ISSN: 2835-7914

Review Article

Biomedical Science and Clinical Research

A Siddha Drug *Flueggea Virosa* (Vellaipula) Extract on Streptozotocin-Induced Diabetic Nephropathy: Protective and Elemental Safety Assessment

Gaddam Dayanand Reddy[1*], Rethinam Ganesan[2], Tadicharla Varalakshmi[1], Arshad Ali[1], Ashok Kumar and Jayaprasanna Kumar[1]

[1]*Department of Pharmacology, Siddha Central Research Institute, Min of Ayush, Govt of India, Arumbakkam, 600106 T.N. India*

[2]*Department of Biochemistry, Siddha Central Research Institute, Min of Ayush, Govt of India, Arumbakkam, 600106 T.N. India*

[*]Corresponding Author
G. Dayanand Reddy, Assistant Director Pharmacology S-IV & HOD, Department of Pharmacology Siddha Central Research Institute (CCRS) Min. of Ayush, Govt of India Arumbakkam, 600106, T.N. India.

Submitted: 2026, Jun 04; **Accepted:** 2026, Jun 25; **Published:** 2026, Jun 29

Citation: Reddy, G. D., Ganesan, R., Varalakshmi, T., Ali, A., Kumar, A., et al. (2026). A Siddha Drug *Flueggea Virosa* (Vellaipula) Extract on Streptozotocin-Induced Diabetic Nephropathy: Protective and Elemental Safety Assessment. *Biomed Sci Clin Res, 5*(2), 01-15.

Abstract

One of the main microvascular consequences of diabetes mellitus and a major global cause of end-stage renal disease is diabetic nephropathy. The goal of the current investigation was to assess the nephroprotective effect of Flueggea virosa extract in experimental rats with diabetic nephropathy caused by streptozotocin (STZ). Animals were split into normal control, disease control, standard (metformin), and treatment groups that received low, mid, and high dosages of Flueggea virosa extract after a single intraperitoneal injection of STZ caused diabetes. To examine glycaemic state and renal function, biochemical markers such as blood glucose, HbA1c, serum creatinine, urea, and uric acid were measured. Antioxidant activity was assessed using oxidative stress markers as superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO), and nitric oxide (NO).

To evaluate structural changes, kidney samples were examined histopathologically. Significant hyperglycemia, higher HbA1c, elevated renal function indicators, increased oxidative stress, and severe histopathological alterations were observed in STZ-induced diabetic rats. In order to ensure safety within allowable limits, heavy metal analysis of the extract was also performed using ICP-OES to ascertain the quantities of hazardous elements like lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). In a dose-dependent way, Flueggea virosa extract treatment dramatically lowered blood glucose and HbA1c levels, enhanced renal function indicators, restored antioxidant enzyme activities, and reduced LPO and NO levels. Histological results, which demonstrated the restoration of normal renal architecture and decreased tissue damage, further supported the protective effect. In conclusion, Flueggea virosa extract exhibits significant nephroprotective activity, likely mediated through its antioxidant and anti-inflammatory properties. These findings suggest its potential as a natural therapeutic agent for the management of diabetic nephropathy.

Keywords: Hyperglycemia, Heavy Metals, Inflammatory Cytokines, Kidney Damage and Oxidative-Nitrosative Stress

1. Introduction

Persistent hyperglycemia brought on by deficiencies in insulin secretion, action, or both is a hallmark of diabetes mellitus (DM), a chronic metabolic disease. Diabetes is now a serious global public health concern due to the sharp rise in its prevalence in recent decades. One of the most serious and potentially fatal microvascular consequences of diabetes is diabetic nephropathy (DN), which eventually results in end-stage renal disease (ESRD) and chronic kidney disease (CKD) [1,2]. Diabetes is a major cause of renal failure worldwide, with an estimated 30–40% of patients developing nephropathy during the course of the illness [3]. Clinical features of diabetic nephropathy include increased blood creatinine, hypertension, gradual reduction in glomerular filtration rate (GFR), and persistent albuminuria.

It is characterised histopathologically by tubulointerstitial fibrosis, podocyte damage, basement membrane thickening, glomerular hypertrophy, and mesangial enlargement [4-6]. Hyperfiltration and microalbuminuria are the first phases of DN development, which ultimately results in irreparable renal damage and fibrosis [7]. Alternative therapeutic techniques must be investigated because existing medicines, such as angiotensin-converting enzyme inhibitors and antidiabetic medications, are unable to totally stop the course of the disease despite advancements in therapeutic strategies.

Metabolic, haemodynamic, and inflammatory mechanisms interact intricately in the multifactorial aetiology of diabetic nephropathy. A number of metabolic changes, including the activation of the polyol pathway, the production of advanced glycation end products (AGEs), and the activation of protein kinase C (PKC), are thought to be caused by chronic hyperglycemia [8]. Together, these mechanisms raise the generation of reactive oxygen species (ROS), which causes oxidative stress, a major factor in the onset and advancement of DN.

An imbalance between the production of reactive oxygen species and the antioxidant defence system leads to oxidative stress. Overproduction of ROS damages cellular macromolecules, such as proteins, lipids, and DNA, which eventually affects renal cell function [9]. ROS are produced in diabetes circumstances by a number of processes, including glucose autoxidation, NADPH oxidase activation, and mitochondrial dysfunction. In addition to directly harming renal tissues, this oxidative stress serves as a crucial mediator that connects hyperglycemia to inflammation and fibrosis in the diabetic kidney [10].

Another important element in the pathophysiology of diabetic nephropathy is inflammation. Increased production of pro-inflammatory cytokines like interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) results from hyperglycemia-induced oxidative stress, which activates multiple inflammatory signalling pathways, including nuclear factor-kappa B (NF-κB). These cytokines encourage mesangial growth, extracellular matrix formation, and inflammatory cell infiltration, all of which lead to glomerulosclerosis and renal fibrosis [11,12]. Moreover, oxidative stress and inflammation are mutually reinforcing processes that create a vicious cycle that speeds up kidney damage in diabetic nephropathy [13]. Haemodynamic changes have a major role in the development of DN in addition to oxidative damage and inflammation.

Changes in renal haemodynamics brought on by hyperglycemia worsen kidney injury by causing glomerular hyperfiltration and elevated intraglomerular pressure [14,15]. Fibrosis and structural damage in renal tissues are largely caused by the activation of signalling pathways like MAP kinases, TGF-β, and the renin-angiotensin system [16]. In the end, these pathological alterations cause renal function to decline and end-stage renal disease to develop. The use of natural products and herbal medications as alternative treatment agents is becoming more popular due to the complicated pathophysiology of diabetic nephropathy. Due to their anti-inflammatory, antihyperglycemic, and antioxidant qualities, which target many pathways involved in the development of disease, medicinal plants have been extensively studied [17,18]. By lowering oxidative stress, preventing inflammatory reactions, and enhancing renal function, phytochemicals such flavonoids, alkaloids, and phenolic compounds have shown notable nephroprotective benefits [19,20].

A significant medicinal plant in the Phyllanthaceae family, *Flueggea virosa* has long been utilised in many medical systems to treat renal problems, diabetes, and inflammation. Alkaloids, flavonoids, tannins, and saponins are among the bioactive components that are said to contribute to its pharmacological actions [21,22]. *Flueggea virosa's* strong antioxidant, anti-inflammatory, hepatoprotective, and anticancer qualities have been shown in earlier research, indicating a possible therapeutic function in the treatment of diabetes problems [23-25]. Because plant extracts can scavenge free radicals, increase endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), and lower lipid peroxidation, their antioxidant activity is especially important in the context of diabetic nephropathy. Its anti-inflammatory properties may also aid in reducing renal tissue damage and inhibiting pro-inflammatory cytokines [26,27]. Nevertheless, there is little scientific data expressly assessing its nephroprotective effects in diabetic nephropathy models, despite these encouraging pharmacological characteristics. An established technique for researching diabetic consequences, such as nephropathy, is the experimental production of diabetes using streptozotocin (STZ). STZ mimics the pathophysiological circumstances of diabetes in humans by selectively destroying pancreatic β-cells, resulting in insulin insufficiency and chronic hyperglycemia [28,29]. The effectiveness of possible treatment medicines in avoiding or lessening diabetic nephropathy is frequently assessed using this model.

Due to the possibility of contamination with heavy metals like lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), which can result from environmental pollution, soil composition, and processing conditions, the safety assessment of medicinal plants has become more significant in recent years. When these harmful substances build up in herbal medicines, there might be major health hazards. Long-term exposure can especially harm important organs like the liver and kidney [30]. As a result, determining the heavy metal concentration of plant-based medicines has become essential to their standardisation and quality assurance. For the sensitive and precise identification of trace elements, analytical methods like Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) are frequently used. Therefore, assessing *Flueggea virosa's* heavy metal profile is crucial to guaranteeing its safety and appropriateness for medicinal uses.

Thus, the goal of this investigation was to assess the nephroprotective potential of *Flueggea virosa* extract in experimental rats with

diabetic nephropathy caused by streptozotocin. Its effects on biochemical measures, oxidative stress indicators, inflammatory mediators, and histological changes in renal tissues are to be examined. *Flueggea virosa* may be a promising natural medicinal agent for the treatment of diabetic nephropathy because it targets several pathways involved in the disease's progression.

