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ORIGINAL ARTICLE **OPEN ACCESS**

Toxicological Effect of Ekpeteshi® (A Polyherbal Drug Used as Immune Booster) on Liver, Kidney and Heart of Albino Rats

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ABSTRACT
Previous investigations into the pharmacological activities of Ekpeteshi®, a polyherbal formulation comprising Ginger (40%), Garlic (40%) and Clove (20%) popularly used in Nsukka community as an immune booster revealed a wide spectrum of noteworthy medicinal properties. This study was designed to investigate the toxicological effect of this formulation on the liver, kidney and heart. Twenty rats were stratified into four distinct groups, denoted as groups A, B, C, and D. Blood samples were collected on day zero to establish baseline liver and kidney enzyme biomarker levels. The animals were administered with Ekpeteshi® extract (100, 200, and 400 mg/kg) for a duration of 21 days. The control group received 5ml/kg of distilled water. The following were examined on zero day and after 21 days: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Blood Urea Nitrogen (BUN) and Creatinine levels. Histopathological analysis of the liver, kidney, and heart were also conducted and the impact of the extract on body weight was also accessed. Histopathological evaluations showed that at the highest administered dose of 400 mg/kg, the hearts exhibited evidence of cardiomyocyte degeneration. In terms of body weight dynamics, a dose-dependent increase was observed, attaining statistical significance ($p \leq 0.05$). The liver also exhibited a dose-dependent increase in hepatocellular swelling across all administered doses but this swelling was not significant. These results signifies that the formulation has a commendable safety profile concerning hepatic and renal functions. It is noteworthy that while the marginal increase in body weight achieved statistical significance ($p \leq 0.05$), the variations in liver and kidney enzyme biomarkers did not reach statistical significance ($p \leq 0.05$), affirming the safety profile of Ekpeteshi®. This study, therefore, contributes a nuanced understanding of the safety profile of Ekpeteshi®, shedding light on the toxicological profile of this formulation.
Keywords: Ekpeteshi®, Toxicological effect, Liver, Kidney, Heart

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INTRODUCTION

Plants, as the primary source of life on Earth, play a crucial role in various ecological systems(1). Their significance extends to medicine, where their potent impact on human physiology is evident. However, the application of medicinal plants requires careful consideration, as certain species exhibit an uncanny similarity to potentially lethal relatives, emphasizing the need for caution(2).

Numerous plant species possess toxic properties, with toxic attributes classified into groups based on their active chemical constituents, such as alkaloids, glycosides, tannins, and saponins(3). Plant materials are complex mixtures of chemical substances and biological structures, necessitating the extraction of bioactive compounds for biological and toxicological evaluations(4). Recent studies, aided by modern methodologies and advances in analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS), have enhanced our ability to identify and quantify these compounds precisely(5).

The interplay between genetic variations in plants and their toxicological profiles has also been explored, shedding light on how factors like geographical location, climate, and soil composition influence the concentration and potency of bioactive compounds within plant species(6). A comprehensive understanding of plants and their toxicological activities is pivotal for ensuring their safe and effective use in traditional and modern medicine.

In the dynamic landscape of healthcare, the exploration of herbal remedies has intensified globally, driven by a resurgence of interest in traditional medicine(7). One such polyherbal blend, EKPETESHI®, composed of ginger, garlic, and clove, has gained recognition for its noteworthy medicinal properties. However, the imperative to comprehensively assess the safety of such formulations remains paramount.

This study delves into the toxicological effects of EKPETESHI® on the liver, kidney, and heart of albino rats. Administered at doses of 100, 200, and 400 mg/kg body weight, alongside a control group receiving water, this research endeavors to unravel the impact of EKPETESHI® on vital organs. Blood samples collected for liver and kidney function tests, coupled with subsequent histopathological studies on the 21st day, provide a holistic evaluation of potential toxicological ramifications.

As the popularity of herbal interventions continues to soar, a nuanced understanding of their safety profiles becomes indispensable. This investigation not only contributes insights into the specific toxicological effects of EKPETESHI® but also underscores the broader imperative of navigating the risks and benefits associated with polyherbal drugs. In doing so, this research enriches the discourse surrounding evidence-based healthcare practices and facilitates informed decision-making in the realms of both traditional and modern medicine.

MATERIAL AND METHODS

Place and Duration of Study

The work was carried out at the Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu State Nigeria for a period of two months.

Collection/Identification and Preparation of Plant Materials

In January 2022, fresh *Zingiber officinale* (Ginger) and *Allium sativum* (Garlic) samples were obtained from Ogige Market in Nsukka, Enugu State, Nigeria. *Syzygium aromaticum* (Cloves) were also sourced from Kano State Central Market during the same period. Mr. Felix Nwafor, a botanist from the Department of Pharmacognosy and Environmental Medicine at the University of Nigeria, Nsukka, authenticated these plant materials. Voucher specimens with reference numbers PCG/UNN/0422 (*Zingiber officinale*), PCG/UNN/O424 (*Allium sativum L.*), and PCG/UNN/O424 (*Syzygium aromaticum*) were deposited in the herbarium of the University of Nigeria, Nsukka.

Experimental Animals

Albino mice (22-30g) and adult Wister albino rats (170 – 210g) of both sexes were purchasd from the Department of Zoology, University of Nigeria Nsukka. The animals were kept and maintained under laboratory conditions of temperature, humidity, and light, and allowed free access to standard pelletized feed (Top Feeds Nig. Ltd) and water *ad libitum*. The use and care of the laboratory animals were in accordance with internationally accepted best practices as contained in the National Code of Conduct for Animal Research Ethics (NCARE) and approved by the local Ethics Committee of our institution (FPSRA/UNN/23/0055) (8).

