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Histopathological Evaluation 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 CEO, PCC and UIE 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 at investigating the effect of methanol extract of *Napoleonae imperialis* leaves against methotrexate renal histology 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 received feed and water only and Group B was induced with methotrexate without treatment. 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 28 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 28 days, and the kidney were carefully dissected from the abdominal region. They

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were fixed in normal saline for 72 hours and sliced into a thickness of 2.1mm samples of and processed for histopathological examination.

Results: The photomicrographs result showed that in group A, (normal control group) evenly distributed glomeruli of smaller size, with normal mesangial cellularity. In group B, (positive control group) there is a significant pathology and mild interstitial inflammation. In groups (C and D) (tests group that received 250 and 500 mg/kg b.wt of the extract) there is no significant pathology, in group E, there is no significant pathology.

Conclusion: The results of this study indicate that the leaves extract may have exerted nephroprotective effects in albino rats, and may also be used pharmacologically in the management of organ toxicity.

Keywords: Renal histology; methotrexate; histopathological examination; Napoleonae imperialis; kidney.

1. INTRODUCTION

Homoeopathic plants are plants which comprise ingredients that could be used for healing purposes or which are intermediates for the production of important medicines [1]. Homoeopathic plants, since ancient time have been applied in almost all traditions as a source of medicine. More than five thousand plants are identified to exhibit therapeutic properties in the world, but not many have been evaluated or examined [2]. Natural substances derived from plants could be alternative active ingredient for the invention of important biological function, that is for the treatment for cancer and free radical scavengers [3].

Napoleonae imperialis is a small, evergreen tropical West African tree in the family lecythidaceae, native to Africa [4], it grows to some 6m height, with a dense, and low branching crown. The showy flowers have two inner rows of petal and vary in colour, usually creamy yellow, along the circumference, with the centre varying from red to apricot to purple, they develop either as tender trunks or develop mainly from the ancient wood of the branch. The fruit is a berry, dark orange or reddish brown containing kink a kidney shaped seed. The specie is popularly cultivated as an ornamental tree [5].

Extracts from the leaves and toxic seeds display bactericidal activity and contain various glycosides, tannins, proteins and saponins, with flavonoids, resins and steroids absent. E. coli, B. subtilis and Pseudomonas aeruginosa which are repressed, is affirming the applications of the organisms in traditional medicine. In another experiment Bilgin et al. [6], prepared an herbal ointment of the methanol solution of *Napoleonae imperialis* and examined its wound healing effect by the excision wound model on guinea pigs. The result of the experiment indicates that *Napoleonae imperialis* extract possess a better wound healing property as compared to the antibiotic used as control. The bark and fruit pulp are chewed to alleviate pulmonary problem [7].

Consequently, in this study we monitored the effects of histopathological examination of methanol extract of *Napoleonae imperialis* against methotrexate (MTX) renal damage in albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material

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. 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.

2.2 Extraction

The powdered leaves of *Napoleonae imperialis* (120 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^{o}C to dryness and then used for the study.

2.3 Animals

Healthy male albino rats of 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*.

2.4 Induction of Nephrotoxicity

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

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 (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 28 days and after which the animals were sacrificed on day 15 under mild anesthesia (10% formasaline).

2.6 Histopathological Examination

The method described by Odula et al. [8] 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.

3. RESULTS AND INTERPRETATION

3.1 Histological Effects of Methanol Extract of *Napoleonae imperialis* on the Kidney

In Group 1, (normal control group) photomicrographs show 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 haemorrhage into the interstitium.