2. Materials and Methods

2.1 Chemicals and Kits

Streptozutasin (purity ≥ 96.0%) from Bio Corporals Chennai Tamil Naidu India, Metformin (purity 98.0–101.5%) from Apollo pharmacy Chennai and other chemicals, assay kits and reagents from Sigma - Aldrich, USA. All of the other compounds were of analytical grade. The following rat ELISA assay kits, such as glutathione (GSH), Lipid peroxidation (LPO), superoxide dis mutase (SOD), nitric oxide (NO) assay kits, and TNF-α, IL-6, IL-1β, were bought from Synergy scientific services Pvt Ltd, Chennai, India.

2.2 Procurement and Preparation of *Flueggea Virosa* Leaves Extract

Leaves of *Flueggea virosa* were collected from Aralvaymozhi, Kanyakumari district (August 2021), and authenticated (Voucher No. F16092101V). The leaves were washed, shade-dried, and powdered. About 100 g of the powder was subjected to Soxhlet extraction using 500 mL methanol at ~65 °C for 24 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator, followed by drying to obtain a semisolid mass (yield: 73 g). The extract was stored safely and suspended in normal saline at required concentrations for pharmacological studies.

2.3 Heavy Metal Analysis by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Instrument

Take about 20 to 50 mg of sample into the Teflon microwave digestion vessel and add 1 mL of ultrapure nitric acid to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared for 2 µg/mL to 10 µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF power 1.2 kW, a plasma gas flow rate 12 L min-1, and a nebulizer gas flow rate 0.70 L/min. The samples are introduced into the plasma using nebulizer and spray chamber for the analysis [31].

2.4 Experimental Animals

All animal experiments were performed using healthy male Wistar albino rats weighing 150–200 g. The animals were procured from TANUVAS, Madhavaram, Chennai, India (Registration No: 190/GO/Re/SL/2000/CPCSEA). Prior to the initiation of the study, the animals were acclimatized to laboratory conditions for a period of 7 days. The study was conducted in the animal house facility of the Siddha Central Research Institute, Arumbakkam, Chennai, India (Registration No: 512/GO/R/S/01/CPCSEA). The animals were housed under standard laboratory conditions with a 12 h light/dark cycle, controlled temperature (22 ± 2 °C), and relative humidity of 40–70%. Rats were provided with standard pellet diet and water ad libitum throughout the experimental period. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC No: 198/PHARMA/SCRI,2018), and all procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.5 Development of Streptozotocin-Induced Diabetic Nephropathy Model and Treatment Regimen in Rats

Streptozotocin (STZ)-induced diabetic nephropathy model was developed in Wistar albino rats. Healthy rats (150–200 g) were fasted overnight (18 h) with free access to water prior to induction of diabetes. Diabetes was induced by a single intraperitoneal injection of STZ (45 mg/kg body weight), freshly dissolved in 0.05 M citrate buffer (pH 4.5). To prevent initial drug-induced hypoglycemia, rats were provided with 10% glucose solution overnight after STZ administration [32,33]. After 72 h of STZ injection, fasting blood glucose (FBG) levels were measured using a glucometer.

Rats with FBG levels ≥250 mg/dL were considered diabetic and included in the study (Day 0). The total duration of the experimental study was 21 days. *Flueggea virosa* was administered orally suspended in 0.5% CMC at three different dosages as 200, 400 and 600 mg/kg BW per animal, whereas standard drug Metformin was administered at a dose of 500 mg/kg BW. All dosages were given to the animals from Day 0 to day 21 by oral route administration except STZ. Six groups of six rats each (n=6) were randomly selected from the grouping of rats.

Group I: Normal control (received normal diet and distilled water)
Group II: Diabetic control (received vehicle only, STZ alone without *Flueggea virosa*)
Group III: STZ+ 0.5% CMC standard drug (Metformin 500 mg/kg, oral)
Group IV: STZ + Low dose of 0.5% CMC *Flueggea virosa* extract (200 mg/kg, oral)
Group V: STZ + Mid dose of 0.5% CMC *Flueggea virosa* extract (400 mg/kg, oral)
Group VI: STZ +High dose of 0.5% CMC *Flueggea virosa* extract (600 mg/kg, oral)
All the groups except normal control were challenged with STZ from Day 1 to Day 21

On the 21st day, which was the termination day, blood was drawn aseptically and stored in a freezer at -800C for further biochemical and ELISA analysis. Diabetic and non-diabetic animals were subjected to death by co2 asphyxiation, kidney and pancreas organ weights were measured. A distal portion or segments of kidney and pancreas tissues were preserved at room temperature in 10% buffered formalin for histopathological study.

2.6 Assessment of Histopathological Findings in Kidney and Pancreas

The organ tissues were removed for histological examination, fixed in 10% formalin right away, dehydrated in ethanol (50–100%), cleaned in xylene, and Sections (4-5 mm) were produced and stained with hematoxylin-eosin dye for photomicroscopic studies after being embedded in paraffin25. The sections were assessed by a skilled pathologist, and the Modified Histological Classification of Suzuki's score27 was used to determine the histological degree of kidney injury. Tubular degeneration, tubular necrosis, glomerular alterations, and mononuclear cell infiltration were the four components assessed, and each was scored on a scale of 0 to 4 (Table 1) [34,35].

Score	Pancreas (Islet Damage Score)	Kidney (Renal Damage Score)
0	Normal architecture; intact islets with no cellular damage	Normal glomeruli and tubules; no structural abnormalities
1	Mild changes; slight islet shrinkage or minimal β-cell degeneration	Mild damage; slight glomerular congestion or tubular dilation
2	Moderate damage; reduced islet size with noticeable β-cell loss and mild vacuolation	Moderate damage; mesangial expansion, mild tubular degeneration, and slight inflammatory infiltration
3	Severe damage; marked islet degeneration, extensive β-cell destruction, and inflammatory infiltration	Severe damage; glomerular shrinkage, tubular necrosis, and moderate inflammatory infiltration
4	Very severe damage; complete islet destruction with necrosis and loss of cellular integrity	Very severe damage; extensive glomerular destruction, tubular necrosis, interstitial fibrosis, and heavy inflammatory infiltration

Table 1: Histopathological Scoring System (0–4 Scale)

2.7 Determination of body and organ weights

The weights of animals were recorded until the study's termination. At the end of the experimental period, all animals were fasted overnight and sacrificed under appropriate anesthesia. Following sacrifice, vital organs including the pancreas and kidneys were carefully excised, blotted dry to remove excess blood, and weighed immediately using a calibrated digital weighing balance. The absolute organ weight of each organ was recorded individually and expressed in grams (g).

2.8 Estimation of Glycated Hemoglobin (HbA1c) and Glucose

Using blood samples taken from experimental rats at the end of the trial, blood glucose levels were determined using a standard glucose estimation kit based on the glucose oxidase–peroxidase (GOD-POD) method. Following the manufacturer's instructions, glycated haemoglobin (HbA1c) levels were measured using a commercially available diagnostic kit based on the ion-exchange resin or immunoturbidimetric method. A spectrophotometer was used to measure the absorbance, and the results were reported as percentage (%) for HbA1c and mg/dL for glucose. Both short-term and long-term glycaemic management were evaluated using these metrics [36].

2.9 Estimation of Oxidative Stress Biomarkers

At the end of the experimental period, blood samples were collected from all animals under mild anesthesia via retro-orbital plexus (or cardiac puncture). The collected blood was allowed to clot at room temperature and centrifuged at 3000 rpm for 10 minutes to obtain serum. The separated serum was stored at −20°C until further biochemical analysis. The serum samples were used for the estimation of various oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) using standard assay kits according to the manufacturer's instructions [37,38].

2.10 Estimation of Nitrites and Lipid Peroxidation

Production of nitric oxide (NO) was evaluated by measuring serum nitrite levels using Griess reagent, as described by Green et al. (1982). Briefly, 100 µL of serum sample was mixed with an equal volume of Griess reagent containing 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide, and 2.5% phosphoric acid. The reaction mixture was incubated at room temperature for 10 minutes, and the absorbance was measured at 540 nm using a microplate ELISA reader. The concentration of nitrite, an indicator of NO production, was determined using a sodium nitrite ($NaNO_2$) standard calibration curve and expressed as µM/mL of serum.

Lipid peroxidation in serum was assessed by measuring thiobarbituric acid reactive substances (TBARS), following the method of Wright et al. (1981) with slight modifications. Briefly, the reaction mixture (total volume 3 mL) consisted of 1 mL of serum sample, 1 mL of 10% trichloroacetic acid (TCA), and 1 mL of 0.67% thiobarbituric acid (TBA). The mixture was incubated in a boiling water bath for 45 minutes, cooled, and centrifuged at 2500 × g for 10 minutes. The absorbance of the supernatant was measured at 532 nm using a UV–Visible spectrophotometer. The level of lipid peroxidation was expressed as nmol of malondialdehyde (MDA)/mL of serum, using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} [39].

2.11 Estimation of Inflammatory Cytokines in Serum

Inflammatory factors TNF-α, IL-1β, and IL-6 were measured in the serum using a sandwich ELISA kit. Serum samples were incubated with a biotin conjugate solution, followed by streptavidin-HRP. After incubation, the absorbance was read spectrophotometrically

at a wavelength of 450 nm using a microplate ELISA reader. All cytokines' concentrations were expressed as ng/ml in serum [40,41].

