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Toxicological evaluation of a herbal tea made from the leaves of *Setaria megaphylla*, *Ageratum conyzoides* and *Chromolaena odorata*

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Abstract

The toxicological effect of an herbal tea made from leaf extracts of *Ageratum conyzoides, Chromolaena odorata, and Setaria megaphylla*, plants traditionally used in Nigeria for malaria treatment was studied. Hot aqueous extracts of the ternary combination (1:1:1 ratio) of the plants were administered in varying doses (0, 50, 100, and 200 mg/kg) to assess impact on hepatic, renal and haematologic function parameters, and liver and kidney tissue histology of forty male albino rats divided into four groups of ten animals each. The tea extracts were administered orally daily for 60 days. Biochemical analyses revealed that the extract significantly ($p<0.05$) reduced ALT (25.91 ± 2.35 to 6.84 ± 0.31 U/L), AST (36.39 ± 1.96 to 19.24 ± 1.19 U/L), and ALP (21.94 ± 3.51 to 11.06 ± 2.76 IU/L) activities, with no significant ($p>0.05$) changes in bilirubin, protein and albumin concentrations, suggesting non-hepatotoxic property. Among the kidney function, significant ($p<0.05$) dose-dependent increases was observed only in serum urea ranging from 23.09 ± 0.79 mg/dl (Control group) to 34.90 ± 0.52 mg/dl (200 mg/kg treated group). Increase in WBC count was observed indicative of a possible immune response, while other blood parameters remained stable. Histopathological examination confirmed that lower extract doses were relatively safe, whereas higher doses presented early signs of organ stress. These findings suggest that this herbal tea extract has a favourable safety profile at moderate doses, though higher doses may pose risk of organ toxicity, warranting further studies on dose optimization and elucidation of its folkloric therapeutic potential.

Keywords: Malaria; Goat weed; Siam weed; Ribbon grass; Herbal tea; Biochemical analysis

1. Introduction

Ageratum conyzoides (goat weed) is a widely distributed herbaceous plant in the Asteraceae family, recognized for its extensive use in traditional medicine across various regions. In Nigeria, this plant is known by different names depending on the ethnic group: Imiesu among the Yorubas, Ula ujula among the Igbos, and Ahenhen or Igedes among the Hausas (Okunade, 2002). It has been traditionally utilized for the treatment of malaria, stroke, heart disorders, diabetes, and wounds. Recent research has corroborated its traditional uses, demonstrating significant antimicrobial, analgesic, antispasmodic, and anti-arthritic properties, thus validating its application in managing malaria and other health conditions (Akinmoladun et al., 2019; Akindele et al., 2021).

Chromolaena odorata (Siam weed), another perennial shrub from the Asteraceae family, is prevalent in Southeast Nigeria. It is traditionally known as Siam weed, 'Elizabeth', 'Independence leaf', and 'Awolowo' among the Igbos. This plant is reputed for its antimalarial and wound healing properties. Contemporary studies have highlighted its broad spectrum of pharmacological activities, including antibacterial, antifungal, antidiabetic, anti-inflammatory, and

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antioxidant effects. Several recent studies have validated its effectiveness against malaria and other diseases (Okeke et al., 2018; Nwachukwu et al., 2021).

Setaria megaphylla (Broad-leafed bristle grass) is a robust perennial grass traditionally used in Nigeria for treating malaria, inflammation, and diabetes. Known locally as ribbon grass or broad-leafed bristle grass, it is employed in indigenous medicine as a sedative for coughs and a remedy for urogenital infections. Scientific investigations have revealed its antiplasmodial, hypoglycemic, anti-inflammatory, analgesic, and cytotoxic activities, supporting its potential as an antimalarial agent (Oluwaseun et al., 2018; Ogu et al., 2022).

The global fight against malaria faces significant challenges due to the limitations of current antimalarial drugs. While there are effective treatments available, such as artemisinin-based combination therapies (ACTs), issues like drug resistance, high costs, and limited accessibility persist. Artemisinin resistance has been reported in parts of Southeast Asia, which threatens the efficacy of these treatments. Moreover, the high cost of ACTs makes them inaccessible to many in endemic regions, particularly in sub-Saharan Africa (World Health Organization, 2023).