Chemicals, Solvents, Instruments and Reagents Used

ALT substrate solution; Reagent 1 (R1) working solution: (Bottles 1 and 1a) Tris buffer: 125 mmol/l, pH 7.3; L-alanine: 625 mmol/l; NADH: 0.23 mmol/l (yeast); LDH D 1.5 U/ml (microorganisms); preservative, ALP substrate solution; Reagent 1 (R1) working solution: Buffer/magnesium (bottles 1 and 1a); 2-Amino-2-methyl-1- propanol D 0.93 mol/l, pH 10.5; magnesium-L-aspartate: 1.24 mmol/l; hydrochloric acid; zinc sulfate hepta-hydrate, AST substrate solution; Reagent 1 (R1) working solution: Tris buffer: 100 mmol/l, pH 7,8; L-aspartate: 300 mmol/l; NADH: 0.23 mmol/l (yeast); MDH D 0,53 U/ml (porcine heart); LDH D 0,75 U/ml (microorganisms); preservative, BUN substrate solution; Reagent 1 (R1) working solution: CAPSO buffer: 5 mmol/l, pH 9.65; NADH ≥ 0.23 mmol/l east); preservative, Creatinine substrate solution; (9) Reagent 1 (R1) working solution: Sodium hydroxide: 0.20 mol/l, 10% formalin, Ethanol, Chloroform, Paraffin wax, Water, Haematoxylin, Eosin (H and E)(10)

Extraction of Plant Materials

The washed and air-dried quantities of *Zingiber officinale* (200g), *Allium sativum* (200g), and *Syzygium aromaticum* (100g) were combined and processed with a Silver Crest 5000W blender. This mixture was then macerated in 1.5 liters of absolute ethanol for 72 hours. Subsequently, it was filtered through Whatman No. 1 filter paper, and the resulting filtrate was subjected to air-drying within the laboratory environment(11). The resulting crude dried extract was accurately weighed and securely stored in a cool area for subsequent experiments(12).

Acute Toxicity Test (LD_{50})

The toxicity tests in mice were conducted following the Lorke method (13). Before the test, the animals underwent a 24-hour fast while being provided unrestricted access to water. The mice were divided into three groups, each comprising three animals. Initial doses of 10, 100, and 1000mg/kg of the drug were administered in the first stage, and no fatalities were observed after 24 hours. Subsequently, doses of 1600, 2900, and 5000 mg/kg were administered to another group of animals. No fatalities occurred within the observation period for animals administered with doses of 1600mg/kg and 2900mg/kg. However, one fatality was recorded in the group administered a dose of 5000mg/kg. This indicates that the drug exhibits toxicity, with a calculated lethal dose of 3808.9mg/kg.

Experimental Design

A total of twenty albino rats (58-169g) were selected at random and divided into four (A-D) groups (n=5). Group A-C was the treatment group and received 100, 200, and 400 mg/kg of the extract respectively. Group D was the control group and received 5ml/kg of distilled water. Extract administration was done orally once daily for 21 days. On days 0 and 21, blood (2-3 ml) was withdrawn by ocular puncture into sample bottles. The blood samples which was clotted were used to ascertain the baseline and final AST, ALP, ALT, Creatinine, and BUN respectively. On day 21, one animal was randomly taken from each group, and sacrificed and the kidneys, livers, and hearts were isolated. The organs were placed in 10% phosphate-buffered formalin (pH = 7.2) for histopathological studies. The body weights of these animals were also taken on days 0, 7, and 14 which were used to measure the effect of extract on the weight of the animals.

Statistical Analysis

IBM SPSS 29 was used to analyze all data obtained using One-Way ANOVA and subjected to Dunnett t-tests and least square deviation (LSD) post hoc test for multiple comparisms. Results were expressed as Mean ± Standard error of Mean (SEM) and differences between means were not significant for Liver and Kidney function tests at P≤0.050 and were accepted to be significant at 0.05 (*) level for Effect of Extract on the weight of the animals.

RESULTS

Result of Acute Toxicity Test

The results from the acute toxicity test, conducted using Lorke's method to determine the LD_{50}, indicate that the Ekpeteshi® formulation has a lethal dose of 3808.9 mg/kg. This conclusion is based on the recorded death of an animal administered with a dose of 5000mg/kg of the extract within 6.25 minutes. The LD50 was calculated using the formula $LD50 = \sqrt{(D_0 \times D_{100})}$, with D_0 being 5000mg/kg and D_{100} being 2900 mg/kg.

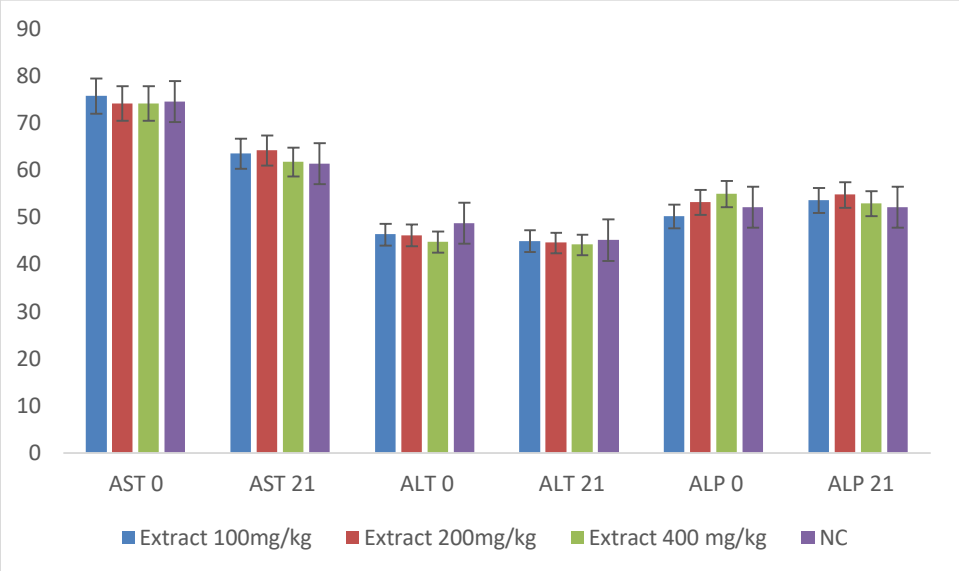

FIGURE 1: The Effect of Ethanol Extract of Ekpeteshi® on the Liver Enzyme Biomarker (Baseline Vs Final Analysis)

