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Protective Effects of Methanolic Extracts of *Calotropis gigantea* Leaves, Stem Bark and Roots against Diabetes-Induced Hepatic and Renal Dysfunction in Streptozotocin-Induced Albino Rats

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Abstract

Calotropis gigantea is widely used in traditional medicine, but comparative evidence concerning the antidiabetic and organ-protective effects of its different anatomical parts remains limited. This study evaluated the phytochemical constituents and therapeutic effects of methanolic extracts derived from the leaves, stem bark, and roots of *C. gigantea* in streptozotocin (STZ)-induced diabetic albino rats. Plant materials were air-dried, pulverized, extracted through cold maceration in methanol for 72 hours, and concentrated using a rotary evaporator. Phytochemical constituents were characterized by gas chromatography–mass spectrometry (GC–MS). Thirty-six male albino rats were randomly allocated to six groups: normal control, diabetic control, metformin-treated (100 mg/kg), and leaf-, stem-bark-, and root-extract-treated groups (100 mg/kg each). Treatments were administered for 14 days. Fasting blood glucose, liver and kidney function indices, and lipid profiles were assessed, alongside histopathological examination of liver and kidney tissues. GC–MS identified several bioactive constituents, including 7-octen-2-ol, α-terpineol, and D-limonene. Diabetes induction

increased fasting blood glucose from 4.93 ± 0.25 to 16.14 ± 0.98 mmol/L, with concentrations remaining elevated in untreated diabetic rats. All extracts significantly reduced blood glucose, while the root extract produced the greatest reduction, from 15.37 ± 1.10 to 8.93 ± 1.94 mmol/L, comparable to the response observed with metformin. The root and leaf extracts produced the strongest improvements in renal function indices. The leaf extract yielded the most pronounced reductions in alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities and improved the lipid profile by reducing serum triglyceride and cholesterol concentrations while increasing high-density lipoprotein concentrations. Histopathological findings corroborated the biochemical results, demonstrating reduced hepatic and renal tissue damage in treated animals. These findings indicate that *C. gigantea* extracts, particularly those obtained from the roots and leaves, possess antidiabetic, hepatoprotective, nephroprotective, and lipid-modulating potential, supporting their further pharmacological evaluation for managing diabetes-associated complications.

Keywords: Antidiabetic Activity; *Calotropis gigantea*; GC–MS; Hepatorenal Protection; Streptozotocin-Induced Diabetes

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2014). It is one of the leading causes of morbidity and mortality worldwide and poses a significant public health challenge (World Health Organization, 2016). The prevalence of diabetes continues to increase globally due to population growth, urbanization, sedentary lifestyles, and dietary changes (International Diabetes Federation, 2019). Persistent hyperglycaemia is associated with disturbances in carbohydrate, lipid, and protein metabolism, leading to the development of both microvascular and macrovascular complications that affect vital organs, including the liver, kidneys, heart, and nervous system (Forouhi and Wareham, 2014).

Among the major complications of diabetes mellitus, hepatic and renal dysfunction are particularly important because of their contribution to disease progression and mortality (Chaudhury *et al.,* 2017). The liver plays a central role in glucose homeostasis through glycogenesis, glycogenolysis, and gluconeogenesis (Rines *et al.,* 2016). Under diabetic conditions, excessive production of reactive oxygen species (ROS) and chronic

oxidative stress disrupt normal hepatic metabolism, resulting in hepatocellular injury and altered liver function (Chen *et al.,* 2020). This damage is often reflected by elevated serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), which serve as important biomarkers of liver dysfunction (Bello *et al.,* 2022).

Similarly, the kidneys are highly susceptible to hyperglycaemia-induced damage (Thomas *et al.,* 2015). Diabetic nephropathy is one of the most common and serious complications of diabetes mellitus and remains a major cause of chronic kidney disease and end-stage renal failure worldwide (Alicic *et al.,* 2017). Persistent elevation of blood glucose promotes oxidative stress, inflammation, glomerular hypertrophy, and tubular degeneration, leading to progressive deterioration of renal function (Atta *et al.,* 2021). Increased serum concentrations of urea and creatinine, together with electrolyte imbalances, are commonly observed indicators of diabetes-induced renal impairment (Khan *et al.,* 2025).

The pathogenesis of diabetic liver and kidney injury is closely linked to oxidative stress and inflammatory responses (Giacco and Brownlee, 2010). Hyperglycaemia enhances the generation of free radicals while simultaneously reducing the efficiency of endogenous antioxidant defence systems (Brownlee, 2005). Excessive oxidative stress damages cellular proteins, lipids, and nucleic acids, ultimately disrupting normal tissue architecture and organ function (Sies, 2017). Consequently, therapeutic agents capable of reducing oxidative stress and preserving tissue integrity may play an important role in the prevention and management of diabetic complications (Newsholme *et al.,* 2016).

Although conventional antidiabetic drugs such as metformin and insulin are effective in controlling blood glucose levels, they may not completely prevent the progression of diabetic complications and are sometimes associated with adverse effects following prolonged use (Inzucchi *et al*., 2015). These limitations have stimulated increasing interest in medicinal plants as alternative or complementary therapeutic agents (Ekor, 2014). Medicinal plants contain diverse bioactive phytochemicals that possess antioxidant, anti-inflammatory, antihyperglycaemic, and organ-protective properties (Newman and Cragg, 2016). Consequently, natural products continue to serve as valuable sources for the discovery of novel therapeutic compounds (Atanasov *et al.,* 2015).

Calotropis gigantea (L.) Dryand., belonging to the family Apocynaceae, is a perennial medicinal shrub widely distributed in tropical and subtropical regions (Kumar *et al.,* 2013). The plant has been extensively used in traditional medicine for the treatment of various ailments, including inflammation, microbial infections, skin diseases, gastrointestinal disorders, and metabolic conditions (Murti *et al.,* 2010). Different parts of the plant, such as the leaves, stem bark, roots, flowers, and latex, have been reported to contain numerous biologically active constituents, including flavonoids, alkaloids, terpenoids, glycosides, saponins, tannins, and phenolic compounds (Singh and Rastogi, 2021; Jain *et al.,* 2022). These phytochemicals have been associated with a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, nephroprotective, and antidiabetic effects (Patil and Jain, 2019; Sharma *et al.,* 2020; Rajput *et al.,* 2020).

Previous studies have demonstrated the antihyperglycaemic potential of *Calotropis gigantea* (Gupta *et al.,* 2021); however, information regarding the comparative protective effects of its leaves, stem bark, and roots against diabetes-induced hepatic and renal dysfunction remains limited (Ram *et al.,* 2023). Furthermore, there is a need for comprehensive investigations that integrate biochemical assessments with histopathological evaluations to establish the extent of organ protection afforded by different plant parts (Devi *et al.,* 2020; Rao *et al.,* 2022).

Therefore, the present study was designed to evaluate the hepatoprotective and nephroprotective effects of methanolic extracts of Calotropis gigantea leaves, stem bark, and roots in streptozotocin-induced diabetic albino rats (Kumar *et al.,* 2021). Specifically, the study assessed changes in liver and kidney function biomarkers, electrolyte balance, and histopathological alterations following treatment with the extracts (Singh *et al.,* 2023). The findings of this study are expected to provide scientific evidence supporting the potential use of *Calotropis gigantea* as a natural therapeutic agent for the management of diabetes-associated hepatic and renal complications.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The fresh leaves, Stem bark and roots of *Calotropis gigantea* were collected from Wukari village in Wukari Local Government Area of Taraba State, Nigeria. The leaves,

Stem bark and roots were washed with water, air dried and then pulverized into fine powder using traditional mortar and pestle.

