



Ameliorative Effect of the Methanol Extract of *Napoleonae imperialis* Leaves against Methotrexate-induced Renal Damage in Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author OJM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GSA and ENU managed the analyses of the study. Author OJM equally managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed to evaluate the effect of methanol extract of *Napoleonae imperialis* leaves against methotrexate renal damage in albino rats.

Methodology: Thirty (30) male albino rats of mean weight 130 g were used for this study. The animals for the study were grouped into five (5) of six (6) rats each. Group A (normal control) received feed and water only and Group B (positive control) was induced with methotrexate without treatment. Test groups (C and D) were orally given 250 mg and 500 mg/kg b.wt of leaves extract, and group E was orally given the extract only (500 mg/kg b.wt) respectively for 14 days. All the rats used in this study were initially subjected to renal damage using 0.5 ml/kg of methotrexate except the normal control group. The rats were sacrificed after 14 days and the blood samples were collected for biochemical analysis.

Results: From the result obtained, there was a significant ($p < 0.05$) decrease in the groups that received 250 mg/kg and 500 mg/kg b.wt of the plant extract in (Urea, Creatinine and Na^+), and a significant increase ($p < 0.05$) in K^+ and Cl^- . Also there was a significant ($p < 0.05$) decrease in (Urea,

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Creatinine, and Na⁺) in comparison with the control groups and the group that received the extract only (500 mg/kg b.wt), and a significant increase (p< 0.05) in (K⁺ and Na⁺).

Conclusion: The study indicates that the methanol extract of *Napoleonae imperialis* leaves may have exerted renal functioning effects in albino rats, and may also be used pharmacologically in the management of organ toxicity.

Keywords: Creatinine; urea; renal damage; methotrexate; sodium ion.

1. INTRODUCTION

Kidney malfunction frequently existing collectively, both as part of multiorgan dysfunction in an unfavourably sick patient, or due to damage of each organ individually. Three principal medical conditions can be recognized in which kidney malfunction coincide; infections concurrently relating the liver and the kidney, or a basic liver disorder with elementary kidney malfunction, or vice versa [1]. Attendant kidney abnormality may exhibit mutual pathogenetic mechanisms. 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, which trigger severe failure of both organs [2]. Kidney dysfunction relative to hepatic malfunction is usually efficient in nature and arises due to the absence of important modifications in kidney histology (pre-renal). However, inherent kidney dysfunction may lead to severe or prolonged hepatic disease (inherent kidney failure) [1]. Preventive uropathy could result to postrenal 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) [3]. Hepatorenal disease is a major type of practical kidney malfunction that frequently result in progressive hepatic pathology, kidney disorder or threshold hypertension [4].

Kidney damages could be triggered by several toxic agents like certain antibiotics, chemotherapeutic agents, carbon tetrachloride (CCl₄), thioacetamide (TAA), methotrexate (MTX), other haloalkanes, excessive alcohol consumption and microbes is well studied.

Consequently, in this present study the effects of methanol extract of *Napoleonae imperialis* against methotrexate renal damage were studied on albino rats

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of the plant *Napoleonae imperialis* were obtained from a local farm in Umuariaga village, Umudike, Abia State, Nigeria, and identified by Dr. Garuba Omosun of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike.

2.2 Experimental Animals

Healthy male albino rats of mean weight 130 g were obtained from Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The rats were freely allowed access to standard feed and water *ad libitum*. All experiments with the Laboratory Animals were conducted in accordance with National Institute of Health Guidelines revised in 1996 (NIH Publications No. 8- 23).

2.3 Drugs and Chemicals

Methanol and methotrexate were purchased from Sigma-Aldrich (Steinheim, Germany). 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.

2.4 Preparation of Extract

The fresh leaves of the plant collected were washed and dried under shade at room temperature and then blended to powdery form using a blender. The powdered leaves 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 then the filtrate was allowed to evaporate using the water bath at a temperature of 40°C to dryness and then used for the study.