In contrast, Africa has a rich repository of folkloric treatment options, which are often more accessible and culturally ingrained in local practices. These traditional remedies, derived from indigenous plants, offer a valuable alternative for malaria management, especially in resource-limited settings. However, the efficacy and safety of these treatments are not always well-documented, underscoring the need for scientific validation and integration into broader health care strategies (Banda et al., 2019; Bode et al., 2022). This study aims to assess the toxicological effects of an herbal combination tea made from *Ageratum conyzoides*, *Chromolaena odorata*, and *Setaria megaphylla* leaves, focusing on its safety and efficacy as a traditional antimalarial remedy.

2. Materials and Methods

2.1. Plant materials

Fresh leaves of the plants (*Setaria megaphylla, Ageratum conyzoides* Linn, and *Chromolaena odarata* Linn.) were collected from a farm at Ihiagwa community, Owerri West Local Government Area, Imo State, Nigeria. The plants were identified by a plant taxonomist at the Department of Forestry and Wildlife, School of Agriculture and Agricultural Technology, Federal University of Technology Owerri (FUTO) with voucher numbers 722, 723 and 724 assigned and documented.

2.2. Preparation of plant samples

The fresh leaves of the plants were collected, washed and dried under shade in the laboratory. The dried leaf samples were separately ground into powder using an electric grinder (Saisho 200W) and labelled. The plants' powders were combined in a ratio of 1:1:1, and 500 g of the powder combination was subjected to hot aqueous extraction using 1000 ml deionized water for 30 minutes with intermittent agitation. The aqueous extract was concentrated, labelled and stored at -4 °C ready for use.

2.3. Experimental animals

Forty (40) male Swiss Albino rats were purchased from the Department of Veterinary Medicine, University of Nigeria Nsukka (UNN) and transported to Federal University of Technology Owerri (FUTO), where the research was carried out. The rats were acclimatized to laboratory condition for a period of 2 weeks.

2.4. Administration of extracts

The animals were randomly divided into four groups of ten (10) rats per group according to their body weights. The extracts were dissolved in normal saline and the experimental groups (I – IV) were respectively administered doses of 0, 50, 100 and 200 mg/kg body weight of the extract. The control group (0 mg/kg) animals did not receive any extract, but were administered only normal saline. The administration was performed orally once daily for sixty days.

2.5. Collection of blood samples

At the end of the experimental period, all the animals were humanely sacrificed and blood drained by cardiac puncture with sterile syringes and needles. The blood was then emptied into labelled test tubes, allowed to clot and sera obtained were used for determination of the biochemical parameters. Another set of blood samples were collected in EDTA bottles for the haematological parameters.

2.6. Biochemical analysis

Assay of alkaline phosphatase activity (ALP) was carried out using the colorimetric endpoint method as described by Roy (1970). Assays for serum aspartate and alanine aminotransferase activities (AST and ALT) were carried out using the kinetic method as described by Reitman and Frankel, (1957). Estimations of serum total bilirubin, total protein and albumin concentrations were by colorimetric, biuret and bromocresol green methods as described by Jendrassik and Grof, (1938), Tietz (1995) and Doumas *et al.,* (1971) respectively.

Cholesterol and triglyceride concentrations were estimated by colorimetric methods (Abell *et al.,* 1952; Trinder, 1969). Serum high density lipoprotein (HDL) concentration was determined by the enzymatic precipitation method of Assmann, (1979). Estimation of serum urea concentration was by the urease-Berthelot method (Weatherburn, 1967). Creatinine was determined using Bartels and Bolumer method (1972). Similarly, the serum concentrations of potassium, sodium, chloride and bicarbonate were determined using colorimetric methods (Teri and Sesin, 1958; Skeggs and Hochstrasser, 1964; Forester *et al.,* 1976).

2.7. Haematological analysis

Haematological analysis was carried out on the whole blood in EDTA with the aid of an automated haematology analyzer (Mindray BC 2300, USA). The instrument performs blood cell count (RBC) by direct current detection method, haemoglobin (HGB) determination by non-cyanide haemoglobin analysis method, and calculates the red blood cell constants automatically from RBC count, HGB, and packed cell volume (PCV) values.

2.8. Histological analysis

Histological analysis was carried out on the liver and kidney tissues extracted from two randomly selected rats from each group immediately after sacrifice. The tissues were initially preserved in formalin until analysis. The method described by Mitchell and Kumar (2009) was used for the histological analysis of the tissue sections.

