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INVESTIGATING THE IN *VITRO* ANTI-INFLAMMATORY PROPERTIES AND THE EFFECT OF *FICUS EXASPERATA* ON KIDNEY PROFILE

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ABSTRACT

Ficus exasperata (FE) possesses a rich heritage of application in indigenous medicinal systems especially in Nigeria. This study investigates the *in vitro* anti-inflammatory properties of this plant and its effect on renal profile in rats. Methanol extract of FE leaves was prepared using standard techniques. Inhibition of protein denaturation, hypotonicity, and heat-induced haemolysis assay were investigated as the anti-inflammatory markers. The toxicity test was assessed through a repeated-dose oral gavage study using doses (125, 250, and 500 mg/kg) administered daily for 28 days. Biochemical markers and histological examination were employed to assess toxicity in the experiments. The results showed that the extract exhibited concentration-dependent inhibition of protein denaturation at different concentrations. There was no significant difference ($p>0.05$) between the extract and control in the red blood cell membrane stabilization assay. Catalase and superoxide dismutase activities showed no significant difference across the groups. However, there was a significant increase ($p<0.05$) in reduced glutathione activity (GSH) in group 2 and GPx activity and total protein level in group 3 experimental rats when compared to the control. Malondialdehyde level was significantly reduced ($p<0.05$) in groups 3 and 4 compared to the control. Urea and creatinine showed no difference statistically across the different groups. Bicarbonate was not statistically different among the groups, but other electrolytes assayed for differed significantly. The histology displayed normal architecture at different doses of FE but active interstitial congestion was noted. The methanol leaf extract of FE possesses high anti-inflammatory potentials and it is relatively safe.

Keywords: *Ficus exasperata*, Renal profile, Anti-inflammation, Toxicity, Histology

INTRODUCTION

Plants have been a breakthrough for medicine in the production of drugs (Olude *et al*., 2020). Novel drug discovery happens daily due to the unique capacity of secondary metabolites present in plants (Omoregie *et al*., 2020). The secondary metabolites are compounds termed phytochemicals. Phytochemicals are potent with inherent pharmacological activity. Flavonoids, tannins, terpenoids, steroids, phenols, anthraquinones, and alkaloids are some of these phytochemicals with huge therapeutic effects (Enoyoze *et al*., 2025) offering potential for direct use in treating or preventing diseases. Among the plants rich in phytochemicals is *Ficus exasperata* (FE). FE commonly known as the African sandpaper tree, holds immense potential (Okon *et al*., 2025). This plant is distributed widely across Africa; Ethiopia, Angola, Zambia, Mozambique even India (Bello *et al*., 2025). Okolie and Olude (2025) reported the presence of some bioactive compounds in this plant; alpha-caryophyllene, isoquinoline, quercetin, kaempferol, rutin, garcin, caffeic acid, luteolin, linalool catechin, epigallocatechin, stigmasterol, sitosterol, orientin, naringerin, hesperidin, isovitexin and isorhamnetin. These bioactive compounds are potent antioxidants, capable of scavenging free radicals and inhibiting lipid peroxidation. They also have diverse pharmacological properties such as immunomodulation, neuroprotection, anti-inflammatory, proapoptotic and anticancer activities (Havelek *et al*., 2016; Alam *et al*., 2018; Choi *et al*., 2018; Zdunska *et al*., 2018; Al-ghazzawi *et al*., 2019; Yun *et al*., 2021). Traditionally, the use of this plant is gaining momentum and Nigeria researchers have also reported the use of different parts of this plant; root, stem, and leaves in *in-vivo* studies in the treatment of inflammatory diseases, cancer, diabetes, hypertension, ulcers, and hemorrhoids. (Adeyomoye *et al*., 2024; Bello *et al*., 2025; Okolie and Olude, 2025; Okon *et al*, 2025; Olude and Emeninwa, 2025,).

While reports have ascertained the potential health benefits of *Ficus exasperata* (FE) extracts, not much has been reported on the potential toxicity of FE on the kidney. Therefore, this research explores the toxicity profile of methanol extract of *F, exasperata* leaves on renal function and its anti-inflammatory capacity.