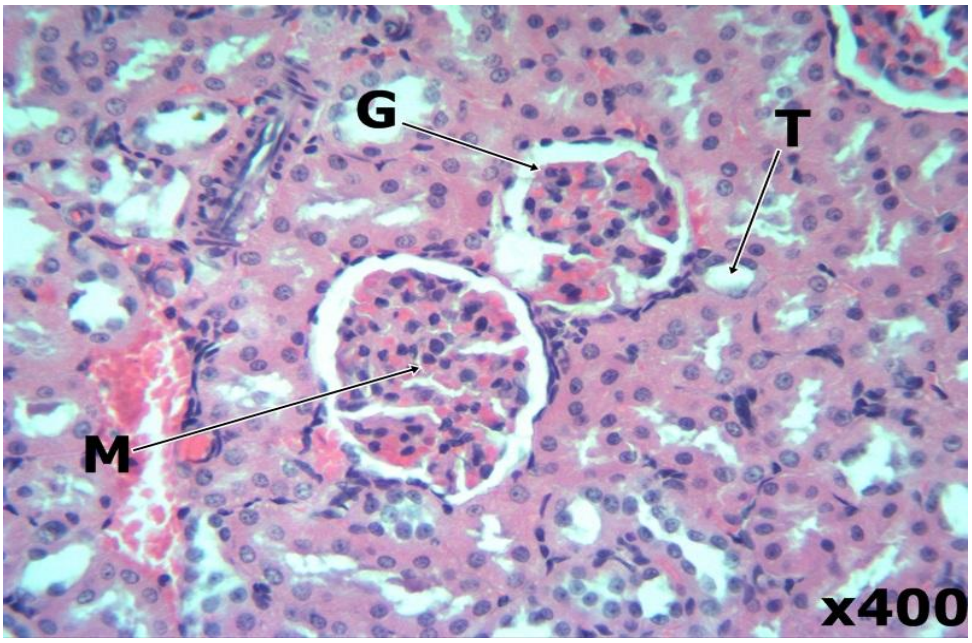

Plate 1. A cross section of the kidney of normal control group H&E x400
M= mesangium, G= glomerulus, T= tubule

In Group 2, (positive control group) there is a significant pathology and mild interstitial inflammation.

Group 3, (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.

In Group 4 (test group that received 500 mg/kg body weight of the plant extract) there is no significant pathology.

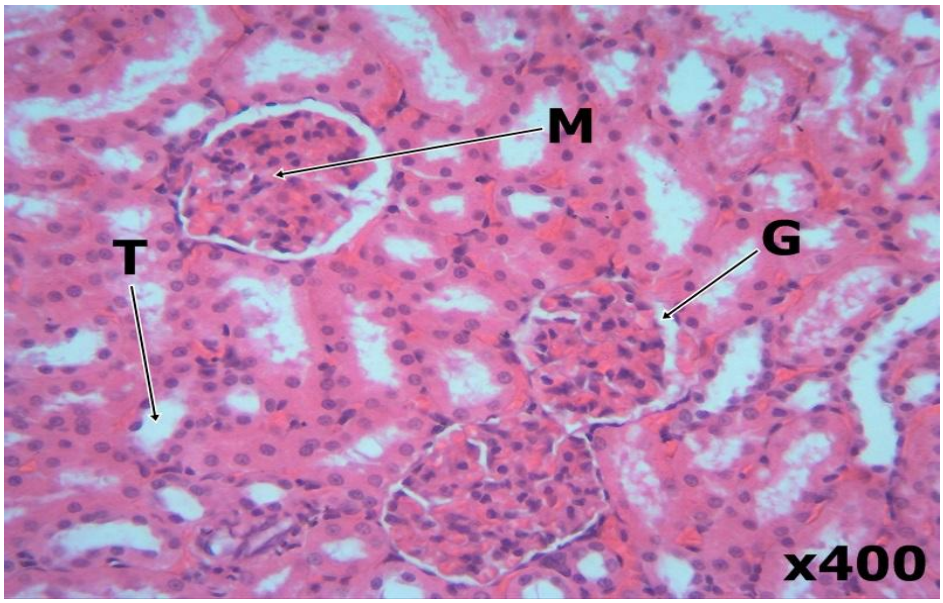

Plate 2. A cross section of the kidney of methotrexate intoxicated rats without treatment
M= mesangium, G= glomerulus, T= tubule

