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GC–MS Phytochemical Characterization and Comparative Antidiabetic Activities of Methanolic Extracts of *Calotropis gigantea* Leaves, Stem Bark, and Roots in Streptozotocin-Induced Diabetic Rats

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Abstract

Calotropis gigantea is widely used in traditional medicine for treating various health conditions. This study evaluated the phytochemical composition and comparative therapeutic effects of methanolic extracts from its leaves, stem bark, and roots in streptozotocin (STZ)-induced diabetic albino rats. Plant materials were air-dried, pulverized, and extracted through cold maceration in methanol for 72 hours, after which the extracts were concentrated using a rotary evaporator and characterized by gas chromatography–mass spectrometry (GC–MS). Thirty-six male albino rats were randomly assigned 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 compounds, including the oxygenated monoterpenes 7-octen-2-ol, α-terpineol, and D-limonene, indicating potential antioxidant relevance. Diabetes induction increased fasting blood glucose from 4.93 ± 0.25 to 16.14

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± 0.98 mmol/L, and concentrations remained elevated in untreated diabetic rats. All extracts significantly reduced blood glucose, with the root extract producing 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 demonstrated the strongest improvements in renal function indices, whereas the leaf extract produced the most pronounced reductions in alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities. The leaf extract also reduced serum triglyceride and cholesterol concentrations while increasing high-density lipoprotein levels. Histopathological findings corroborated the biochemical results by demonstrating reduced liver and kidney tissue damage in treated animals. These findings indicate that *C. gigantea*, particularly its root and leaf extracts, possesses antidiabetic, hepatoprotective, nephroprotective, and lipid-modulating potential, supporting its further investigation as a candidate for managing diabetes mellitus and its associated complications.

Keywords: Antidiabetic Activity; *Calotropis gigantea*; Diabetes Mellitus; GC–MS; Phytochemical Profiling

INTRODUCTION

Diabetes mellitus is one of the most prevalent metabolic disorders worldwide and represents a major public health challenge (American Diabetes Association, 2014). The disease is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (World Health Organization, 2016). Persistent elevation of blood glucose levels leads to disturbances in carbohydrate, lipid, and protein metabolism, ultimately causing serious complications affecting vital organs such as the liver, kidneys, heart, eyes, and nervous system (Forouhi and Wareham, 2014). According to recent global estimates, the incidence of diabetes continues to rise, particularly in developing countries where access to healthcare resources and effective treatment options remains limited (International Diabetes Federation, 2019). Consequently, the search for affordable, safe, and effective therapeutic agents for diabetes management has become increasingly important (Chaudhury *et al.,* 2017).

Streptozotocin (STZ)-induced diabetes remains one of the most widely used experimental models for investigating the pathophysiology of diabetes mellitus and evaluating potential antidiabetic agents (Szkudelski, 2001). Streptozotocin selectively targets

pancreatic β-cells through glucose transporter-2 (GLUT-2)-mediated uptake, resulting in oxidative stress, DNA damage, and destruction of insulin-producing cells (Lenzen, 2008). The consequent reduction in insulin secretion produces persistent hyperglycemia and metabolic disturbances similar to those observed in human diabetes mellitus (Goyal *et al.,* 2016). This experimental model therefore provides a valuable platform for assessing the efficacy of medicinal plants and novel therapeutic compounds (Islam and Choi, 2009).

Despite the availability of several conventional antidiabetic drugs, including insulin, metformin, sulfonylureas, and thiazolidinediones, their long-term use is often associated with adverse effects such as hypoglycemia, gastrointestinal disturbances, weight gain, hepatic dysfunction, and cardiovascular complications (Inzucchi *et al.,* 2015). Furthermore, the high cost of treatment and limited accessibility of medications in many low-income regions have encouraged the exploration of medicinal plants as alternative or complementary therapeutic agents (Ekor, 2014). Plant-derived compounds have attracted considerable scientific attention due to their broad pharmacological activities, natural abundance, and relatively low toxicity (Newman and Cragg, 2016).

Among the medicinal plants utilized in traditional medicine, Calotropis gigantea (L.) Dryand has gained significant interest because of its diverse therapeutic properties (Kumar *et al.,* 2013). The plant belongs to the family Apocynaceae and is widely distributed across tropical and subtropical regions (Kareem *et al.,* 2019). Various parts of the plant, including the leaves, roots, stem bark, flowers, and latex, have been traditionally employed in the treatment of inflammation, microbial infections, wounds, digestive disorders, respiratory diseases, and metabolic abnormalities (Murti *et al.,* 2010). Recent pharmacological investigations have demonstrated that *Calotropis gigantea* possesses antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, nephroprotective, anticancer, and antidiabetic activities (Patil and Jain, 2019; Sharma *et al.,* 2020).

The therapeutic potential of Calotropis gigantea has been largely attributed to its rich phytochemical composition (Singh and Rastogi, 2021). Previous studies have identified numerous secondary metabolites including flavonoids, alkaloids, terpenoids, cardiac glycosides, phenolic compounds, steroids, tannins, and saponins (Jain *et al.,* 2022). These phytoconstituents are known to exert biological effects through multiple mechanisms, including free radical scavenging, inhibition of inflammatory mediators, enhancement of insulin sensitivity, protection of pancreatic β-cells, and modulation of glucose metabolism

(Rajput *et al.,* 2020; Gupta *et al.,* 2021). However, the concentration and distribution of these bioactive compounds may vary among different plant parts, potentially influencing their pharmacological efficacy (Ali *et al.,* 2018).

Gas Chromatography-Mass Spectrometry (GC-MS) has emerged as one of the most powerful analytical techniques for the identification and characterization of volatile and semi-volatile phytochemicals present in plant extracts (Sarker *et al.,* 2020). The technique combines the separation efficiency of gas chromatography with the structural identification capabilities of mass spectrometry, allowing accurate detection of biologically active compounds (Srivastava *et al.,* 2019). GC-MS analysis therefore provides valuable insight into the chemical constituents responsible for the therapeutic properties of medicinal plants and facilitates the correlation between phytochemical composition and biological activity (Gomathi *et al.,* 2018).

Although several investigations have reported the medicinal properties of *Calotropis gigantea*, limited information is available regarding the comparative antidiabetic potential of different plant parts in relation to their phytochemical profiles (Ram *et al.,* 2023). Most previous studies have focused on a single plant organ, thereby limiting the understanding of variations in bioactive constituents and pharmacological effectiveness among different tissues of the plant (Devi *et al.,* 2020). Furthermore, comprehensive studies integrating GC-MS characterization with in vivo antidiabetic evaluation remain relatively scarce (Rao *et al.,* 2022).

Therefore, the present study was designed to investigate the phytochemical constituents of methanolic extracts obtained from the leaves, stem bark, and roots of *Calotropis gigantea* using GC-MS analysis and to comparatively evaluate their antidiabetic activities in streptozotocin-induced diabetic rats (Kumar *et al.,* 2021). The study aims to identify the most biologically active plant part and establish a scientific basis for the potential development of *Calotropis gigantea*-derived phytotherapeutic agents for diabetes management (Singh *et al.,* 2023).

Aim of the Study

The aim of this study is to investigate the GC-MS profile and comparative antidiabetic activities of methanolic extracts of *Calotropis gigantea* parts in streptozotocin-induced diabetic albino rats.

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 Methanol Extracts of *Calotropis gigantea*

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.

GC–MS Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) analysis was carried out on the methanolic leaf extract of Calotropis gigantea to identify its phytochemical constituents. The analysis was performed using a GC–MS system equipped with a capillary column. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250°C, while the oven temperature was programmed from 60°C to 280°C. Identification of compounds was achieved by comparing the mass spectra obtained with those in the National Institute of Standards and Technology (NIST) spectral database. Relative abundance of compounds was determined based on percentage peak areas.

