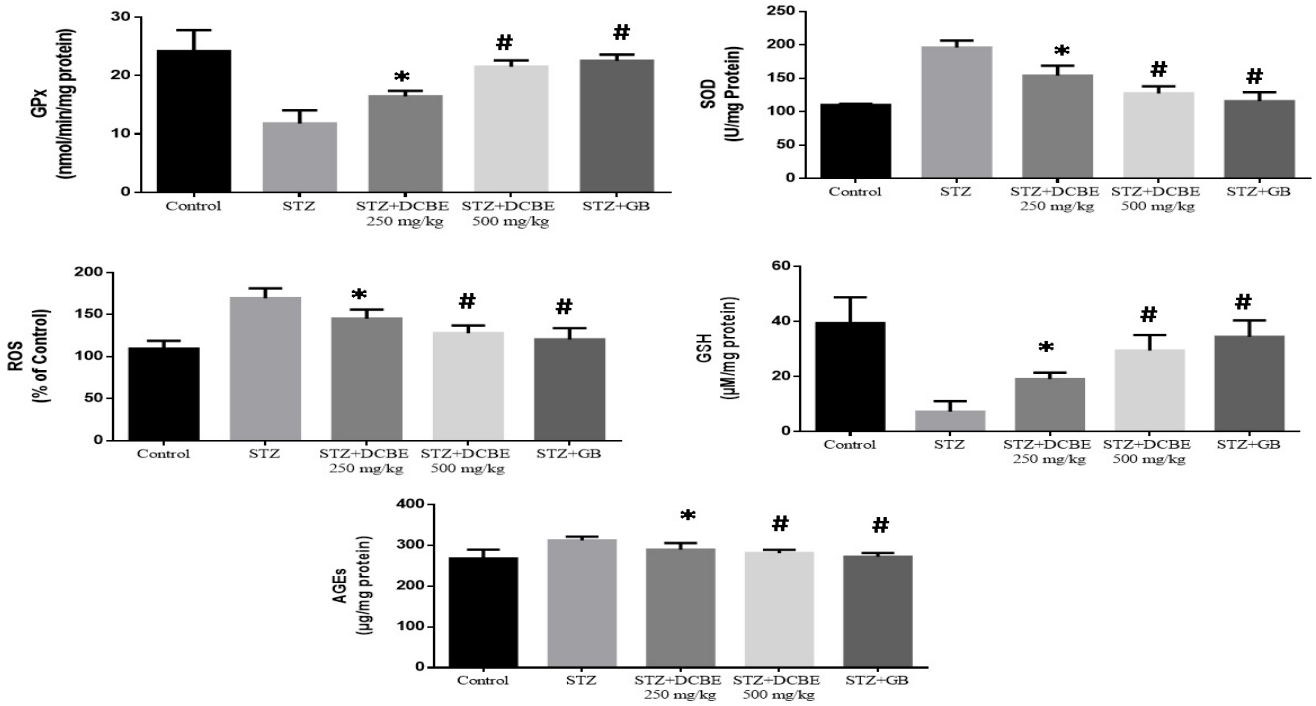

Figure 5: Effect of DCBE on the oxidative stress and antioxidant biomarkers in the experimental rats. Tukey's *post hoc* test and one-way ANOVA were used to statistically evaluate all of the results. Data are expressed as Mean ± SD (*n*=6). "*"Significantly different compared with the control group ($p < 0.05$); "#" significantly different compared with DN-induced rats ($p < 0.01$)..

