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Research Article

Biochemical and Histo-pathological Evaluation of the Methanol Extract of *Napoleonaea imperialis* Leaves against Methotrexate- Induced Renal Damage in Albino Rats

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Abstract

We investigated the biochemical activity of *Napoleonae imperialis leaves* extracts, within the context of its potential to fight human diseases. For the experiment the biochemical and histological effects of methanol extract of *Napoleonae imperialis leaves* against methotrexate (MTX) renal damage in albino rats were evaluated. To do this, sixty male albino rats ((30) each, used for the biochemical and histological experiments), mean weight 130 g. The animals were grouped into five (5), six (6) rats each. Group A (normal control) received feed and water only; Group B (positive control), induced with MTX without treatment. Test groups (C and D) were orally given 250 mg and 500 mg/kg b.wt of leaves extract; group E was orally given the extract only (500 mg/kg b.wt), for 14 days. Similar groupings were done for the histological study. All the experimental animals were subjected to renal damage using 0.5 ml/kg of MTX except the normal control group. The rats were sacrificed after 14 days, blood samples collected for biochemical analysis, for the histological, the kidney was carefully dissected from the abdominal region. The biochemical results, showed significant ($p< 0.05$) decrease in the groups that received 250 mg/kg and 500 mg/kg b.wt of the extract in (Urea, Creatinine and Na^+), significant increase ($p< 0.05$) in K^+ and Cl^-. Also there were significant ($p< 0.05$) decrease in (Urea, Creatinine, and Na^+) in comparison with control groups; the group that received the extract only (500 mg/kg b.wt), and a significant increase ($p< 0.05$) in (K^+ and Na^+). Histologically, photomicrographs showed group A, (normal control group) with evenly distributed glomeruli of smaller size, normal mesangial cellularity; group B, (positive control group) there was significant pathology including interstitial inflammation; groups (C and D) (tests group that received 250 and 500 mg/kg b.wt of the extract), no significant pathology and group E, no significant pathology. Our results indicates, methanol extract of *Napoleonae imperialis leaves,* have exerted positive renal functioning effects in albino rats. Moreover, the histology indicates, the leaves extract may have exerted nephron-protective effects in albino rats, and may be important also for pharmacological usage towards management of organ toxicity.

Keywords: Renal histology, methotrexate, histopathological, biochemical examination, *Napoleonae imperialis,* kidney, plant extracts.

Introduction

Kidney malfunction frequently exists in sick patient, due to individual damage of the organ. Three principal medical conditions have been recognized where kidney malfunction coincide; infections concurrently relating the liver and the kidney, or a basic liver disorder with elementary kidney malfunction, or vice versa (Moreau. *et al.*,

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2002). Kidney malfunction in this scenario often grows gradually. With the exclusion of definite disease such as leptospirosis, some viral hemorrhagic fevers and toxin-mediated damages such as paracetamol injury, could trigger severe failure of this organ (Moreau. *et al*., 2002).

Kidney dysfunction due to hepatic malfunction occurs in nature, arising due to the absence of important pre-renal modifications in kidney histology. However, inherent kidney failure may lead to severe or prolonged hepatic disease (Moreau. *et al*., 2002). Preventive uropathy could result to post-renal severe kidney dysfunction, which often manifest in chronic hepatic disease (papillary necrosis in alcoholic liver disease, haemorrhage in the urinary route as a result of acute coagulopathy). Hepato-renal disease is a major type of practical kidney malfunction that frequently result in progressive hepatic pathology, kidney disorder or threshold hypertension (Guevara and Gines, 2005).

Several toxic agents and certain antibiotics, chemotherapeutic agents, carbon tetrachloride (CCl_4), thioacetamide (TAA), MTX, could trigger kidney damages. Other halo-alkanes involved in kidney damage, and damages due to excessive alcohol consumption and microbes have been well studied.

We recently reported the hepatic protective effects of this extract (Nwachukwu. *et al*., 2020), signifying, the extracts consists ingredients that could be used for healing purposes or, which are intermediates for the production of important medicines (Mba. *et al*., 2020; Abolaji. *et al*., 2007). More than five thousand plants have been identified to exhibit therapeutic properties although, not many have been evaluated or examined (Abolaji. *et al*., 2007). Natural substances derived from plants are alternative sources of active ingredients, which are necessary for medicinal development/food supplements with important biological function, necessary for the treatment of variance of disease phenotypes including; cancer, diabetics, sickle cell disease (SCD), and malaria (Okoh. *et al*., 2019; Adejoh. *et al*., 2021; Okoh. *et al*., 2021).

Napoleonae imperialis is a small, evergreen tropical West African tree in the family of lecythidaceae, native to Africa, cultivated as an ornamental tree (Odugbemi. *et al*., 2007).

Consequently, in these experiments we monitored the effects of methanol extract of *Napoleonae imperialis* against MTX renal damage in albino rats with a view to establish the biochemical parameters it affects, with histopathological examination of the extract against MTX renal damage in albino rats, postulate the molecular and cellular events it may modulate for the methanol extract of *Napoleonae imperialis* to mediate renal repair and functions in albino rats.

Materials and Methods

Materials

Assay kits for the estimation of urea, sodium ion (Na^+), chloride ion (Cl^-), creatinine and potassium (K^+) were purchased from Randox, UK. All other chemicals were of analytical grade.

Plant Material

Fresh leaves of the plant *Napoleonaea imperialis* were obtained in a farm at Umudike, Abia State, Nigeria. The plant was identified at the Plant Science and Biotechnology Department, Michael Okpara Uiversity of Agriculture, Umudike. The plant name was checked with http://www.theplantlist.org. The fresh leaves of the plant were washed and dried under shade at room temperature and blended into a powder using a blender.

Extraction

The leaves powdered of *Napoleonae imperialis* (100 g) were soaked in methanol for 48 hours, after, which the extract was filtered using a Whatman no.1 filter paper and the filtrate, allowed to evaporate to dryness in a water bath (40^oC).

Animals

The experiment was carried out following the internationally accepted principles for laboratory animal use and care. Healthy male albino rats with mean weight of 130 g were

used for the study. All animals were kept in the animal house under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*. The animals were housed in standard animal cage, at room temperature with access to water in accordance with the international guide for the care and use of laboratory animals (Committee for update of the guide for the care and use of laboratory animals, 2011). They were maintained under controlled environmental condition with a 12 hour dark: light cycle.