MATERIALS AND METHODS

Plants collection

The leaf of *Ficus exasperata* was obtained from a local garden in Benin City, Edo State, Nigeria. It was identified by Dr Akinnibosun, of the Department of Plant Biology and Biotechnology at the University of Benin, Benin City. The plant was assigned a voucher number UBH-F319 and a specimen was deposited within the same departmental repository for future reference

Preparation of extracts

Fresh leaves of *Ficus exasperata* were cut and thoroughly washed with clean water to eliminate contaminants. The leaves were then spread out under shade to drain completely. The leaves were air-dried at room temperature. Once dried, the leaves were ground into a fine powder and stored in airtight containers for extraction. For the extraction process, the powdered leaves were soaked in methanol for 72 hrs. After steeping, the mixture was filtered and the filtrate was concentrated using a freeze drier. After the extraction process, the resulting residual material was preserved at a temperature of 4°C.

Anti-Inflammatory Analysis

The inhibition of protein denaturation was assessed following the protocol outlined by Sakat *et al*. (2010). The control solution (50 mL) consisted of 2 mL of egg albumin, 28 ml of phosphate buffer (pH 6.4), and 20 ml of distilled water. The standard drug solution (50 mL) included 2 ml of egg albumin, 28 ml of phosphate buffer, and various concentrations of standard drug (diclofenac) at 100, 200, 400, 800, and 1000 µg/ml. The test solution (50 mL) comprised 2 ml of egg albumin, 28 mL of phosphate buffer, and various concentrations of plant extract at 100, 200, 400, 800, and 1000 µg/ml. The samples were incubated at 37°C for 15 minutes and heated for 5 minutes at 70°C. After cooling, the absorbance of the solutions was measured at 660 nm. The percent inhibition of protein denaturation was calculated using the formula:

% Inhibition= $(A_t/A_c - 1) \times 100$

Where A_t is the absorbance of the test sample, A_c is the absorbance of the control.

Membrane stabilization

Preparation of Red Blood cell (RBC) suspension

RBC suspension was prepared following the procedures outlined by Sadique *et al*. (1987) and Sakat *et al*. (2010). Blood obtained from apparently healthy human volunteers was transferred to centrifuge tubes and centrifuged at 3000 rpm for 10 minutes. The serum was removed and the resulting red blood cells were washed with an equal volume of normal saline. This was done by centrifuging the solution obtained from the mixture of the normal saline and RBC at 3000 rpm for 10 minutes. Immediately after centrifugation the serum was removed, an equal volume of normal saline was added to the red blood cells. This process was repeated three times. The volume of blood was measured and reconstituted as a 10% v/v suspension with normal saline.

Heat-induced haemolysis

The haemolysis inhibition assay was conducted following the protocols outlined by Oyedapo *et al*. (2010) and Sakat *et al*. (2010). In each reaction mixture (2ml), 1ml of plant extract at various concentrations (100 - 500 µg/ml) and 1ml of 10% red blood cell (RBC) suspension were combined. A control test tube, used for comparison, contained saline instead of the plant extract. Diclofenac served as the standard drug. The reaction mixtures were then incubated in a water bath at 56ºC for 30 minutes. After incubation, the tubes were cooled, and centrifuged at 2500 rpm for 5 minutes, and the absorbance of the supernatants was measured at 560 nm. This assay assesses the ability of the plant extract to inhibit haemolysis, a process that can be indicative of its potential protective effect on red blood cells. The experiment was performed in triplicates for all the test samples. The percentage protection was calculated as follows:

Percentage protection = 100- (OD sample/OD control) x 100

Hypotonicity-induced haemolysis

The hypotonicity-induced haemolysis assay, following the method of Azeem *et al*. (2010), involved mixing different concentrations of the extract (100-500µg/ml), a reference sample, and control with 1ml of phosphate buffer, 2ml of hyposaline (0.25% NaCl), and 0.5ml of human red blood cell suspension. Diclofenac sodium (100µg/ml) served as the standard drug. After incubating all assay mixtures at 37°C for 30 minutes and centrifuging at 3000rpm, the supernatant liquid was decanted, and the haemoglobin content was estimated using a spectrophotometer at 560nm. The percentage of haemolysis was calculated, assuming the haemolysis produced in the control as 100%. This assay helps assess the potential protective effect of the plant extract on red blood cells by evaluating its impact on preventing haemolysis.