2.12 Statistical analysis

Using GraphPad Prism, version 11.00 (85) software, one-way ANOVA was used for all statistical analyses. Using the STZ control group as a reference, post hoc Dunnett's multiple comparison procedures were used to compare the groups. The mean ± standard error of the mean (SEM) was used to express the results. When the p-value was less than 0.05, statistical significance was taken into consideration.

3. Results

3.1 Heavy Metal Analysis by ICP-OES Characterization

Using multi-element standards in the concentration range of 2–10 µg/mL, the linearity of the ICP-OES technique was assessed, showing good correlation ($R^2 > 0.995$) for all metals examined (Table 2). At their respective wavelengths, arsenic, cadmium, mercury, and lead demonstrated robust linear correlations between concentration and emission intensity. Cadmium and lead had increased sensitivity, and the calibration curves (figure 1A) verified a direct proportional rise in signal with concentration. The method's excellent precision was demonstrated by the superimposed spectra of standards (figure 1B), which showed distinct peaks free of spectral interference. Additionally, the presence of trace heavy metals in the sample was confirmed by ICP-OES spectral analysis of *Flueggea virosa* extract (figure 1C), which showed different emission peaks corresponding to each element.

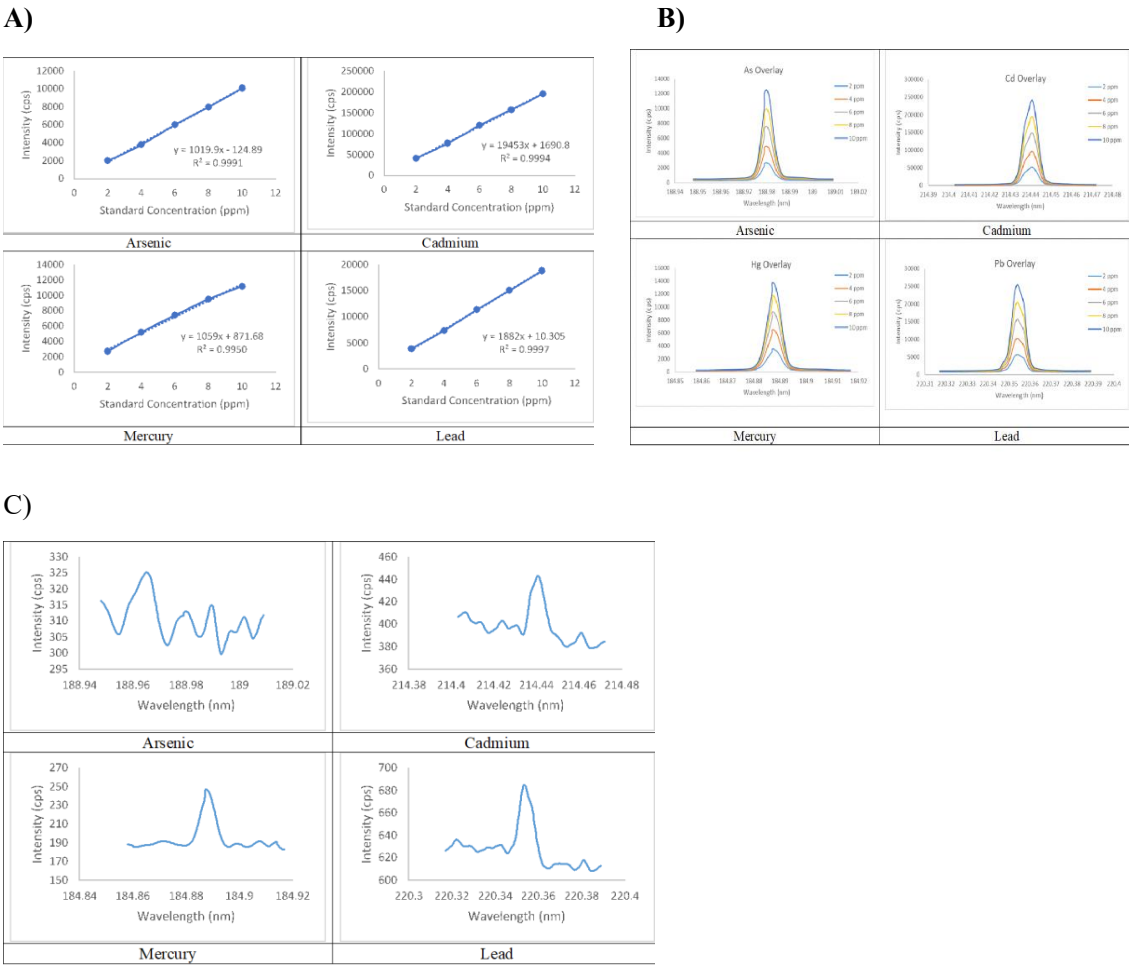


Figure 1: Heavy Metals Analysis of *Flueggea virosa* (A) Calibration Curves of Heavy Metal Standards (*As, Cd, Hg, and Pb*) Obtained by ICP-OES. (B) Overlay of ICP-OES Calibration Spectra for Heavy Metal Standards (*As, Cd, Hg, and Pb*) (C) ICP-OES Spectral Analysis of Heavy Metals in *Flueggea Virosa* Leaf Extract

S.No	Element	Wavelength	R^2 Value
1	Arsenic [As] (µg/ml)	188.980	0.9991
2	Cadmium [Cd] (µg/ml)	226.502	0.9994
3	Mercury [Hg] (µg/ml)	184.887	0.9950
4	Lead [Pb] (µg/ml)	220.353	0.9997

Table 2: Standard Linearity Data for Heavy Metals (As, Cd, Hg, and Pb) Analyzed by ICP-OES.

3.2 Effect of *Flueggea Virosa* on Body and Organ Weights Changes

Rats with diabetic nephropathy caused by streptozotocin were used to assess the impact of *Flueggea virosa* extract on body and organ weights. Severe metabolic and tissue damage was shown by the diabetic control group's considerable decrease in body weight (figure 2A), pancreas, and kidney weights ($p < 0.001$ versus normal control) (figure 2B). Body and organ weights were considerably improved by metformin treatment ($p < 0.01$ vs diabetic control). Comparing *Flueggea virosa* extract to the diabetes control group, the low dose demonstrated a slight improvement ($p < 0.05$), the mid dose demonstrated a moderate restoration ($p < 0.01$), and the high dose demonstrated a large recovery of body and organ weights ($p < 0.001$). These results imply that *Flueggea virosa*, especially at larger doses, significantly reduces organ damage and weight loss brought on by diabetes.