Induction of Nephrotoxicity

All the rats used for this study were initially subjected.
to renal damage using 0.5 ml of MTX intraperitoneal (i.p) except the normal control group.

Experimental design

Sixty (60) male albino rats of mean weight 130 g were used for both the biochemical and histopathological studies. The animals were divided into two equal halves of 30 each and were grouped into five (5) groups of six (6) rats each. Group (A) and (B) were the control groups, group (C) and (D) were the test groups. and group E was the group that receive the extract only. Group (A) represented the normal control group that received feed and water only and Group (B) represented the positive control group that was induced without treatment, test Groups C and D were orally given 250 mg and 500 mg/kg body weight of *Napoleonae imperialis* leaves extract respectively and group E orally received the extract only (500 mg/kg b.wt). All the rats used in this study (renal study) were initially subjected to renal damage using 0.5 ml/kg of MTX except the normal control group. Treatment lasted for 14 days and after which the animals were sacrificed on day 15 under mild anesthesia (10% formasaline).

Evaluation of the Biochemical Parameters

Determination of serum creatinine concentration

Determination of serum creatinine concentration was done by Jaffe's reaction as described by (Peters, 1942). Creatinine is derived from creatinine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine through the kidney. Creatinine was measured to assess kidney function. The spectrophotometric method described by Jaffe's reaction and adopted by (Peters, 1942), was used in the determination of serum creatinine concentration, with standard, picric acid, and sodium hydroxide (NaOH). The serum was calculated using the formula:

$$\text{Serum creatinine concentration} = \Delta A \text{ X STD} \quad \frac{\text{Conc. X } 0.005}{\Delta A \text{ Standard}}$$

Determination of serum urea concentration

Serum urea concentration was determined by the method of Varley and Alan (1984). Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically using Berthelot reaction, with Urease, Sodium nitropuriside, phenol concentrate, Hypochlorite concentrate and standard. The serum urea concentrate was calculated thus;

$$\text{Serum urea Conc} = \frac{\text{A of sample}}{\text{A of standard}} \text{ X Standard concentration (Mmol/dl)}$$

Where A = Absorbance.

Tests for Electrolyte Activity

Estimation of serum sodium ion (Na^+) and potassium ion (K^+)

The serum sodium and potassium ion concentration were determined using the method of Terri and Sesin (1958). Flame

photometric method was used. Stock sodium (58.48 g NaCl in 1 L H_2O) and Stock potassium (74.55 g KCl in 1 L H_2O) were prepared.

The contents of the tubes were well mixed then placed in the flame photometer. The air compressor was switched on and air pressure adjusted. Deionized water was introduced through the atomizer. The gas was turned on and adjusted to give fine sharp cones. Appropriate filters were then placed for simultaneous sodium and potassium estimation. The diluted test serum was introduced and the reading taken and recorded using flame photometer.

Estimation of serum chloride concentration

Serum chloride ion concentration was determined using the method of (Skeggs and Hochstrasser, 1964), using mercuric nitrate. It involves titrating chloride ion with mercury ions, forming soluble but non-ionized mercury chloride. The end product is reached when excess Hg^+ forms a complex with an indicator such as diphenylcarbazone producing a pale violet colour. The serium chloride concentration was calculated using;

$$\text{Serum chloride concentration} = \frac{\text{Titration of test}}{\text{Titration of standard}} \times \text{conc. of standard}$$

Statistical Analysis

The data were expressed as mean ± standard deviation and analyzed using statistical package for the social sciences (SPSS 22.0). Comparisons were made between the test groups and the control groups using One-Way Anova and $p \leq 0.05$ was considered statistically significant.

Histopathological Examination

The method described by (Oduola. *et al*., 2007) was followed. After blood collection, the kidney was carefully dissected from the abdominal region. They were fixed in normal saline for 72 hours and sliced into a thickness of 2.1mm. The tissues were dehydrated with alcohol of graded concentration. They were further treated with paraffin wax and cast into blocks; sections of the kidney were then cut on a microtome of 5µm. these were later attached to a slide and allowed to dry. The samples were subsequently stained in haematoxylin and eosin (H&E) and examined under a light microscope. Photomicrographs of the samples were recorded.

Results

The current experiments were designed to monitor the effects of methanol extract of *Napoleonae imperialis* on induced renal damage of albino rats using MTX by analyzing some biochemical parameters, corroborating the same with histopathological examination. The biochemical analyses are presented in the Fig 1-10.

Figure legends

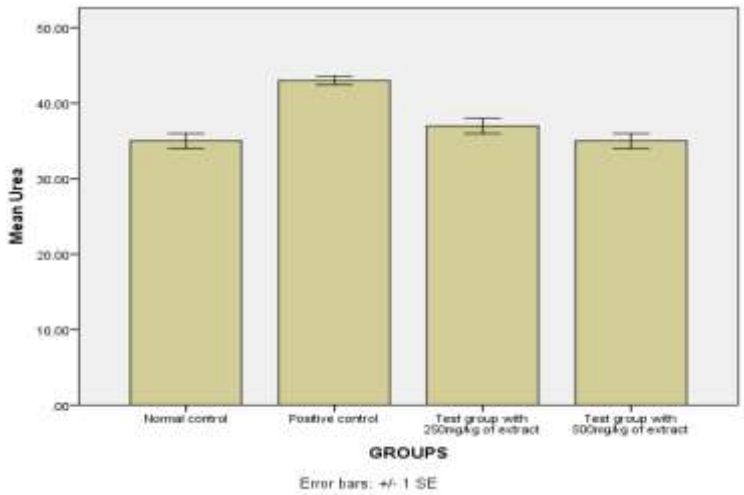

Figure 1: The comparative mean values of urea, the control and test groups

The Fig 1, is the effects of methanol extract of *Napoleonae imperialis* on urea showing the mean values of urea, the control and the test groups, with a significant decrease (p> 0.05) between the positive control and the test groups that received 250 mg/kg and 500 mg/kg body weight of the leaves extract,