Percentage protection = 100- (OD sample/OD control) x 100

Sub-Acute Toxicity Assessment

The subacute toxicity assessment of the methanol extract of FE was performed according to the methodological frameworks explained by the Organization for Economic Co-operation and Development (OECD) guideline 407. The experimental animals (24 rats divided into four groups weighing 150-200 g) were subjected to oral administrations (with the aid of a gavage) of graded doses of the FE plant extract; 125, 250, and 500 mg/kg body weight, respectively. A control group labeled group 1 was only fed with feed. the experiment lasted for 28 days. Written approval for the study was obtained from the Research Ethics Committee Guideline Principles on Handling of Animals of the Faculty of Life Sciences, University of Benin, Benin City, and was strictly adhered to.

After the 28-day treatment, the animals were fasted overnight and sacrificed. Blood was collected via cardiac puncture for biochemical analysis, the kidneys were excised and washed in ice-cold saline, and some portions were preserved in 10% formalin for histopathology. The remaining tissues were homogenized in normal saline, and centrifuged. The supernatant was immediately used for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), total protein (TP), and malondialdehyde (MDA) assays.

Biochemical Analysis

Malondialdehyde (MDA) levels were estimated by the method of Beuge and Aust (1978). Total protein (TP) was determined using the method of lowry (1951) and catalase (CAT) was assayed according to the method of Cohen *et al*. (1970). Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich (1972). Glutathione Peroxidase (GPx) was determined according to the method of Paglia and Valentine (1969) and reduced glutathione (GSH) was estimated according to the method of Ellman (1959).

Electrolytes, creatinine, and urea were assayed using commercial kits. Urea method by Tietz (1987). The quantification of creatinine in serum followed the procedure outlined by Bartels and Bohmer (1972). Sodium, chloride bicarbonate, and potassium followed the method of Tietz (1999).

Statistical Analysis of Data

The obtained data underwent statistical analysis, with values presented as Mean ± standard error of mean. Comparisons between samples were conducted using ANOVA, Tukey's multiple comparison test (SPSS, version 17), and GraphPad Prism. Significance was determined at p-values below 0.05 ($p<0.05$).

Histopathological Evaluation

Tissue fixation followed the method of Kiernan (2008): kidney tissue was fixed in 10% buffered formalin, dehydrated, and paraffin-embedded. Sections were obtained, mounted on slides, and haematoxylin-eosin stained for light microscopic examination. Slides were coded and analyzed by a histopathologist

RESULTS AND DISCUSSION

In Vitro Anti-Inflammatory Activities of Methanol Leaf Extract of *F. Exasperata*

F. exasperata exhibited significant antiproteinase activity at different concentrations as displayed in Figure 1. Inhibition of protein denaturation leads to the protection of protein's activity and integrity. The results showed that the extracts exhibited concentration-dependent inhibition of protein denaturation throughout the concentration. There are two common RBC membrane stabilization assays; heat and hypotonicity-induced haemolysis, displayed in Figures 2 and 3 respectively. The result displayed by *F. exasperata* showed a remarkable red blood cell membrane stabilization activity. In the hypotonicity and heat-induced haemolysis assay; the standard drug and plant followed the same trend between the concentration of 100-500µg/ml. The extract was effective in inhibiting hypotonicity-induced haemolysis even at its lowest concentrations.

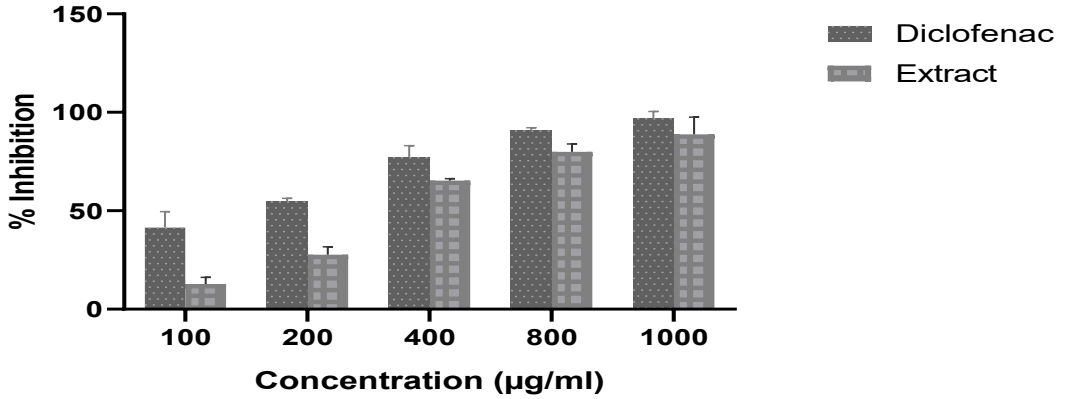

Figure 1: Inhibition of Protein Denaturation Activity of Methanol Extract of *Ficus Exasperata* Leaves. Values are Expressed as mean ± SEM, n=3/ group. Data were Considered to be Statistically Significantly Different at P < 0.05