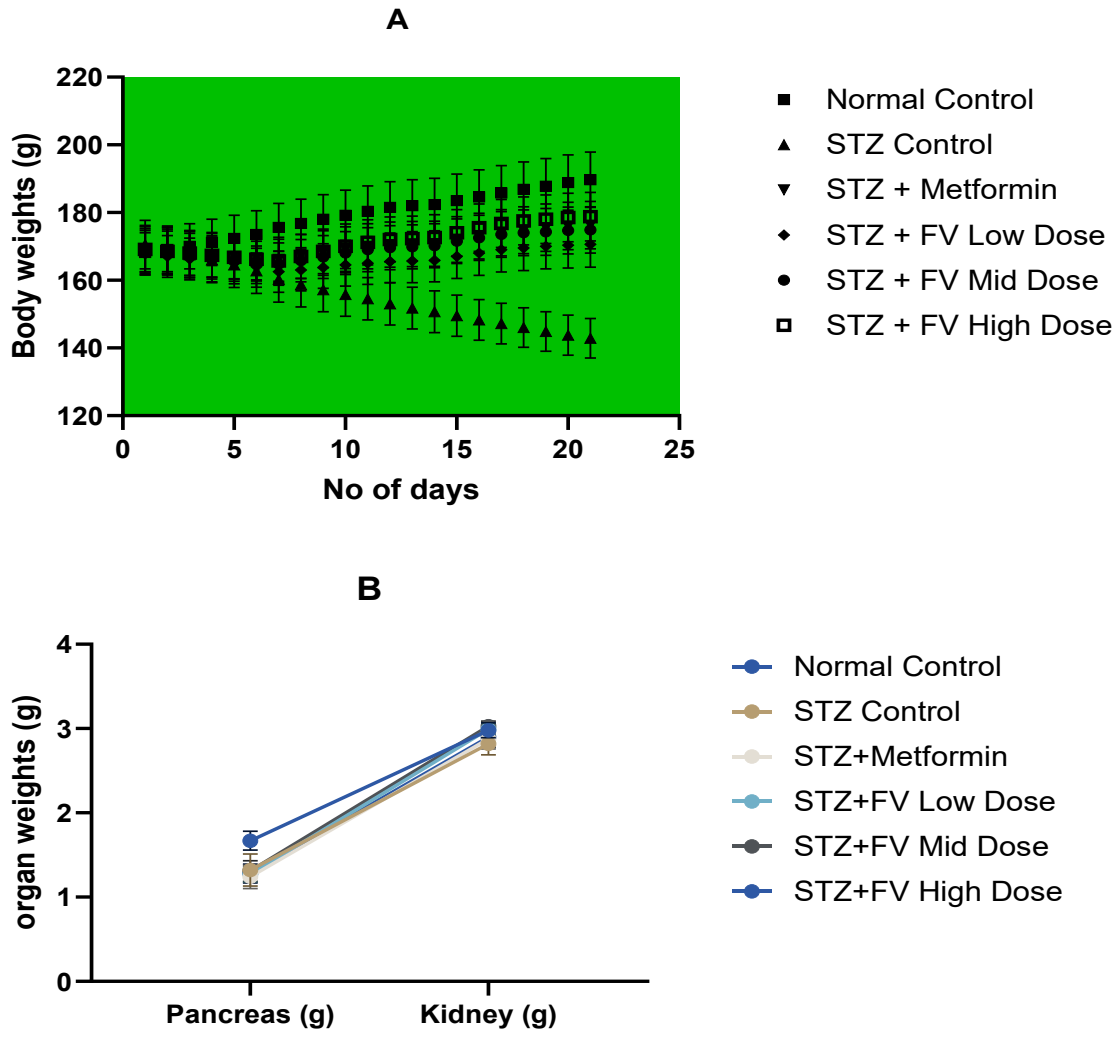


Figure 2: Effect of *Flueggea Virosa* on Body and Organ Weight Changes in Streptozotocin-Induced Diabetic Nephropathy Rats. Treatment with *Flueggea Virosa* Extract at Different Doses (Low, Mid, And High) Along with The Standard (Metformin) Significantly Improved Body Weight and Organ Weights in Experimental Animals Compared to The Diabetic Control Group. (A) Effect on Body Weight Changes Observed During the Experimental Period. (B) Pancreas and Kidney Weights Showing Restoration in Treated Groups. Results Were Expressed as Mean ± SEM (n = 6). ###$p < 0.001$; ##$p < 0.01$; #$p < 0.05$ Compared with The Normal Control Group; *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ Compared with The Diabetic Control Group; Ns, Non-Significant.

3.3 Effect of *Flueggea virosa* on Glucose & HbA1c levels

The effect of *Flueggea virosa* extract on blood glucose and HbA1c levels in streptozotocin-induced diabetic nephropathy rats is presented in (figure 3 (A–B)). The STZ control group showed a significant elevation in blood glucose and HbA1c levels ($p < 0.001$) compared to the normal control group, indicating severe hyperglycemia and poor glycemic control. Treatment with metformin significantly reduced both blood glucose ($p < 0.01$) and HbA1c ($p < 0.01$) levels compared to the STZ control group. Similarly, *Flueggea virosa* extract exhibited dose-dependent effects. The low-dose group showed non-significant (ns) changes in both parameters, whereas the mid-dose group demonstrated a moderate reduction in HbA1c ($p < 0.05$) with non-significant changes in blood glucose. Notably, the high-dose group showed a significant reduction in both blood glucose and HbA1c levels ($p < 0.001$) compared to the STZ control group. Overall, *Flueggea virosa* extract, particularly at the high dose, effectively improved glycemic control in diabetic nephropathy rats, as evidenced by reduced blood glucose and HbA1c levels.

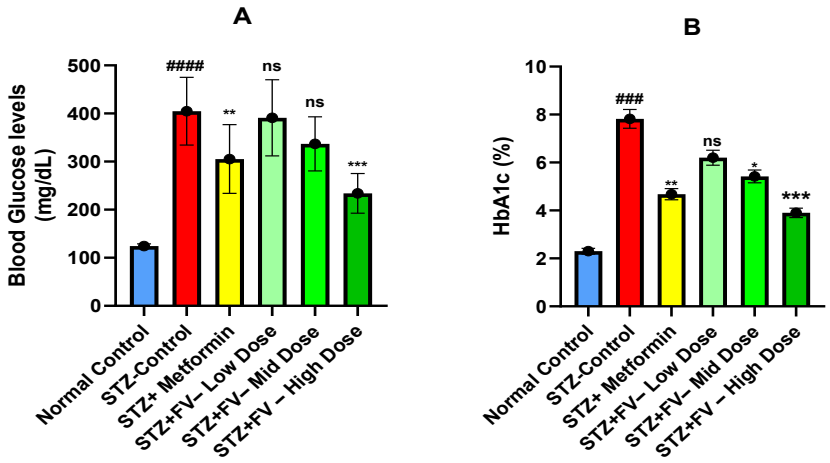


Figure 3: Effect of *Flueggea Virosa* On Blood Glucose and HbA1c levels in Streptozotocin-Induced Diabetic Nephropathy Rats. (A) Blood Glucose Levels (mg/dL) and (B) HbA1c (%) were Measured to Evaluate Glycemic Control. The STZ Control Group Showed A Significant Increase in Blood Glucose and HbA1c Levels Compared to The Normal Control Group (####$p < 0.001$). Treatment with Metformin Significantly Reduced Both Parameters (**$p < 0.01$), whereas *Flueggea Virosa* Treatment Exhibited Dose-Dependent Effects. The Low-Dose Group Showed Non-Significant (ns) Changes in Glucose and HbA1c levels, While the Mid-Dose Group Showed Moderate Reduction (*$p < 0.05$). Notably, the High-Dose Group Showed A Significant Decrease in Blood Glucose and HbA1c levels (***$p < 0.001$) Compared to the STZ Control Group. Results are Expressed as Mean ± SEM ($n = 6$). Statistical Significance: ####$p < 0.001$ vs Normal Control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs STZ Control; Ns: Non-Significant.

3.4 Effect of *Flueggea Virosa* on Pancreas, Kidney Histology of STZ Induced Rats

Histopathological examination of pancreas and kidney tissues (figure 4) revealed normal architecture in the control group, with well-defined pancreatic islets and intact renal glomeruli and tubules. In contrast, the STZ control group showed marked pathological alterations, including pancreatic islet degeneration, β-cell damage, glomerular degeneration, tubular necrosis, and inflammatory infiltration, indicating severe tissue injury. Treatment with metformin and *Flueggea virosa* extract demonstrated improvement in tissue architecture. The low-dose group showed mild restoration, whereas the mid-dose group exhibited moderate recovery with reduced cellular damage. Notably, the high-dose *Flueggea virosa* group showed near-normal histological features with preserved pancreatic islets and improved renal structure, indicating significant protective effects against diabetic-induced tissue damage.

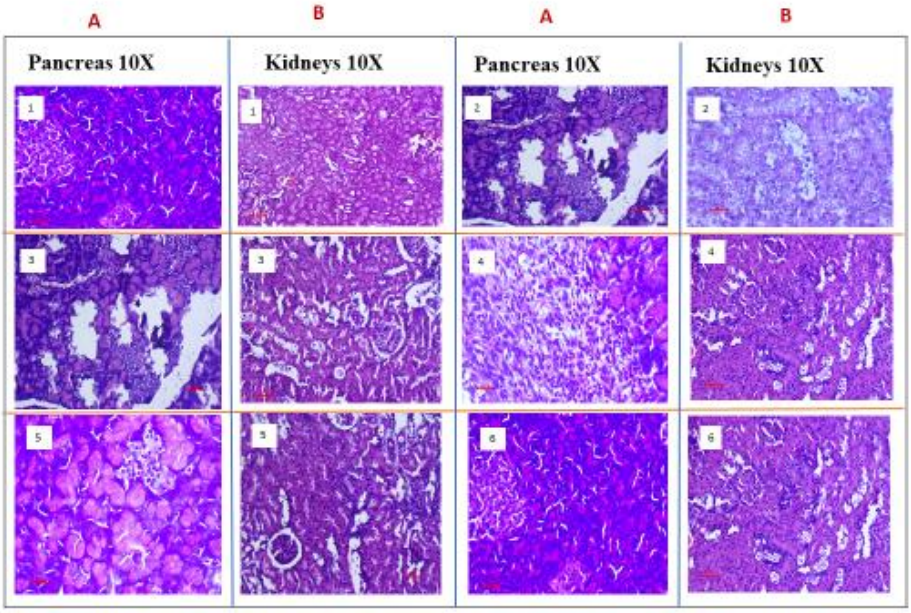


Figure 4: Effect of *Flueggea Virosa* on Histopathological Changes in Pancreas and Kidney of Streptozotocin-Induced Diabetic Nephropathy Rats (H&E staining, 10×). (A) Pancreas Sections and (B) Kidney Sections Showing Morphological Alterations Across Experimental Groups. (1) Normal Control Showing Intact Pancreatic Islets and Normal Renal Architecture. (2) STZ Control Showing Severe Pathological Changes Including Islet Degeneration, β-cell Damage, Glomerular Distortion, Tubular Necrosis, and Inflammatory Infiltration. (3) Standard (Metformin) Group Showing Moderate Restoration of Pancreatic and Renal Structures. (4) *Flueggea Virosa* Low-Dose Group Showing Mild Improvement with Partial Restoration of Tissue Architecture. (5) *Flueggea Virosa* Mid-Dose Group Showing Moderate Recovery with Reduced Cellular Damage and Improved Organization. (6) *Flueggea Virosa* High-Dose Group Showing Near-Normal Architecture with Well-Preserved Pancreatic Islets and Renal Structures, Indicating Significant Protective Effect.