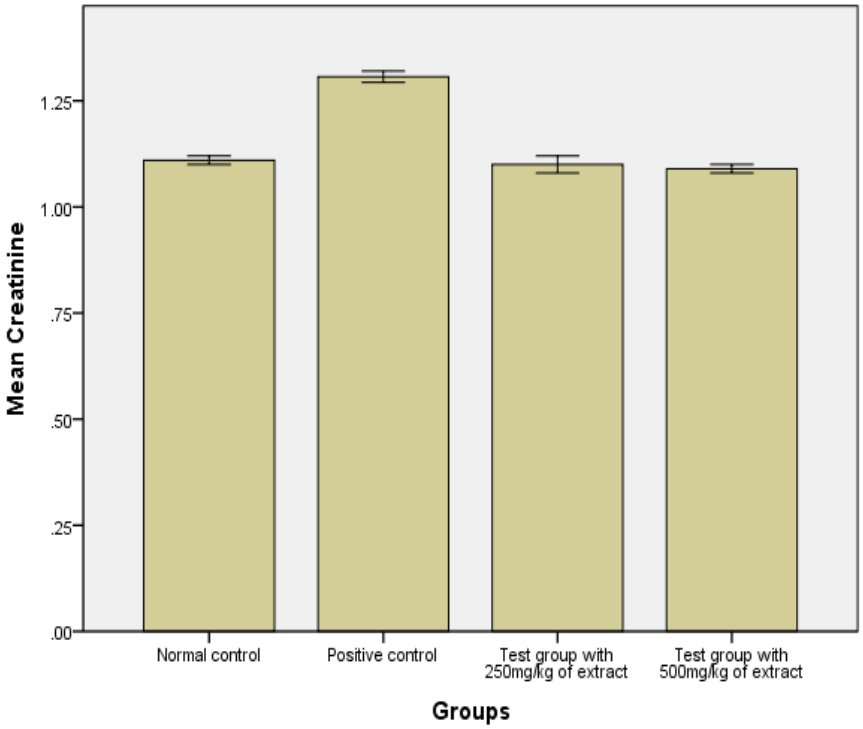

Fig 2: Comparative means values of creatinine, the control and the test groups

The Fig 2 is the effect of methanol extract of *Napoleonae imperialis* on creatinine. It shows the comparative mean values of creatinine, control and test groups, with a significant decrease (p< 0.05) between the positive control and the test groups that received 250 mg/kg and 500-mg/kg body weight of the leaves extract.

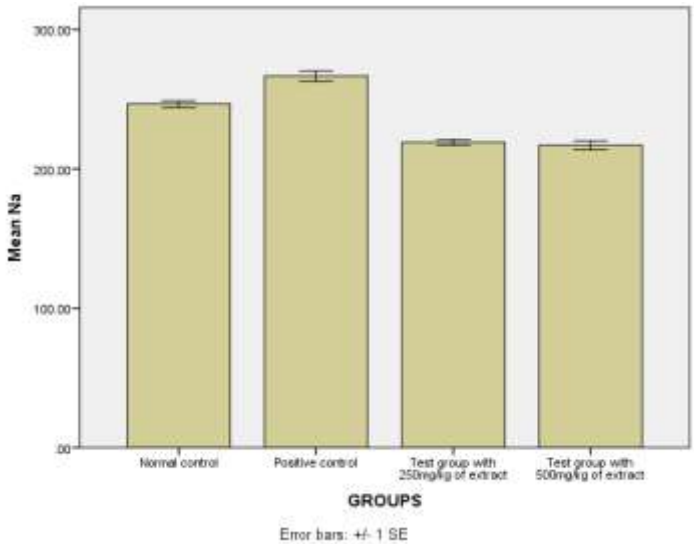

Fig 3: Mean values comparison of Na^+ between the control groups and the test groups

Fig 3 is the results of the effects of methanol extract of *Napoleonae imperialis* on sodium ion (Na^+). The figure show the comparative values for the Na^+ with a significant decrease ($p< 0.05$) between the control groups (normal and positive control) and the test groups that received 250 mg/kg and 500-mg/kg-body weight of the leaves extract.

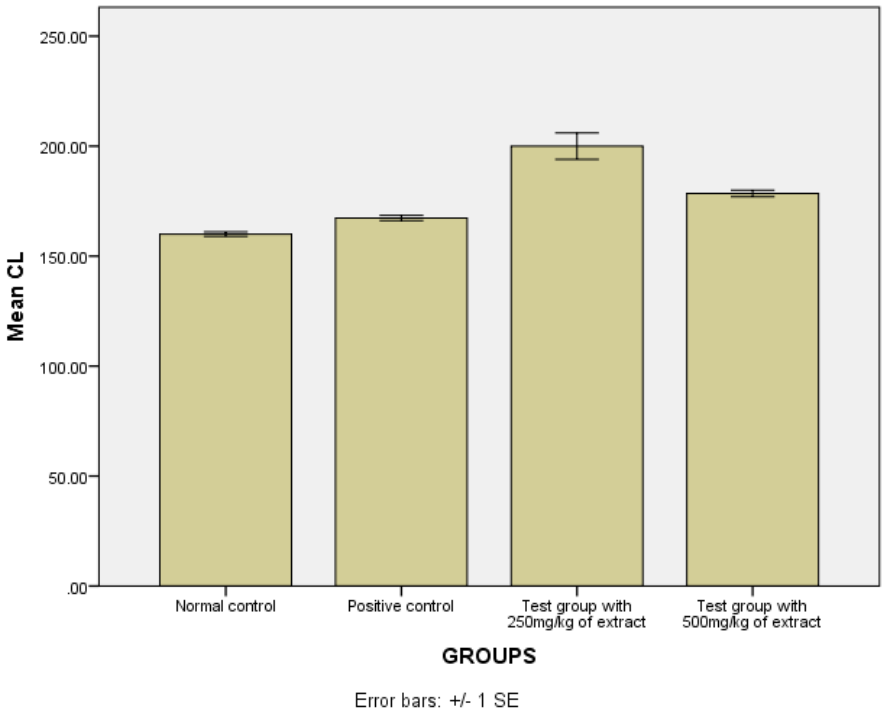

Fig 4: Mean values comparison of Cl^- between the control groups and test groups

The Fig 4 is showing the comparative values of Cl^- the control and the test group with consequent significant increase ($p< 0.05$) between the control groups (normal and positive control) and the test groups that received 250 mg/kg and 500 mg/kg body weight of the leaves extract.