Preparation of Plant Extracts

The dried leaves, stem bark, and roots of Calotropis gigantea were first pulverized into fine powder using a laboratory mortar and pestle as well as an electric grinder. The resulting powdered materials were then stored in clean, properly labeled, dry sample containers and kept for subsequent analyses. Extraction was performed using a conventional solvent extraction technique as described by (Umaru *et al.*, 2019), with minor modifications. One kilogram (1 kg) of the powdered leaf sample was measured and placed in separate plastic containers, after which 2000 mL of methanol was added (1:4 w/v ratio). The mixture was left to macerate for 72 hours, with intermittent shaking to enhance extraction efficiency. After the soaking period, the mixture was first filtered using muslin cloth and subsequently passed through Whatman No. 1 filter paper.

The filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborota 4000 efficient) under reduced pressure at temperatures above 75 °C to obtain the crude methanol extract. The dry weight and percentage yield of the extract were then calculated. The concentrated extracts were stored in airtight containers and preserved under refrigeration until needed. Prior to administration, the extracts were reconstituted in distilled water.

Experimental Animals

Male albino rats with body weights ranging from 120 g to 200 g were selected for use in the study. The animals were kept in cages lined with sawdust bedding and maintained within the animal house facility of the Department of Biochemistry, Faculty of Biosciences, Federal University Wukari. All experimental procedures involving the laboratory rats were conducted in accordance with ethical guidelines and were approved by the Animal Ethical Committee of the Department of Biochemistry, Federal University Wukari. The animals were acclimatized and maintained under standard laboratory conditions, including a 12-hour light/12-hour dark cycle and a controlled room temperature of 25°C ± 3°C. They were provided with unrestricted access to standard laboratory feed and clean drinking water throughout the study period.

Acute Toxicity Study

Acute toxicity studies of the methanolic leaf, stem bark, and root extracts were conducted according to the Organisation for Economic Cooperation and Development (OECD) guideline 423. Experimental animals were observed for behavioural changes, signs of toxicity, morbidity, and mortality for fourteen days following oral administration of the extracts.

Induction of Experimental Diabetes

Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg body weight dissolved in freshly prepared cold citrate buffer (0.1 M, pH 4.5). Following induction, rats were provided with 5% glucose solution for 24 h to prevent sudden hypoglycaemia. After 72 h, fasting blood glucose levels were determined using a glucometer. Rats with fasting blood glucose levels above 11.1 mmol/L were considered diabetic and selected for the study.

Experimental Design

Thirty-six male albino rats were acclimatized for a period of two weeks before being randomly assigned into seven experimental groups. Group 1 served as the normal control and received only distilled water without diabetes induction, while Group 2 functioned as the diabetic control, in which diabetes was induced using streptozotocin (STZ) but no treatment was administered. Group 3 was treated with the standard antidiabetic drug metformin at a dose of 100 mg/kg body weight. Groups 4, 5, and 6 received methanolic extracts of Calotropis gigantea stem bark, root, and leaves respectively, each administered at 200 mg/kg body weight. Group 7 was given a combined treatment consisting of stem bark, root, and leaf extracts at a total dose of 200 mg/kg body weight. This grouping arrangement enabled the comparison of the individual effects of different plant parts, as well as their combined effect, against both the standard drug (metformin) and the untreated diabetic control.

GROUPS	TREATMENT
Group 1	Normal control (non-diabetic; distilled water only)
Group 2	Diabetic control (STZ only; no treatment)
Group 3	Diabetic + Metformin (100 mg/kg b.w.)
Group 4	Diabetic + C. gigantea stemback extract (100 mg/kg b.w.)
Group 5	Diabetic + C. gigantea leave extract (100 mg/kg b.w.)
Group 6	Diabetic + C. gigantea Root extract (100mg/kg b.w.)

Animal Sacrifice and Collection of Blood Samples

At the end of the treatment period, the rats were anaesthetized using chloroform vapour and subsequently sacrificed. Blood samples were collected through cardiac puncture into appropriate sample containers for different analyses. Whole blood was collected into EDTA bottles for haematological assessment. Blood samples collected in plain tubes were allowed to clot for 10 minutes and then centrifuged at 3000 rpm for 10 minutes to separate the serum from cellular components and fibrinogen. The serum was carefully extracted using a Pasteur pipette and stored for biochemical analysis. The liver and kidneys were also harvested and preserved in 10% formalin for histological examination.

Determination of Serum Biochemical Parameters

Serum biochemical parameters were determined spectrophotometrically using standard Randox diagnostic kits. The parameters assessed included liver function enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as total bilirubin (TB), direct bilirubin (DB), total protein (TP), and albumin (ALB). Renal function indices including creatinine and urea were also measured.

Histopathological Examination

Histopathological analysis was conducted following the procedures described by (*Choji et al.* 2015) and Avwioro 2011). Tissues were fixed in 10% formalin for three days and then trimmed into small sections measuring approximately 5 mm × 2 mm × 1 mm. The samples were processed and embedded in molten paraffin wax using embedding moulds and cassettes on a Tissue-Tek Embedding Centre, followed by rapid cooling.

The embedded tissues were sectioned using a rotary microtome at 4 micrometres thickness, mounted on slides, and prepared for staining. The sections were dewaxed in xylene, rehydrated through graded alcohol concentrations (100%, 80%, and 70%), and rinsed in water. Staining was carried out using Harris' haematoxylin for 5 minutes, followed by washing in running water. Differentiation was done using 1% acid alcohol, then rinsed and blued in Scott's tap water substitute for 5 minutes. Counterstaining was performed using 1% aqueous eosin for 2 minutes.

The stained sections were dehydrated through ascending grades of alcohol, cleared in xylene, and mounted in DPX mountant. Slides were dried in an oven at 40°C overnight and subsequently examined under a light microscope at magnifications of ×100 and ×400.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 27 was used for this analysis. Statistical analysis was carried out using one-way Analysis of Variance (ANOVA) followed by Duncan multiple comparison test. The results were expressed as Mean ± standard deviation (SD). (n = 5) with $p < 0.05$ being statistically significant.

RESULTS

Effect of Methanol Extract of Calotropis gigantea on Kidney Function Parameters

The effect of methanol extract of Calotropis gigantea on kidney function parameters in streptozotocin-induced diabetic Wistar rats is presented in Table 1.

Urea increased in diabetic control (24.54 ± 6.61 mg/dL) compared to normal (13.63 ± 7.06 mg/dL), indicating renal impairment. Creatinine increased from 1.27 ± 1.02 to 2.93 ± 0.91 mg/dL, confirming reduced filtration. Leaves extract reduced urea to 14.12 ± 0.96 and creatinine to 2.00 ± 0.11 mg/dL. Root extract showed near-normal urea (13.80 ± 0.60) and improved creatinine (2.05 ± 0.10). Sodium dropped from 147.49 ± 16.54 to 125.61 ± 5.15 (leaves), restoring electrolyte balance.

Table 1. Concentration of selected Kidney parameters in streptozotocin-induced diabetic Wistar rats administered methanol extracts of *Calotropis gigantea* parts (over 14 days treatment)

TREATMENT	UREA (mg/dL)	CREATININE (mg/dL)	SODIUM (mmol/L)	POTASSIUM (mmol/L)
Normal control	13.63 ± 7.06^{a}	1.27 ± 1.02^{a}	130.75 ± 8.70^{a}	96.37 ± 32.22^{b}
Diabetic control (STZ only no ntreatment)	24.54 ± 6.61^{b}	2.93 ± 0.91^{c}	147.49 ± 16.54^{b}	137.75 ± 36.25^{c}
Positive control (Diabetic + Metformin 100 mg/kg b.w.)	18.01 ± 1.05^{a}	1.96 ± 0.27^{b}	146.61 ± 8.06^{b}	85.71 ± 6.78^{a,b}
Diabetic + C. gigantea stem bark extract 100mg/kg b.w.	16.49 ± 1.49^{a}	2.21 ± 0.30^{b}	135.42 ± 8.09^{a}	77.74 ± 2.22^{a,b}
Diabetic + C. gigantea leaves extract 100mg/kg b.w.	14.12 ± 0.96^{a}	2.00 ± 0.11^{b}	125.61 ± 5.15^{a}	63.67 ± 6.28^{a}
Diabetic + C. gigantea root extract 100mg/kg b.w.	13.80 ± 0.60^{a}	2.05 ± 0.10^{b}	125.61 ± 2.37^{a}	59.97 ± 3.54^{a}

Results are expressed as mean ± standard deviation. Means on the same column with same superscript are not statistically significant, while means on the same column with different superscript are statistically significant ($p<0.05$).