3.5 Effect of *Flueggea Virosa* on Renal Function Tests

The effect of Flueggea virosa extract on renal function parameters in streptozotocin-induced diabetic nephropathy rats is presented in (figure 5(A–F)). The STZ control group showed a significant increase in urea, serum creatinine, uric acid, LDH, and total bilirubin levels along with a decrease in calcium levels ($p < 0.01$ / $p < 0.001$) compared to the normal control group, indicating renal dysfunction and metabolic imbalance. Treatment with metformin significantly reduced urea ($p < 0.01$), creatinine ($p < 0.05$), uric acid ($p < 0.001$), LDH ($p < 0.01$), and total bilirubin ($p < 0.001$) levels, while improving calcium levels. Similarly, *Flueggea virosa* extract showed dose-dependent improvement in renal markers. The low-dose group showed non-significant (ns) changes in some parameters, whereas the mid-dose group demonstrated moderate improvement ($p < 0.05$). Notably, the high-dose group showed significant reduction in urea, creatinine, uric acid, LDH, and total bilirubin levels ($p < 0.001$ / $p < 0.01$) along with restoration of calcium levels compared to the STZ control group.

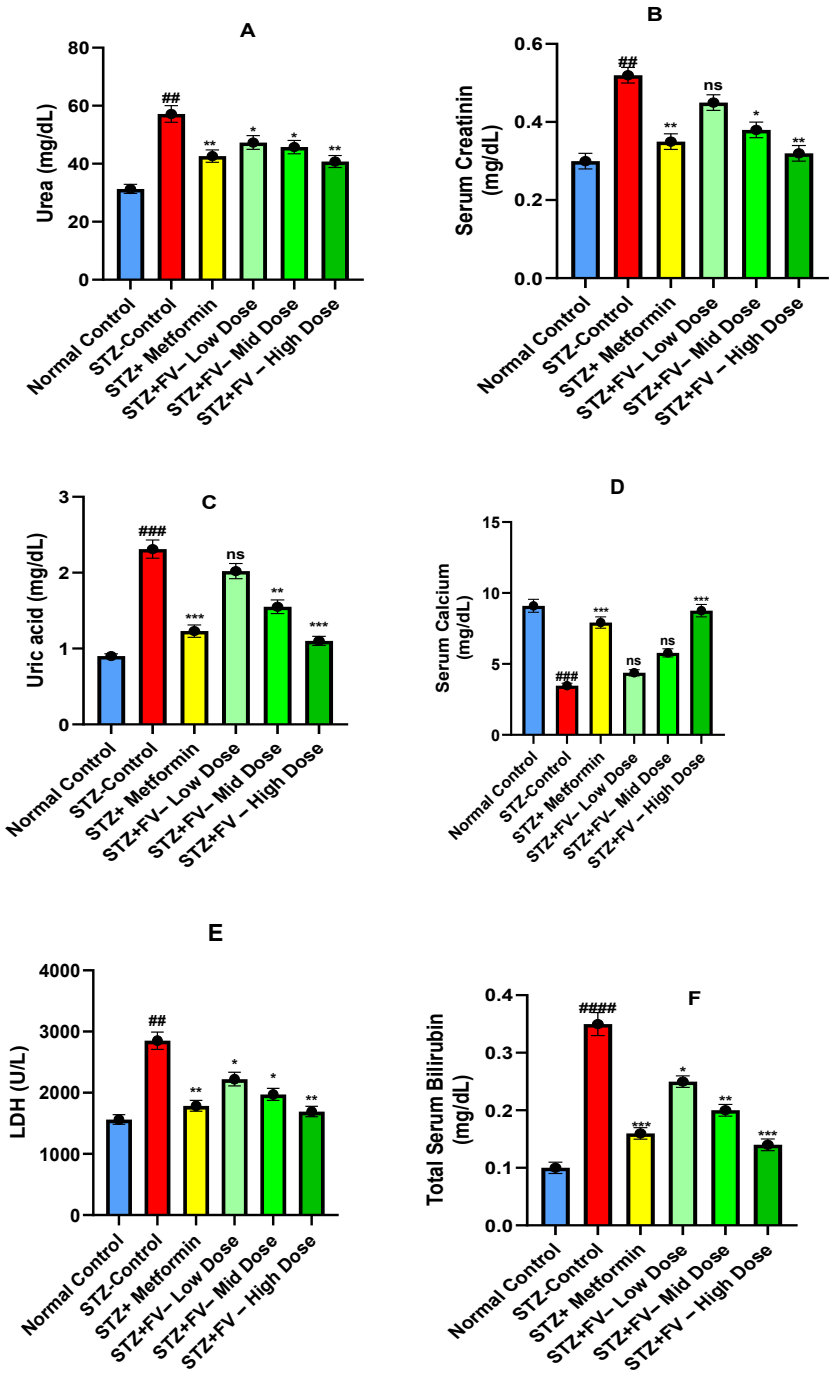


Figure 5: Effect of *Flueggea Virosa* on Renal Function Markers in Streptozotocin-Induced Diabetic Nephropathy Rats. (A) Urea (mg/dL), (B) Serum Creatinine (mg/dL), (C) Uric Acid (mg/dL), (D) Serum Calcium (mg/dL), (E) Lactate Dehydrogenase (LDH, U/L), and (F) Total Serum Bilirubin (mg/dL) Were Evaluated to Assess Renal Function and Metabolic Status. The STZ Control Group Showed A Significant Increase in Urea, Creatinine, Uric Acid, LDH, and Total Bilirubin Levels Along with A Decrease in Calcium Levels Compared to The Normal Control Group (##p < 0.01, ###p < 0.001). Treatment with Metformin Significantly Improved These Parameters, Showing Reduced Levels of Urea, Creatinine, Uric Acid, LDH, and Bilirubin, Along with Restoration of Calcium Levels (*p < 0.05, **p < 0.01, ***p < 0.001). Similarly, *Flueggea Virosa* Extract Exhibited Dose-Dependent Effects, Where the Low-Dose Group Showed Non-Significant (ns) Changes in Some Parameters, While the Mid- and High-Dose Groups Showed Significant Improvement in Renal Markers Compared to The STZ Control Group. The High-Dose Group Demonstrated Maximum Restoration of Renal Function Parameters. Results are Expressed as Mean ± SEM (n = 6) Statistical Significance: ##p < 0.01, ###p < 0.001 vs Normal Control; *p < 0.05, **p < 0.01, ***p < 0.001 vs STZ Control; Ns: Non-Significant.

3.6 Effect of *Flueggea Virosa* on Oxidative Stress Markers

The effect of *Flueggea virosa* extract on antioxidant enzyme levels in streptozotocin-induced diabetic nephropathy rats is presented in (figure 6 (A–C)). The STZ control group showed a significant decrease in superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels ($p < 0.001$) compared to the normal control group, indicating enhanced oxidative stress. Treatment with metformin significantly increased SOD, CAT, and GSH levels ($p < 0.01$ / $p < 0.001$) compared to the STZ control group. Similarly, *Flueggea virosa* extract exhibited dose-dependent improvement in antioxidant status. The low-dose group showed mild or non-significant (ns) changes, while the mid-dose group demonstrated moderate enhancement ($p < 0.05$ / $p < 0.01$). Notably, the high-dose group showed significant restoration of SOD, CAT, and GSH levels ($p < 0.001$), approaching normal values. These findings indicate that *Flueggea virosa* effectively enhances antioxidant defense mechanisms and reduces oxidative stress in diabetic nephropathy.