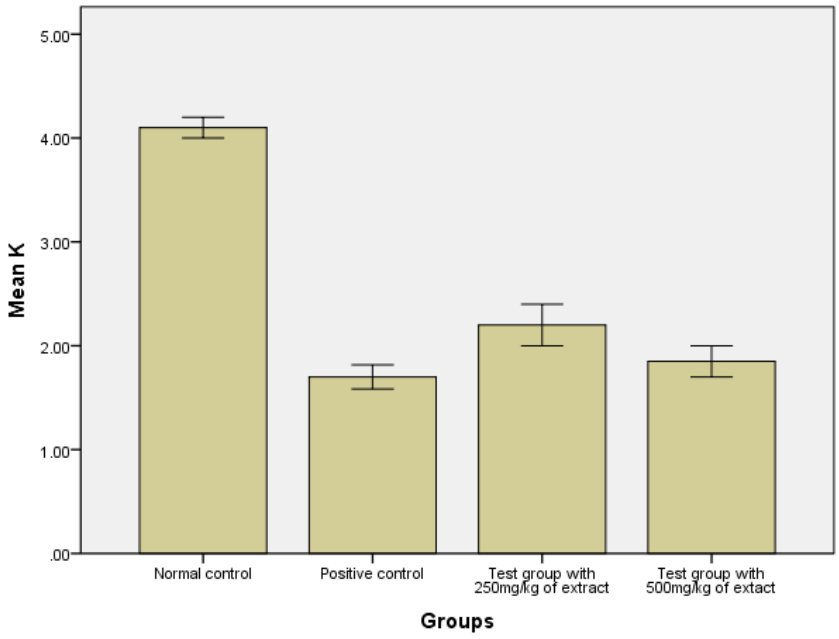

Fig 5: Mean values comparison of K^+ between the control groups and the test groups

The Effects of methanol extract of *Napoleonae imperialis* on potassium ion (K^+) is represented in the Fig 5. The figure is the comparative values of K^+ with a significant decrease ($p< 0.05$) between the normal control and the test groups that received 250 mg/kg and 500-mg/kg-body weight of the leaves extract.

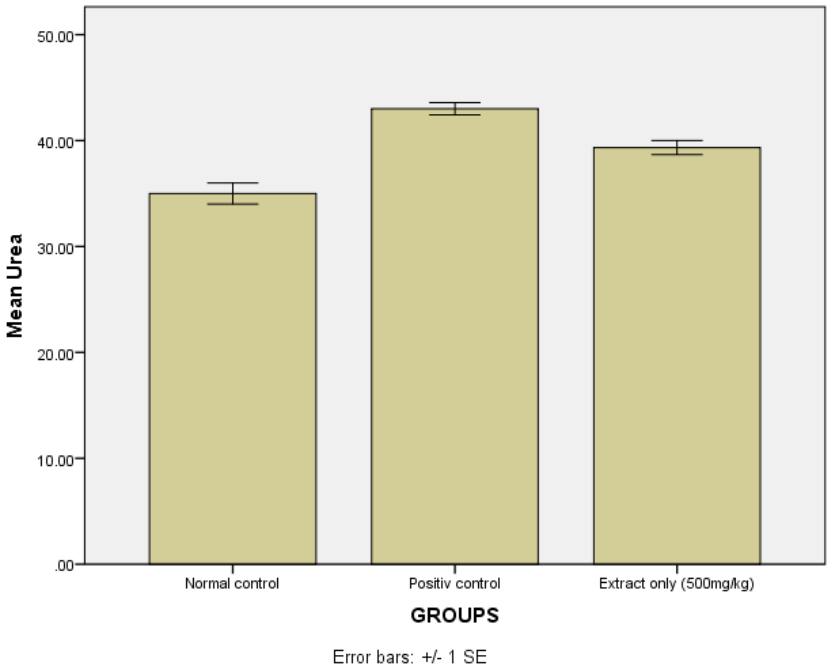

Fig 6: Mean values comparison of urea between the control groups and the group that received the extract only (500 mg/kg body weight)

This is the result on the effects of methanol extract of *Napoleonae imperialis* on urea. This Fig 6 indicates some significant increase ($p< 0.05$) between the control groups (normal and positive control) and the group that received the leaves extract only (500 mg/kg body weight).

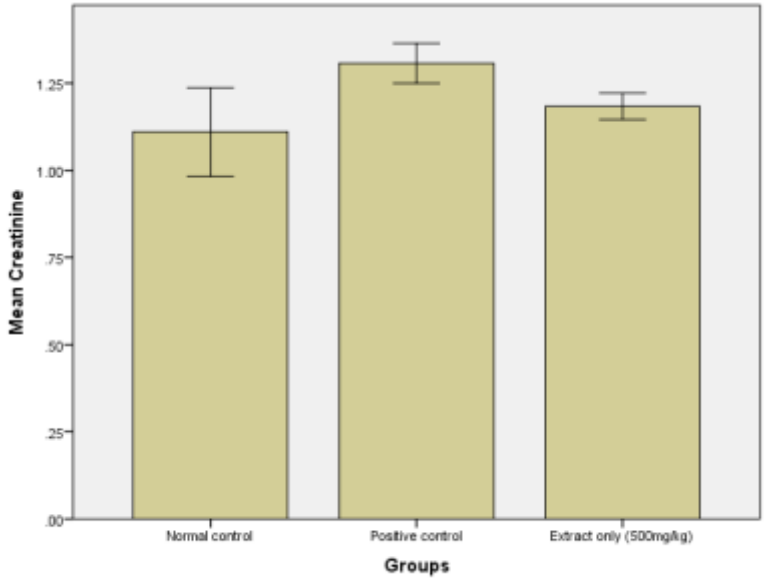

Fig 7: Mean values comparison of creatinine, the control groups and the group that received the extract only (500 mg/kg body weight)

The Fig 7 is the result on the effects of the methanol extract of *Napoleonae imperialis* on creatinine. There is a significant increase (p< 0.05) between the normal control and the group that received the extract only (500 mg/kg body weight).