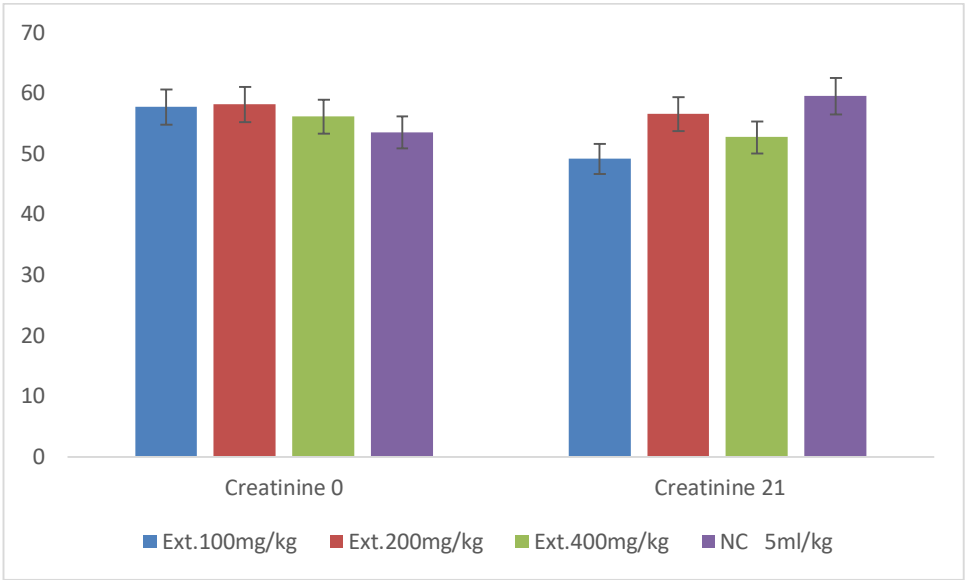

FIGURE 2: Chart showing the Effect of Ethanol Extract of Ekpeteshi® on Creatinine (Baseline Vs Final Analysis)

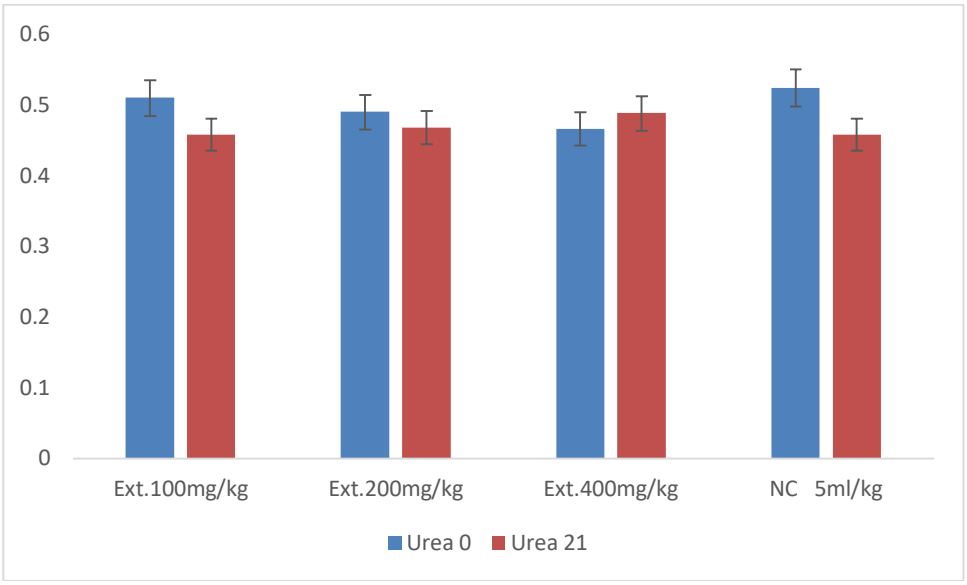

FIGURE 3: Chart showing the Effect of Ethanol Extract of Ekpeteshi® on Urea (Baseline Vs Final Analysis)