Concentration of selected Liver indices parameters in streptozotocin-induced diabetic Wistar rats administered methanol extracts of Calotropis gigantea parts (ovter 14 days treatment)

ALT increased in diabetic control (68.58 ± 10.59) compared to normal (42.03 ± 12.88). Leaves extract reduced ALT to 49.77 ± 2.79, closest to normal. AST reduced from 84.93 ± 15.69 (diabetic) to 70.47 ± 5.45 (leaves). ALP reduced from 114.34 ± 3.15 to 76.86 ± 4.33. Total protein decreased in diabetic (4.72 ± 0.15) but restored partially in treated groups.

Table 2. Concentration of selected Liver indices parameters in streptozotocin-induced diabetic Wistar rats administered methanol extracts of Calotropis gigantea parts (over 14 days treatment)

TREATMENT	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dL)	ALBUMIN (g/dL)	GLOBULIN (g/dL)
Normal control	42.03 ± 12.88^{a}	56.06 ± 20.92^{a}	70.77 ± 27.18^{a}	5.44 ± 0.26^{c}	4.12 ± 0.05^{b}	1.39 ± 0.34^{c,d}
Diabetic control (STZ only no treatment)	68.58 ± 10.59^{d}	84.93 ± 15.69^{c}	114.34 ± 3.15^{b}	4.72 ± 0.15^{b}	3.98 ± 0.37^{b}	0.80 ± 0.41^{a,b}
Positive control (Diabetic + Metformin 100 mg/kg b.w.)	61.45 ± 5.35^{c,d}	75.65 ± 10.00^{b,c}	84.81 ± 12.00^{a}	5.13 ± 0.38^{b,c}	3.44 ± 0.11^{a}	1.68 ± 0.49^{d}
Diabetic + C. gigantea stem bark extract 100 mg/kg b.w.	58.58 ± 5.94^{b,c}	80.40 ± 3.59^{b,c}	86.67 ± 6.86^{a}	4.72 ± 0.58^{b}	3.48 ± 0.42^{a}	1.13 ± 0.76^{b,c}
Diabetic + C. gigantea leaves extract 100 mg/kg b.w.	49.77 ± 2.79^{a,b}	70.47 ± 5.45^{a,b,c}	76.86 ± 4.33^{a}	3.61 ± 0.72^{a}	3.45 ± 0.12^{a}	0.49 ± 0.07^{a}
Diabetic + C. gigantea root extract 100 mg/kg b.w.	51.87 ± 3.74^{b,c}	69.14 ± 1.79^{a,b}	77.98 ± 1.19^{a}	3.98 ± 0.22^{a}	3.37 ± 0.13^{a}	0.61 ± 0.11^{a,b}

Note: Results are expressed as mean ± SD. Value with same superscript within column are statistically not significant while values with different superscripts within a column are statistically significant ($P<0.05$).

Legend: ALT = Alanin transaminase, AST = Aspertate transaminase, ALP = Alkaline phosphatase, TP = Total protein.

Histological analysis results of the Kidney of the animals after 14 days treatment

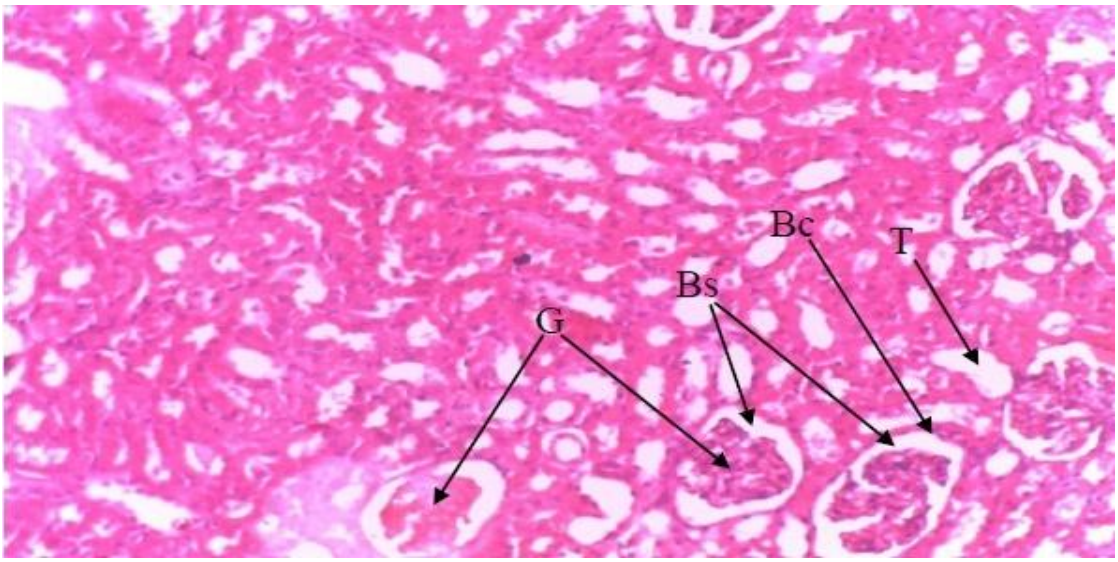

Figure 1. Photomicrograph of kidney section of normal control rat (group 1) showing normal glomerulus, Bowman's space, Bowman's capsule and normal tubules.

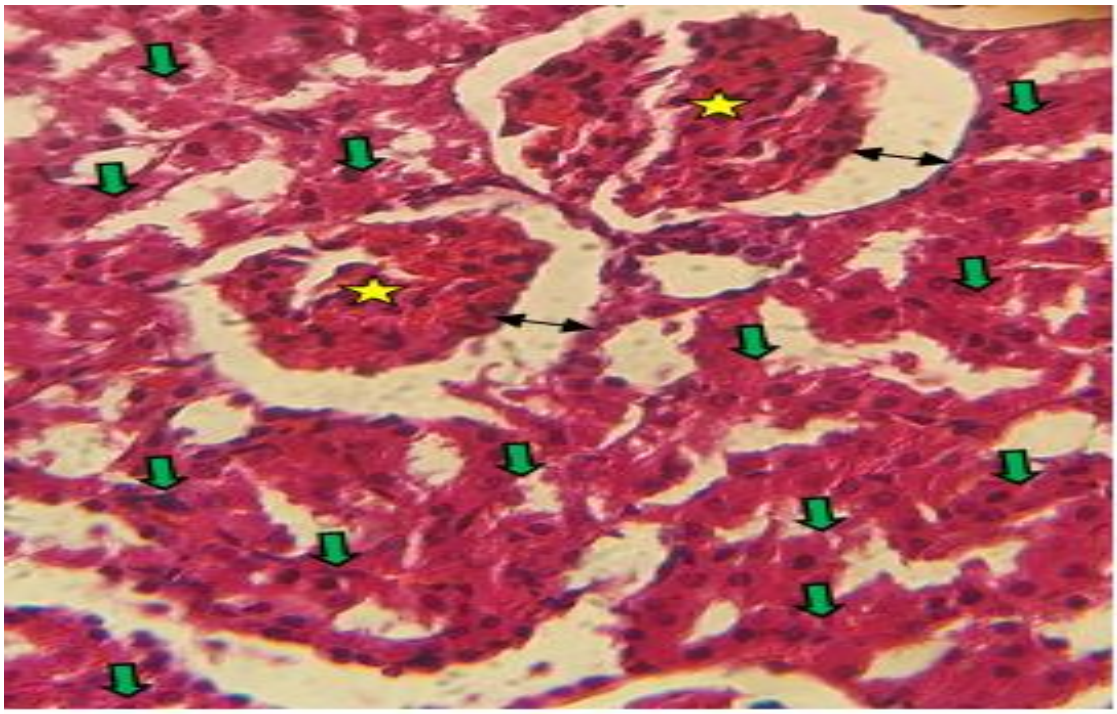

Figure 2. Photomicrograph of kidney section of group 2, showing congestion of the renal blood vessels; necrosis of the renal tubules (green arrows); necrosis and atrophy renal corpuscles (yellow stars) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400.