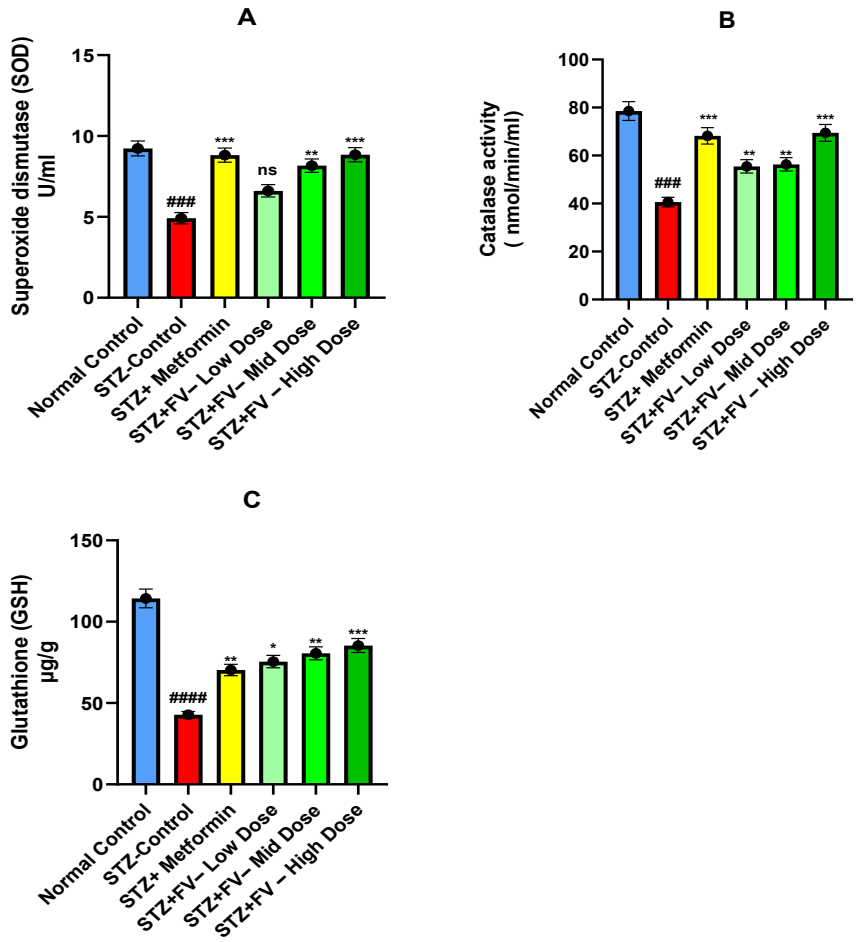


Figure 6: Effect of *Flueggea Virosa* on Antioxidant Enzymes in Streptozotocin-Induced Diabetic Nephropathy Rats. (A) Superoxide Dismutase (SOD), (B) Catalase (CAT), and (C) Reduced Glutathione (GSH) Levels Were Measured to Assess Antioxidant Status. The STZ Control Group Showed A Significant Decrease In SOD, CAT, and GSH Levels Compared to The Normal Control Group (###$p < 0.001$), Indicating Increased Oxidative Stress. Treatment with Metformin Significantly Increased SOD, CAT, and GSH levels (**$p < 0.01$, *$p < 0.001$) Compared to the STZ Control Group. Similarly, *Flueggea Virosa* Extract Exhibited Dose-Dependent Effects, Where the Low-Dose Group Showed Non-Significant (ns) Changes, the Mid-Dose Group Showed Moderate Improvement (*$p < 0.05$, **$p < 0.01$), and the High-Dose Group Showed Significant Restoration of Antioxidant Enzyme Levels (***$p < 0.001$) Compared to the STZ Control Group. Results are Expressed as Mean ± SEM (n = 6). Statistical Significance: ###$p < 0.001$ vs Normal Control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs STZ Control; Ns: Non-Significant.

3.7 Effect of Lipid Peroxidation and Nitrites

The effect of *Flueggea virosa* extract on oxidative stress markers, lipid peroxidation (LPO) and nitric oxide (NO), in streptozotocin-induced diabetic nephropathy rats is presented in (figure 7 (A–B)). The STZ control group showed a significant increase in LPO and NO levels ($p < 0.001$) compared to the normal control group, indicating enhanced oxidative stress and nitrosative damage. Treatment with metformin significantly reduced LPO ($p < 0.001$)

and NO ($p < 0.01$) levels compared to the STZ control group. Similarly, *Flueggea Virosa* extract demonstrated dose-dependent effects. The low-dose group showed non-significant (ns) changes in both LPO and NO levels, whereas the mid-dose group showed a significant reduction ($p < 0.01$). Notably, the high-dose group exhibited a marked decrease in LPO and NO levels ($p < 0.001$) compared to the STZ control group.

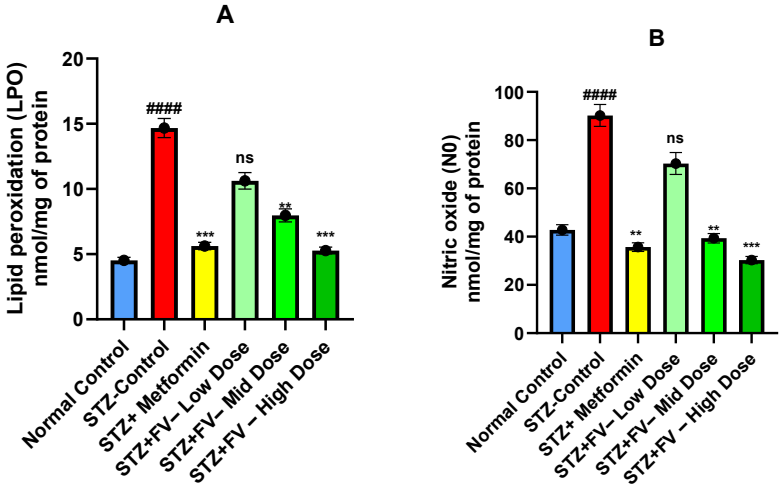
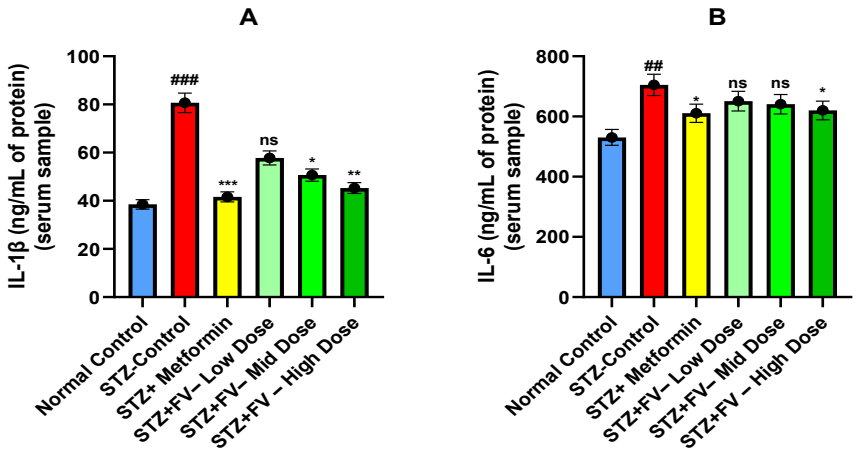


Figure 7: Effect of *Flueggea virosa* on Lipid Peroxidation (LPO) and Nitric Oxide (NO) Levels in Streptozotocin-Induced Diabetic Nephropathy Rats. (A) Lipid Peroxidation (LPO) Levels (nmol/mg protein) and (B) Nitric Oxide (NO) Levels (nmol/mg protein) Were Evaluated as Markers of Oxidative Stress. The STZ Control Group Showed A Significant Increase in LPO and NO Levels Compared to the Normal Control Group (####$p < 0.001$). Treatment with Metformin Significantly Reduced LPO (***$p < 0.001$) and NO (**$p < 0.01$) Levels. Similarly, *Flueggea virosa* Extract Exhibited Dose-Dependent Effects, Where the Low-Dose Group Showed Non-Significant (ns) Changes, the Mid-Dose Group Showed Moderate Reduction (**$p < 0.01$), and the High-Dose Group Showed Significant Reduction in both LPO and NO Levels (***$p < 0.001$) Compared to the STZ control group. Results are Expressed as Mean ± SEM (n = 6).

3.8 Effect of Treatments on Inflammatory Cytokines in Diabetic Rats

The effect of *Flueggea virosa* extract on inflammatory cytokines in streptozotocin-induced diabetic rats is shown in (figure 8(A–C)). The STZ control group showed a significant increase in IL-1β, IL-6, and TNF-α levels ($p < 0.001$ / $p < 0.01$) compared to the normal control group, indicating enhanced inflammatory response. Treatment with metformin significantly reduced IL-1β ($p < 0.001$), IL-6 ($p < 0.05$), and TNF-α ($p < 0.01$) levels. Similarly, *Flueggea virosa* extract exhibited dose-dependent effects, where the low-dose group showed non-significant (ns) changes in some parameters, while mid- and high-dose groups showed significant reduction in IL-1β ($p < 0.05$, $p < 0.01$), IL-6 ($p < 0.05$), and TNF-α ($p < 0.01$) compared to the STZ control group. Overall, *Flueggea virosa* demonstrated anti-inflammatory activity, with better effects observed at higher doses.



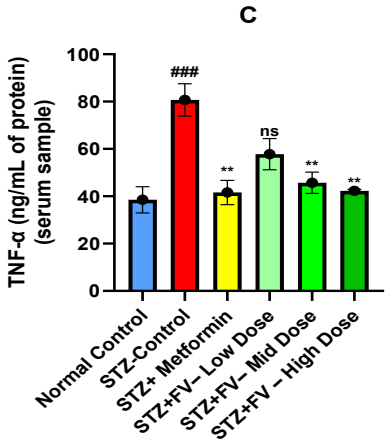


Figure 8: Effect of *Flueggea virosa* On Inflammatory Cytokines in Streptozotocin-Induced Diabetic Rats. (A) IL-1β (ng/mL of protein), (B) IL-6 (ng/mL of protein), and (C) TNF-α (ng/mL of protein) Levels Were Measured in Serum Samples to Evaluate Inflammatory Response. The STZ Control Group Showed A Significant Increase in IL-1β and TNF-α Levels (###$p < 0.001$) and IL-6 Levels (##$p < 0.01$) Compared to The Normal Control Group. Treatment with Metformin Significantly Reduced IL-1β (***$p < 0.001$), IL-6 (*$p < 0.05$), and TNF-α (**$p < 0.01$) Levels. Similarly, *Flueggea virosa* Extract Showed Dose-Dependent Anti-Inflammatory Effects, Where the Low-Dose Group Showed Non-Significant (ns) Changes in Selected Cytokines, While the Mid- And High-Dose Groups Showed Significant Reductions in IL-1β (*$p < 0.05$, **$p < 0.01$), IL-6 (*$p < 0.05$), and TNF-α (**$p < 0.01$) Compared to the STZ Control Group. Results Are Expressed as Mean ± SEM (n = 6). Statistical Significance: ###$p < 0.001$, ##$p < 0.01$ vs normal control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs STZ Control; Ns: Non-Significant.