2.5 Experimental Design

Thirty (30) male albino rats of mean weight 130 g were used for this study. The animals for the

study 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 with methotrexate (MTX) 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 methotrexate (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% formosaline). Blood samples were collected in the plain bottle for the analyses of the effect of the methanol extract on the kidney biochemical parameters.

2.6 Evaluation of the Various Parameters Studied

2.6.1 Determination of serum creatinine concentration

Determination of serum creatinine concentration was done by Jaffe's reaction as described by [5].

2.6.2 Determination of serum urea concentration

Serum urea concentration was determined by the method of [6].

2.6.3 Tests for electrolyte activity

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

The serum sodium and potassium ion concentration was determined using the method of [7].

2.6.3.2 Estimation of serum chloride concentration

Serum chloride ion concentration was determined using the method of [8].

2.7 Statistical Analysis

The data were expressed as Mean \pm Standard error of Mean (Mean \pm SEM) and presented as figures. Data were analysed using statistical package for the social sciences (SPSS 22.0). Comparison was made between the test groups

and the control groups using One way Anova and $P = 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effects of Methanol Extract of *Napoleonae Imperialis* on Urea

In Fig. 1, there is 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.

3.2 Effects of Methanol Extract of *Napoleonae imperialis* on Creatinine

In Fig. 2, there is 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.

3.3 Effects of Methanol Extract of *Napoleonae imperialis* on Sodium Ion (Na^+)

There is 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.

3.4 Effects of Methanol Extract of *Napoleonae imperialis* on Chloride ion (Cl^-)

There is a 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.

3.5 Effects of Methanol Extract of *Napoleonae imperialis* on Potassium Ion (K^+)

In Fig. 5, there is 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.

3.6 Effects of Methanol Extract of *Napoleonae imperialis* on Urea

In Fig. 6, there is 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).

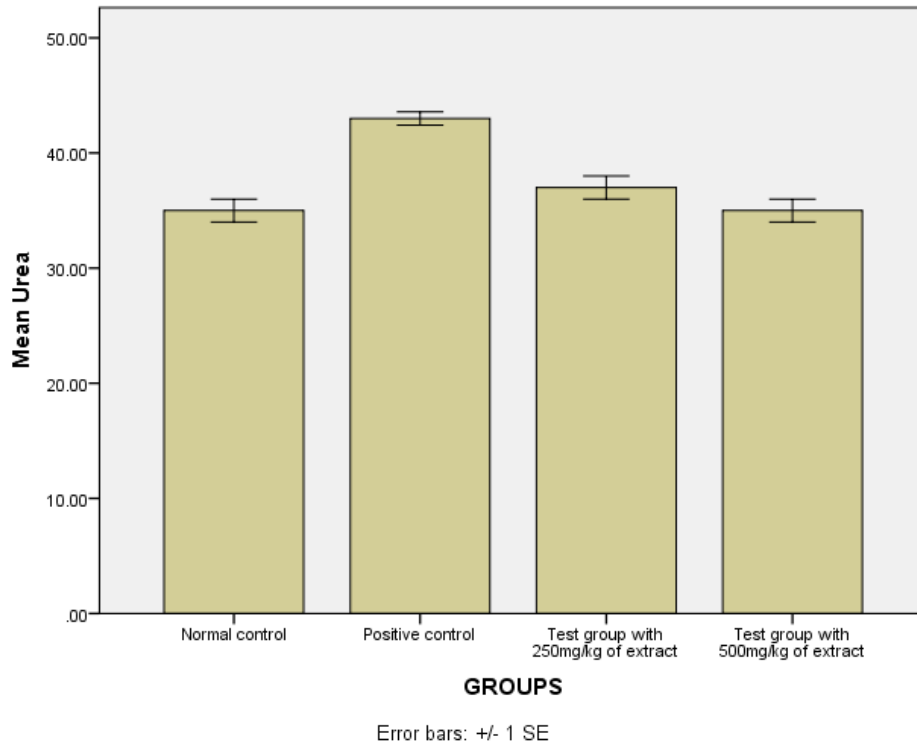


Fig. 1. Mean values comparison of urea between the control groups and test groups