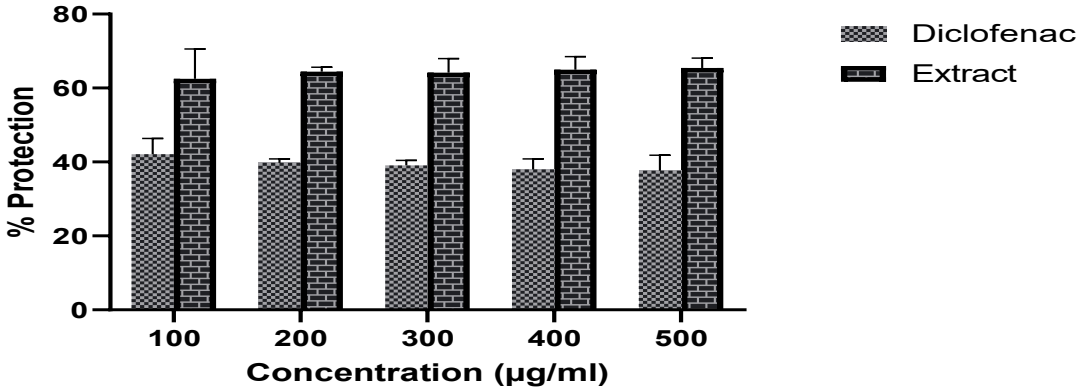

Figure 2: Heat-induced haemolysis; Percentage Protection Activity of Methanol Extract of *Ficus Exasperata* Leaves. Values are Expressed as mean ± SEM, n=3/ group. Data were Considered to be Statistically Significantly Different at P < 0.05

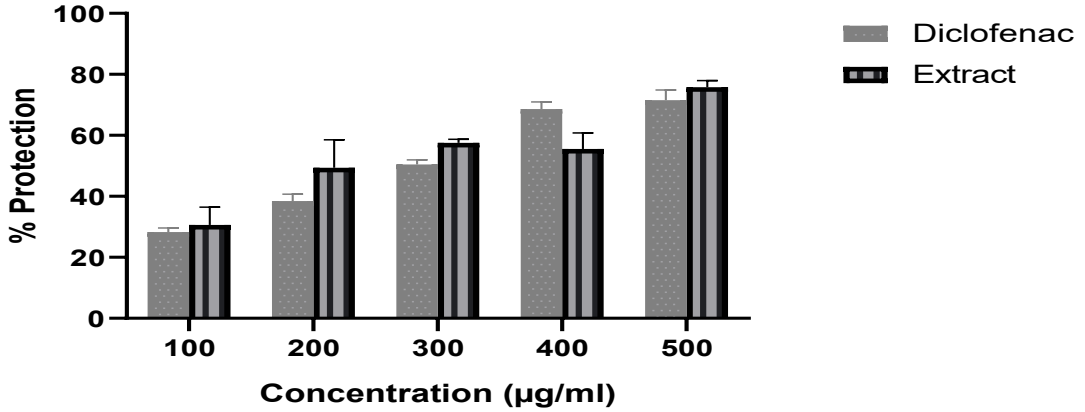

Figure 3: Hypotonicity induced haemolysis; Percentage protection activity of methanol extract of *Ficus exasperata* leaves. Values are expressed as mean ± SEM, n=3/ group. Data were considered to be statistically significantly different at P < 0.05

Protein denaturation, characterized by the loss of a protein's structure, can result from factors such as radiation, heat, or stress, leading to the disruption of biological activity and potential cell death (Nwaehujor *et al*., 2020). Leukocyte proteinase has been implicated in tissue damage during inflammatory reactions, and proteinase inhibitors have been

shown to provide significant protection (Das and Chatterjee, 1995). *Ficus exasperata* demonstrated substantial antiproteinase activity at various concentrations. Inhibiting protein denaturation is crucial for preserving the activity and integrity of proteins. The results indicated that the extract exhibited concentration-dependent inhibition of protein (egg albumin) denaturation across different concentrations. This result showed that *F. exasperata* methanol leaf extract has good inhibition of protein denaturation. Flavonoids, terpenoids, tannins, and alkaloids, known for their anti-inflammatory properties, have demonstrated significant efficacy both in vitro and in vivo (Ghasemian *et al*., 2016). The compounds isolated from the plant extract, belonging to these classes of secondary metabolites, may contribute to the observed anti-inflammatory activities of the extract.