4. Discussion

The results of ICP-OES analysis for *Flueggea virosa* leaf extract revealed the presence of heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). All detected elements showed good linearity and reliable quantification within the tested range. The concentrations of these metals were found to be within permissible limits when compared with standard regulatory guidelines, indicating the safety of the plant material for further pharmacological use. The absence of abnormal peak interference and the consistency in calibration curves confirm the accuracy and precision of the analytical method [42]. Overall, the findings suggest that the plant sample does not pose significant toxicological risk with respect to heavy metal contamination.

One of the most serious side effects of diabetes mellitus is diabetic nephropathy (DN), which is characterised by structural abnormalities, increasing renal dysfunction, and ultimately renal failure [43]. With an emphasis on biochemical, oxidative stress, and histological characteristics, the current study assessed the nephroprotective efficacy of *Flueggea virosa* extract in streptozotocin-induced diabetic rats. The results showed that *Flueggea virosa* considerably reduced kidney damage in a dose-dependent manner, indicating that it may be used as a treatment for diabetic nephropathy. The main cause of diabetic nephropathy is hyperglycemia. Multiple metabolic pathways, including as the polyol pathway, protein kinase C (PKC) activation, and advanced glycation end-product (AGE) production, are activated when blood glucose levels are persistently elevated. These processes all contribute to kidney damage [44]. Reactive oxygen species (ROS), which are crucial to the pathophysiology of DN, are ultimately produced in excess as a result of these metabolic disruptions. Diabetic rats in this study showed markedly higher glucose and HbA1c levels, indicating that diabetes and its related metabolic problems were successfully induced.

One of the main mechanisms underlying diabetic nephropathy has been identified as oxidative stress. Cellular damage is caused by an imbalance between ROS production and antioxidant defence mechanisms. According to earlier research, too much ROS destroys DNA, lipids, and proteins, which eventually impairs renal cell function and speeds up the course of the disease. In agreement with these findings, the present study showed increased lipid peroxidation (LPO) and nitric oxide (NO) levels in diabetic rats, indicating enhanced oxidative stress [45,46]. Simultaneously, antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were significantly reduced, reflecting compromised antioxidant defense. Antioxidant enzyme levels were considerably recovered and oxidative stress markers were decreased when *Flueggea virosa* extract was administered. This implies that the extract's antioxidant qualities may be responsible for its nephroprotective action.

Previous research has demonstrated that plant-derived polyphenols can lower oxidative stress by scavenging free radicals and boosting endogenous antioxidant systems [47]. These effects are probably caused by the phytochemical components of *Flueggea virosa*, such as flavonoids and phenolic compounds, which are known to protect cellular structures and neutralise ROS.

The development of diabetic nephropathy is significantly influenced

by inflammation in addition to oxidative stress. Oxidative stress brought on by hyperglycemia triggers inflammatory signalling pathways like nuclear factor-kappa B (NF-κB), which increases the production of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. These cytokines encourage mesangial growth, extracellular matrix formation, and inflammatory cell infiltration, which eventually lead to glomerulosclerosis and fibrosis. Renal injury is made worse by the interaction of oxidative stress and inflammation [48,49]. Since NO is frequently linked to inducible nitric oxide synthase (iNOS) activity during inflammation, elevated nitric oxide levels in diabetic rats in the current study may also indicate inflammatory activation. *Flueggea virosa* extract treatment dramatically decreased NO levels, suggesting that it may have anti-inflammatory properties. This observation is consistent with previous reports suggesting that antioxidant compounds can suppress inflammatory pathways by inhibiting NF-κB activation and reducing cytokine production.

Serum creatinine, urea, and uric acid are examples of renal function markers that are crucial indicators of kidney disease. Due to decreased glomerular filtration and renal dysfunction, these indicators are markedly increased in diabetes circumstances. The current investigation confirmed the development of nephropathy by showing a significant increase in these parameters in the disease control group. Nevertheless, *Flueggea virosa* extract treatment dramatically decreased these levels, suggesting improved renal function. Its capacity to lower oxidative stress and inflammation, so maintaining kidney structure and function, may be the cause of this protective action. The biochemical results were further corroborated by histopathological investigation. The hallmarks of diabetic nephropathy, such as tubular degradation, mesangial enlargement, glomerular hypertrophy, and inflammatory cell infiltration, were seen in diabetic rats. The main causes of these structural anomalies are inflammatory processes and cellular damage brought on by oxidative stress. According to earlier research, ROS-mediated damage causes extracellular matrix buildup, basement membrane thickening, and podocyte dysfunction, all of which contribute to renal fibrosis [50].

Renal histoarchitecture significantly improved after *Flueggea virosa* extract treatment, showing less tubular necrosis, glomerular damage, and inflammatory infiltration. The restoration of nearly normal kidney structure indicates that the extract successfully guards against hyperglycemia-induced structural damage. Its bioactive components' combination anti-inflammatory and antioxidant properties may be the cause of this protective effect.

The dose-dependent impact of *Flueggea virosa* is another significant finding of this investigation. When compared to low and mid doses, the high dose demonstrate the highest level of protective action, suggesting a positive relationship between dose and therapeutic efficacy. This result is in line with earlier research on herbal extracts, which showed stronger pharmacological effects at higher bioactive ingredient concentrations.

This work used a well-established STZ-induced diabetic model that closely resembles diabetic nephropathy in humans. Insulin insufficiency and hyperglycemia result from streptozotocin's selective destruction of pancreatic β-cells. This model is frequently used to assess possible treatment agents and investigate the mechanisms underlying diabetic complications. The current study's biochemical and histological alterations are in line with earlier findings in models of STZ-induced diabetic nephropathy [51]. Overall, this study's results demonstrate *Flueggea virosa* extract's strong nephroprotective properties. The extract reduces hyperglycemia, improves antioxidant defence, inhibits oxidative stress, and suppresses inflammatory reactions, among other methods. It is a potential option for the treatment of diabetic nephropathy because of these diverse activities.

5. Conclusion

The results of this study show that *Flueggea virosa* extract administration successfully reduces the oxidative, structural, and biochemical changes linked to diabetic nephropathy. The high dose demonstrated the strongest nephroprotective benefit among the studied doses, with the mid dose coming in second and the low dose showing a moderate improvement. In a dose-dependent way, the extract improved glycaemic management, boosted endogenous antioxidant defences, restored renal function indices, and decreased oxidative stress and inflammatory mediators. By maintaining normal kidney architecture and reducing glomerular and tubular damage, especially in the high-dose group, histopathological data further validated its protective role.

These findings imply that *Flueggea virosa* nephroprotective action is mainly mediated by its anti-inflammatory and antioxidant qualities, with higher doses offering greater efficiency. *Flueggea virosa* may therefore be a viable natural therapeutic agent for the management of diabetic renal problems; however, additional molecular and clinical research is needed to determine its ideal dosage and translational application.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

Acknowledgments

We thank to all Pharmacology and Administrative staffs from SCRI (CCRS), Chennai for their support and timely completion of studies.

References

1. Asmat, U., Abad, K., & Ismail, K. (2016). Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharmaceutical Journal, 24*(5), 547–553.
2. John, S. (2016). Complication in diabetic nephropathy. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 10*(4), 247–249.
3. Stanton, R. C. (2014). Clinical challenges in diagnosis and management of diabetic kidney disease. *American Journal of Kidney Diseases, 63*(2 Suppl. 2), S3–S21.
4. Kshirsagar, A. V., Joy, M. S., Hogan, S. L., Falk, R. J., & Colindres, R. E. (2000). Effect of ACE inhibitors in diabetic