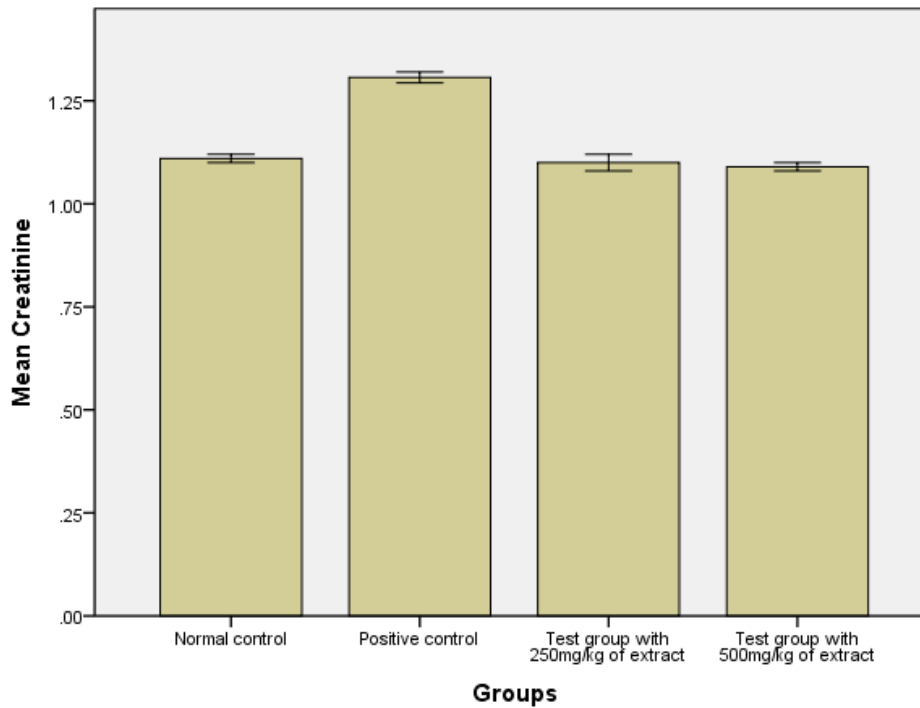


Fig. 2. Mean values comparison of creatinine between the control groups and the test groups

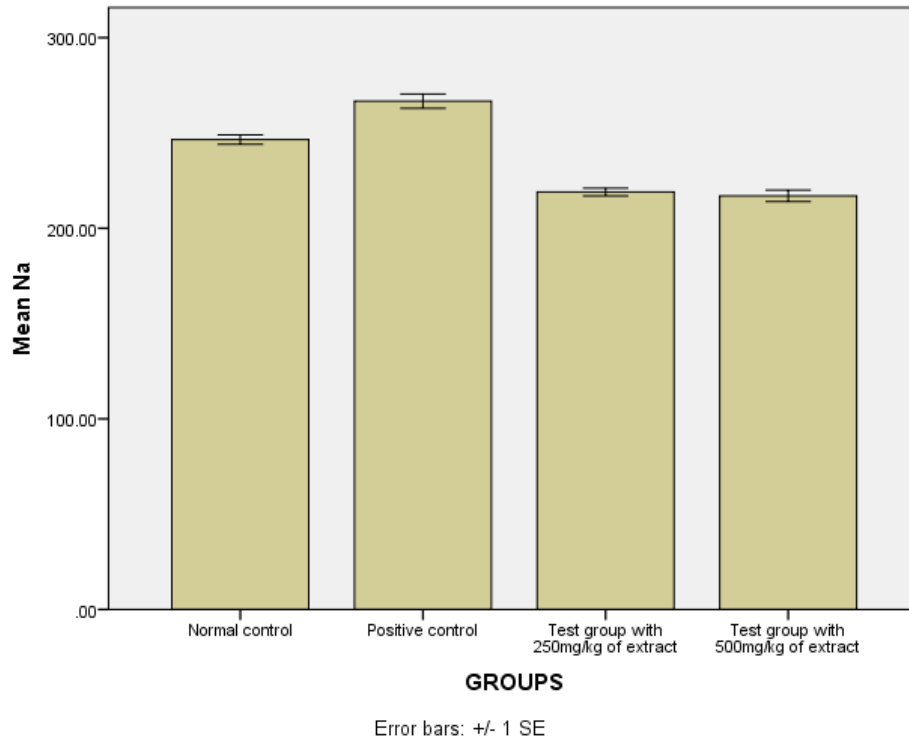


Fig. 3. Mean values comparison of Na⁺ between the control groups and the test groups