Protein denaturation, linked to tissue injury and inflammation, is inhibited by drugs or compounds with potential anti-inflammatory activity (Osman *et al*., 2016; Anokwah *et al*., 2022). Additionally, the erythrocyte membrane's resemblance to the lysosomal membrane makes red blood cell (RBC) membrane stabilization an intrinsic in vitro anti-inflammatory assay. Stabilizing the RBC membrane suggests potential lysosomal membrane stabilization, a crucial mechanism in limiting inflammation by preventing the release of inflammatory constituents (Shenoy *et al*., 2010). Inflammation, both acute and chronic, involves the release and activities of lysosomal enzymes, and this cascade can be mitigated by lysosomal enzyme inhibition or lysosomal membrane stabilization achieved by non-steroidal anti-inflammatory drugs (Rajendran *et al*., 2018). *Ficus exasperata* demonstrated significant red blood cell (RBC) membrane stabilization activity through both heat-induced haemolysis and hypotonicity-induced haemolysis assays. In the heat-induced haemolysis assay (Figure 2), the extract effectively inhibited haemolysis even at its lowest concentrations (100 µg/ml), following a similar trend to the standard drug. The hypotonicity-induced haemolysis assay revealed that the extract, at concentrations of 100-500 µg/ml, provided protection against RBC lysis induced by the hypotonic solution, with a maximum protection of 74% at 500µg/ml. (Sumathi and Anuradha, 2016). The inhibition of protein denaturation, hypotonicity, and heat-induced haemolysis ascertained that *F. exasperata* has a potent anti-inflammatory activity (Amponsah *et al*., 2015; Anokwa *et al*., 2022).

Tissue Enzymatic Antioxidants and Lipid Peroxidation Marker

The results for the antioxidant parameters, total protein and lipid peroxidation marker (MDA) of the toxicity test on the kidney are presented in Table 1. Catalase (CAT) and superoxide dismutase (SOD) activities in the kidney showed no significant difference across the groups. Though SOD activity was high in group 1 but statistically it was not significant. However, there was a significant increase in reduced glutathione activity (GSH) in group 2 and GPx activity in group 3 experimental rats when compared to the control. Total protein (TP) showed a significant difference, a significant increase was observed in group 3 compared to control. Malondialdehyde level was significantly reduced in group 3 and 4 compared to the control.

Table 1: Kidney Antioxidants, Total Protein and MDA Levels

Assays	GRP 1 (Control)	GRP 2 (125mg/kg bw extract)	GRP 3 (250mg/kg bw extract)	GRP 4 (500mg/kg bw extract)
SOD (units/g wet tissue)	19.1 ± 9.6	5.4 ± 0.76	5.14 ± 1.00	6.3 ± 1.4
CAT (units/g wet tissue)	1.94 ± 0.89	0.51 ± 0.22	0.68 ± 0.13	0.94 ± 0.39
GPX (units/g wet tissue)	7.42 ± 2.41	15.40 ± 2.49	16.79 ± 2.80[a]	10.17 ± 2.72
GSH (Mm/g tissue)	115.62 ± 23.5	180.55 ± 33.36[a]	173.95 ± 33.55	138.54 ± 21.27
TP (g/dl)	18.30 ± 5.96	38.03 ± 6.04	41.86 ± 7.02[a]	34.51 ± 5.70
MDA ($\times 10^{-4}$) mmol/g tissue	19.32 ± 7.77[cd]	6.11 ± 2.99	4.41 ± 1.12[a]	5.49 ± 1.16[a]

Values are expressed as mean ± SEM, n=6/group. Lowercase letters represent a significant difference at $P < 0.05$. Comparison was done across columns

Reactive nitrogen species and reactive oxygen species can both be deleterious and at the same time beneficial to human health (Sies *et al*., 2022). The imbalance between these free radicals production and the antioxidants system is called oxidative stress (Wang *et al*., 2024) which is the pivot factor for many chronic ailments such as cardiovascular diseases, cancer, neurological disorders, and metabolic disorders (Jomova *et al*., 2024). To combat the deleterious and harmful reactions of the free radicals are enzymatic antioxidants such as; superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPx) (Wei *et al*., 2020).