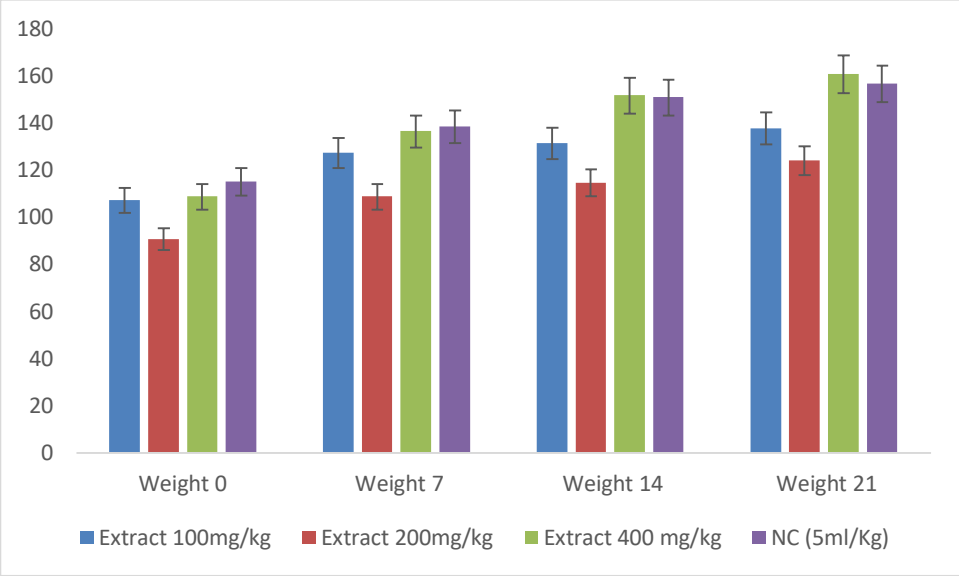

FIGURE 5: The Effect of ethanol Extract of Ekpeteshi® on the Weight of Animals

GROUP A

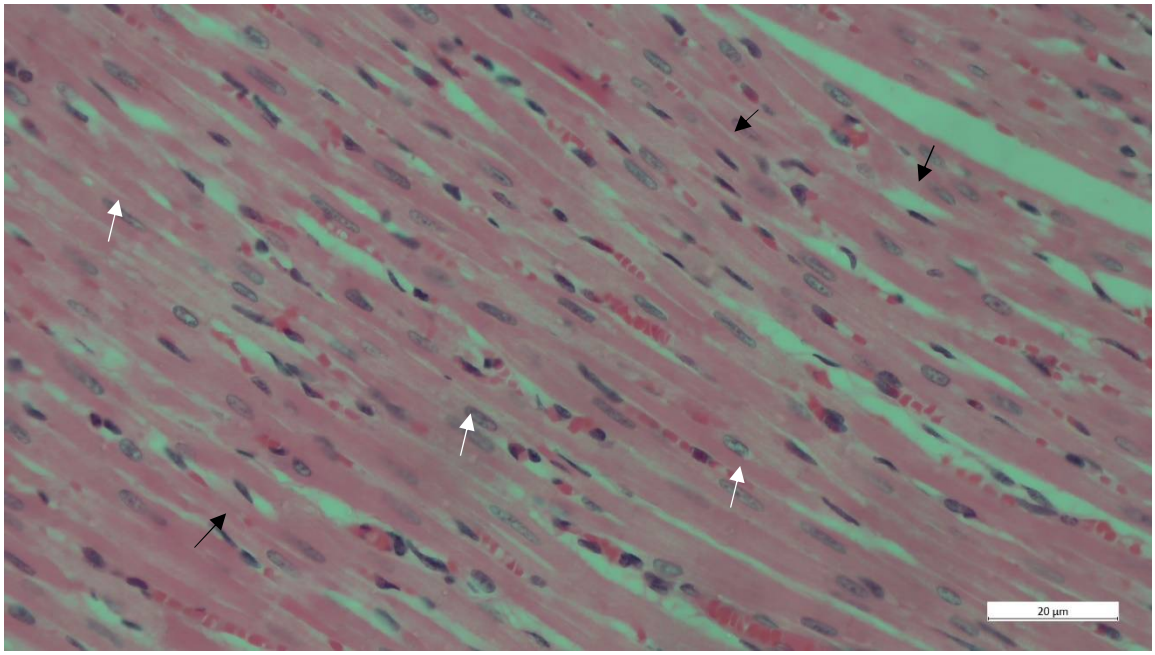

PLATE 1: Photomicrograph of Heart sections from 100 mg/kg extract treated rats on day 21 showing the normal myocardial histomorphology.

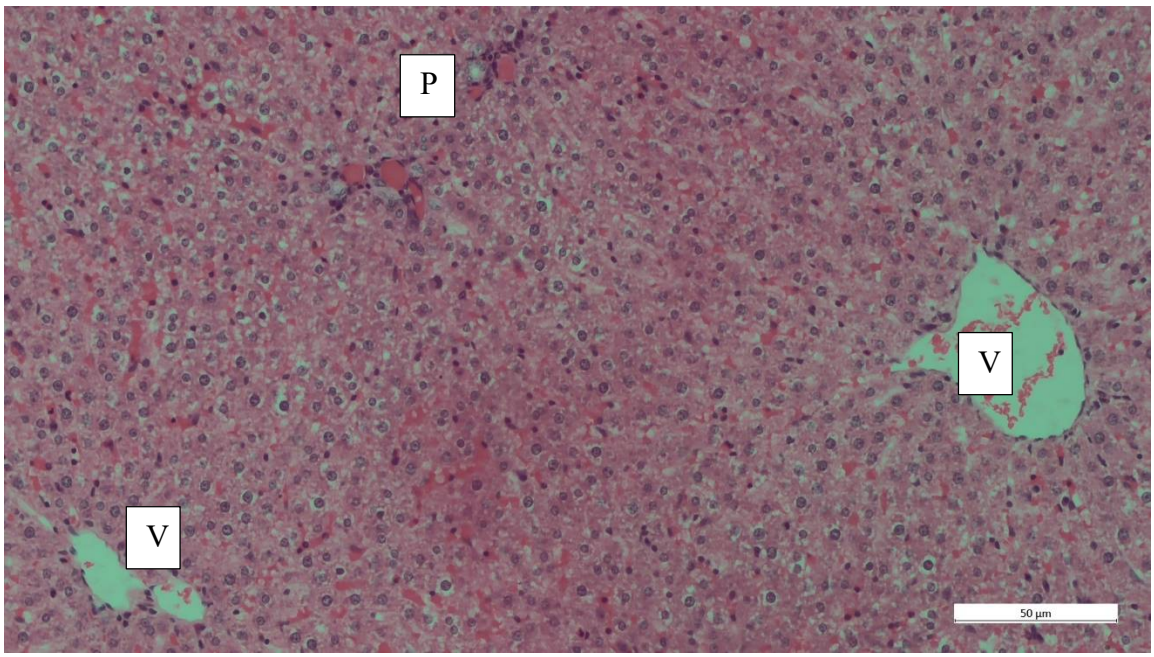

Plate 2: Photomicrograph of Liver sections from 100 mg/kg extract treated rats on day 21 showing mild periportal hepatocellular swelling.

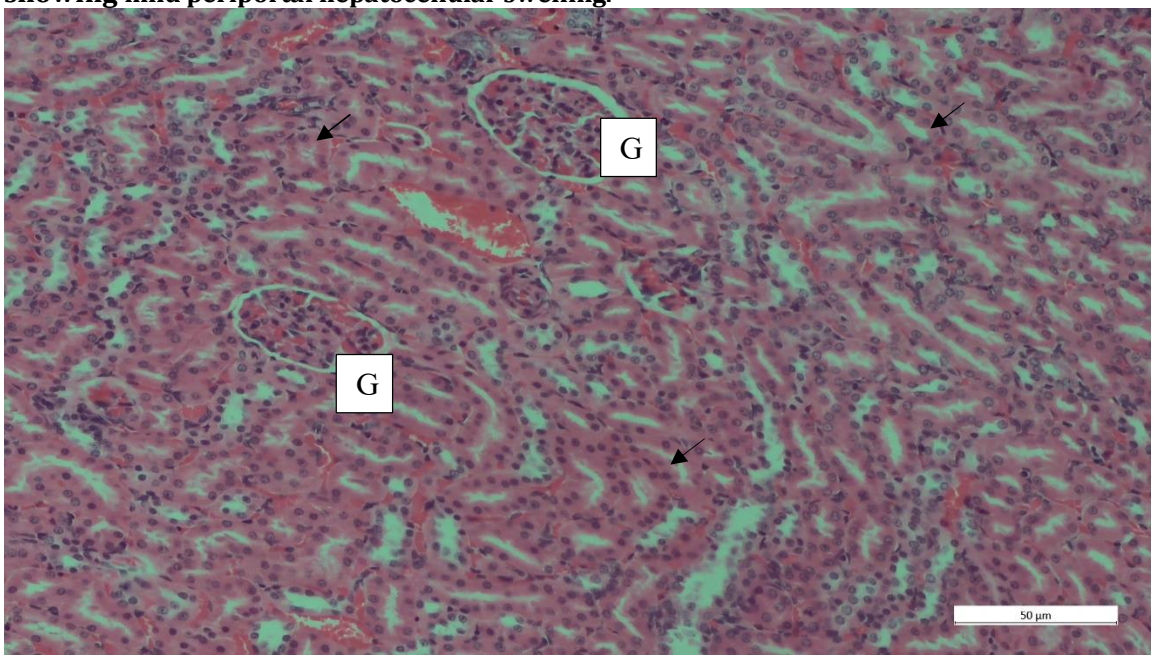

Plate 3: Photomicrograph of Kidney sections from 100 mg/kg extract treated rats on day 21 showing the normal renal histo-architecture for laboratory rodents.