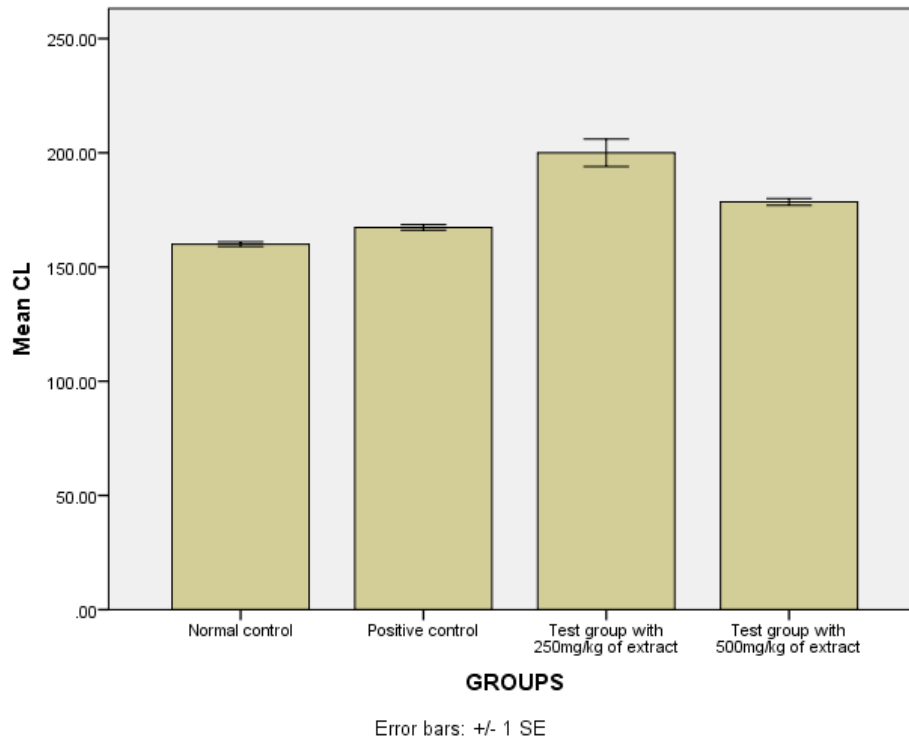


Fig. 4. Mean values comparison of Cl⁻ between the control groups and test groups

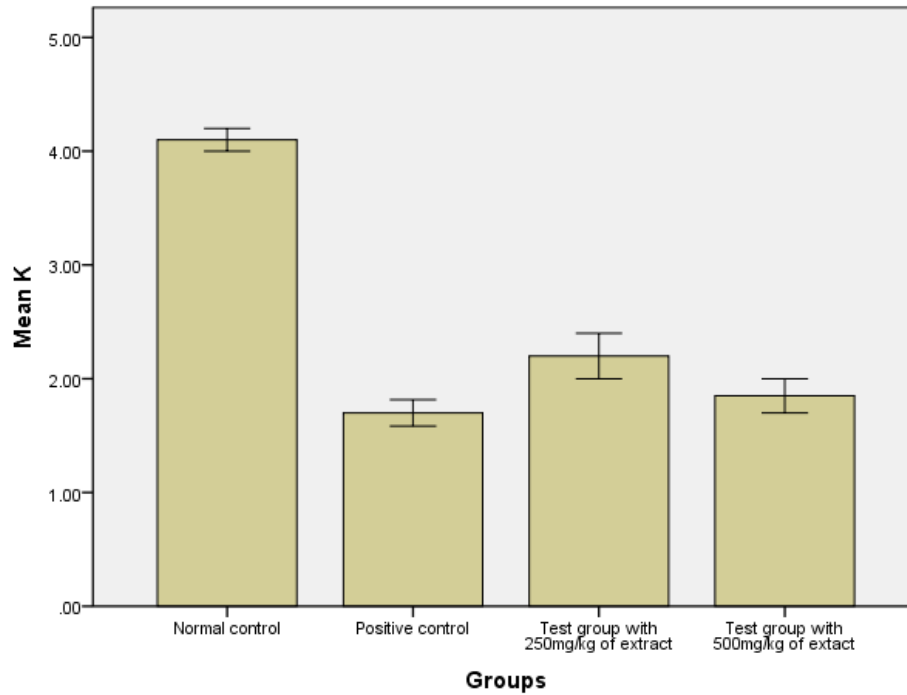


Fig. 5. Mean values comparison of K^+ between the control groups and the test groups

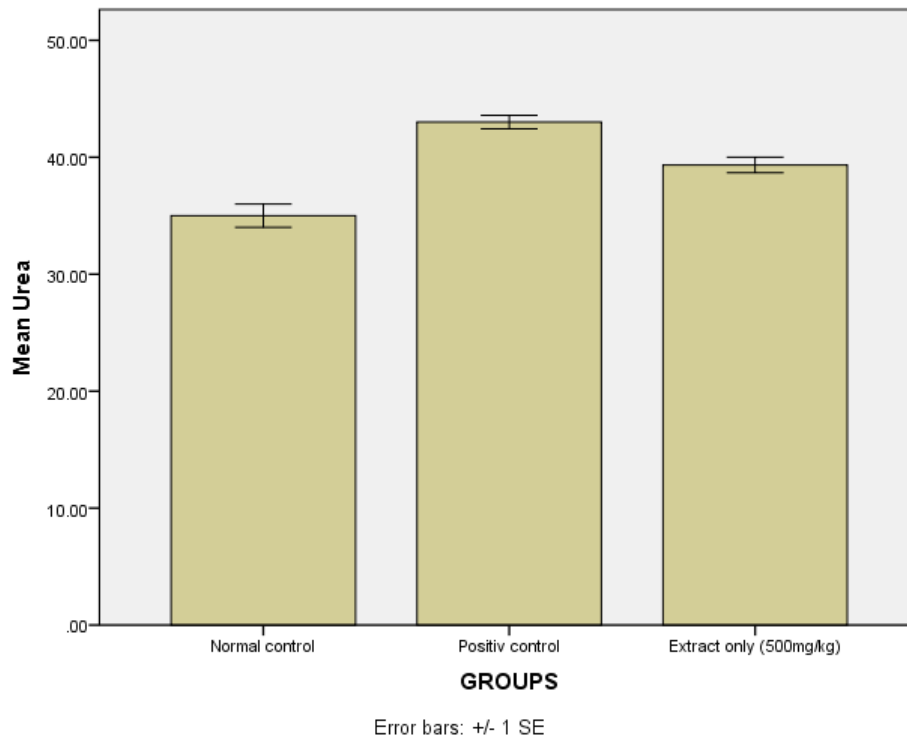


Fig. 6. Mean values comparison of urea between the control groups and the group that received the extract only (500 mg/kg body weight)

3.7 Effects of Methanol Extract of *Napoleonae imperialis* on Creatinine

In Fig. 7, 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).

3.8 Effects of Methanol Extract of *Napoleonae imperialis* on Sodium Ion (Na^+)

In Fig. 8, 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).

3.9 Effect of Methanol Extract of *Napoleonae imperialis* on Potassium Ion (K^+)

In Fig. 9, there is a significant decrease ($P = 0.05$) between the normal control and the group that received the extract only (500 mg/kg body weight).

3.10 Effects of Methanol Extract of *Napoleonae imperialis* on Chloride Ion (Cl^-)

In Fig. 10, there is 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).