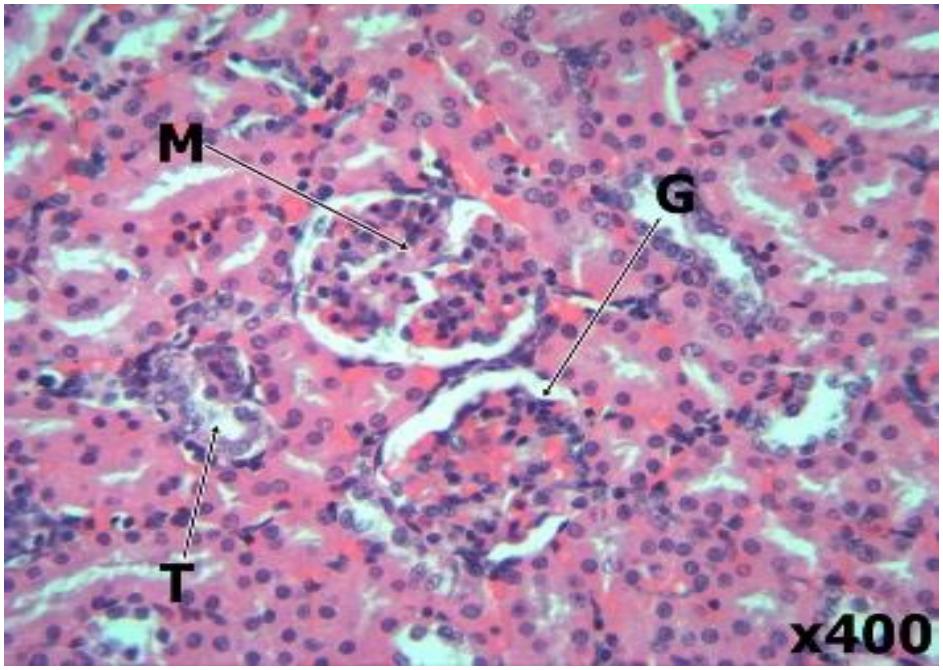

Plate 3. A cross section of the liver of methotrexate intoxicated rats treated with 250 mg/kg body weight of *Napoleonae imperialis* leaf extract. H&E x400
M= mesangium, G= glomerulus, T= tubule

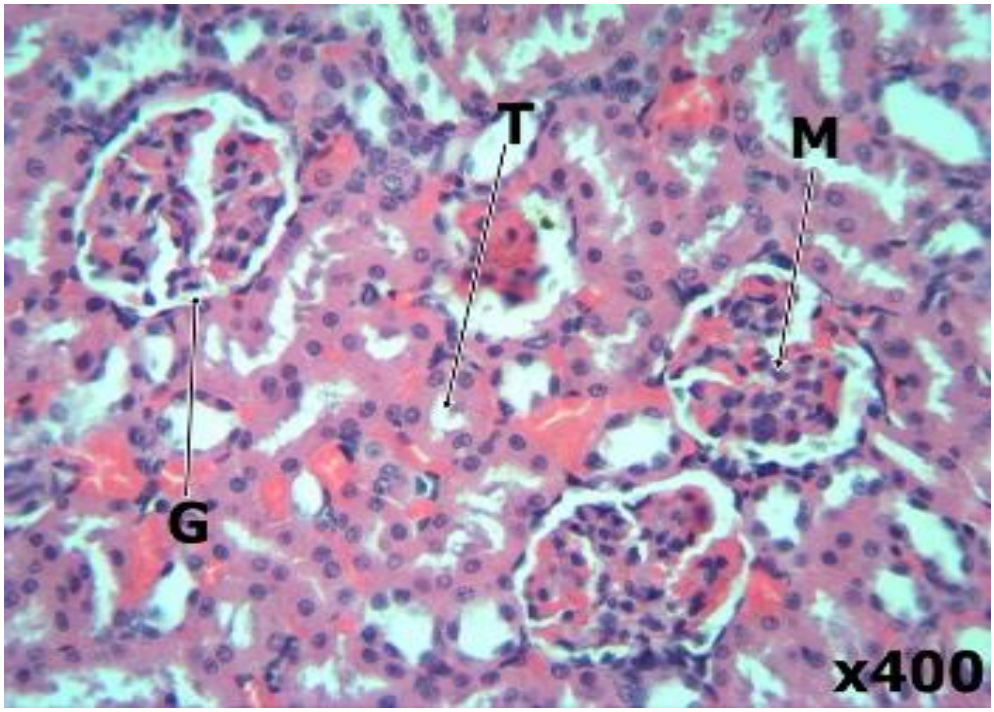

Plate 4. A cross section of the liver of methotrexate intoxicated rats treated with 500 mg/kg body weight of *Napoleonae imperialis* leaf extract. H&E x400
M= mesangium, G= glomerulus, T= tubule