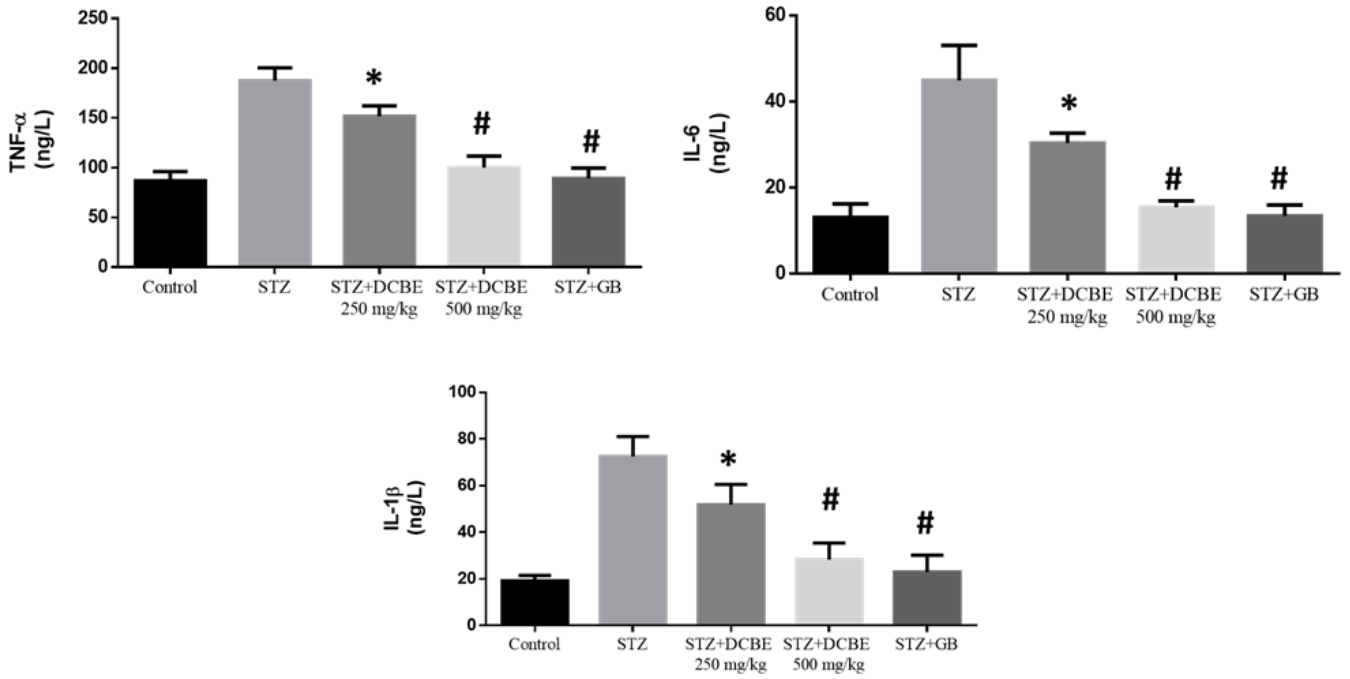

Figure 6: Effect of DCBE on the pro-inflammatory markers in the experimental rats. (a) TNF-α, (b) IL-6, (c) IL-1β. Tukey's *post hoc* test and one-way ANOVA were used to statistically evaluate all of the results. Data are expressed as Mean ± SD (*n*=6)."*"Significantly different compared with the control group ($p < 0.05$); "#" significantly different compared with DN-induced rats ($p < 0.01$)..