In this study; the plant did not alter the enzymatic antioxidant ability of the renal tissue but rather enhanced the antioxidant potential of the kidney tissue without eliciting overproduction of free radicals. Lipid peroxidation marker (MDA) was statistically reduced in groups 3 and 4 compared to the control. The result of the MDA level is another marker buttressing that FE is rich in antioxidant levels (Olude and Emeninwa, 2025). The high level of total protein and antioxidant activity expressed in this plant could also be linked to the high levels of quercetin, kaempferol, rutin, and isoquinoline present in the methanol extract of *F. exasperata* as reported by Okolie and Olude (2025).

Effect of the Extract on Electrolytes, Creatinine, and Urea

Urea and creatinine which are markers for kidney function tests showed no difference statistically across the four different groups. Bicarbonate was not statistically different among the different groups, but other electrolytes assayed for differed significantly. The sodium and potassium levels of the control group were significantly higher when compared to the other groups.

Table 2: Serum Assays of Some Renal Function Parameters

Assays	GRP 1 (Control)	GRP 2 (125mg/kg bw extract)	GRP 3 (250mg/kg bw extract)	GRP 4 (500mg/kg bw extract)
Creatinine (mg/dl)	0.94 ± 0.094	0.92 ± 0.04	1.01 ± 0.06	0.98 ± 0.02
Urea (mg/dl)	32.09 ± 2.39	30.32 ± 4.71	34.03 ± 6.88	38.33 ± 5.38
K^+ (mmol/L)	15.32 ± 0.87	10.73 ± 2.32[a]	9.47 ± 1.4[a]	11.02 ± 3.4
Na^+ (mmol/L)	182.44 ± 11.99	151.22 ± 5.8[a]	157.22 ± 7.11[a]	154.85 ± 6.4[a]
Chloride (mmol/L)	51.25 ± 17	9.3 ± 2.8[a]	23 ± 6.76	31 ± 13.1
Bicarbonate (mmol/L)	22.88 ± 4.27	27.59 ± 0.69	24.02 ± 1.2	22.82 ± 1.13

Values are Expressed as mean ± SEM, n=6/group. Lowercase Letters Represent a Significant Difference at $P < 0.05$. Comparison was Across Column

Histology Result of the Effect of *F. Exasperata* on Kidney Tissue

Plates 1 to 4 show the effect of the methanol extract of the plant on the kidney tissue. The plant appeared non-toxic to the tissues even at 500mg/kg body weight. The kidney tissues displayed normal architecture at different doses of MEFE but active interstitial congestion was noted.

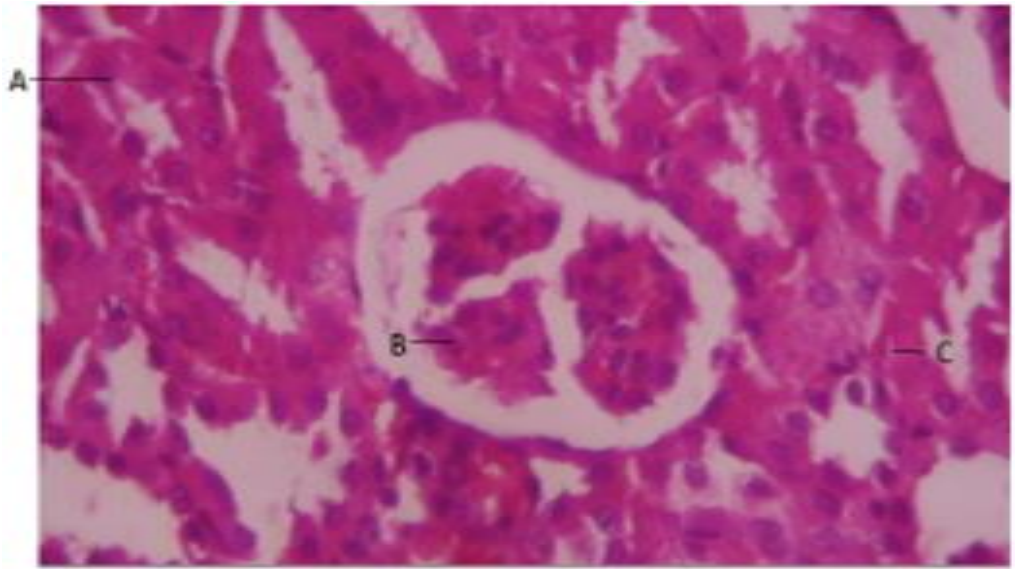

Plate 1: Kidney Section of Rat (Control) Composed of Normal Tissue: A. Tubules, B. Glomerulus, C. Interstitial Space (H&E x400)