Experimental Animals

Thirty-six healthy male Wistar albino rats weighing between 150 and 200 g were obtained from the Animal House Unit of the Department of Biochemistry, Federal University Wukari. The animals were housed in standard laboratory cages under controlled

environmental conditions (25 ± 2°C, 12 h light/dark cycle) and allowed free access to standard pellet feed and clean water. The animals were acclimatized for two weeks before commencement of the experiment.

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)

GROUPS	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.)

Determination of Fasting Blood Glucose

Fasting blood glucose determination will be carried out according to the method described by Yakubu et al. (2013). Blood samples will be collected via tail puncture from overnight-fasted rats. A drop of blood will be placed on an assay strip, and the fasting blood glucose levels will be measured using an Accu-Chek Active glucometer. This procedure will be performed weekly for

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. In addition, electrolyte levels such as sodium (Na^+) and potassium (K^+), as well as lipid profile parameters including high-density lipoprotein (HDL), total cholesterol, and triglycerides, were evaluated.

Determination of Haematological Indices

Haematological parameters were analysed using an automated haematology analyser (Agile S30 Haematology Autoanalyzer V2.0, China). Whole blood samples were used to determine red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), white blood cell (WBC) count, and platelet count.

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

GC-MS Phytochemical Profile of methanol extracts of *Calotropis gigantea* Leaves Extract

A total of 24 compounds, representing 100% of the total extract composition were identified from the GC-MS analysis of methanol extracts of *Calotropis gigantea* Leaves Extract showing various phytochemical activities. The phytochemical constituents with their retention time (RT), area (%), Molecular formula and Molecular weight are presented in table 1 while the chromatogram is presented in figure 1. The following are some of the phytoconstituents present in the GC-MS analysis Squalene, 4,7-methano-1H-inden-5-, 1-Propanol, 2-(2-hydroxypropoxy)-, D-Limonene.

Table 1: GC-MS Phytochemical Profile of methanol extract of *Calotropis gigantea* leaves

S/N	Compound Name	Retention Time (min)	Area %	Molecular Formula	Molecular Weight
1	α-Pinene	7.912	0.31	$C_{10}H_{16}$	136
2	β-Myrcene	9.542	0.02	$C_{10}H_{16}$	136
3	7Oxabicyclo(2.2.1)heptane, 1-methyl- 0.01 0.04	10.406	0.01	$C_{10}H_{18}O$	154
4	D-Limonene	10.987	16.85	$C_{10}H_{16}$	136
5	3-Acetoxy-p-menthan-1-ol	11.045	0.02	$C_{12}H_{22}O_3$	214
6	2-Propanol, 1,1'-oxybis-	11.658	0.13	$C_6H_{14}O_3$	134
7	γ-Terpinene	11.784	0.23	$C_{10}H_{16}$	136
8	2-Propanol, 1,1'-oxybis-	11.965	0.56	$C_6H_{14}O_3$	134
9	7-Octen-2-ol, 2,6-dimethyl-	12.590	39.97	$C_{10}H_{20}O$	156
10	(+)-2-Carene	12.662	0.39	$C_{10}H_{16}$	136
11	1-Propanol, 2-(2-hydroxypropoxy)-	13.050	2.53	$C_6H_{14}O_3$	134
12	1-Propanol, 2-(2-hydroxypropoxy)-	13.213	1.77	$C_6H_{14}O_3$	134
13	1-Propanol, 2-(2-hydroxypropoxy)-	13.894	0.50	$C_6H_{14}O_3$	134
14	3-Cyclohexen-1-ol, 1-methyl-4-(1-methyl	14.402	0.27	$C_{10}H_{18}O$	154
15	Cyclohexanol, 1-methyl-4-(1-methylheptan-	14.922	0.96	$C_{15}H_{30}O$	226
16	Bicyclo(3.1.0)hexan-3-ol, 4-methyl-1-	15.574	0.38	$C_{10}H_{18}O$	154
17	α-Terpineol	16.545	27.11	$C_{10}H_{18}O$	154
18	2,6-Octadienal, 3,7-dimethyl-, (Z)-	16.980	0.47	$C_{10}H_{16}O$	152
19	Citronellol	17.194	0.69	$C_{10}H_{20}O$	156
20	2,6-Octadienal, 3,7-dimethyl-, (Z)-	17.580	15.09	$C_{10}H_{16}O$	152
21	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	17.874	0.72	$C_{10}H_{18}O$	154
22	4,7-methano-1H-inden-5-ol, 3a,4,5,6,7,	22.463	0.78	$C_{12}H_{16}O_2$	192
23	Octanal, 2-(phenylmethylene)-	30.323	10.19	$C_{15}H_{20}O$	216
24	Squalene	20.092	0.89	$C_{30}H_{50}$	410

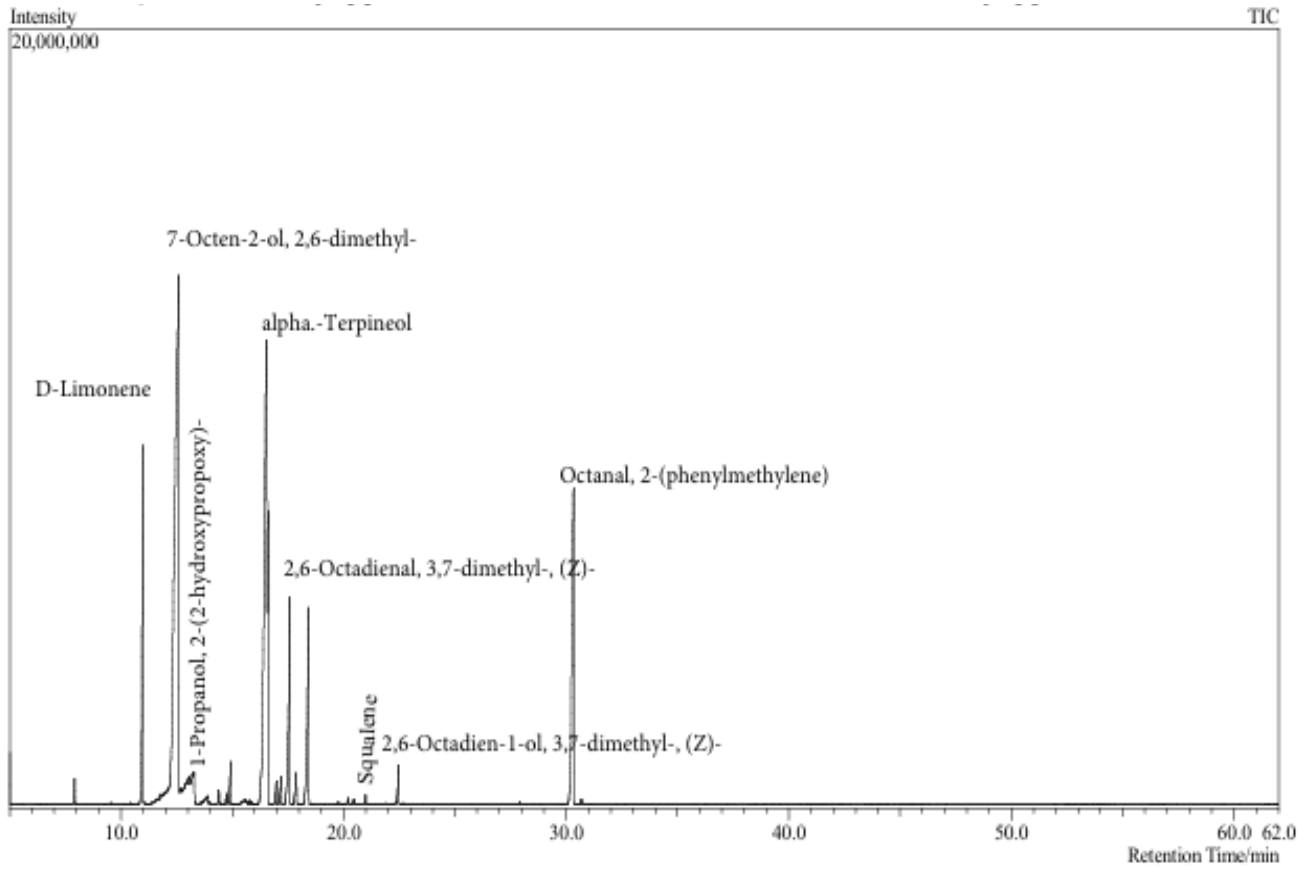

Figure 1: GC-MS Phytochemical Profile of methanol extracts of *Calotropis gigantea* Leaves Extract.