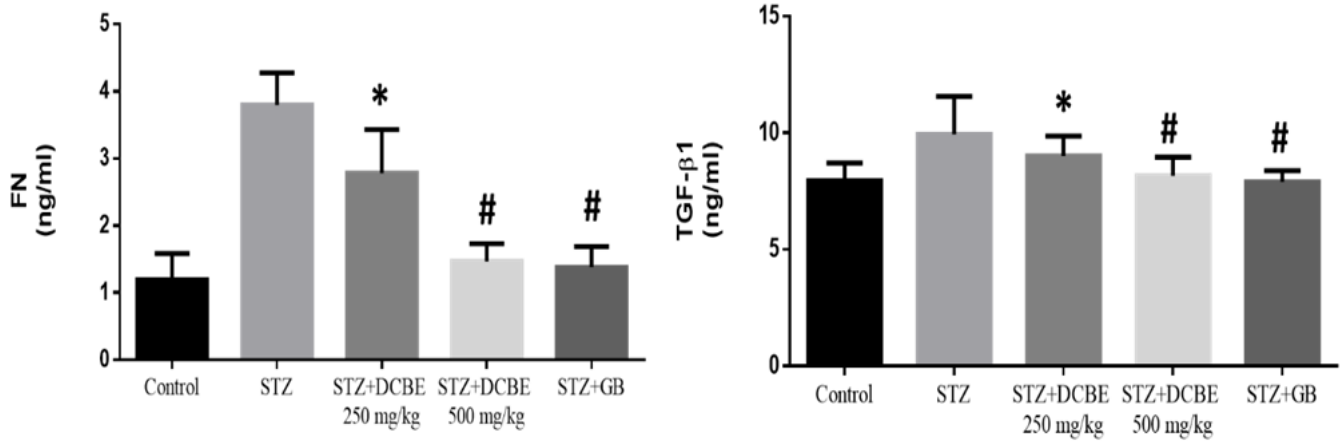

Figure 7: Effect of DCBE on the renal biochemical markers in experimental rats. (a) Fibronectin (FN) (b) TGF-β1. Tukey's *post hoc* test and one-way ANOVA were used to statistically evaluate all of the results. Data are expressed as Mean ± SD (*n*=6)."*"Significantly different compared with the control group ($p < 0.05$); "#" significantly different compared with DN-induced rats ($p < 0.01$)..

promising therapeutic targets. Furthermore, diabetic nephropathy can be prevented or lessened by inhibiting the inflammatory mediators.

The findings of research on SOD in CKD patients are inconsistent and challenging to understand. While some authors found no significant difference in SOD between CKD patients and controls, others found that plasma SOD activity increased in CKD patients as renal insufficiency progressed, or that SOD activity was lower in hemodialysis and peritoneal dialysis patients compared to controls.[36] The author showed that prooxidant/antioxidant mechanisms are out of balance in DN patients. Glutathione peroxidase's enzymatic activity is reduced by ROS, which significantly lowers GSH levels in DN.[37] Serum AGEs and reduced kidney function in T2D were examined in a 2021 study by Nishad. In individuals with T2D, they discovered a favourable association between AGEs and reduced kidney function, and they proposed that AGEs could be used as prognostic indicators for DN.[38]

The relevance of IL-6 in T2D and DN was further highlighted in this study. In contrast to those without nephropathy, they found that type 2 diabetics with nephropathy had higher serum IL-6 levels.[39] Prior research indicates that the type 2 diabetes group had higher serum TNF-α levels, while the type 2 DN group had the highest levels.[40] According to clinical research, diabetic individuals with renal impairment have higher serum and urine levels of TNF-α, which rise as the condition worsens.[15] Furthermore, oxidative stress markers, including TOS, TAR, OSI, NO, MDA, and total thiols, as well as inflammatory markers like IL-6, IL-1β, and TNF-α, were measured to assess the antioxidant and anti-inflammatory potential of DC fruit extract high in polyphenols. This was done because polyphenols can suppress pro-inflammatory signalling, interfere with oxidative stress signalling, and decrease inflammation through their antioxidant activities.[11]

A major contributing factor to diabetic nephropathy, hyperglycemia increases inflammation and encourages macrophage infiltration in the kidneys by producing glycation end products and glucose breakdown products. TGF-β1 (secreted by macrophages) and Intracellular Cell Adhesion Molecule-1 (ICAM-1, generated by renal tubular cells) are two examples of immune complexes and cytokines that are essential to this process.[18]

DN is a progressive kidney disease brought on by changes in the structure and function of the tubules and glomerular capillaries, brought on by a disruption in glucose homeostasis. Despite substantial progress in detecting and treating DN patients over the past few decades, we have yet to dramatically lower the mortality rate among this population.[41]

CONCLUSION

The findings of this study demonstrated that DCBE exhibited nephroprotective effects comparable to glibenclamide in STZ-induced diabetic nephropathy. According to the results, DCBE treatment significantly reduced oxidative stress, inflammatory mediators, and renal injury biomarkers while restoring antioxidant defense systems. This could suggest that the danger of the disease progressing is lower. It might therefore be employed as a possible treatment for diabetic nephropathy in the future. There is still more research needed in the future to fully understand how DCBE treats diabetic nephropathy.