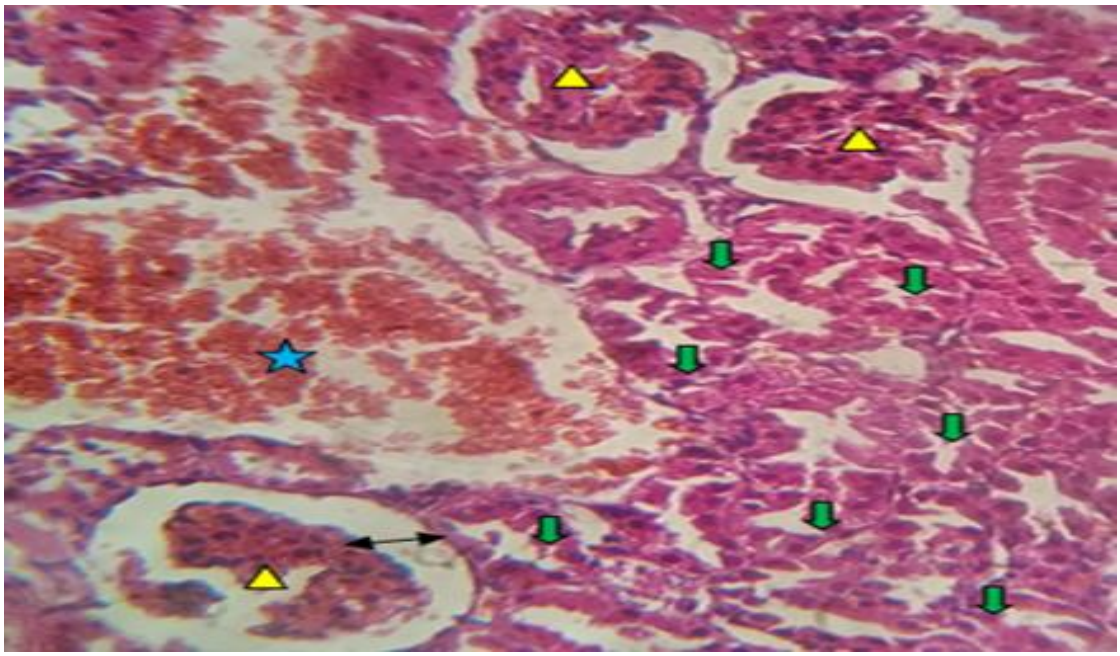

Figure 3. Photomicrograph of kidney section of group 3 showing congested renal blood vessels (blue stars); necrosis of the renal tubules (green arrows); necrosis and atrophy of the renal corpuscles (yellow triangles) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400

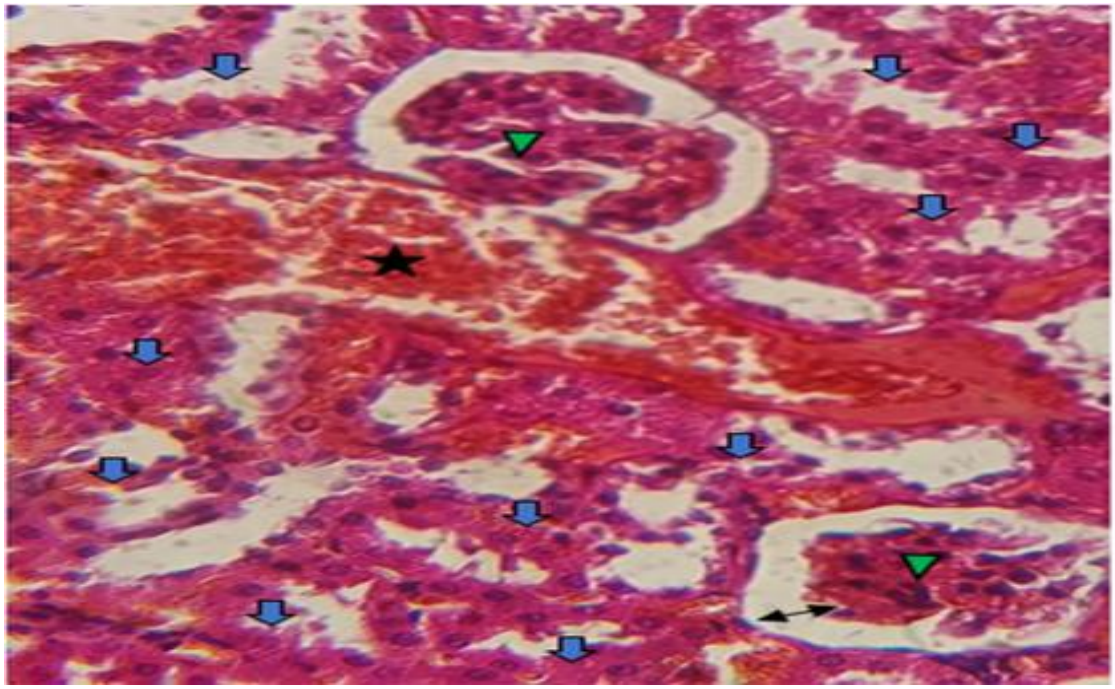

Figure 4. Photomicrograph of kidney section of group 4 showing congestion of the renal blood vessels (black stars); necrosis of the renal tubules (blue arrows); atrophy and

necrosis of the renal corpuscles (green triangles) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400

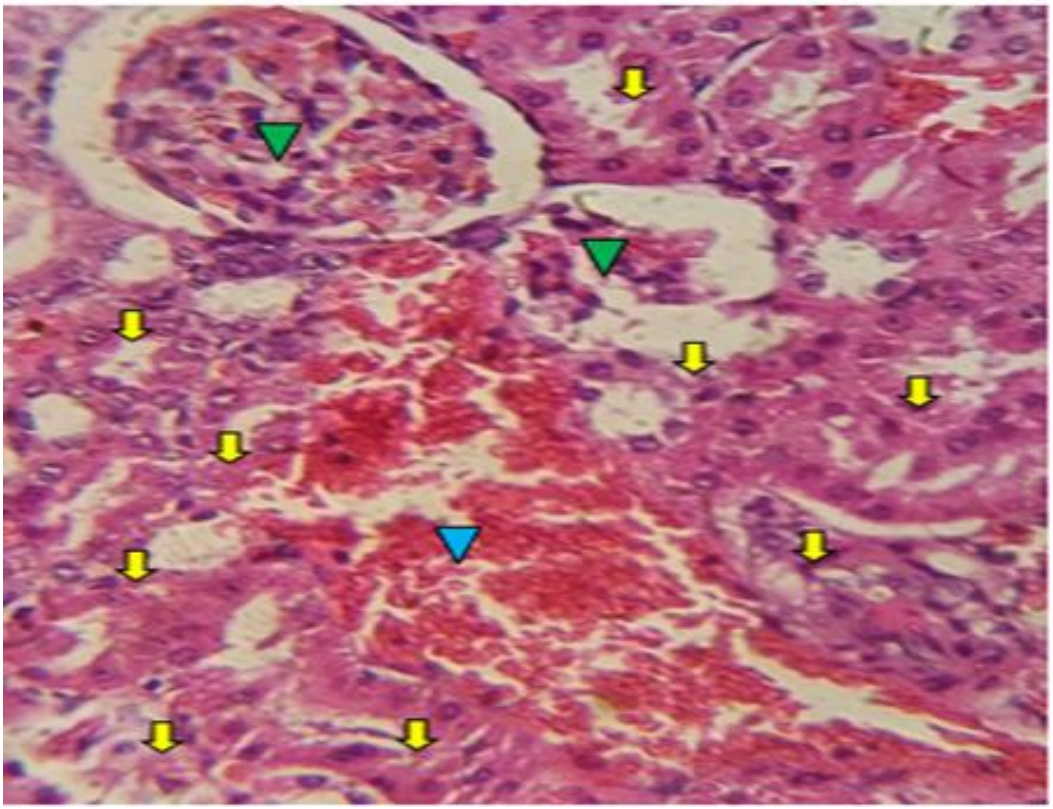

Figure 5. Photomicrograph of kidney section of group 5 showing congestion of the renal blood vessels (blue triangles); necrosis of the renal tubules (yellow arrows); renal corpuscles (green triangles). Hematoxylin and eosin stain. Magnification x400