Fasting Blood glucose (FBG) of streptozotocin-induced diabetic Wistar rats administered methanol extracts of *Calotropis gigantea* parts (over 14 days treatment)

Normal control values remained stable from 4.91 ± 0.46 to 4.72 ± 0.64 mmol/L, indicating normal glucose homeostasis. Diabetic control increased sharply from 4.93 ± 0.25 to 16.14 ± 0.98 mmol/L after induction and remained elevated (17.00 ± 0.85 and 16.63 ± 0.05), confirming persistent hyperglycemia.

Metformin group reduced from 15.65 ± 1.24 to 7.72 ± 0.86 mmol/L by week 2, showing significant ($p<0.05$) recovery. Stem bark extract reduced glucose from 14.82 ± 1.93 to 11.73 ± 1.19 mmol/L, showing moderate effect. Leaves extract reduced from 15.92 ± 0.99 to 11.68 ± 1.59 mmol/L, similar to stem bark. Root extract reduced from 15.37 ± 1.10 to 8.93 ± 1.94 mmol/L.

Table 2: Fasting blood glucose level concentration (mg/dl) of daibetilc Wistar rats administered methanol extracts of *Calotropis gigantea* parts (over 14 days treatment).

TREATMENT	BEFORE INDUCTION (mmol/L)	AFTER INDUCTION (mmol/L)	WEEK 1 TREATMENT (mmol/L)	WEEK 2 TREATMENT (mmol/L)
Normal control	4.91 ± 0.46^{b}	5.12 ± 0.57^{a}	4.81 ± 0.40^{a}	4.72 ± 0.64^{a}
Diabetic control (STZ only no ntreatment)	4.93 ± 0.25^{b}	16.14 ± 0.98^{b}	17.00 ± 0.85^{d}	16.63 ± 0.05^{d}
Positive control (Diabetic + Metformin 100 mg/kg b.w.)	4.31 ± 0.29^{a}	15.65 ± 1.24^{b}	7.52 ± 0.84^{b}	7.72 ± 0.86^{b}
Diabetic + C. gigantea stem bark extract 100 mg/kg b.w.	4.75 ± 0.28^{a,b}	14.82 ± 1.93^{b}	11.64 ± 1.36^{c}	11.73 ± 1.19^{c}
Diabetic + C. gigantea leaves extract 100 mg/kg b.w.	4.81 ± 0.42^{b}	15.92 ± 0.99^{b}	11.06 ± 1.12^{c}	11.68 ± 1.59^{c}
Diabetic + C. gigantea root extract 100 mg/kg b.w.	5.09 ± 0.54^{b}	15.37 ± 1.10^{b}	10.94 ± 1.21^{c}	8.93 ± 1.94^{b}

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 ($p<0.05$).

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 3.

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 3: 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 5: 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 ±	56.06 ±	70.77 ±	5.44 ±	4.12 ± 0.05^{b}	1.39 ± 0.34^{c,d}

TREATMENT	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dL)	ALBUMIN (g/dL)	GLOBULIN (g/dL)
	12.88[a]	20.92[a]	27.18[a]	0.26[c]		
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.

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

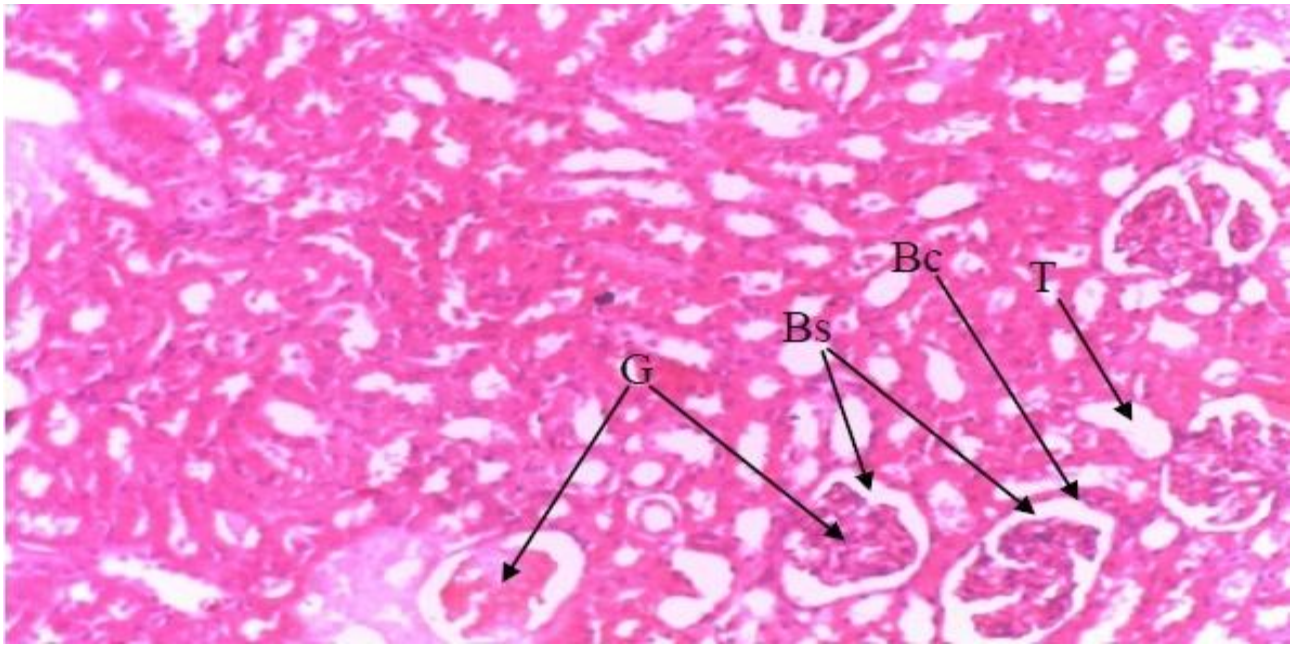

Figure 2: Photomicrograph of kidney section of normal control rat (group 1) showing normal glomerulus, Bowman's space, Bowman's capsule and normal tubules.