ACKNOWLEDGEMENT

Thanks DST-FIST (SR/FST/COLLEGE/2022/1290) and DST-CURIE (DST/CURIE-PG/2024/28, dt: 02.11.24), Ministry of Science and Technology, India, for financial support.

ABBREVIATIONS

STZ: Streptozotocin; **DCBE:** *Dichrostachys cinerea* Bark Extract; **DMSO:** Dimethyl Sulfoxide; **BW:** Body Weight; **FBG:** Fasting Blood Glucose; **HOMA-IR:** Homeostasis Model Assessment of Insulin Resistance; **ELISA:** Enzyme-Linked Immunosorbent Assay; **BUN:** Blood Urea Nitrogen; **SCr:** Serum Creatinine; **NAG:** N-Acetyl-β-D-Glucosaminidase; **U-mAlb:** Urinary Microalbumin; **SOD:** Superoxide Dismutase; **GSH:** Reduced Glutathione; **GPx:** Glutathione Peroxidase; **AGEs:** Advanced Glycation End Products; **DCFDA:** 2′,7′-Dichlorofluorescein Diacetate; **DCF:** Dichlorofluorescein; **ROS:** Reactive Oxygen Species; **PBS:** Phosphate-Buffered Saline; **IL-6:** Interleukin-6; **TNF-α:** Tumor Necrosis Factor-Alpha; **IL-1β:** Interleukin-1 Beta; **TGF-β1:** Transforming Growth Factor-Beta 1; **H&E:** Hematoxylin and Eosin; **ANOVA:** Analysis of Variance; **SD:** Standard Deviation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

The experimental protocol entitled "Investigating the potential of *Dichrostachys cinerea* bark extract and its derived fractions on diabetes and diabetes-related complications in a chemically induced diabetic animal model" was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Muthayammal Centre for Advanced Research, Muthayammal College of Arts and Science, Rasipuram, Namakkal, Tamil Nadu, India (Approval No: MCAS/IAEC/03/05; dated 04.04.2024). All experimental procedures were conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

SUMMARY

In the present research, the preventive properties of DCBE and Glibenclamide towards rats with diabetes caused by Streptozotocin (STZ) were investigated in detail. In the course of the study, the treatment with DCBE reduced the levels of oxidative stress, renal biochemical indicators, and the activity of pro-inflammatory markers was also lowered. The antioxidant levels were elevated upon the treatment with DCBE. The glibenclamide treatment also had comparable results with the DCBE. Therefore, it could be employed as a potential treatment for diabetic nephropathy in the future.

REFERENCES

1. Papadopoulou-Marketou N, Kanaka-Gantenbein C, Marketos N, Chrousos GP, Papassotiriou I. Biomarkers of diabetic nephropathy: A 2017 update. Crit Rev Clin Lab Sci. 2017;54(5):326-42. doi:10.1080/10408363.2017.1377686. PMID: 28984563.
2. Rout P, Jialal I. Diabetic Nephropathy. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534200/
3. Pandarekandy ST, Sreejesh PG, Harikumaran Thampi BS, Sreekumaran E. Hypoglycaemic effect of glibenclamide: A critical study based on creatinine and

lipid peroxidation status of streptozotocin-induced diabetic rat. Indian J Pharm Sci. 2017;79(5):768-77. doi:10.4172/pharmaceutical-sciences.1000289. PMID: 29599581.