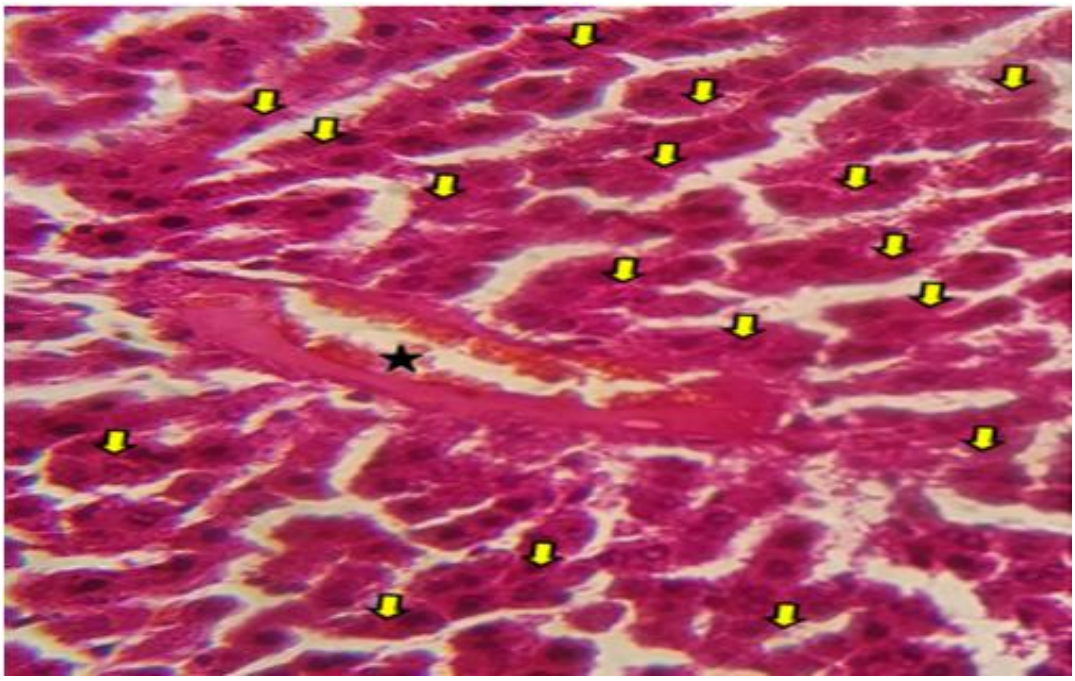

Figure 6. Photomicrograph of kidney section of group 6 showing congestion of the renal blood vessels (blue stars); necrosis of the renal tubules (yellow arrows) and renal corpuscles (black stars) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400

Histological analysis results of the Liver of the animals after 14 days treatment

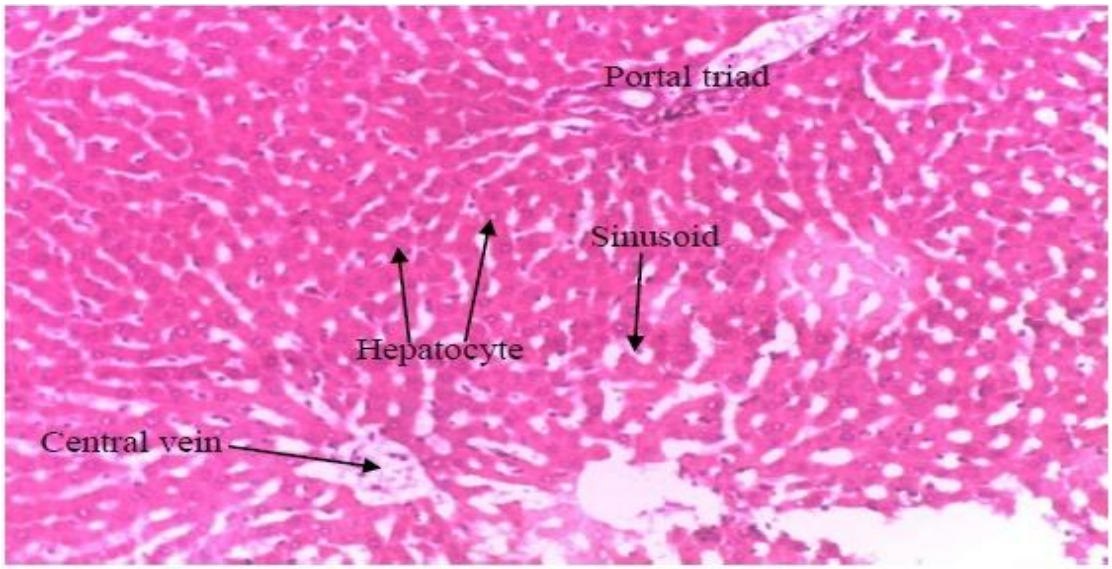

Figure 7. Photomicrograph of liver section of normal control rat (group 1) showing central vein, hepatocytes, sinusoid and portal triad.

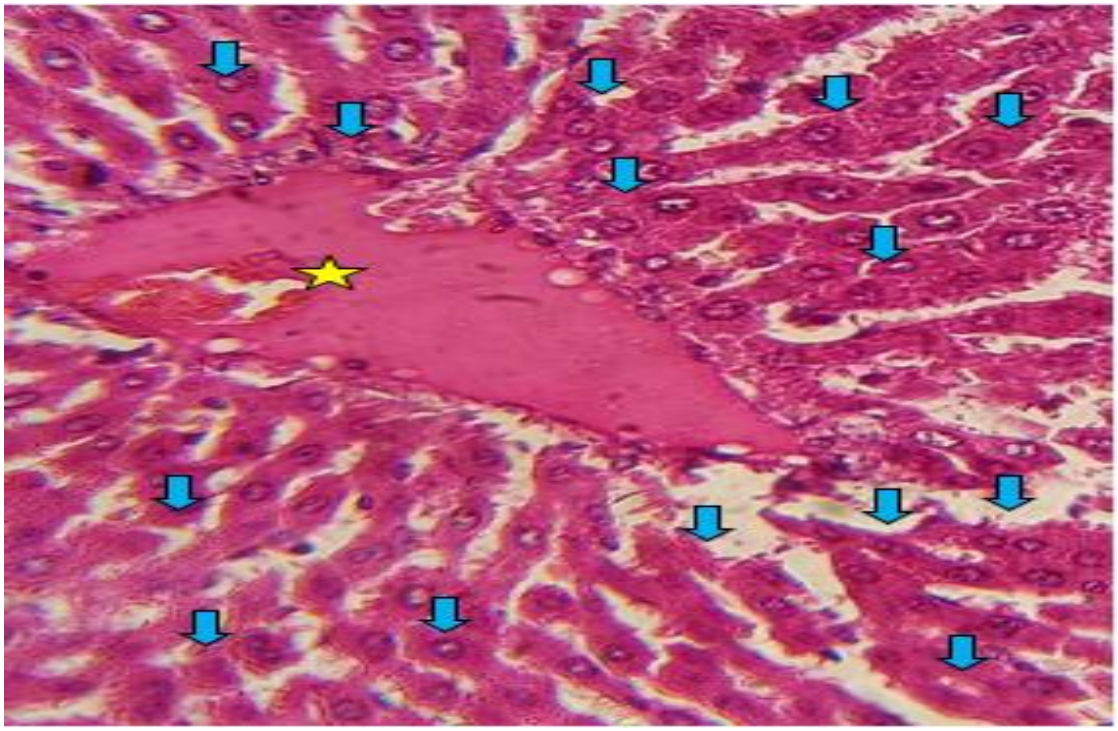

Figure 8. Photomicrograph of liver section of group 2 showing atrophy and necrosis of the hepatocytes (blue arrows); congestion of the central veins (yellow stars). Hematoxylin and eosin stain. Magnification x400.