Methotrexate is an antimetabolite and an analogue of folic acid that 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 [9]. Numerous adverse effects have been stated. Studies in rheumatoid arthritis, juvenile idiopathic arthritis, sarcoidosis, and psoriasis have shown that vomiting, nausea, diarrhea, and leucopenia are the most frequent side effects that usually respond to dose reduction [10].

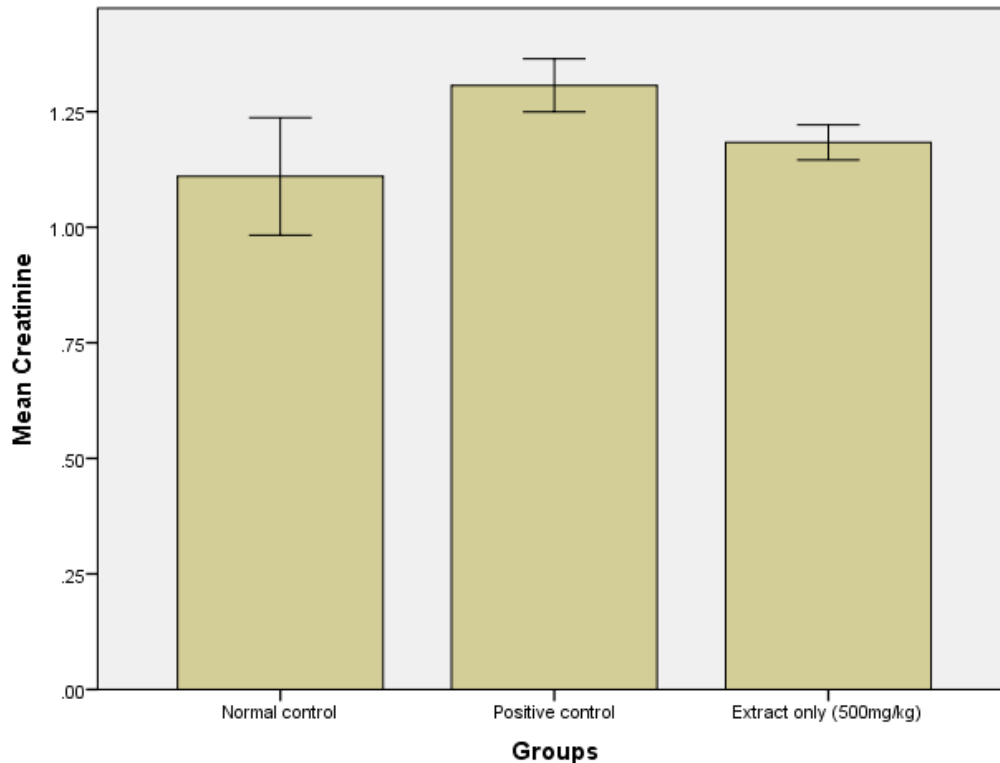


Fig. 7. Mean values comparison of creatinine between the control groups and the group that received the extract only (500 mg/kg body weight)