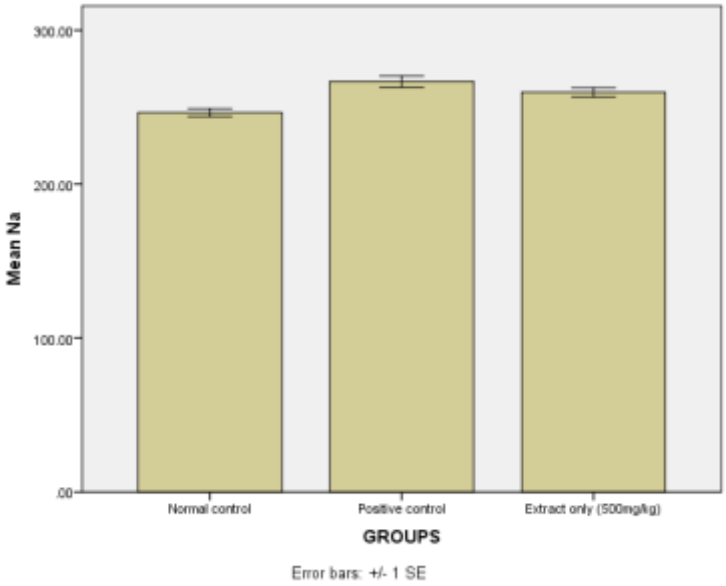

Fig 8: Mean values comparison of Na^+ between the control groups and the group that received the extract only (500 mg/kg body weight)

The Fig 8 shows the result on the effect of methanol extract of *Napoleonae imperialis* on sodium ion (Na^+). There is a significant increase (p< 0.05) between the normal control and the group that received the extract only (500 mg/kg body weight).

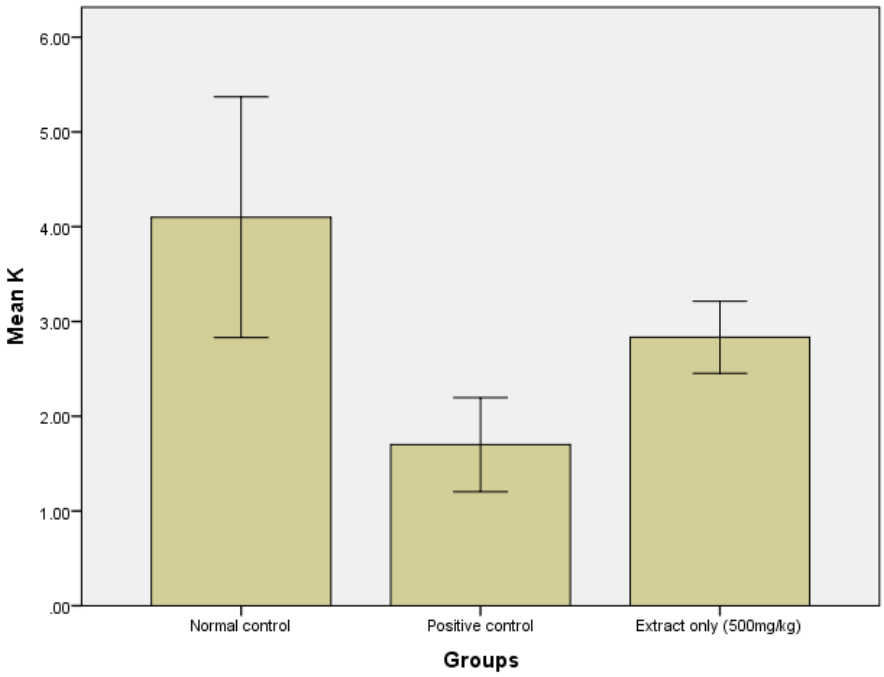

Fig 9: Mean values comparison of K^+ between the control groups and the group that received the extract only (500 mg/kg body weight)

The Fig 9 is the result on the effect of methanol extract of *Napoleonae imperialis* on potassium ion (K^+). The figure shows a significant decrease (p< 0.05) between the

normal control and the group that received the extract only (500 mg/kg body weight).

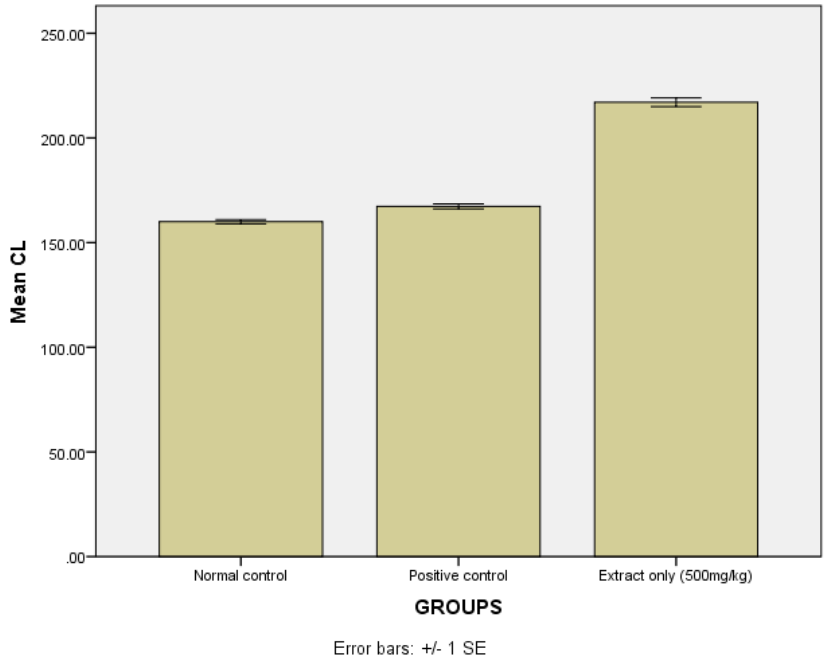

Fig 10: Mean values comparison of Cl^- between the control groups and the group that received the extract only (500 mg/kg body weight)

The Fig 10 is the effect of methanol extract of *Napoleonae imperialis* on chloride ion (Cl^-). The result shows a significant increase ($p< 0.05$) between the control groups (normal and positive control) and the group that received the leaves extract only (500 mg/kg body weight).

Histological Findings

The results from the light microscopic findings are shown in plate 11-15. The photomicrographs of group 11, (normal control group) show an evenly distributed glomeruli of smaller size, with normal mesangial cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. There is mild hemorrhage into the interstitium.