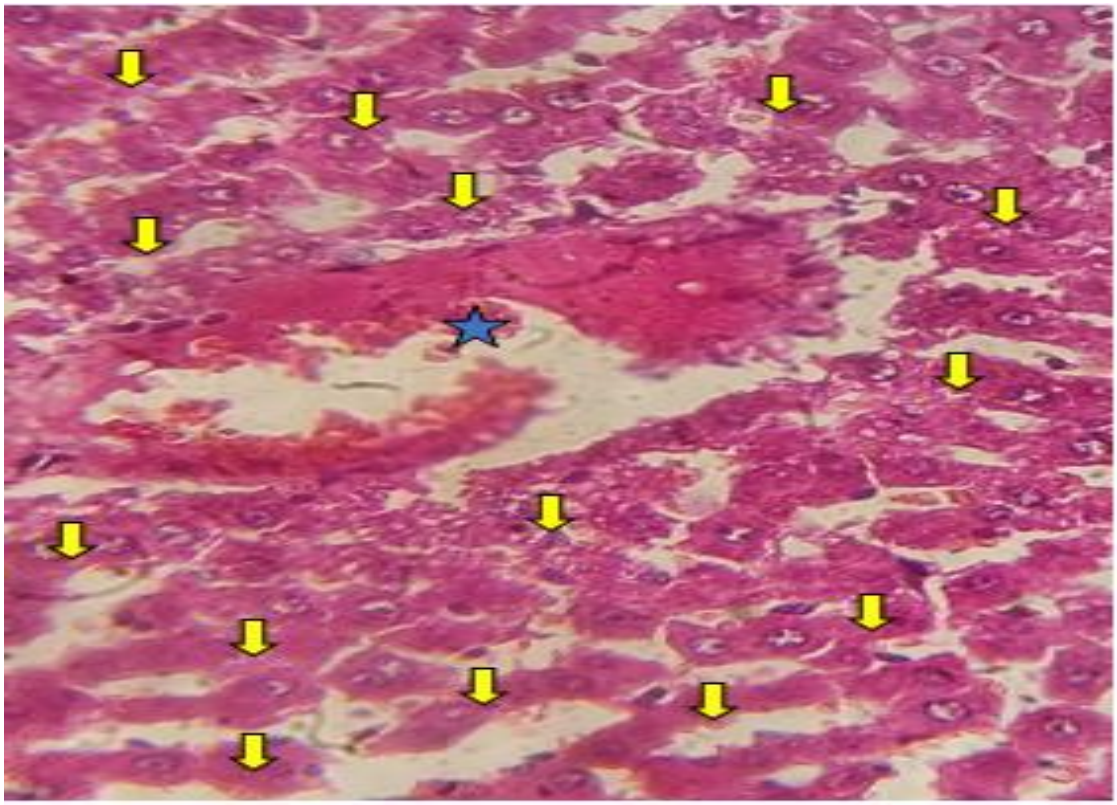

Figure 9. Photomicrograph of liver section of group 3 showing necrosis of the hepatocytes (yellow arrows); congestion of the central veins (blue stars). Hematoxylin and eosin stain. Magnification x400.

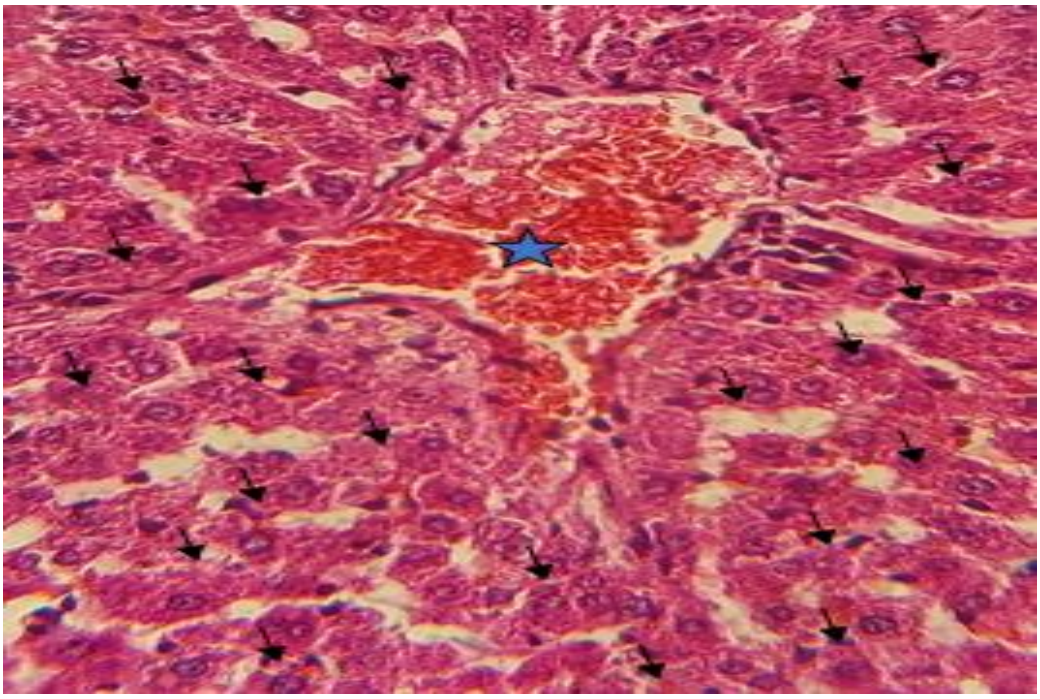

Figure 10. Photomicrograph of liver group 4 showing section of necrosis of the liver parenchyma i.e. the hepatocytes (black arrows); congested central vein (blue star) and portal vein (yellow star). Hematoxylin and eosin stain. Magnification x400

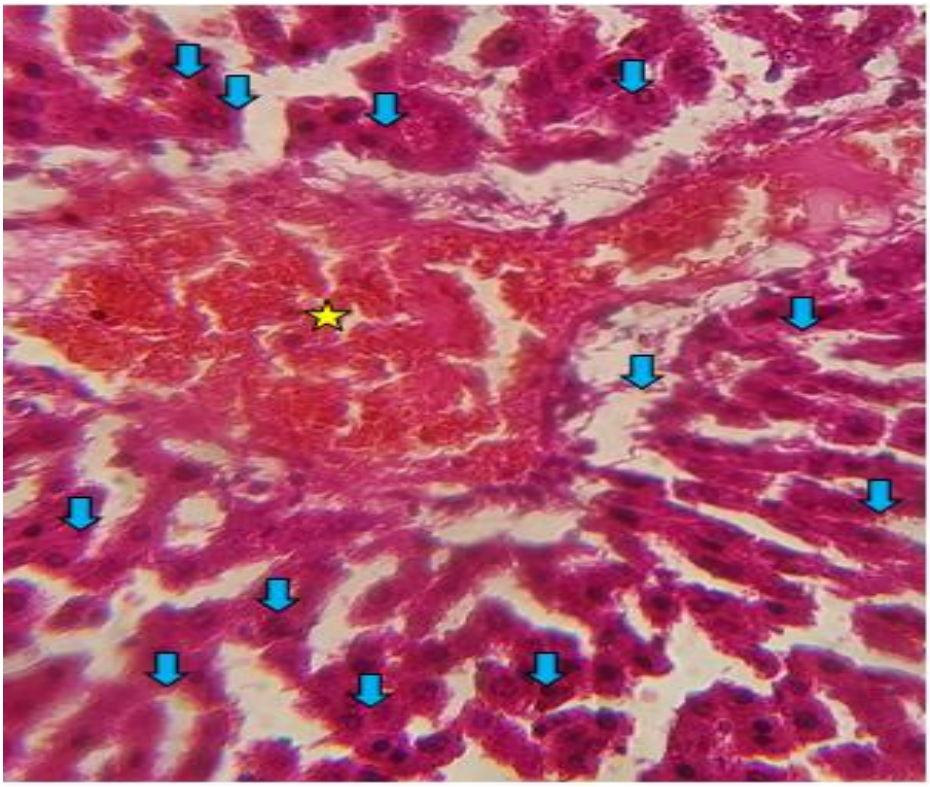

Figure 11. Photomicrograph of liver section of group 5 showing necrosis and atrophy of the hepatocytes (blue arrows); congestion of the central veins (yellow stars). Hematoxylin and eosin stain. Magnification x400.