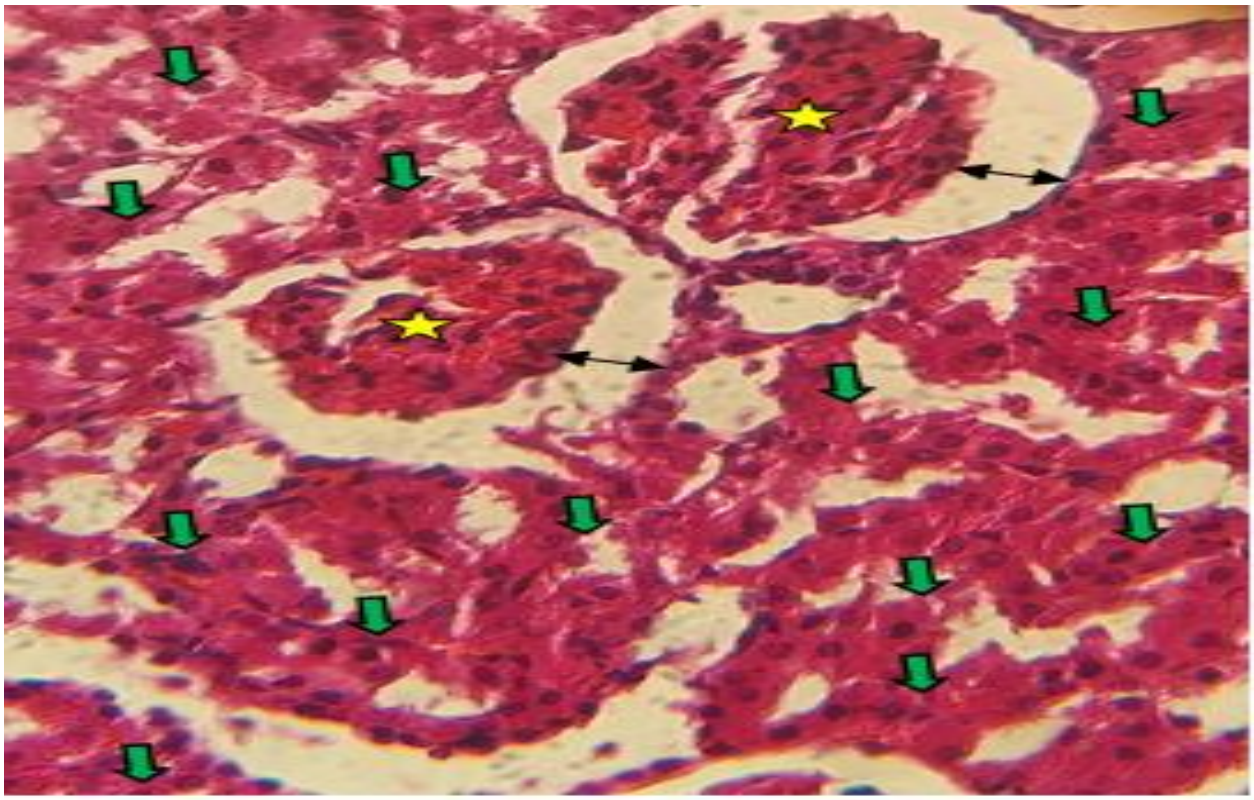

Figure 3: Photomicrograph of kidney section of group 2, showing congestion of the renal blood vessels; necrosis of the renal tubules (green arrows); necrosis and atrophy renal corpuscles (yellow stars) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400

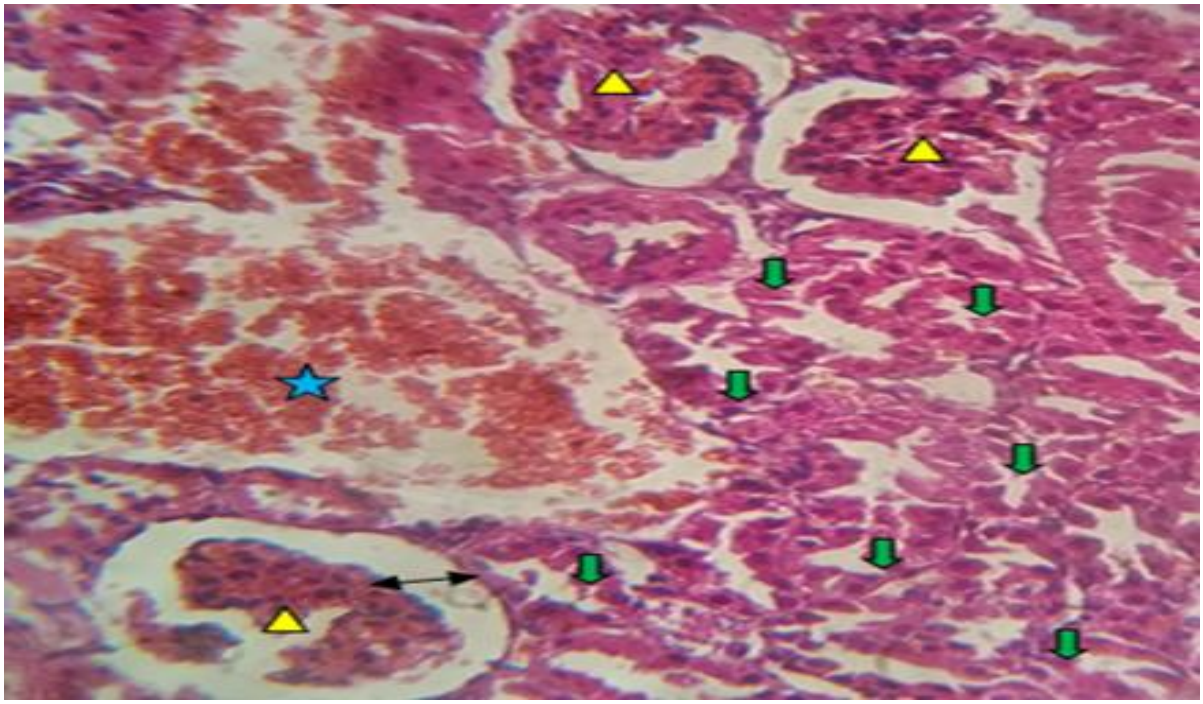

Figure 4: Photomicrograph of kidney section of group 3 showing congested renal blood vessels (blue stars); necrosis of the renal tubules (green arrows); necrosis and atrophy of the renal corpuscles (yellow triangles) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400

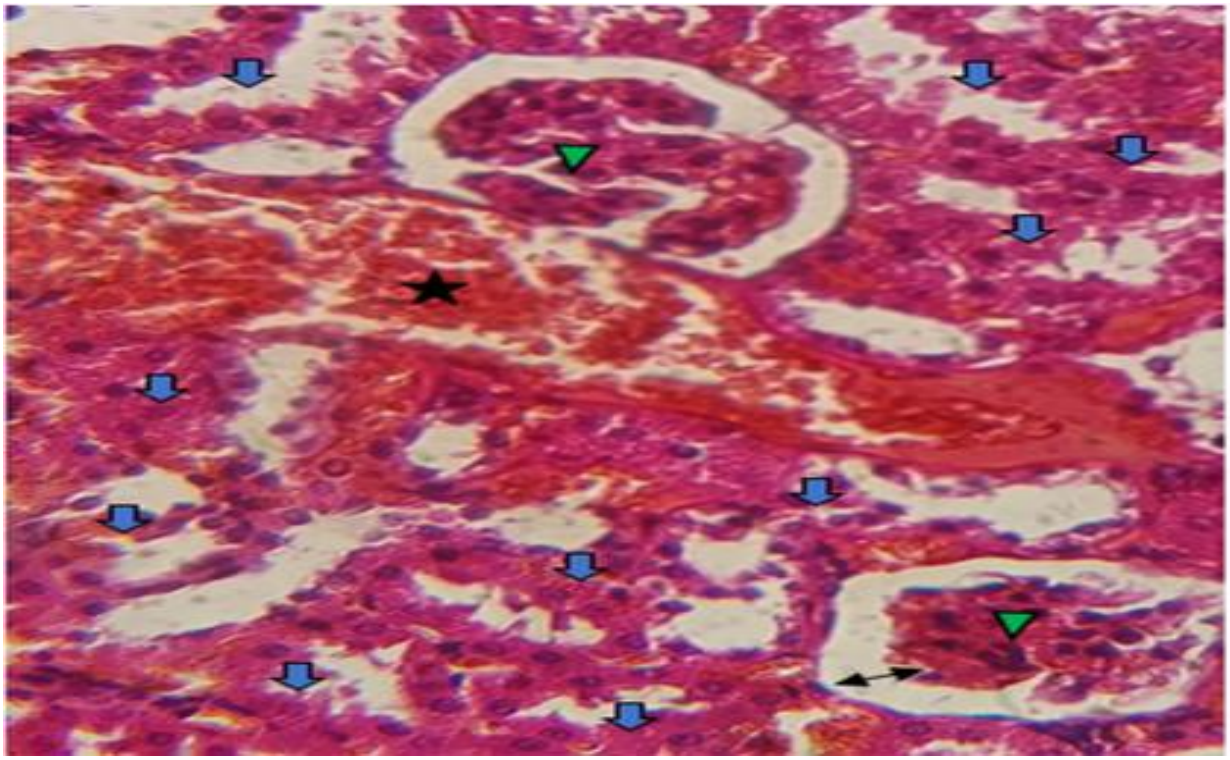

Figure 5: Photomicrograph of kidney section of group 4 showing congestion of the renal blood vessels (black stars); necrosis of the renal tubules (blue arrows); atrophy and necrosis of the renal corpuscles (green triangles) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400