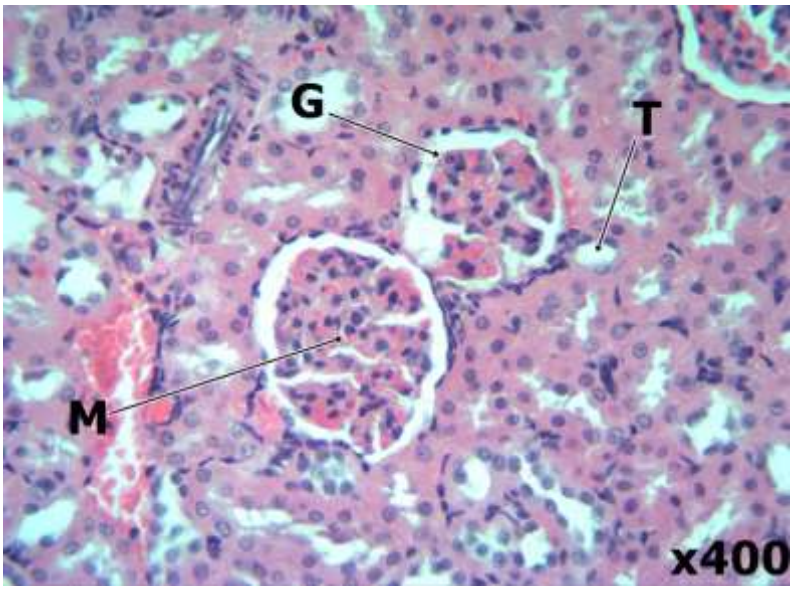

Plate 11: A cross section of the kidney of normal control group H&E x400.

Key: M= mesangium, G= glomerulus, T= tubule

However, in-group 12, which is the positive control group (was induced with CCl_4 without treatment), there is a significant pathology and mild interstitial inflammation.

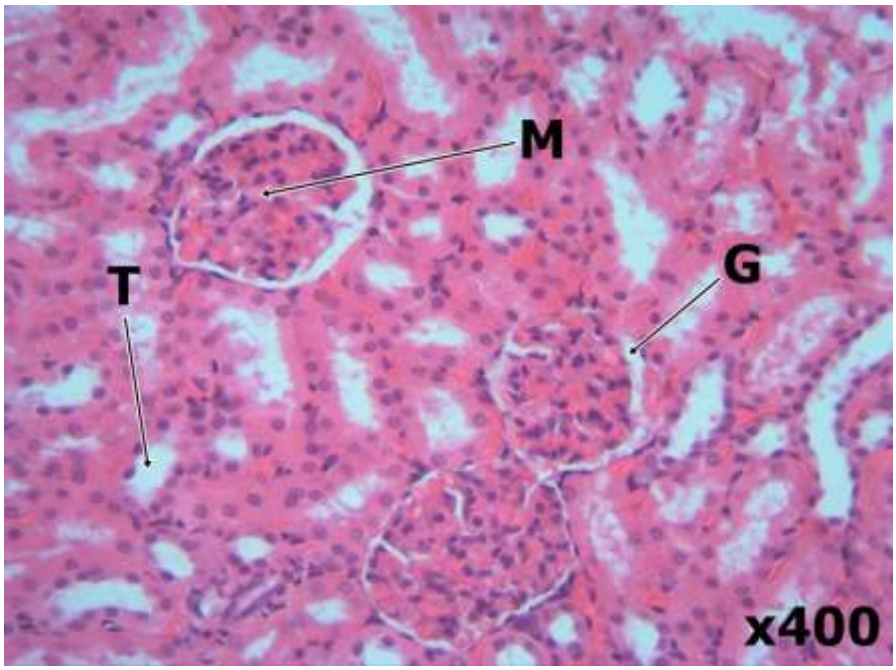

Plate 12: A cross section of the kidney of methotrexate intoxicated rats without treatment
Key: M= mesangium, G= glomerulus, T= tubule

The group 13, (test group that received 250 mg/kg body weight of the plant extract) there is no significant pathology compared to the normal and positive control.

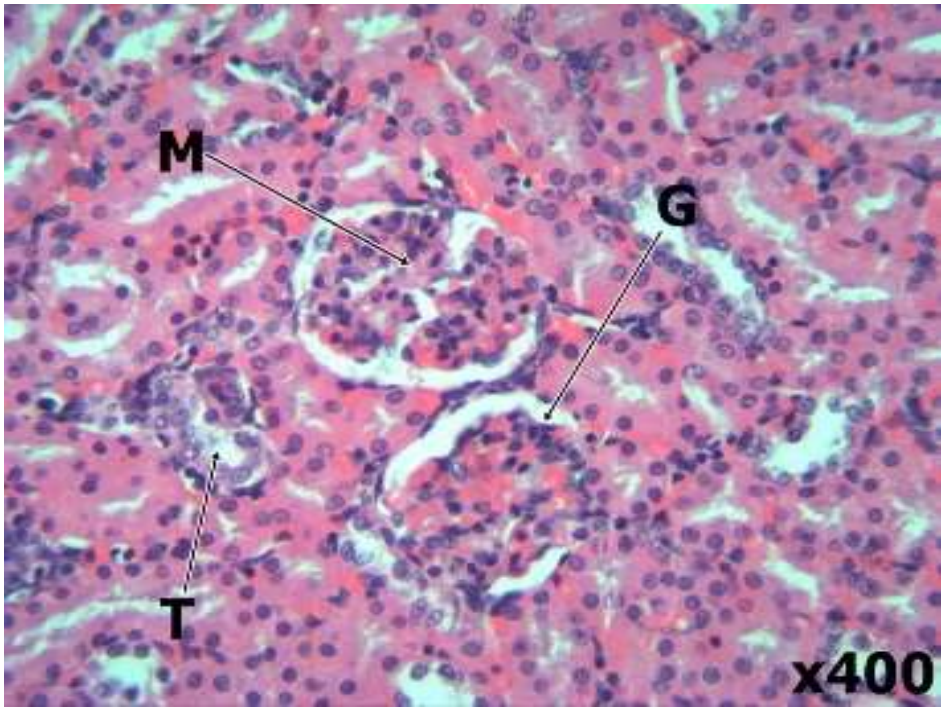

Plate 13: A cross section of the liver of methotrexate intoxicated rats treated with 250 mg/kg body weight of *Napoleonae imperialis* leaf extract. H&E x400.
Key: M= mesangium, G= glomerulus, T= tubule

The group 14 is the test group that received 500-mg/kg body weight of the plant extract; we can observe that there is no significant pathology.