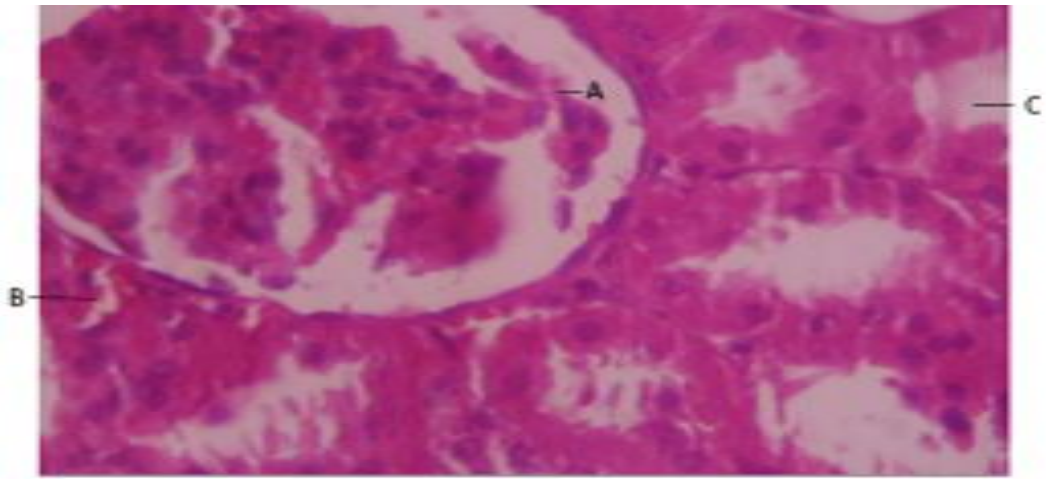

Plate 2: Kidney Section of Rat Given 125mg/kg bw Extract Showing Normal Architecture: A. Glomerulus, B. Active Interstitial Congestion, C. Tubules (H&E x 400)

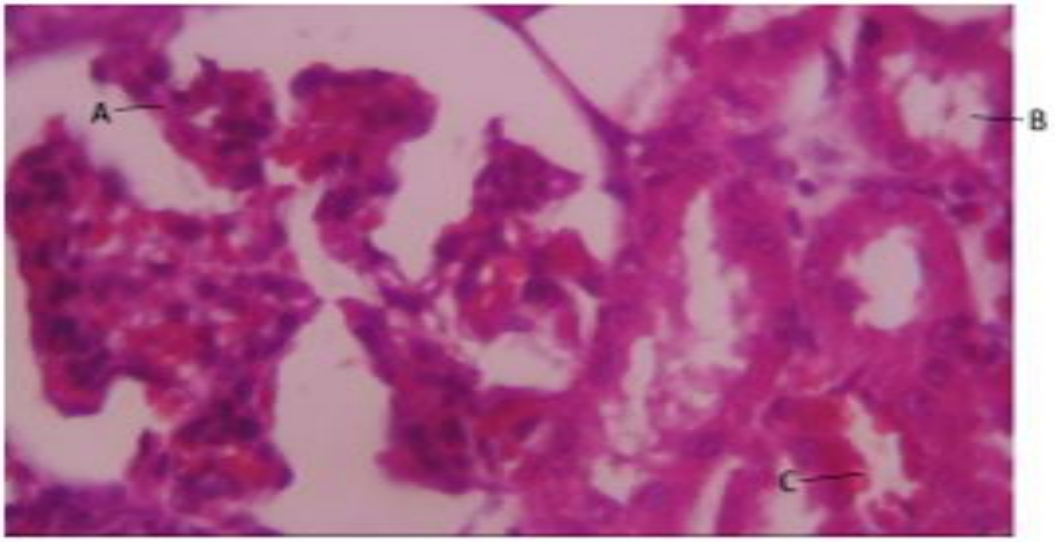

Plate 3: Kidney Section of Rat given 250mg/kg bw Extract Showing Normal Architecture: A. Glomerulus, B, Tubule, C. Active Interstitial Congestion and Vasodilatation (H&E x 400)