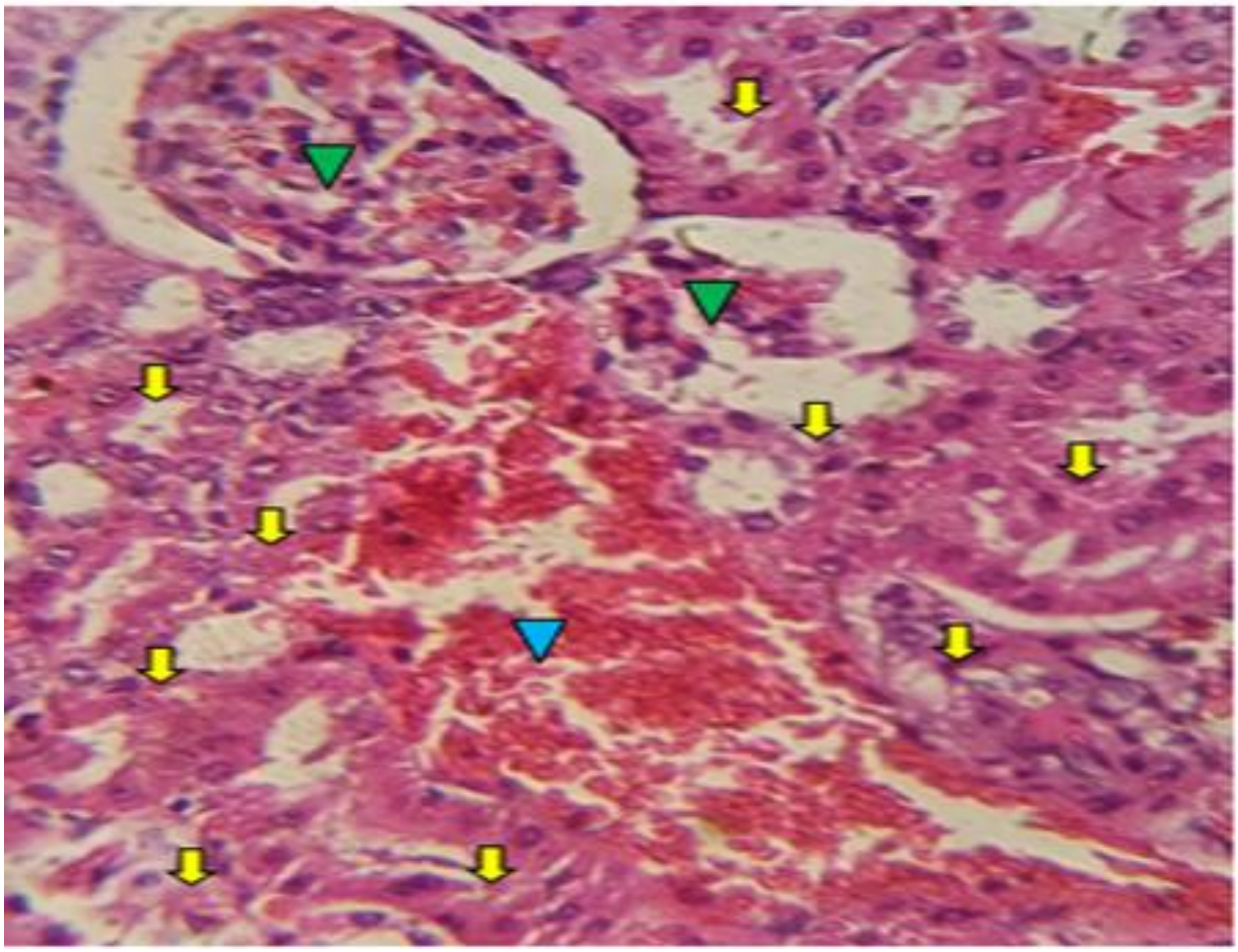

Figure 6: Photomicrograph of kidney section of group 5 showing congestion of the renal blood vessels (blue triangles); necrosis of the renal tubules (yellow arrows); renal corpuscles (green triangles). Hematoxylin and eosin stain. Magnification x400

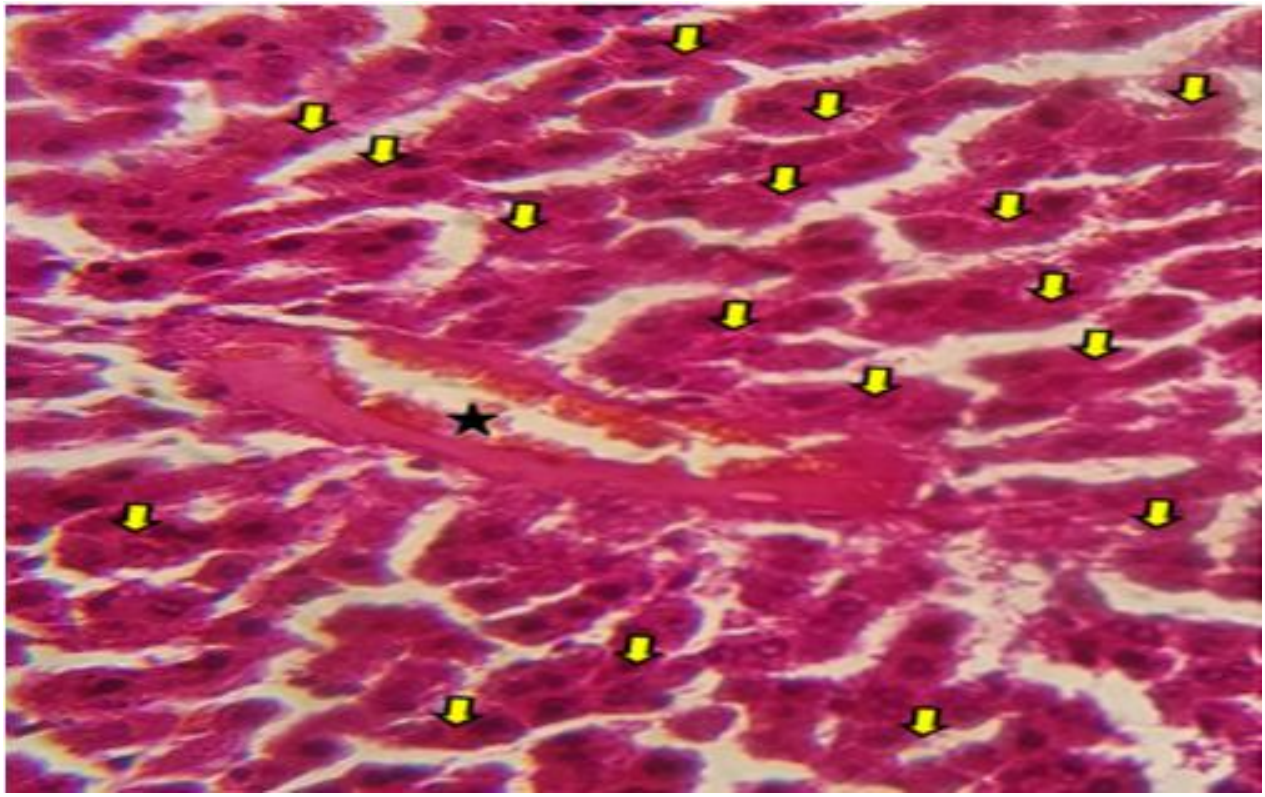

Figure 7: Photomicrograph of kidney section of group 6 showing congestion of the renal blood vessels (blue stars); necrosis of the renal tubules (yellow arrows) and renal corpuscles (black stars) with widened Bowman's space (line arrow: double). Hematoxylin and eosin stain. Magnification x400