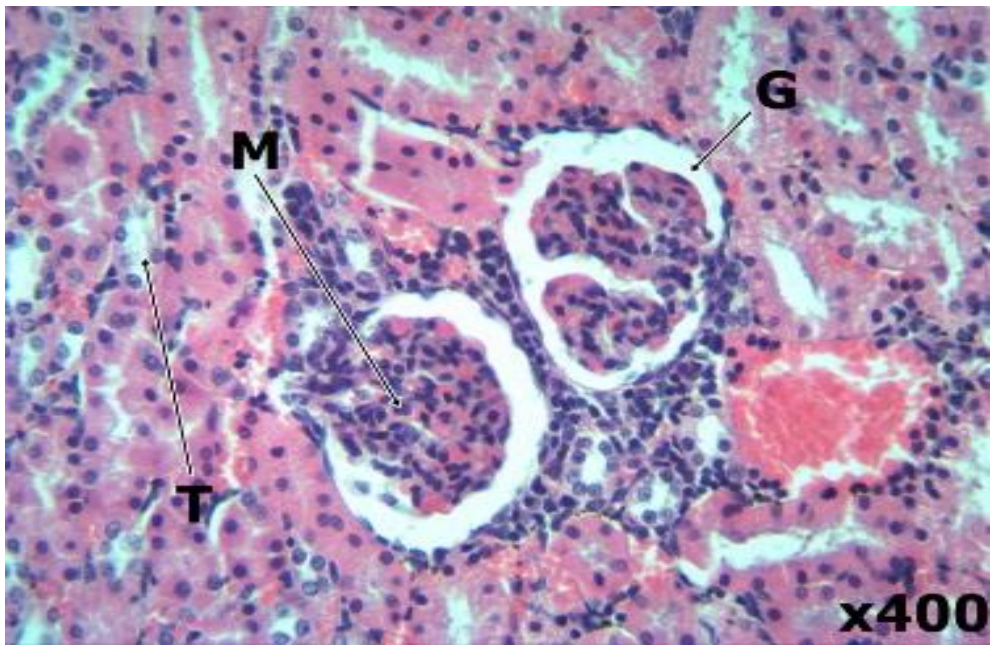

Plate 5. A cross section of the kidney treated with 500 mg/kg body weight of *Napoleonae imperialis* leaf extract only. H&E x400
M= mesangium, G= glomerulus, T= tubule

Group 5 (the group that received the extract only), there is no significant pathology.

4. DISCUSSION

From the present study, it was shown that the tissue segment of kidney of control animals were fundamentally normal. Histopathological study displayed that oral administration of the extract to rat at various doses appears to have restored the damage by methotrexate (MTX) on the kidneys. The pathological changes observed in these organs also appear to be dose dependent. The existence of uncontrollable damage to the kidney is not amazing because the kidney is the main organ of excretion. So, it can be as a result of the exposure of these organs to the waste products present in the extract. This report seems to concur with the findings of Abdulrahman et al. [9]. They published a work on renal and hepatic damage of albino rats administered aqueous root bark extract of *Vitex doniana* and rats fed with feeds composing anti-Nutritive compounds like saponins and tannins. Increased sodium creatinine and urea levels have been reported [10], when rats were treated with aqueous root bark extract of *Vitex doniana* extract for 14 days. Our report still confirms the reason for the organ liaison observed.

5. CONCLUSION

The findings of this study indicate 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 may contain other components that could have interfered with its nephroprotective property.

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 appropiate ethics committe.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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