GROUP B

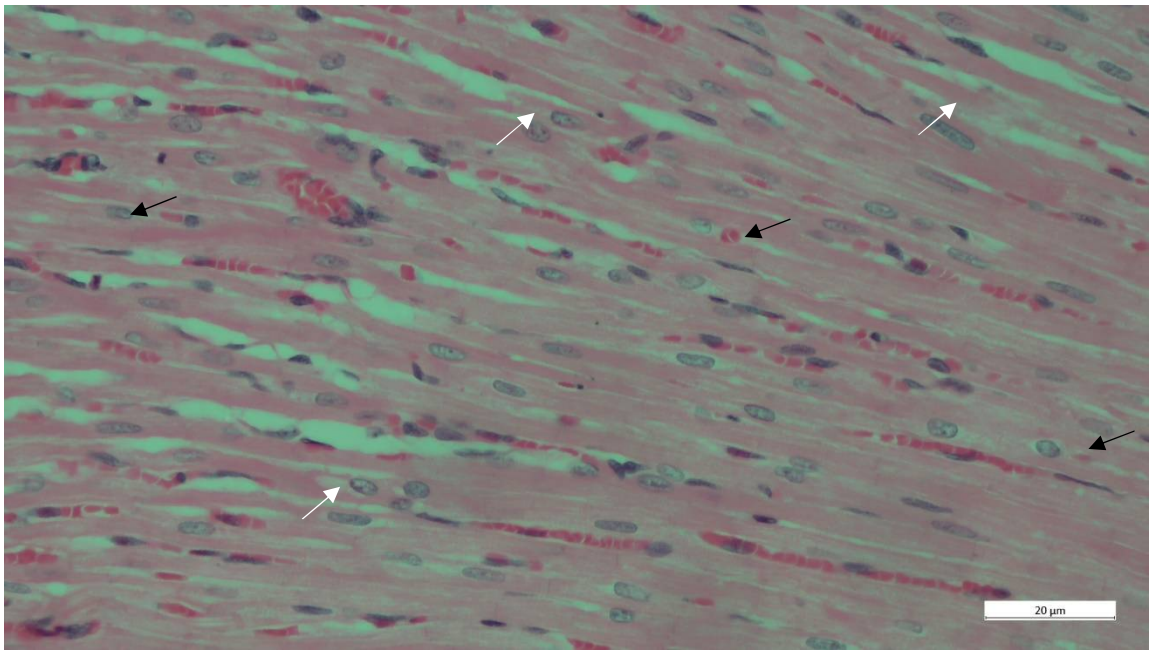

Plate 4: Photomicrograph of Heart sections from 200 mg/kg extract treated rats on day 21 showing the normal myocardial histomorphology.

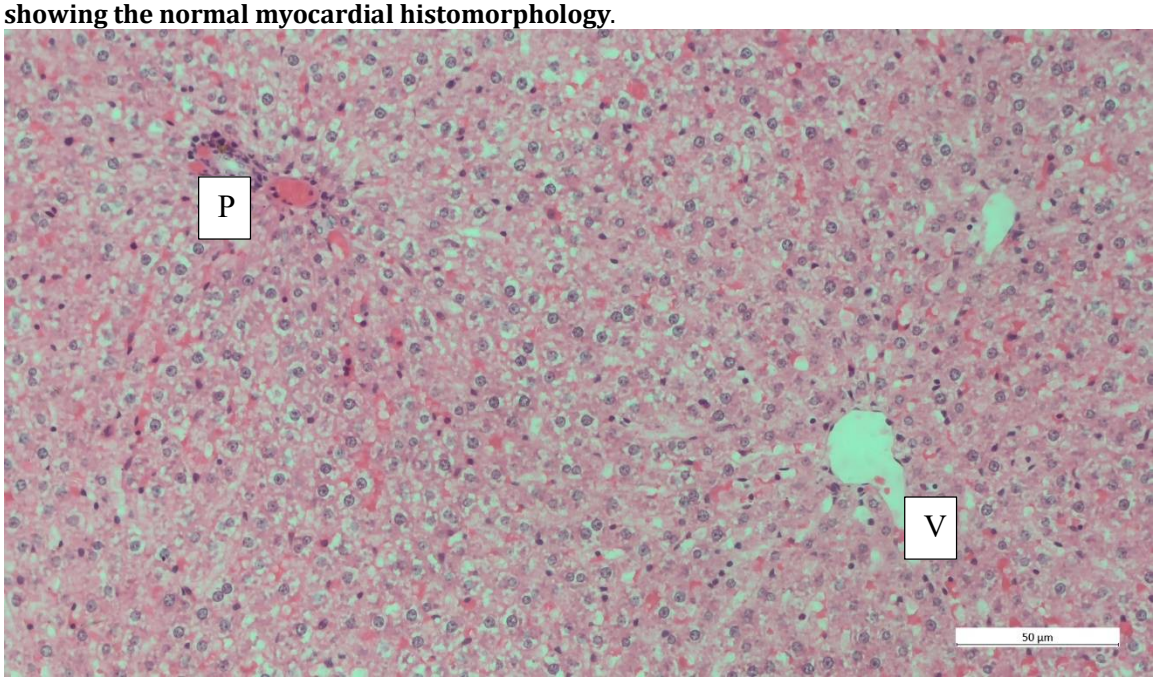

Plate 5: Photomicrograph of Liver sections from 200 mg/kg extract treated rats on day 21 showing mild to moderate diffuse hepatocellular swelling.