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

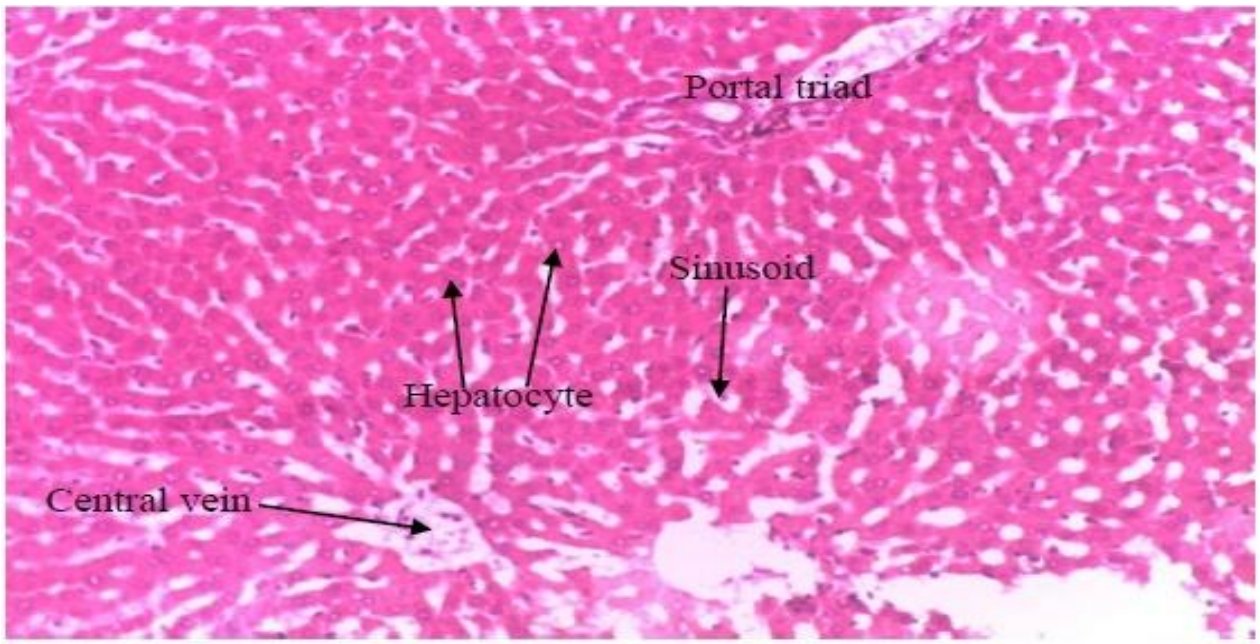

Figure 8: Photomicrograph of liver section of normal control rat (group 1) showing central vein, hepatocytes, sinusoid and portal triad.

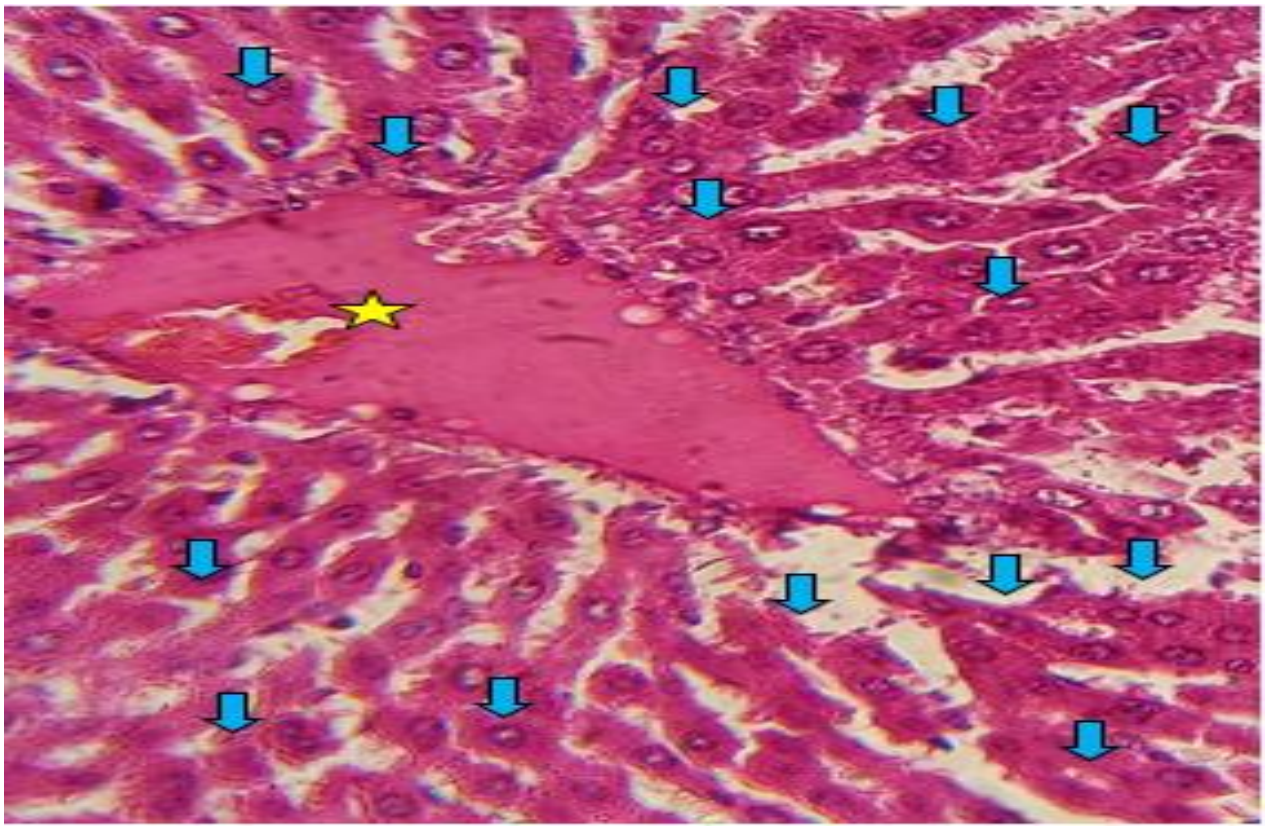

Figure 9: Photomicrograph of liver section of group 2 showing atrophy and necrosis of the hepatocytes (blue arrows); congestion of the central veins (yellow stars). Hematoxylin and eosin stain. Magnification x400.