and nondiabetic chronic renal disease: A systematic overview of randomized placebo-controlled trials. *American Journal of Kidney Diseases, 35*(4), 695–707.
5. Rout, P., & Jialal, I. (2025). Diabetic nephropathy. In StatPearls. StatPearls Publishing. PMID: 30480939
6. Qasim, H., Hayajneh, A., Khattab, K., Leoni, M. L., & Varrassi, G. (2025). Histopathology of diabetic nephropathy: Beyond glomerular basement membrane thickening. *Cureus, 17*(9).
7. Vallon, V., & Komers, R. (2011). Pathophysiology of the diabetic kidney. *Comprehensive Physiology, 1*(3), 1175–1232.
8. González, P., Lozano, P., Ros, G., & Solano, F. (2023). Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. *International Journal of Molecular Sciences, 24*(11), Article 9352.
9. Juan, C. A., Pérez D. I. L., J. M., Plou, F. J., & Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences, 22*(9), Article 4642.
10. Fakhruddin, S., Alanazi, W., & Jackson, K. E. (2017). Diabetes-induced reactive oxygen species: Mechanism of their generation and role in renal injury. *Journal of Diabetes Research, 2017,* Article 8379327.
11. Hou, G., Dong, Y., Jiang, Y., Zhao, W., Zhou, L., et al. (2025). Immune inflammation and metabolic interactions in the pathogenesis of diabetic nephropathy. *Frontiers in Endocrinology, 16*, Article 1602594.
12. Rezaee, A., Rahmanian, P., Nemati, A., Sohrabifard, F., Karimi, F., et al. (2024). NF-κB axis in diabetic neuropathy, cardiomyopathy and nephropathy: A roadmap from molecular intervention to therapeutic strategies. *Heliyon, 10*(9), e29871.
13. Jin, Q., Liu, T., Qiao, Y., Liu, D., Yang, L., et al. (2023). Oxidative stress and inflammation in diabetic nephropathy: Role of polyphenols. *Frontiers in Immunology, 14,* Article 1185317.
14. Wu, J. (2023). Paradigm shift in hyperglycemic glomerular hyperfiltration: Blunted tubuloglomerular feedback or preglomerular vasodilation? *Hypertension, 80*(8), 1611–1613.
15. Schrijvers, B. F., De Vriese, A. S., & Flyvbjerg, A. (2004). From hyperglycemia to diabetic kidney disease: The role of metabolic, hemodynamic, intracellular factors and growth factors/cytokines. *Endocrine Reviews, 25*(6), 971–1010.
16. Yuan, Q., Tang, B., & Zhang, C. (2022). Signaling pathways of chronic kidney diseases: Implications for therapeutics. *Signal Transduction and Targeted Therapy, 7*(1), Article 182.
17. Rahman, M. M., Dhar, P. S., Anika, F., Ahmed, L., Islam, M. R., Sultana, N. A., Cavalu, S., Pop, O., & Rauf, A. (2022). Exploring plant-derived bioactive substances as antidiabetic agents: An extensive review. *Biomedicine & Pharmacotherapy, 152*, Article 113217.
18. Putra, I. M., Fakhrudin, N., Nurrochmad, A., & Wahyuono, S. (2023). A review of medicinal plants with renoprotective activity in diabetic nephropathy animal models. *Life, 13*(2), Article 560.
19. Negi, K., & Mirza, A. (2020). Nephroprotective and therapeutic potential of traditional medicinal plants in renal diseases. *Journal of Drug Research in Ayurvedic Sciences, 5*(3), 175–183.
20. Alsawaf, S., Alnuaimi, F., Afzal, S., Thomas, R. M., Chelakkot, A. L., et al. (2022). Plant flavonoids on oxidative stress-mediated kidney inflammation. *Biology, 11*(12), Article 1717.
21. Bailly, C. (2024). Traditional uses, pharmacology and phytochemistry of the medicinal plant *Flueggea virosa* (Roxb. ex Willd.) Royle. *Future Pharmacology, 4*(1), 77–102.
22. Ajaib, M., Pullaiah, T., & Shah, S. (2022). Phytochemistry and pharmacology of Flueggea virosa (Roxb. ex Willd.) Royle. In T. Pullaiah (Ed.), Bioactives and pharmacology of medicinal plants (pp. 187–198). Apple Academic Press.
23. Mayakrishnan, V., Thirupathi, A., Ramamoorthy, K., Annadurai, K., Prakasam, R., et al. (2024). Chemical composition analysis and assessment of antioxidant and anti-inflammatory activities of crude extract of Flueggea leucopyrus on carrageenan-induced paw edema in Wistar albino rats. *Antioxidants, 13*(8), Article 976.
24. Chao, C. H., Cheng, J. C., Shen, D. Y., Huang, H. C., Wu, Y. C., et al. (2016). Terpenoids from *Flueggea virosa* and their anti-hepatitis C virus activity. *Phytochemistry, 128,* 60–70.
25. Mustaqim, W. A. (2021). *Flueggea virosa* (Roxb. ex Willd.) Royle (Phyllanthaceae). In Ethnobotany of the mountain regions of Southeast Asia (pp. 493–498). Springer.
26. Al-Waili, N., Al-Waili, H., Al-Waili, T., & Salom, K. (2017). Natural antioxidants in the treatment and prevention of diabetic nephropathy: A potential approach that warrants clinical trials. Redox Report, 22(3), 99–118.
27. Sun, Y., Jin, D., Zhang, Z., Zhang, Y., Zhang, Y., et al . (2023). Effects of antioxidants on diabetic kidney diseases: Mechanistic interpretations and clinical assessment. Chinese Medicine, 18(1), Article 3.
28. Ghasemi, A., & Jeddi, S. (2023). Streptozotocin as a tool for induction of rat models of diabetes: A practical guide. *EXCLI Journal, 22*, 274–294.
29. Wu, J., & Yan, L. J. (2015). Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β-cell glucotoxicity. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 8,* 181–188.
30. Abdelmonem, B. H., Kamal, L. T., Elbaz, R. M., Khalifa, M. R., & Abdelnaser, A. (2025). From contamination to detection: The growing threat of heavy metals. *Heliyon, 11*(1), e41713.
31. Khan, S. R., Sharma, B., Chawla, P. A., & Bhatia, R. (2022). Inductively coupled plasma optical emission spectrometry (ICP-OES): A powerful analytical technique for elemental analysis. *Food Analytical Methods, 15*(3), 666–688.
32. Mestry, S. N., Dhodi, J. B., Kumbhar, S. B., & Juvekar, A. R. (2017). Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract.

Journal of Traditional and Complementary Medicine, 7(3), 273–280.
33. Kumar, H., Sharma, S., & Vasudeva, N. (2022). Plant Calotropis gigantea: Management of diabetic nephropathy in experimentally induced diabetes in rats. *Research Journal of Pharmacy and Technology, 15*(3), 1090–1096.
34. An, Y., Xu, F., Le, W., Ge, Y., Zhou, M., et al. (2015). Renal histologic changes and the outcome in patients with diabetic nephropathy. Nephrology Dialysis Transplantation, 30(2), 257–266.
35. Reddy, D. G., Mefi, B. M., Bharathy, H., Kumar, N. G., Pullaiah, C. P., et al. (2018). Hepatoprotective activity of Aruvadha churnam, a traditional Siddha formulation, against paracetamol-induced liver damage in rats. Research Journal of Pharmacy and Technology, 11(8), 3380–3384.
36. Dalan, R., Earnest, A., & Leow, M. K. (2013). Ethnic variation in the correlation between fasting glucose concentration and glycated hemoglobin (HbA1c). *Endocrine Practice, 19*(5), 812–817.
37. Vodošek Hojs, N., Bevc, S., Ekart, R., & Hojs, R. (2020). Oxidative stress markers in chronic kidney disease with emphasis on diabetic nephropathy. *Antioxidants, 9*(10), Article 925.
38. Chou, S. T., & Tseng, S. T. (2017). Oxidative stress markers in type 2 diabetes patients with diabetic nephropathy. *Clinical and Experimental Nephrology, 21*(2), 283–292.
39. Aloud, A. A., Veeramani, C., Govindasamy, C., Alsaif, M. A., El Newehy, A. S., et al. (2017). Galangin, a dietary flavonoid, improves antioxidant status and reduces hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. *Redox Report, 22*(6), 290–300.
40. Rhodus, N. L., Cheng, B., Myers, S., Bowles, W., Ho, V. U., et al. (2005). A comparison of the pro-inflammatory, NF-κB-dependent cytokines: TNF-α, IL-1α, IL-6, and IL-8 in different oral fluids from oral lichen planus patients. *Clinical Immunology, 114*(3), 278–283.
41. Xie, H., Zhang, G., Xia, Y., Pan, J., Shen, H., et al. (2026). Establishment of reference intervals for serum TNF-α, IFN-α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17 and IFN-γ in healthy Chinese adults using flow cytometry. *Journal of Immunological Methods, 114034.*
42. Nandanwadkar, S. M., Hurkadale, P. J., Bidikar, C. M., & Godbole, M. M. (2021). Multielemental analysis and in vitro evaluation of free radical scavenging activity of natural phytopigments by ICP-OES and HPTLC. *Frontiers in Pharmacology, 12*, Article 620996.
43. Samsu, N. (2021). Diabetic nephropathy: Challenges in pathogenesis, diagnosis, and treatment. *BioMed Research International, 2021,* Article 1497449.
44. Ma, Y., Wang, X., Lin, S., King, L., & Liu, L. (2025). The potential role of advanced glycation end products in the development of kidney disease. *Nutrients, 17*(5), Article 758.
45. Gwozdzinski, K., Pieniazek, A., & Gwozdzinski, L. (2021). Reactive oxygen species and their involvement in red blood cell damage in chronic kidney disease. *Oxidative Medicine and Cellular Longevity, 2021*, Article 6639199.
46. Tejchman, K., Kotfis, K., & Sieńko, J. (2021). Biomarkers and mechanisms of oxidative stress—Last 20 years of research with an emphasis on kidney damage and renal transplantation. *International Journal of Molecular Sciences, 22*(15), Article 8010.
47. Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *Bio Factors, 48*(3), 611–633.
48. Zamanian, M. Y., Alsaab, H. O., Golmohammadi, M., Yumashev, A., Jabba, A. M., et al. (2024). NF-κB pathway as a molecular target for curcumin in diabetes mellitus treatment: Focusing on oxidative stress and inflammation. Cell Biochemistry and Function, 42(4), e4030.
49. Su, H., Wan, C., Song, A., Qiu, Y., Xiong, W., et al. (2019). Oxidative stress and renal fibrosis: Mechanisms and therapies. In Renal Fibrosis: Mechanisms and Therapies (pp. 585–604). Springer.
50. Fakhruddin, S., Alanazi, W., & Jackson, K. E. (2017). Diabetes-induced reactive oxygen species: Mechanism of their generation and role in renal injury. *Journal of Diabetes Research, 2017,* Article 8379327.
51. Alaofi, A. L. (2020). Sinapic acid ameliorates the progression of streptozotocin (STZ)-induced diabetic nephropathy in rats via NRF2/HO-1 mediated pathways. *Frontiers in Pharmacology, 11*, Article 1119.