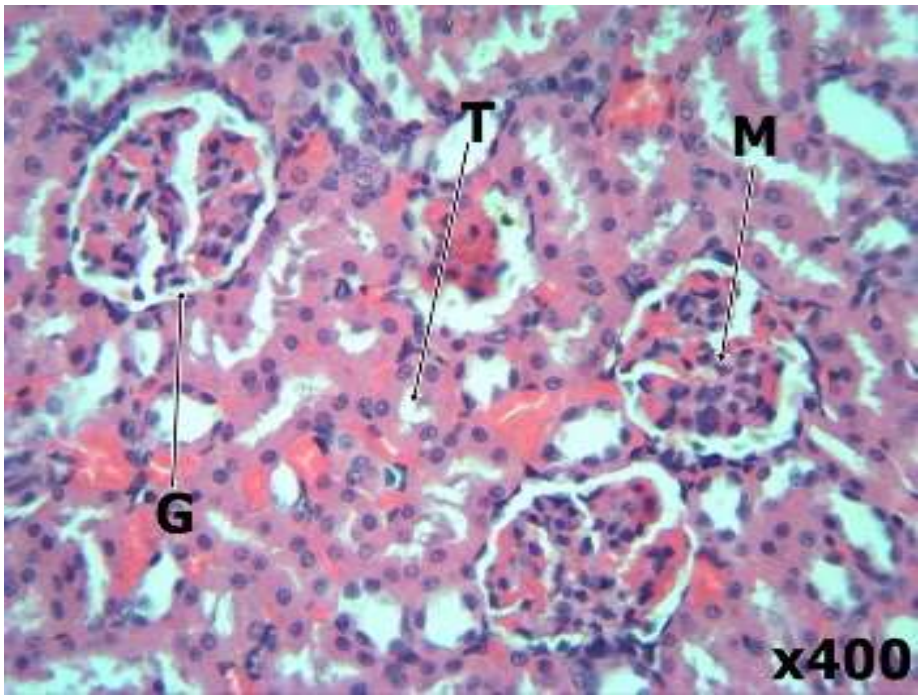

Plate 14: A cross section of the liver of methotrexate intoxicated rats treated with 500 mg/kg body weight of *Napoleonae imperialis* leaf extract. H&E x400.
Key: M= mesangium, G= glomerulus, T= tubule.

The group 15 was the group that received the extract only, and we observe that there was no significant pathology.

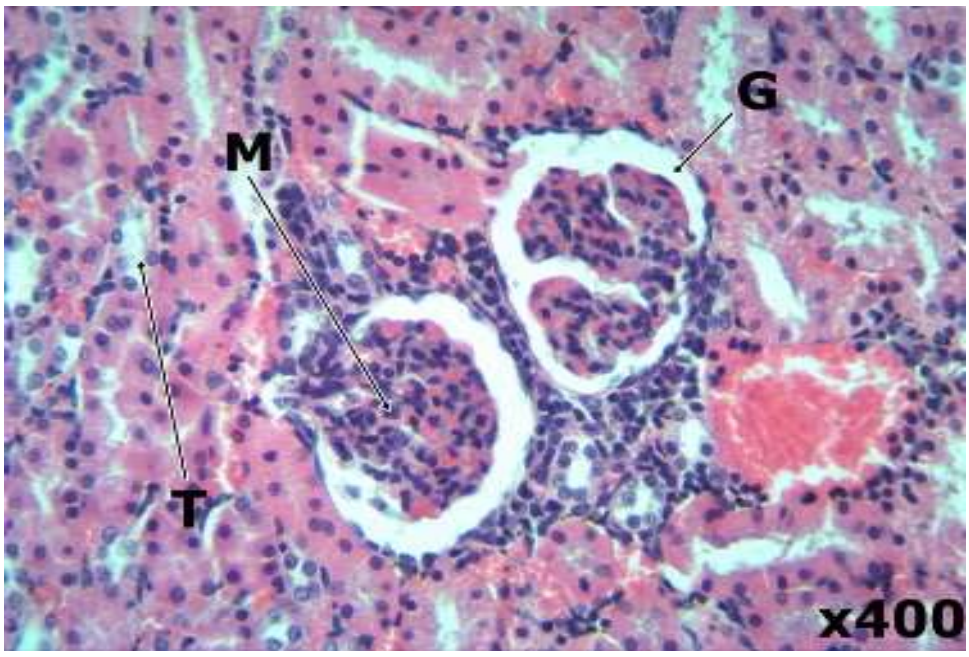

Plate 15: A cross section of the kidney treated with 500 mg/kg body weight of *Napoleonae imperialis* leaf extract only. H&E x400.
Key: M= mesangium, G= glomerulus, T= tubule.

Discussion

Methotrexate is an antimetabolite and it is an analogue of folic acid, used to cure autoimmune disorders such as psoriasis, rheumatoid arthritis and as a medicinal substance to heal several malignant neoplastic infections such as breast, skin, head, neck, lung, lymphoma, osteosarcoma and leukemia (Tousson. *et al.,* 2014). Numerous adverse effects of this drug have also been stated (Tousson. *et al*., 2014. Studies in rheumatoid arthritis, juvenile idiopathic arthritis, sarcoidosis, and psoriasis have shown that vomiting, nausea, diarrhea, and leucopenia are some of the side effects with a dose reduction response (Tousson. *et al*., 2014).

Methotrexate gets into the cell through the active transport beside the reduced folate carrier, where it is carried out of the cell by various ATP-binding cassette (ABC) transporters, usually ABCC1-5, ABCG2 and ABCB1 (Johovic. *et al.,* 2003). Following such transport, the concentration of both enzymatic and non-enzymatic activities, anti-oxidants are reduced and the amounts of free radicals are elevated in the testes, heart, liver, kidney, and gut tissues of experimental albino rats treated with MTX (Ozogula. *et al.,* 2013).