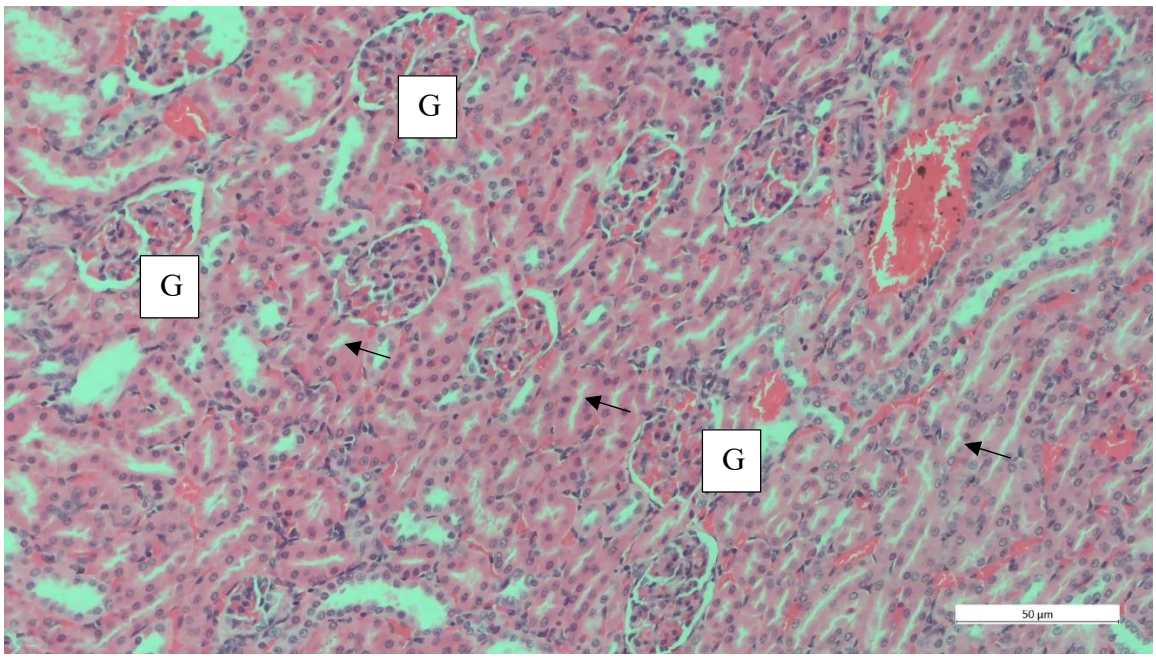

Plate 6: Photomicrograph of kidney sections from 200 mg/kg extract treated rats on day 21 showing the normal renal histo-architecture for laboratory rodents.

GROUP C

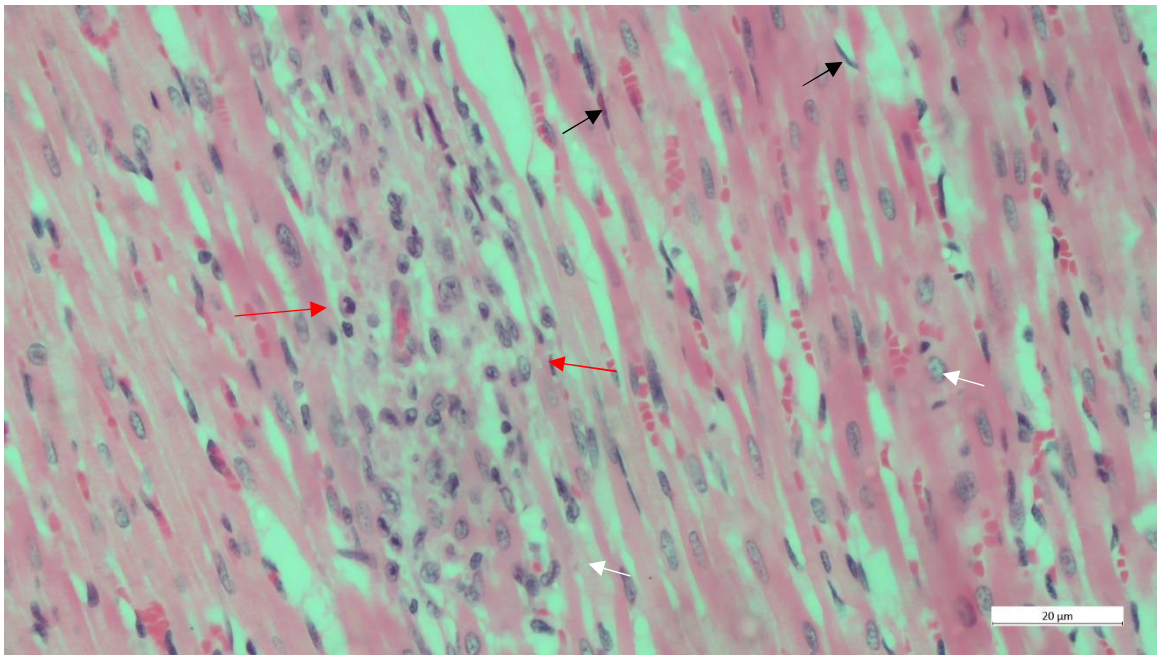

Plate 7: Photomicrograph of Heart sections from 400 mg/kg extract treated rats on day 21 showing multifocal areas myocardial degeneration and necrosis.

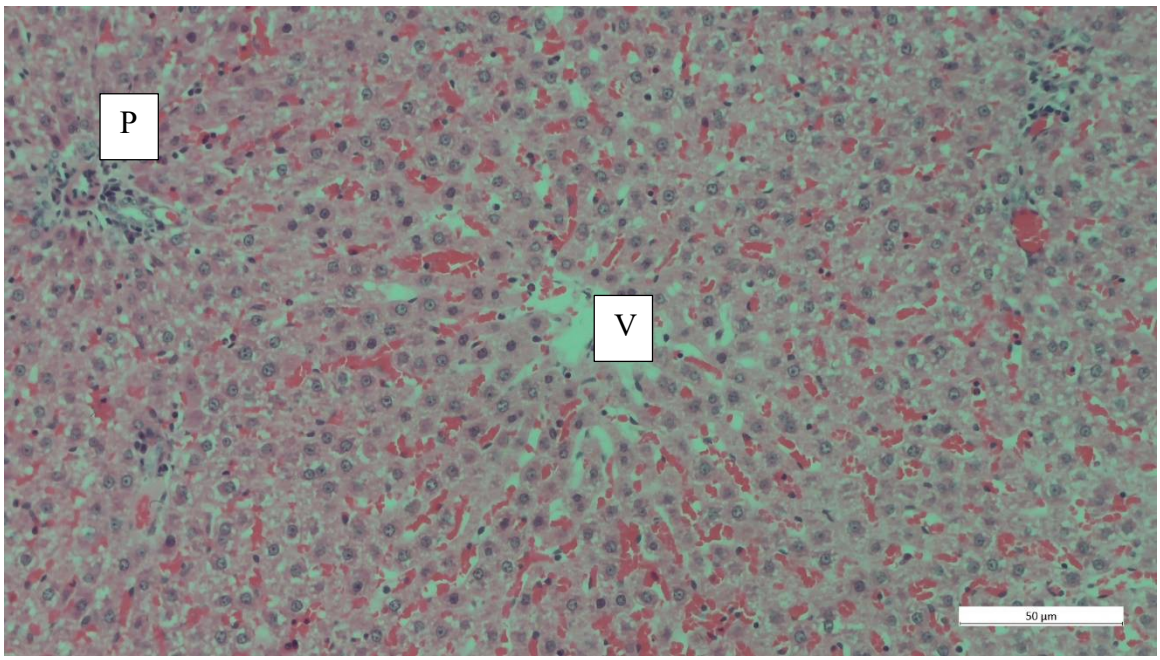

Plate 8: Photomicrograph of Liver sections from 400 mg/kg extract treated rats on day 21 showing mild periportal hepatocellular swelling.