4. El-Sharawy RT, Elkhateeb A, Marzouk MM, Abd El-Latif RR, Abdelrazig SE, El-Ansari MA. Antiviral and antiparasitic activities of clovamide: The major constituent of *Dichrostachys cinerea*. J Appl Pharm Sci. 2017;7(9):219-23. doi:10.7324/JAPS.2017.70931.
5. Ambujakshi HR, Pooja S, Gowtham Kalyan HR, Sachin Gowda NT, Adhil M, Gowda S, *et al. Dichrostachys cinerea*: A comprehensive review on its phytochemical and pharmacological profile. Int J Pharm Sci. 2025;3(4):1425-37. doi:10.5281/zenodo.15198532.
6. Oladele K. Hepatotoxic and nephrotoxic activities of aqueous root extracts of *Dichrostachys cinerea* in male Wistar rats. Biokemistri. 2021;33(2):123-33.
7. Akinmusire OA, Dahunsi AA, Dahunsi AE. Chemical characterization of *Dichrostachys cinerea* extracts using GC-MS and column chromatography. MIR J. 2025;12(1):19-29. doi:10.18527/20251211929.
8. Kiki GA, Pop RM, Sabin O, Bocsan IC, Chedea VS, Socaci SA, *et al.* Polyphenols from *Dichrostachys cinerea* fruits: Anti-inflammatory, analgesic, and antioxidant capacity in Freund's adjuvant-induced arthritic rat model. Molecules. 2022;27(17):5445. doi:10.3390/molecules27175445. PMID: 36080212.
9. Campion CG, Sanchez-Ferras O, Batchu SN. Potential role of serum and urinary biomarkers in diagnosis and prognosis of diabetic nephropathy. Can J Kidney Health Dis. 2017;4:2054358117705371. doi:10.1177/2054358117705371. PMID: 28413663.
10. Lou W, Cheng Q, Liang Y, Xia D. Urinary N-acetyl-β-D-glucosaminidase levels and risk of cardiovascular events in diabetic patients. Int J Gen Med. 2021;14:10495-502. doi:10.2147/IJGM.S337874. PMID: 35002305.
11. Gonzalez-Sanchez E, Perez MJ, Nytofte NS, Briz O, Monte MJ, Lozano E, *et al.* Protective role of biliverdin against bile acid-induced oxidative stress in liver cells. Free Radic Biol Med. 2016;97:466-77. doi:10.1016/j.freeradbiomed.2016.06.030. PMID: 27374986.
12. Sifuentes-Franco S, Padilla-Tejeda DE, Carrillo-Ibarra S, Miranda-Díaz AG. Oxidative stress, apoptosis, and mitochondrial function in diabetic nephropathy. Int J Endocrinol. 2018; 2018:1875870. doi:10.1155/2018/1875870. PMID: 30607083.
13. Dilworth L, Stennett D, Facey A, Omoruyi F, Mohansingh S, Omoruyi FO. Diabetes and the associated complications: The role of antioxidants in therapy. Biomed Pharmacother. 2024;181:117641. doi:10.1016/j.biopha.2024.117641.
14. Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008;19(3):433-42. doi:10.1681/ASN.2007091048. PMID: 18256353.
15. Wada J, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. Clin Sci (Lond). 2013;124(3):139-52. doi:10.1042/CS20120198. PMID: 23083301.
16. Currie G, Delles C, Dominiczak AF. Fibronectin and its role in diabetic nephropathy and vascular complications. Diabetes Care. 2014;37(5):1416-23. doi:10.2337/dc13-2763. PMID: 24757294.
17. Wang L, Wang HL, Liu TT, Lan HY. TGF-β as a master regulator of diabetic nephropathy. Int J Mol Sci. 2021;22(15):7881. doi:10.3390/ijms22157881. PMID: 34360942.
18. Huang B, Han R, Tan H, *et al.* Scutellarin ameliorates diabetic nephropathy via the TGF-β1 signaling pathway. Nat Prod Bioprospect. 2024;14:25. doi:10.1007/s13659-024-00446-y.
19. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Pharmacol Ther. 2021;226:107834. doi:10.1016/j.pharmthera.2021.107834. PMID: 34087291.
20. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2022;179(5):877-94. doi:10.1111/bph.15679. PMID: 34693947.
21. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2023. Diabetes Care. 2023;46(Suppl 1):S19-S40. doi:10.2337/dc23-S002. PMID: 36507649.