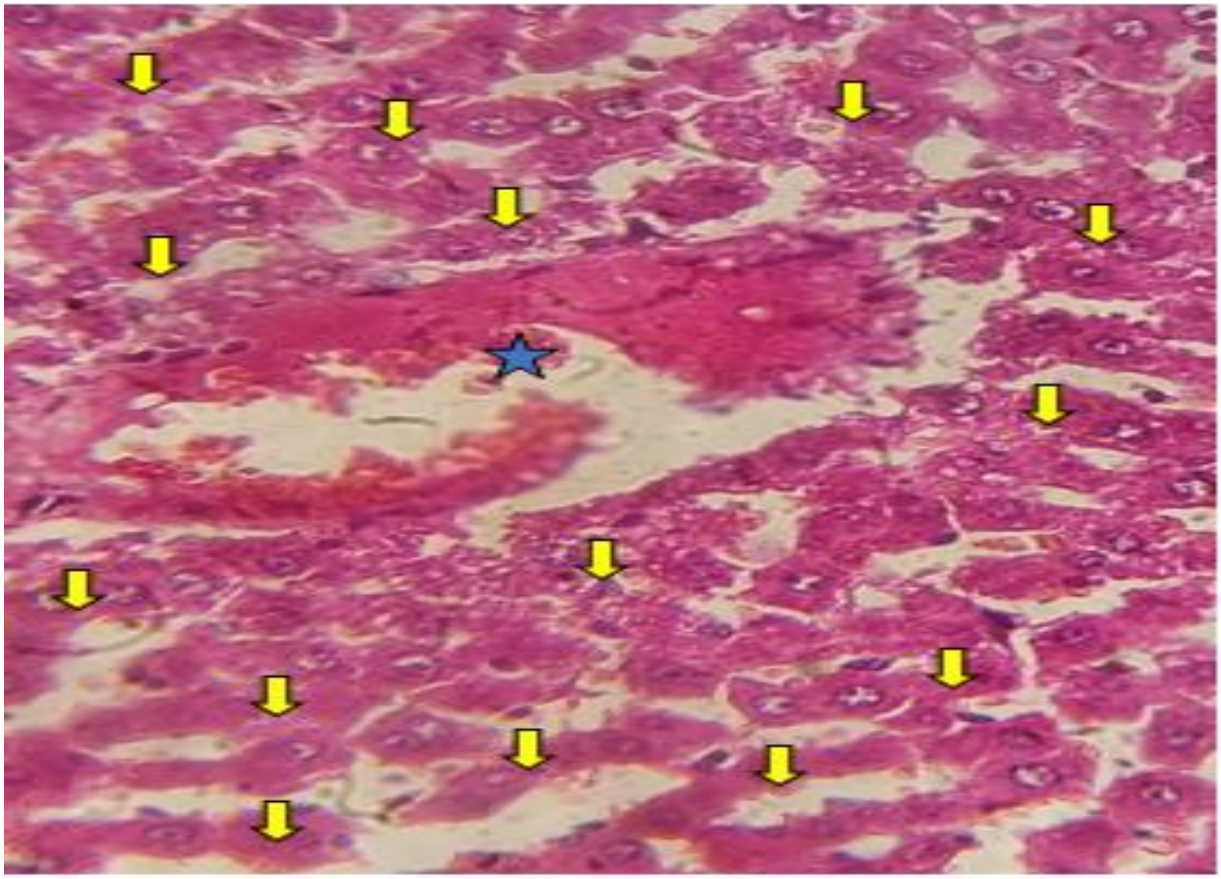

Figure 10: Photomicrograph of liver section of group 3 showing necrosis of the hepatocytes (yellow arrows); congestion of the central veins (blue stars). Hematoxylin and eosin stain. Magnification x400.

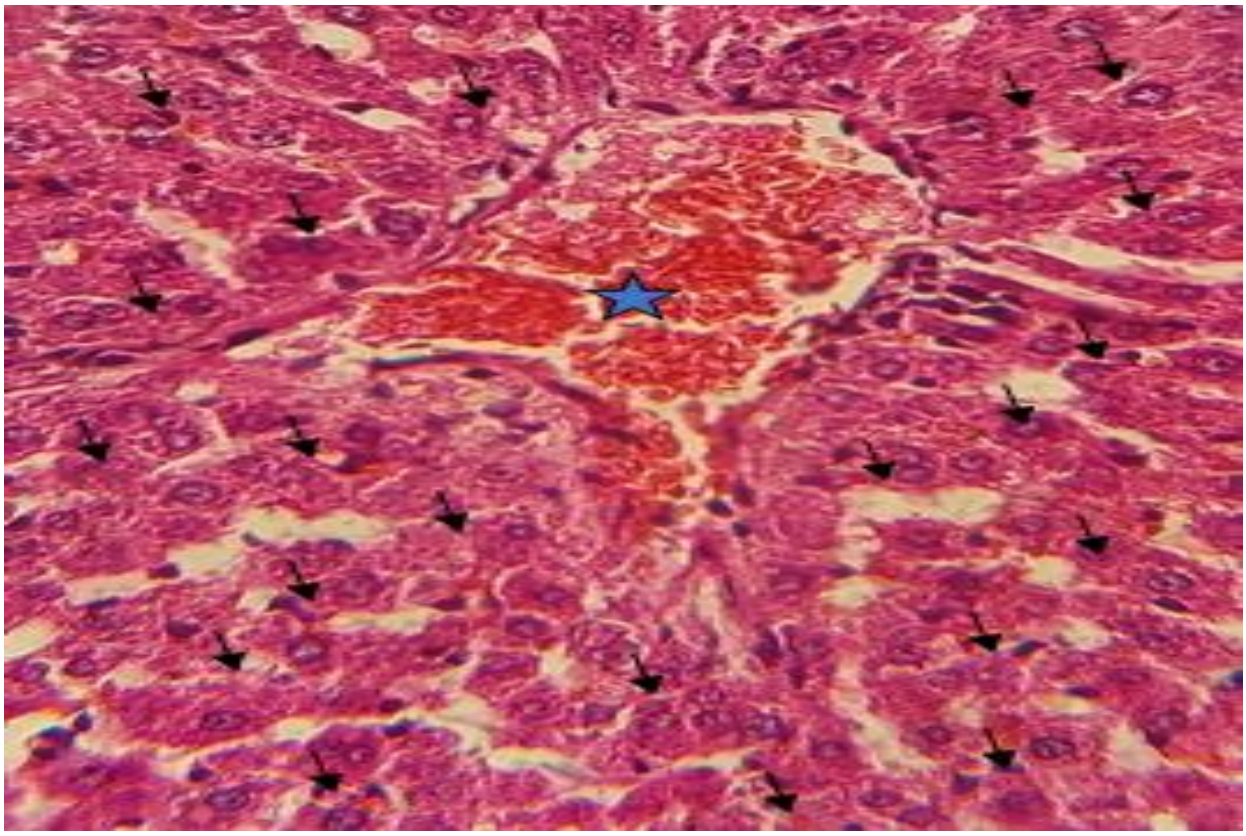

Figure 11: Photomicrograph of liver group 4 showing section of necrosis of the liver parenchyma i.e. the hepatocytes (black arrows); congested central vein (blue star) and portal vein (yellow star). Hematoxylin and eosin stain. Magnification x400