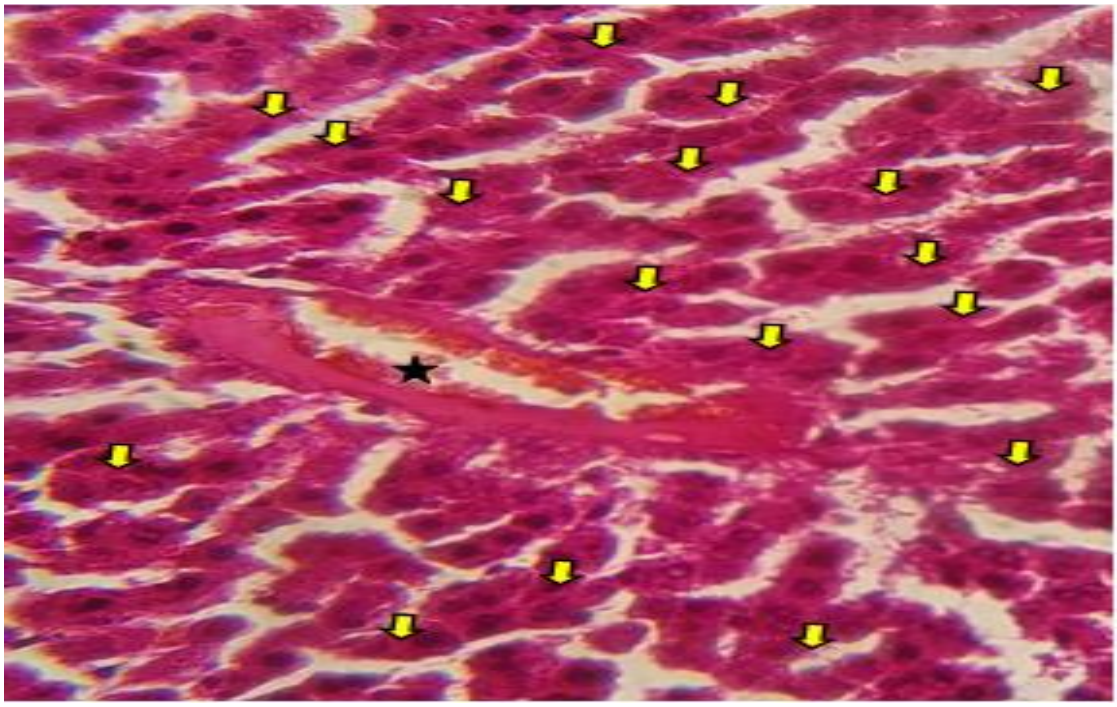

Figure 12. Photomicrograph of liver section of group 6 showing necrosis and atrophy of the hepatocytes (yellow arrows); congestion of the central vein (black star) and portal vein (blue star). Hematoxylin and eosin stain. Magnification x400.

DISCUSSION

The evaluation of liver function parameters revealed significant alterations in the diabetic control group, characterized by elevated levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, indicating hepatocellular injury and compromised liver function. These elevations are indicative of increased membrane permeability and leakage of intracellular enzymes into the bloodstream, a hallmark of hepatic damage associated with diabetes-induced oxidative stress. The observed hepatotoxicity is consistent with reports that hyperglycemia promotes lipid peroxidation, mitochondrial dysfunction, and inflammatory responses in hepatic tissues (Chen *et al.,* 2020 and Bello *et al.,* 2022).

Treatment with methanol extracts of *C. gigantea* resulted in significant reductions in liver enzyme lev els, indicating restoration of hepatocellular integrity and improved liver function. The leaf extract, in particular, demonstrated the most pronounced hepatoprotective effect, with enzyme levels approaching those of the normal control group. This suggests that the bioactive compounds present in the extract effectively

stabilize hepatocyte membranes and reduce oxidative damage. Recent studies have shown that phytochemicals from *C. gigantea* activate antioxidant defense systems, including superoxide dismutase and catalase, thereby mitigating oxidative stress and preventing cellular damage (Alafnan *et al.,* 2021).

In addition to enzyme normalization, improvements in total protein and albumin levels were observed in the treated groups, indicating enhanced synthetic function of the liver. This is significant because hepatic protein synthesis is often impaired in diabetic conditions due to metabolic dysregulation. The restoration of these parameters suggests that the extract not only protects hepatocytes from damage but also supports functional recovery. Mechanistically, these effects may be attributed to the activation of the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway and inhibition of nuclear factor kappa B (NF-ϰB) signaling, which are key regulators of oxidative stress and inflammation (Singh *et al.,* 2023). Overall, the liver function results demonstrate that *C. gigantea* exerts potent hepatoprotective effects, likely mediated through antioxidant and anti-inflammatory mechanisms that preserve cellular integrity and restore metabolic function.

The kidney function results revealed significant impairment in the diabetic control group, as evidenced by elevated levels of urea and creatinine, along with disturbances in electrolyte balance. These findings are indicative of reduced glomerular filtration rate and impaired tubular function, which are characteristic features of diabetic nephropathy. The accumulation of urea and creatinine in the bloodstream reflects the inability of the kidneys to effectively filter metabolic waste products, while electrolyte imbalance suggests disruption of tubular reabsorption processes. These observations are consistent with reports that chronic hyperglycemia induces oxidative stress, inflammation, and structural damage in renal tissues, leading to progressive loss of kidney function (Atta *et al.,* 2021 and Khan *et al.,* 2025).

Treatment with *C. gigantea* extracts resulted in significant reductions in urea and creatinine levels, indicating improved renal function and enhanced clearance of metabolic waste products. The root extract demonstrated the most pronounced nephroprotective effect, with values approaching those of the normal control group. This suggests that the extract effectively protects renal tissues from oxidative damage and supports functional recovery. The normalization of electrolyte levels further indicates restoration of tubular function and improved regulation of ion balance.

The nephroprotective effects observed in this study are likely mediated through multiple mechanisms, including antioxidant activity, which reduces oxidative stress and prevents lipid peroxidation in renal tissues, and anti-inflammatory effects that limit tissue injury and fibrosis. Additionally, phytochemicals in *C. gigantea* may enhance renal blood flow and improve glomerular filtration efficiency. Recent studies have reported that plant-derived antioxidants can attenuate renal damage by modulating signaling pathways such as transforming growth factor-beta (TGF-β) and reducing the expression of pro-inflammatory cytokines (Dwivedi *et al.,* 2024). These findings highlight the potential of *C. gigantea* as a nephroprotective agent capable of mitigating diabetes-induced renal dysfunction through both structural and functional restoration.

The histological evaluation of renal tissues across the experimental groups provides compelling morphological evidence that strongly corroborates the biochemical findings reported earlier. In the normal control group, the kidney sections exhibited well-preserved architecture characterized by intact glomeruli, clearly defined Bowman's capsules, normal Bowman's spaces, and structurally intact renal tubules. This structural integrity is consistent with the observed biochemical values of urea and creatinine within normal physiological limits, indicating efficient glomerular filtration and tubular reabsorption. Such normal renal histoarchitecture has been widely reported as a hallmark of healthy renal physiology in experimental models (Sharma *et al.,* 2023).