22. Matthews DR, *et al.* Homeostasis model assessment: Updated applications. Diabetologia. 2021;64:1127-35. doi:10.1007/s00125-021-05385-6.
23. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2022;45(2):262-7. doi:10.2337/dc21-1592. PMID: 35039377.
24. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. Kidney Int. 2022;102(6):1224-36. doi:10.1016/j.kint.2022.08.019. PMID: 36113485.
25. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2023;103(1):1-47. doi:10.1152/physrev.00045.2021. PMID: 36327255.
26. Chen X, *et al.* Fibronectin and extracellular matrix remodeling in diabetic nephropathy. Int J Mol Sci. 2024;25:1123. doi:10.3390/ijms25021123.
27. Bancroft JD, Gamble M. Theory and practice of histological techniques. 8th ed. Elsevier; 2021.
28. Motulsky H. Intuitive biostatistics. 4th ed. Oxford University Press; 2022.
29. Muhl L, Moessinger C, Adzemovic MZ, Dijkstra MH, Nilsson I, Zeitelhofer M, *et al.* Expression of VEGF-B and VEGFR1 in murine tissues. Cell Tissue Res. 2016;365(1):51-63. doi:10.1007/s00441-016-2396-9. PMID: 26941059.
30. Singh JCH, Kakalij RM, Kshirsagar RP, Kumar BH, Komakula SSB, Diwan PV. Cognitive effects of vanillic acid in STZ-induced neurodegeneration. Pharm Biol. 2015;53(5):630-6. doi:10.3109/13880209.2014.923003. PMID: 25148656.
31. Zhang Y, Ding R, Wang S, Ren Z, Xu L, Zhang X, *et al.* Effects of STZ on learning and memory in mice. Exp Ther Med. 2018;16(3):2375-80. doi:10.3892/etm.2018.6458. PMID: 30116452.
32. Adeyomoye OI, Akintayo CO, Omotuyi KP, Adewumi AN. Biological roles of urea: A review. Indian J Nephrol. 2022;32(6):539-45. doi:10.4103/ijn.ijn_88_21. PMID: 36452859.
33. Rani K, Gavel P, Bharti S. MDA as oxidative stress marker in diabetic nephropathy. Indian J Appl Res. 2019;6:128-130.
34. Nagarchi K, Ahmed S, Sabus A, Saheb SH. Effect of streptozotocin on glucose levels in Wistar rats. J Pharm Sci Res. 2015;7(2):67.
35. Mansoor G, Tahir M, Maqbool T, Abbasi SQ, Hadi F, Shakoori TA, *et al.* Stress markers and cytokines in diabetic nephropathy. Medicina (Kaunas). 2022;58(11):1604. doi:10.3390/medicina58111604. PMID: 36361289.
36. Vodošek Hojs N, Bevc S, Ekart R, Hojs R. Oxidative stress markers in CKD with emphasis on DN. Antioxidants (Basel). 2020;9(10):925. doi:10.3390/antiox9100925. PMID: 33003652.
37. Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, Andrade-Sierra J. Oxidative stress in diabetic nephropathy. J Diabetes Res. 2016; 2016:7047238. doi:10.1155/2016/7047238. PMID: 27840765.
38. Kreiner FF, Kraaijenhof JM, von Herrath M, Hovingh GKK, von Scholten BJ. Interleukin-6 in diabetes and kidney disease. Expert Rev Clin Immunol. 2022;18(4):377-89. doi:10.1080/1744666X.2022.2037345. PMID: 35045841.
39. Nazari A, Sardoo AM, Fard ET, Hassanshahi G, Goujani R, Bagheri E, *et al.* IL-6 in type 2 diabetes mellitus patients. J Endocrinol Diabetes Obes. 2017;5(2):1102.
40. Abou-Elela DH, Emara MM, Abo El-Khair NT, El-Edel RH, Fathy WM. Role of TNF-α in diabetic nephropathy. Menoufia Med J. 2020;33(3):920-25.
41. Sun T, *et al.* Molecular mechanisms of diabetic nephropathy: A narrative review. Cell Biol Int. 2024;48(9):1240-53. doi:10.1002/cbin.12168.

Cite this article: Kandasamy K, Ahmed ZAGA, Abudayeh ZH, Hussein-Al-Ali SH, Narasimhamoorthi SP, Arulselvan P, *et al*. Nephroprotective effect of *Dichrostachys cinerea* Bark Extract against Streptozotocin Induced Diabetic Nephropathy Via Alteration of Oxidative Stress and Inflammation. Indian J of Pharmaceutical Education and Research. 2026;60(4):1720-9.