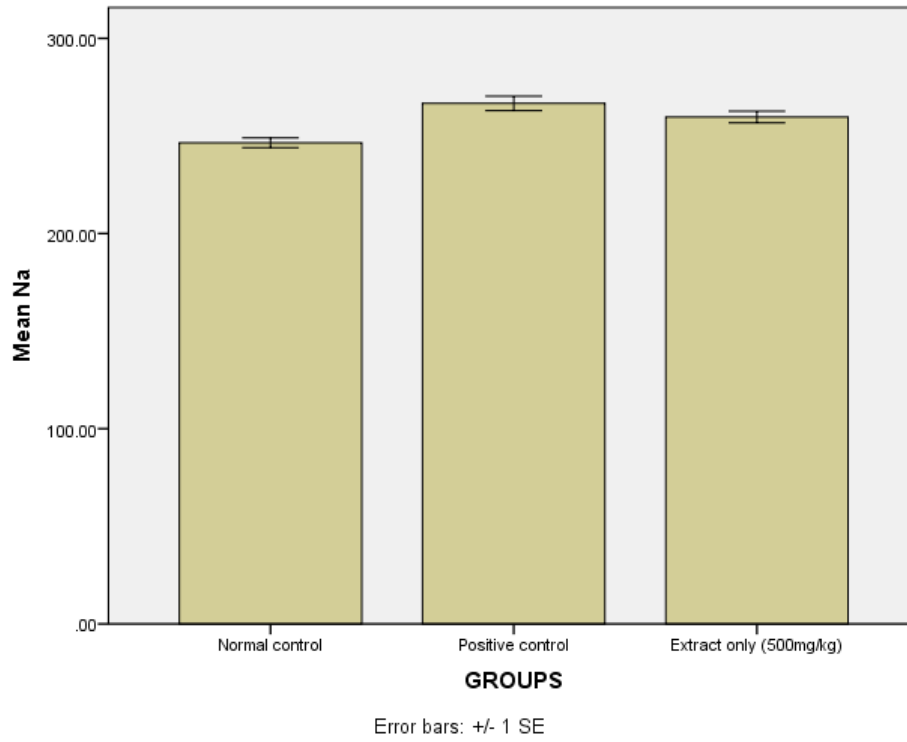


Fig. 8. Mean values comparison of Na⁺ between the control groups and the group that received the extract only (500 mg/kg body weight)

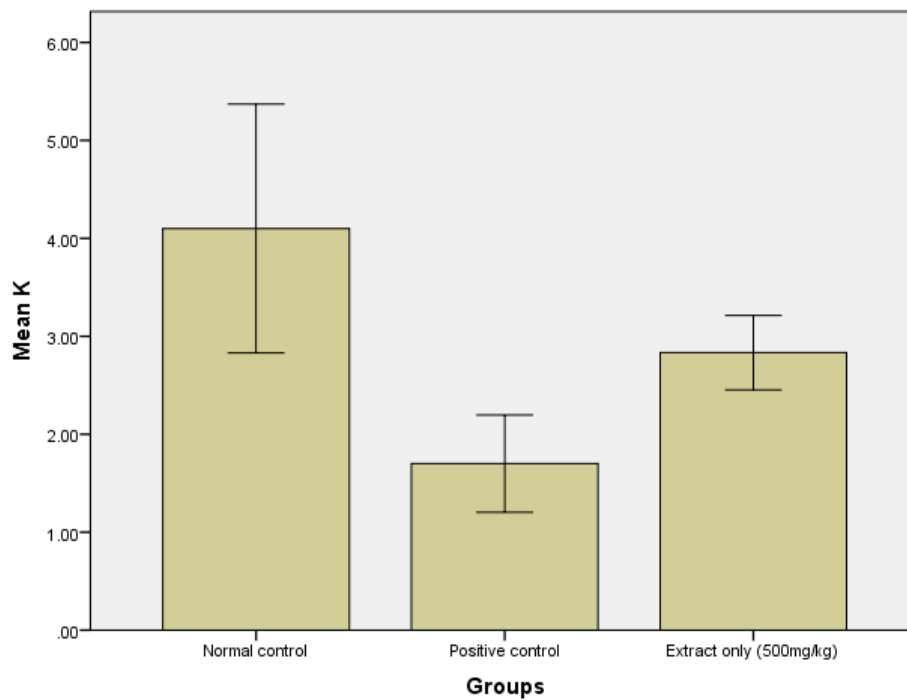


Fig. 9. Mean values comparison of K⁺ between the control groups and the group that received the extract only (500 mg/kg body weight)