In contrast, the diabetic untreated group displayed marked histopathological alterations, including vascular congestion, extensive tubular necrosis, atrophy of renal corpuscles, widening of Bowman's space, and inflammatory cell infiltration. These structural abnormalities reflect severe renal injury induced by hyperglycemia and oxidative stress. Tubular necrosis suggests impaired reabsorptive capacity, while glomerular atrophy and widened Bowman's space indicate compromised filtration efficiency. These findings are in agreement with reports that diabetic nephropathy is characterized by glomerular degeneration, tubular damage, and inflammatory infiltration driven by oxidative stress and metabolic dysregulation (Alafnan *et al.,* 2021).

The elevated levels of urea and creatinine observed in this group are therefore direct consequences of structural damage to the nephron, particularly the glomerulus and renal tubules. Furthermore, electrolyte imbalance observed in the diabetic group reflects tubular dysfunction, as impaired tubular reabsorption disrupts sodium and potassium

homeostasis. This relationship between structural renal damage and biochemical derangement has been consistently reported in recent studies on diabetic renal injury (Khan *et al.,* 2025).

The metformin-treated group demonstrated partial histological recovery, although features such as mild vascular congestion and residual tubular necrosis persisted. The corresponding reduction in urea and creatinine levels suggests that functional recovery may precede complete histological restoration. This observation aligns with previous findings that pharmacological interventions often restore renal biochemical parameters before full structural regeneration is achieved, due to early improvement in filtration efficiency and reduced oxidative stress (Sharma *et al.,* 2023).

The stem bark extract-treated group showed moderate improvement in renal histology, although residual vascular congestion and tubular degeneration were still evident. This partial recovery corresponds with the moderate reduction in urea and creatinine levels, indicating that the extract confers some degree of nephroprotection but does not completely reverse renal injury within the experimental duration. Similar partial protective effects have been reported in studies where plant extracts reduce oxidative damage but require longer duration for full tissue regeneration (Dwivedi *et al.,* 2024).

More pronounced improvements were observed in the leaf extract-treated group, where histological examination revealed reduced necrosis, relatively preserved glomerular structures, and minimal vascular congestion. These structural improvements align closely with near-normal biochemical parameters, indicating effective restoration of renal function. This supports previous findings that *C. gigantea* exhibits strong antioxidant and anti-inflammatory activities capable of protecting renal tissues from oxidative damage (Alafnan *et al.,* 2021).

The root extract-treated group exhibited mild residual necrosis but showed considerable restoration of glomerular architecture and reduced vascular congestion. Notably, the biochemical parameters in this group approached normal values, suggesting effective functional recovery. This observation may be explained by the presence of bioactive phytochemicals that enhance cellular repair and improve renal function despite minor structural irregularities. Recent reviews have emphasized that *C. gigantea* contains bioactive compounds capable of promoting tissue regeneration and mitigating oxidative stress (Nexres Review, 2026).

Overall, the renal histopathological findings demonstrate a clear relationship between structural integrity and functional performance, where severe tissue damage corresponds to elevated biochemical markers, and progressive tissue restoration is associated with normalization of renal function parameters. The histological examination of liver tissues further substantiates the biochemical findings related to hepatic function. In the normal control group, the liver exhibited normal histoarchitecture characterized by well-arranged hepatocytes, intact central veins, and organized sinusoidal spaces. These structural features are consistent with normal levels of liver enzymes, indicating intact hepatocellular integrity and proper metabolic function.

In contrast, the diabetic untreated group showed significant histopathological alterations, including hepatocyte necrosis, cellular atrophy, and congestion of the central vein. These changes reflect severe hepatic injury induced by oxidative stress and metabolic disturbances associated with diabetes. Hepatocyte necrosis leads to the leakage of intracellular enzymes into circulation, thereby explaining the elevated levels of ALT, AST, and ALP observed in this group. Similar histological features of hepatocellular degeneration and vascular congestion have been reported in experimental models of liver injury (Sawong *et al.,* 2022).

The metformin-treated group demonstrated partial histological recovery, although mild necrosis and vascular congestion persisted. The corresponding reduction in liver enzyme levels indicates that metformin exerts hepatoprotective effects by improving cellular integrity and reducing oxidative stress. This finding is consistent with studies showing that antioxidant-mediated protection plays a central role in restoring liver function (Sharma *et al.,* 2023).

The stem bark extract-treated group exhibited moderate improvement, with residual necrosis and vascular congestion still evident. The partial reduction in liver enzyme levels reflects moderate hepatoprotective activity, suggesting that the extract mitigates but does not completely reverse hepatic damage within the study period. Similar findings have been reported in phytochemical studies where partial restoration of liver structure accompanies biochemical improvement (Dwivedi *et al.,* 2024).

The leaf extract-treated group showed substantial histological improvement, with reduced hepatocyte necrosis, mild cellular atrophy, and decreased vascular congestion. These structural changes correspond closely with the reduction in liver enzyme levels,

indicating strong hepatoprotective activity. The ability of *C. gigantea* to stabilize hepatocyte membranes and reduce enzyme leakage has been attributed to its antioxidant and anti-inflammatory phytochemicals (Alafnan *et al.,* 2021).

Similarly, the root extract-treated group demonstrated significant improvement, with only mild residual necrosis and moderate vascular congestion observed. The corresponding biochemical recovery suggests effective restoration of hepatic function, although complete structural normalization may require prolonged treatment. Recent studies have highlighted that bioactive compounds in *C. gigantea* enhance antioxidant enzyme activity and reduce lipid peroxidation, thereby protecting hepatic tissues from damage (Khan *et al.,* 2025).

Overall, the liver histopathological findings demonstrate a clear association between structural damage and enzyme leakage, where hepatocyte necrosis leads to elevated liver enzymes, and tissue recovery results in their reduction..

The combined histological and biochemical evidence from kidney and liver analyses provides a comprehensive understanding of the therapeutic effects of *C. gigantea*. The study demonstrates a strong correlation between tissue structure and organ function, where structural damage induced by diabetes results in significant biochemical alterations, and treatment with plant extracts promotes both morphological restoration and functional recovery. These protective effects are likely mediated through multiple mechanisms, including antioxidant activity, which reduces oxidative stress and prevents cellular damage, anti-inflammatory effects that limit tissue injury and membrane stabilization that prevents leakage of intracellular enzymes. These mechanisms are well supported by recent studies highlighting the pharmacological potential of *C. gigantea* in mitigating oxidative stress-related diseases (Sharma *et al.,* 2023 and Nexres Review, 2026).

Furthermore, the differential efficacy observed among plant parts, with the root extract showing superior nephroprotective and antidiabetic effects and the leaf extract demonstrating strong hepatoprotective activity, highlights the importance of phytochemical variability in determining therapeutic outcomes. This observation aligns with contemporary research emphasizing the role of plant-part-specific phytochemical distribution in pharmacological activity (Dwivedi *et al.,* 2024).

The histopathological findings provide definitive morphological evidence supporting the biochemical data, confirming that *C. gigantea* exerts significant multi-organ protective effects through structural preservation and functional restoration.

CONCLUSION

The findings of this study demonstrate that *Calotropis gigantea* possesses significant antidiabetic and multi-organ protective properties. The plant extracts effectively reduced hyperglycemia, improved renal and hepatic function, corrected lipid abnormalities, and enhanced hematological parameters in streptozotocin-induced diabetic rats. Histopathological evidence confirmed that these biochemical improvements were accompanied by structural restoration of kidney and liver tissues, indicating true tissue repair rather than mere symptomatic relief. The observed therapeutic effects are likely mediated through antioxidant, anti-inflammatory, and metabolic regulatory mechanisms associated with the plant's phytochemical constituents. Among the plant parts studied, the root extract exhibited superior efficacy in glycemic control and renal protection, while the leaf extract showed greater hepatoprotective potential. These findings underscore the importance of plant-part-specific bioactivity and suggest targeted therapeutic applications.

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