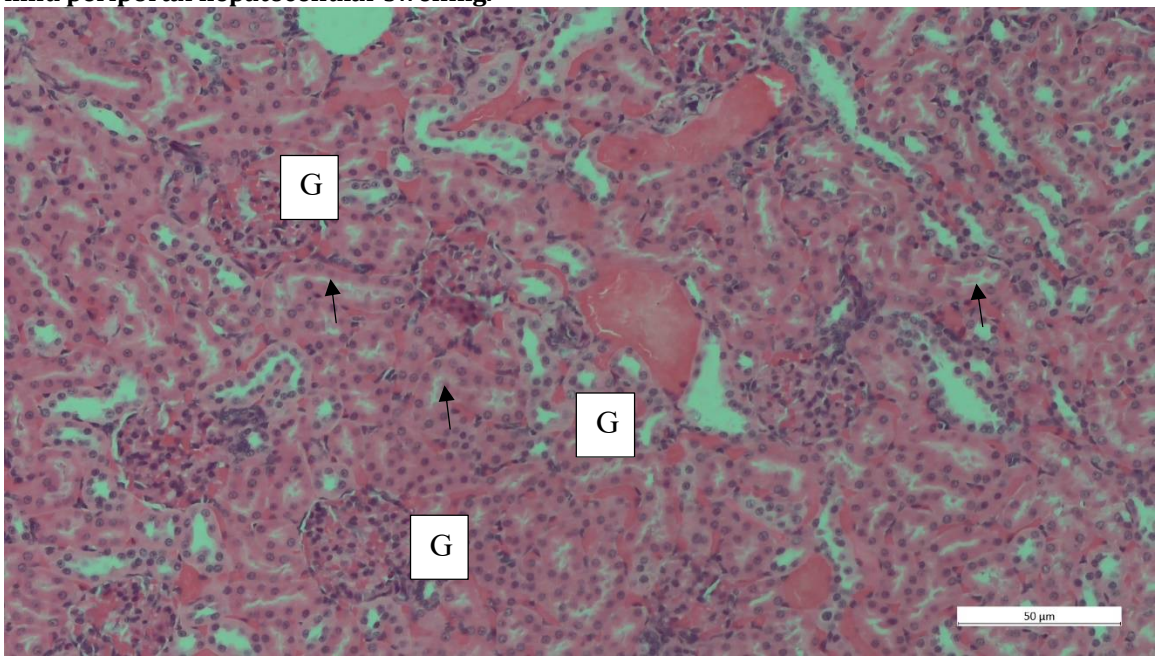

Plate 9: Photomicrograph of kidney sections from 400 mg/kg extract treated rats on day 21 showing the normal renal histo-architecture for laboratory rodents.

GROUP D

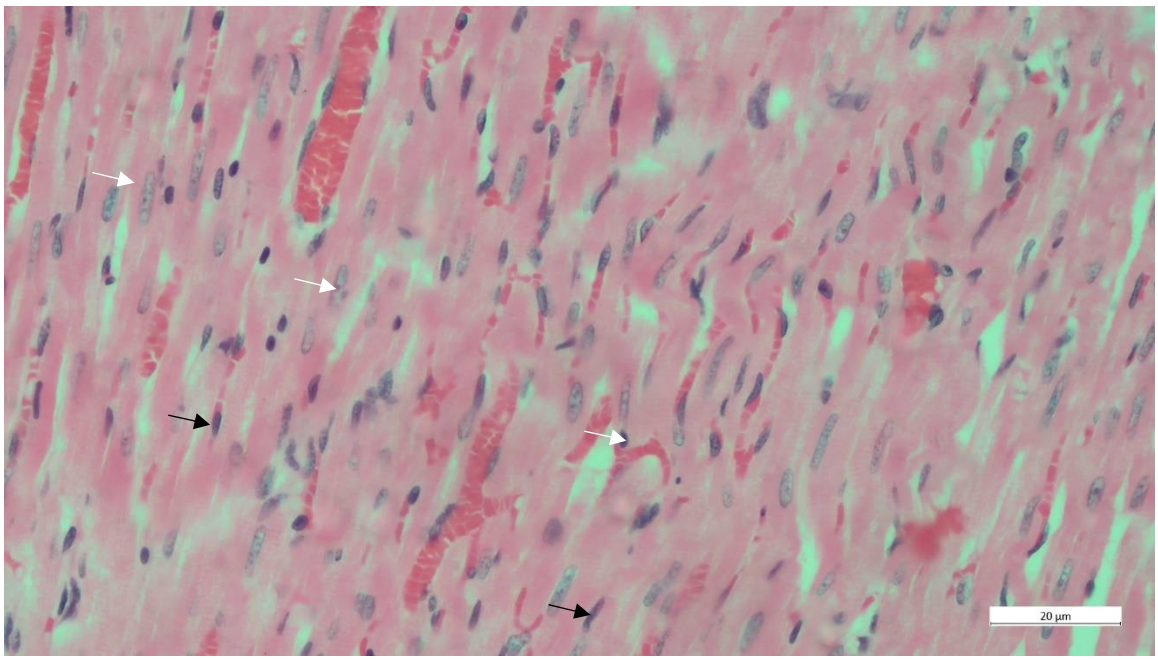

Plate **10: Photomicrograph of Heart sections from 5 ml/kg water treated rats on day 21 showing the normal myocardial histomorphology.**