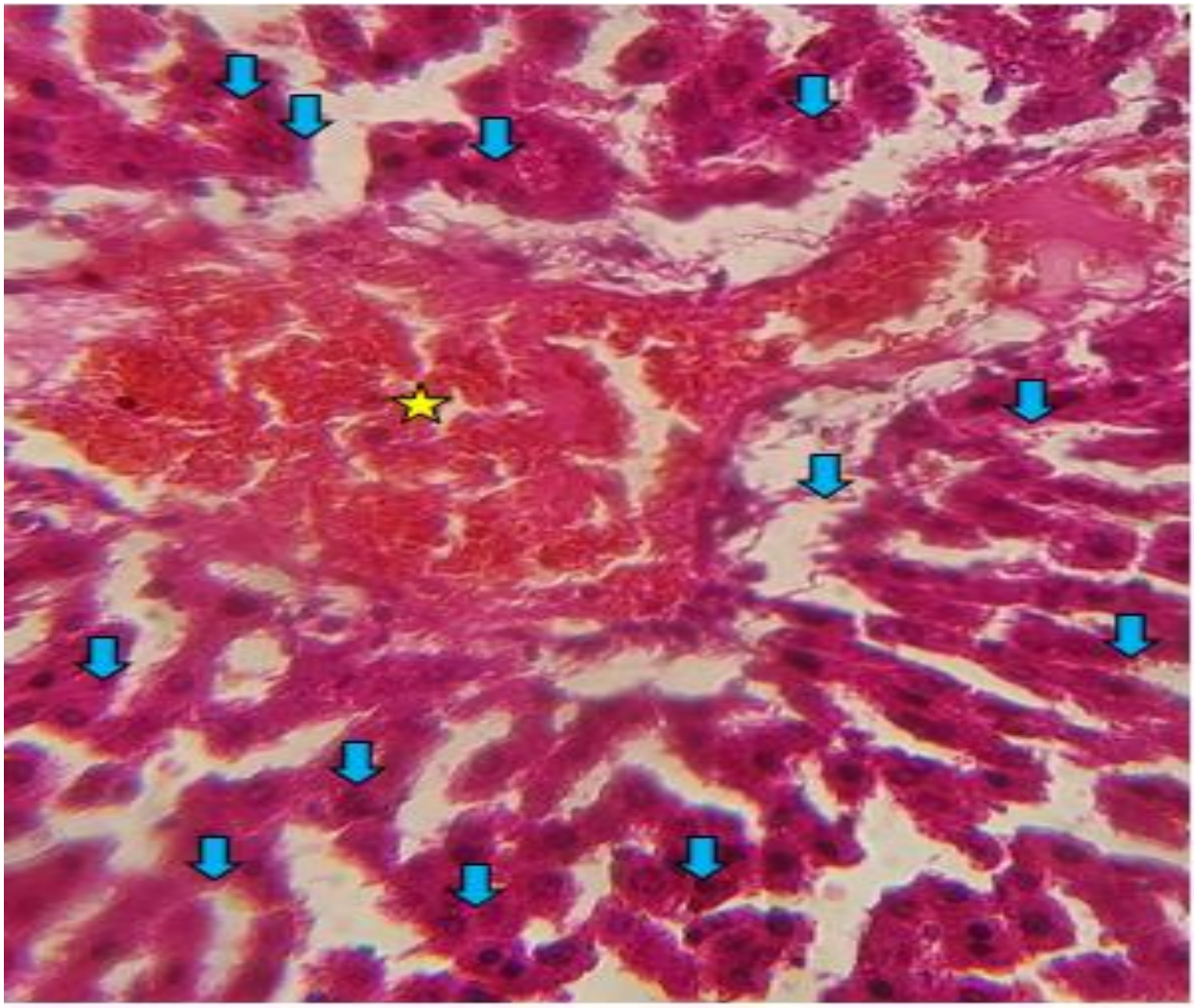

Figure 12: Photomicrograph of liver section of group 5 showing necrosis and atrophy of the hepatocytes (blue arrows); congestion of the central veins (yellow stars). Hematoxylin and eosin stain. Magnification x400.

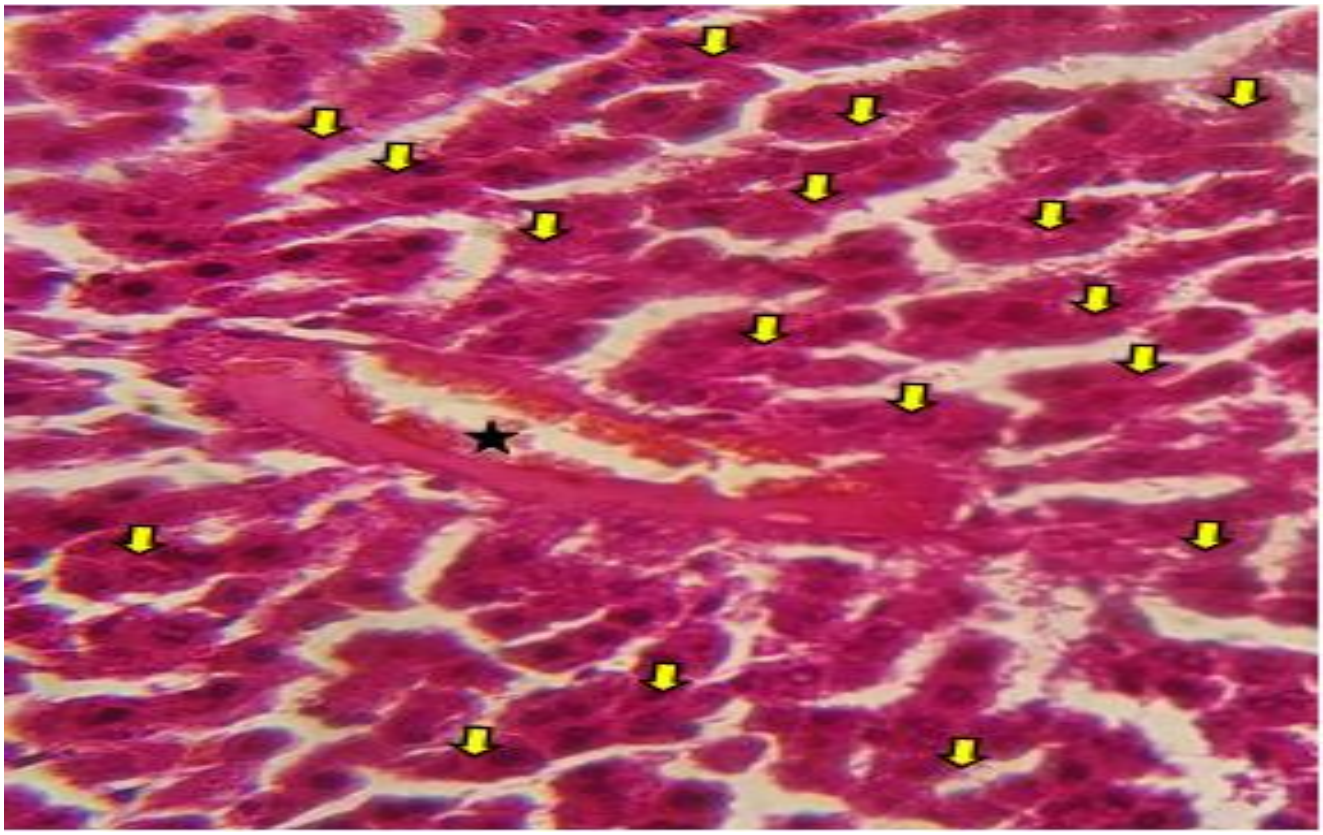

Figure 13: Photomicrograph of liver section of group 6 showing necrosis and atrophy of the hepatocytes (yellow arrows); congestion of the central vein (black star) and portal vein (blue star). Hematoxylin and eosin stain. Magnification x400.

DISCUSSION

The GC–MS analysis of the methanol extract of *Calotropis gigantea* revealed a complex and chemically diverse profile consisting of twenty-four phytoconstituents, with a marked predominance of oxygenated monoterpenes such as 7-octen-2-ol, α-terpineol, D-limonene, and 2,6-octadienal. The high relative abundance of these compounds suggests that they play a central role in mediating the biological activities observed in this study. Oxygenated monoterpenes are widely recognized for their potent antioxidant, anti-inflammatory, and metabolic regulatory properties, which are critical in the management of diabetes and its associated complications. Recent studies have demonstrated that terpenoid-rich extracts from *C. gigantea* exhibit strong free radical scavenging activity and enzyme inhibitory effects, particularly against α-amylase and α-glucosidase, thereby contributing to glycemic control (Singh *et al.,* 2023 and Jena *et al.,* 2025).

The dominance of 7-octen-2-ol in the present study is particularly noteworthy, as this compound has not been extensively reported as a major constituent in earlier phytochemical analyses of *C. gigantea*, suggesting possible geographical or extraction-related variability. This observation aligns with recent findings indicating that environmental factors, solvent systems, and plant part selection significantly influence phytochemical composition and bioactivity (Dwivedi *et al.,* 2024). Furthermore, α-terpineol and D-limonene have been reported to modulate oxidative stress pathways by enhancing endogenous antioxidant enzyme activity and suppressing pro-inflammatory cytokines, thereby protecting tissues from oxidative damage (Gupta *et al.,* 2024).

The presence of minor constituents, although quantitatively low, may contribute to the overall pharmacological effect through synergistic interactions, a phenomenon well documented in phytomedicine. This synergism enhances the therapeutic potential of plant extracts by targeting multiple pathways simultaneously, which is particularly advantageous in complex metabolic disorders such as diabetes. Therefore, the GC–MS findings not only confirm the presence of bioactive compounds but also provide a mechanistic basis for the multi-target therapeutic effects observed in this study.

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 levels, 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.

Overall, this study establishes *Calotropis gigantea* as a promising natural candidate for the management of diabetes and its associated complications.

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