The conversion of MTX to its major extracellular metabolite, 7-hydroxy methotrexate, takes place in the kidney, where it is oxidized by a soluble enzymatic mechanism. In the evaluation of hepatic damage, the examination of enzyme concentration like alanine amino transferase (ALT) and aspartate amino transferase (AST) is highly employed, bearing their presences in the cytosol of liver (Madu. *et al*., 2021). They play a vital role in the metabolism of amino acids into α-ketoacids (Mesbah. *et al.,* 2004). The outcome of this research shows that there was a significant increase in the amount of serum ALT in MTX test groups which is in agreement with previous reports (Fu. *et al.,* 2008; Hemeida and Omar, 2008; Vaghasiya. *et al.,* 2009), who evaluated the effect of polyherbal formulation on MTX induced hepatotoxicity in rats.

The results from the present study demonstrated that methanol extract of *Napoleonae imperialis* has positive effects in animals treated with MTX to induced renal damage. There were some significant (P<0.05) increases in urea, creatinine and uric acid in MTX group when compared with control, this elevation is decreased in treated groups with methanol extract of *Napoleonae imperialis*. Our results are in agreement with previous findings, Kolli. *et al., 2008*, reported that MTX elevated urea and creatinine, Tousson. *et al.,* 2014, reported that such elevation induced renal toxicity.

Renal function depends on the integrity of absorption, reabsorption and excretion of these markers (urea, creatinine, sodium ion, chloride ion and potassium ion, among others). The observed lower concentrations of urea and creatinine in all the test groups when compared to the normal control indicated that the extract possesses renal protective/ameliorative properties comparable to the standard drug that could have improved glomerular filtration rate of urea and creatinine concentrations in the renal damage rats. The general non-significant differences observed in serum levels of urea, creatinine, sodium, and chloride ions of groups 7 and 8 treated with different doses (250 and 500 mg/kg b.wt) of the extract compared to the positive control (group 5) tend to support the efficacy of the extract to protect the nephrons from damage, which is exacerbated in nephrosis, previously reported (Udobre. *et al.,* 2012).

Furtherance, the biochemical parameters as analyzed were corroborated with the histological findings, which clearly indicates that methanol leaves extract of *Napoleonae imperialis* possess renal functioning properties capable of maintaining kidney functions through stabilization of membrane as noticed in the decreased amount of renal parameters and also promote proper kidney functions. The extract was most useful in the treatment of kidney damage at a lower dose 250-mg/kg body weights, as the renal functioning activity decreases with increasing doses, which could

be an indication that the extract may contain other components that may have interfered with its renal protective activity. The results from the study are indicative of synergistic effects of bioactive flavonoids, total phenolics, saponins, and alkaloids present in the methanol extract of the plant *Napoleonae imperialis* leaves that are beneficial in maintaining kidney integrity and functions. However, further studies are required to home in on the specific bioactive compound that may be most active.

Further, we show that the tissue segment of kidney of control animals were fundamentally normal. However, the histopathological study displayed that oral administration of the extract to rat at various doses appears to have restored the damage consequence, of MTX action on the kidneys. The pathological changes observed in these organs also appear to be dose dependent. The uncontrollable damage to the kidney is not surprising or amazing because the kidney is the main organ of excretion. Our report concurs with the findings of Abdulrahan. *et al*., 2007, as they had earlier published some work on renal and hepatic damage of albino rats administered with aqueous root bark extract of *Vitex doniana* and rats fed with feeds comprising anti-Nutritive compounds of plants origin such as; saponins and tannins. Increased sodium creatinine and urea levels have been reported (Ogunmike, 2002), when rats were treated with aqueous root bark extract of *Vitex doniana* extract for 14 days.

The mechanism by which MTX causes nephrotoxicity results from binding to the enzyme dihydrofolic reductase, this prevents the conversion of folic acid to its active form, folinic acid, blocking the synthesis of nucleic acids, certain amino acids and proteins. This can cause damage to cell organs and cell membranes of kidney connective tissues interfering with their function and allowing leakage of enzymes (Penalva. *et al.,* 2002). Further, it may also be probable that MTX mechanistically, share some similarity with glycerol induced nephrotoxicity, which is thought to be mediated by renal ischemia and myoglobin nephrotoxicity involving the rearrangement of some cellular events (Hsu. *et al*., 1997). Following, we postulate, the reason for the organ liaison observed, may be due to the derangement of cellar phospholipid membranes. Such action have potential to trigger some sequences of biochemical events that may lead to irreversible cellular injury, presumably due to enhanced generation of oxygen derived free radicals (Nwachukwu. *et al*., 2020), and activation of tissue phospholipases (Al Asmari. *et al*., 2017).

This study further lead credence, phyto-compounds fully harness, may provides series of pharmacophores whose endogenous role can be directly exploited to treat human infections or conditions of aberrant cell proliferation such as cancer, health diseases, hepatic cells repairs and other genetics diseases including SCD.

Most recently, scientific understanding of the molecular biology of the epigenes that underlie many disease states has grown, helping to increase the scope of potential drug targets. The advances in genome sequencing including; bioinformatics and the maturing of heterologous expression platforms should increase a renaissance in phyto-medicinal plants. Also, the increasing numbers of genomes and transcriptomes of different plant species producing diverse chemistries are becoming available. Hence, natural product chemistry combined with biophysical tools will be essential in the attempt to generating, wealth of interesting structures, the development of powerful tools that would allow the combination of disparate biophysical methods for the exploration of the biosynthesis and mechanistic pathways of these plant based phyto-compounds are imperative for a robust processes towards enhancing the proper harnessing bioactive compounds for disease management (Okoh. *et al*., 2021).

Conclusion

The results and findings of this study indicated that methanol leaves extract of *Napoleonae imperialis* possess nephroprotective

properties capable of maintaining renal functions through stabilization of membrane. The extract was most useful in the treatment of kidney damage at a lower dose 250 mg/kg body weight, as the nephroprotective activity decreases with increasing doses, which could be an indication that the extract contain other components that could have interfered with its nephroprotective property. Further studies are required enabling the formulation of more effective renal drugs of plant origin that will improve human health. Thus, new approaches in the use of plant extracts and possibly diet in the treatment and management of renal malfunction should be advanced to improve the lives of patients.

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