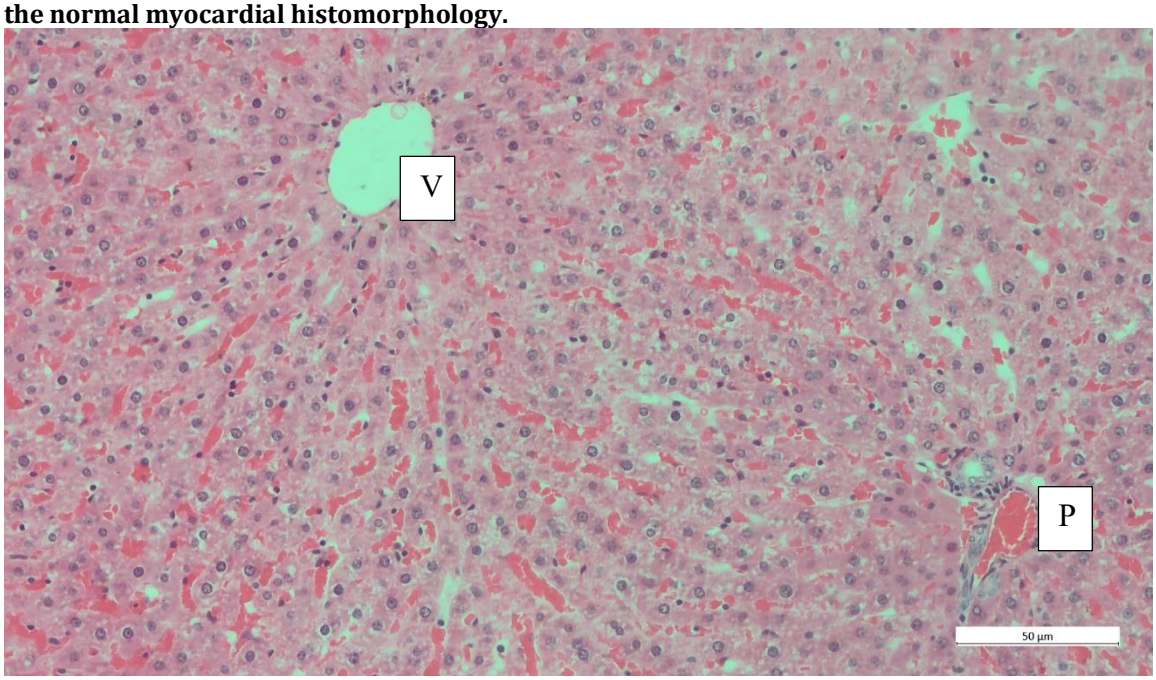

Plate 11: Photomicrograph of Liver sections from 5 ml/kg water treated rats on day 21 showing the normal hepatic histo-architecture.

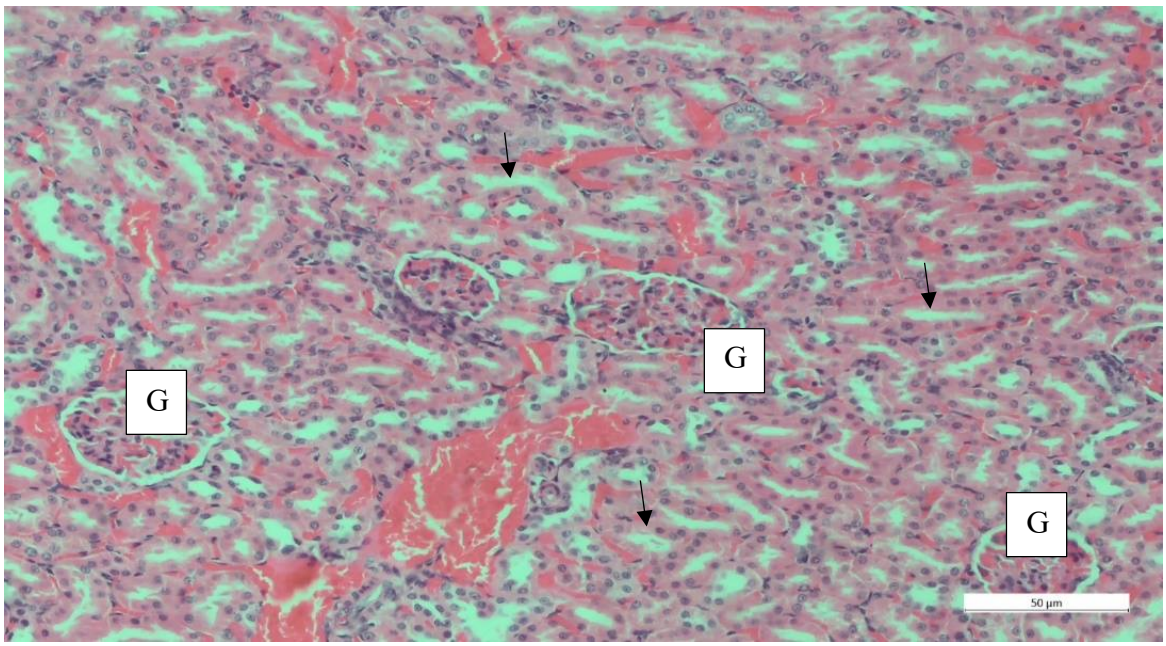

Plate 12: Photomicrograph of Kidney sections from 5 ml/kg water treated rats on day 21 showing the normal renal histo-architecture for laboratory rodents.

DISCUSSION

Herbal medicines are often perceived as natural and safe, leading to self-prescription and lack of control in usage(14). Ekpeteshi® gained popularity during the Covid-19 pandemic for its antimicrobial, antioxidant, hematopoietic, and analgesic effects. However, the potential toxic effects remained unassessed. Toxicity assessment focused on renal and hepatic functions. Urea and creatinine levels indicated improved kidney function with Ekpeteshi® administration. Liver function markers (ALT, AST and ALP) showed no significant differences, suggesting no induction of enzyme activities. Body weight gain in rats exposed to Ekpeteshi® demonstrated a dose-dependent increase, indicating a potential effect on lipid breakdown. Histopathological examinations revealed protective effects on the kidneys, while at high doses (400mg/kg) showed adverse effects on the heart, suggesting the need for cautious use. In the liver, hepatocellular swelling indicated potential stress or injury. Prolonged use of Ekpeteshi® may compromise liver functionality, emphasizing the importance of careful administration.

CONCLUSION

The Results indicate that Ekpeteshi® extract at 100, 200, and 400 mg/kg for 21 days did not adversely affect liver and kidney functions. While there was a slight increase in weight, further lipid profiling is necessary to understand the pharmacological implications. Histopathological examinations revealed no kidney damage at any dose, no heart issues at 100 and 200 mg/kg, but degeneration at 400 mg/kg, emphasizing the need for studies to determine a safe dose for heart tissues. Additionally, investigations should identify a safe dosage to prevent swelling of the liver histo-architecture.

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CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

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