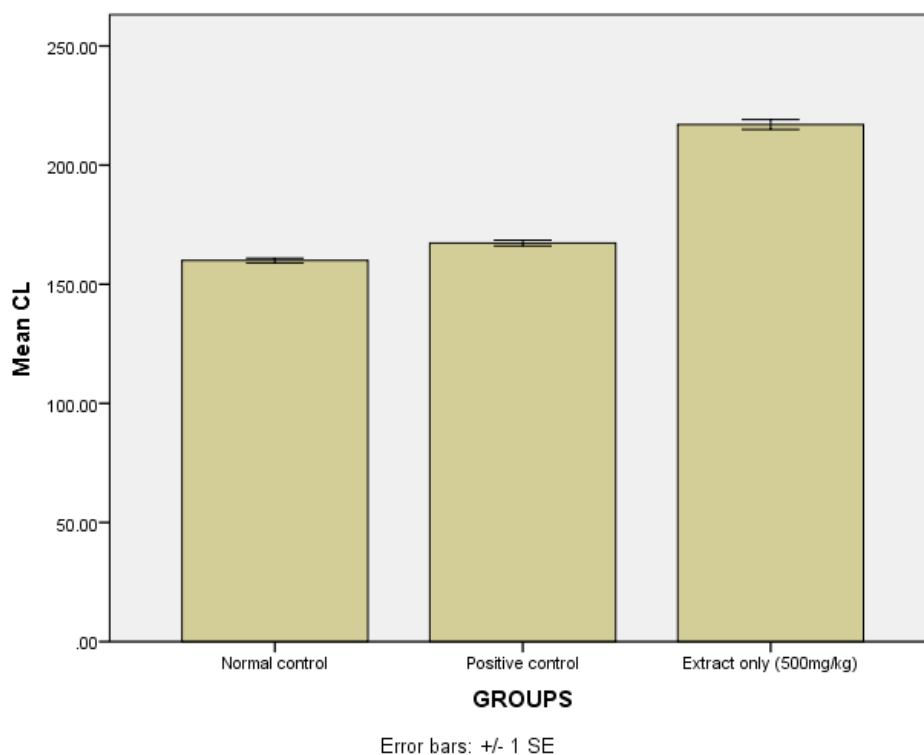


Fig. 10. Mean values comparison of Cl⁻ between the control groups and the group that received the extract only (500 mg/kg body weight)

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 [11]. The concentration of both enzymatic and non-enzymatic 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 methotrexate [12].

In kidney, the conversion of MTX to its major extracellular metabolite, 7-hydroxy methotrexate, takes place where it is oxidized by a soluble enzymatic system. In the evaluation of hepatic damage, the examination of enzyme concentration like ALT and AST is highly employed and present in the cytosol of liver. They play a vital role in the metabolism of amino acids into α -ketoacids [13]. The outcome of this research shows that there was a significant increase in the amount of serum ALT in methotrexate test groups which is in agreement with [14,15,16], who evaluated the effect of polyherbal formulation on methotrexate induced hepatotoxicity in rats.

The mechanism by which MTX causes nephrotoxicity results from binding to the enzyme dihydrofolic reductase, thus preventing conversion of folic acid to its active form, folinic acid. This in turn blocks the synthesis of nucleic acids, certain amino acids and indirectly proteins. This can cause damage to cell organs and cell membranes of kidney connective tissues interfering with their function and allowing leakage of enzymes [17].

In this present research, there was a significant ($P < 0.05$) increase in urea, creatinine and uric acid in MTX group when compared with control, this elevation decreased in treated groups with methanol extract of *Napoleonae imperialis* and increased in methotrexate self-healing when compared with MTX group. Our result is in agreement with [18] who, reported that Methotrexate elevated urea and creatinine effects and also with [9], who discovered that MTX raised urea and creatinine levels which 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 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. Similar results have been reported by several other researchers [19].

4. CONCLUSION

The findings of this study indicate 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 weight, as the renal functioning activity decreases with increasing doses which could be an indication that the extract may contain other components that could have interfered with its renal activity. The study discovers the possible synergistic effect of bioactive flavonoids, total phenolics, saponins, and alkaloids present in the methanol extract of *Napoleonae imperialis* leaves that can be beneficial in maintaining kidney integrity and functions in methotrexate induced kidney damage.

The result of this study, shows that methanol extract of *Napoleonae imperialis* possesses renal functioning effect. The plant should therefore be employed in the formulation of more effective renal drugs that will improve human health.

Thus, a new approach 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.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-

23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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