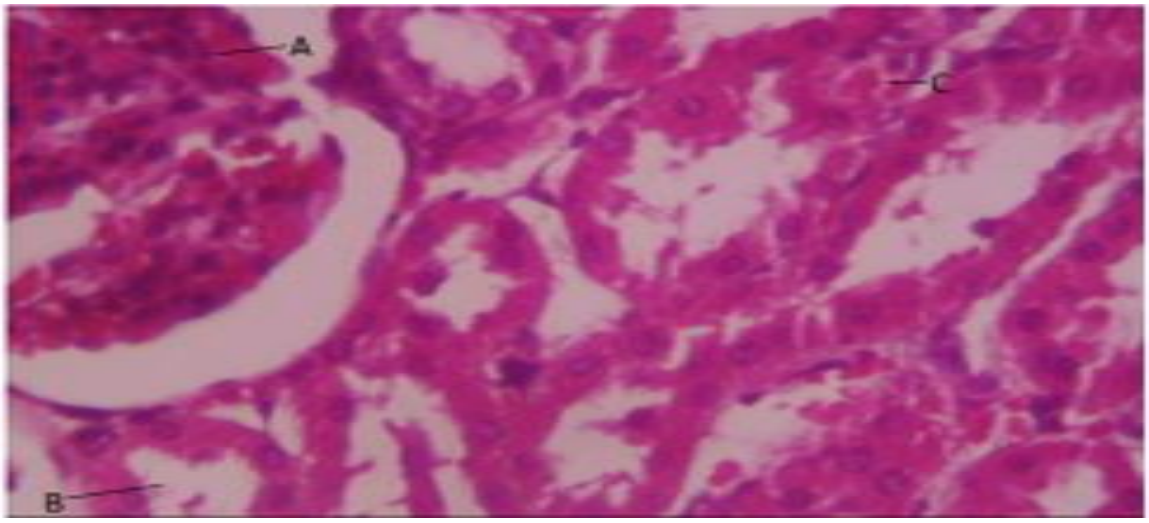

Plate 4: Kidney Section of Rat given 500mg/kg bw Extract Showing Normal Architecture: A. Glomerulus, B. Tubules, C. Active Interstitial Congestion (H&E x 400)

Creatinine and urea are used as a biomarker for the periodic assessment of kidney function (You *et al*., 2018; Peng *et al*., 2021). Creatine biosynthesis involves the production of guandinoacetate in the kidneys followed by methylation in the liver (Wyss and Kaddurah-Daouk, 2000). Creatine is essential in muscle energy metabolism (Wallimann and Harris, 2016). Creatinine is the end product of creatine metabolism which diffuses out of the cell and is excreted unchanged in the urine through glomerular filtration (Post *et al*., 2024). Blood urea nitrogen (BUN) is the end product of amino acid catabolism in the liver (Arihan *et al*., 2018; Du *et al*., 2024) and it is completely excreted by the kidney due to its toxic effect (Li *et al*., 2019; Mao *et al*., 2022). In this study creatinine and urea level showed that FE has no toxic effects on the kidney tissues within the dose range of 125-500 mg/kg body weight. This aligns with the findings of Oviosun *et al*. (2023), who reported that *F exasperata* is not nephrotoxic and may even restore kidney functions in worn-out kidney tissues. The major electrolytes necessary for regular metabolic processes and maintaining homeostasis are potassium, sodium, calcium chloride, magnesium, and bicarbonate (Onyiriuka and Oyenusi, 2018; Tassew *et al*., 2024). Reports have shown that electrolyte disorders can result into morbidity and mortality (Goldberg *et al*., 2004; Palmer *et al*., 2015; Timerga *et al*.,2020). There were discrepancies in the levels of some of the electrolytes across the groups. Potassium levels in groups 2 and 3, sodium levels in groups 2, 3, and 4, and chloride levels in group 2 were significantly reduced compared to the control. FE may be explored in the treatment of hyperkaleamia, hypernatreamia, and hypercholeraemia

The result from the photomicrographs clearly shows that FE did not alter the kidney architecture.

CONCLUSION

Methanol extract of *Ficus exasperata* leaves is not nephrotoxic, it possesses high anti-inflammatory properties and antioxidant potentials hence, it can be used in the treatment of diseases associated with inflammation and oxidative stress.

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