2.9. Statistical analysis

The results generated were presented as mean ± standard deviation and analyzed using one-way analysis of variance with the aid of GraphPad Prism version 5.0. Statistical significance of values were considered at $p<0.05$ using Turkey and Duncan homogeneity of variance test.

3. Results

The liver enzymes (ALT, AST and ALP) results showed significant ($p<0.05$) dose-dependent reductions in activities ranging from 25.91 ± 2.35 to 6.84 ± 0.31 U/L, 36.39 ± 1.96 to 19.24 ± 1.19 U/L, and 21.94 ± 3.51 to 11.06 ± 2.76 IU/L in the normal control to the 200 mg/kg treatment group for ALT, AST and ALP respectively (Table 1). On the other hand, there were no observed changes in the bilirubin, total protein and albumin concentrations, though the albumin concentration showed minimal elevation with increase in extract dosage.

Results of the kidney function and electrolyte profile of the animals (Table 2) showed significant dose-dependent increases in the serum urea concentration ranging from 23.09 ± 0.79 mg/dl in the control animals to 34.90 ± 0.52 mg/dl in the 200 mg/kg treated group. On the other hand, serum sodium ion concentration decreased significantly with extract treatment, while the creatinine, potassium, chloride and bicarbonate ion concentrations showed no significant ($p>0.05$) variations.

Generally, there were observed no significant ($p>0.05$) changes in the haematological parameters of the extract treated animals in comparison with the control group (Table 3).

There were also no lesions observed in the liver (Figures 1 – 4) and kidney (Figures 5 – 8) tissues of rats treated with the tea extracts at 50mg/kg and 100 mg/kg compared to the control. However, in the groups treated with 200 mg/kg extract, a slight cloudy swelling of the liver hepatocytes (Figure 4) was observed, while in the kidney, the glomeruli appeared closely adherent to the Bowman's capsule and some of the tubules appeared enlarged (Figure 8).

Table 1 Liver function profile of albino rats administered the antimalarial tea extract

Parameter	Normal control	Tea extract		
		50 mg/kg	100 mg/kg	200 mg/kg
ALT (U/L)	25.91 ± 2.35[a]	18.71 ± 1.74[b]	17.27 ± 2.35[b]	6.84 ± 0.31[c]
AST (U/L)	36.39 ± 1.96[a]	28.07 ± 1.56[b]	28.85 ± 4.87[b]	19.24 ± 1.19[c]
ALP (IU/L)	21.94 ± 3.51[a]	13.73 ± 2.66[b]	11.63 ± 2.20[b]	11.06 ± 2.76[b]
Total bilirubin (mg/dl)	0.32 ± 0.01[a]	0.35 ± 0.02[a]	0.37 ± 0.02[a]	0.37 ± 0.03[a]
Total protein (g/dl)	6.40 ± 0.84[a]	6.45 ± 0.25[a]	6.23 ± 0.50[a]	5.15 ± 1.24[a]
Albumin (g/dl)	4.13 ± 0.08[a]	3.95 ± 0.10[a]	4.20 ± 0.02[a]	4.30 ± 0.28[a]

Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$).

Table 2 Kidney function and electrolyte profiles of albino rats administered the antimalarial tea extract

Parameter	Normal control	Tea extract		
		50 mg/kg	100 mg/kg	200 mg/kg
Urea (mg/dl)	23.09 ± 0.79[a]	26.82 ± 1.38[a]	32.29 ± 1.58[b]	34.90 ± 0.52[b]
Creatinine (mg/dl)	0.18 ± 0.02[a]	0.16 ± 0.03[a]	0.16 ± 0.03[a]	0.07 ± 0.01[b]
Sodium ion (mmol/L)	137.14 ± 3.64 [a]	125.64 ± 0.95 [a]	109.75 ± 11.37 [b]	79.85 ± 1.21[c]
Potassium ion (mmol/L)	4.58 ± 0.27[a]	4.67 ± 0.38[a]	3.97 ± 0.44[a]	3.97 ± 0.44[a]
Chloride ion (mmol/L)	66.20 ± 4.24[a]	66.05 ± 8.41[a]	68.22 ± 5.27[a]	70.10 ± 6.13[a]
Bicarbonate ion (mmol/L)	44.87 ± 3.12[a]	44.99 ± 6.18[a]	43.38 ± 3.87[a]	42.01 ± 4.50[a]

Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$).

Table 3 Haematologic profile of albino rats administered the antimalarial tea extract

Parameter	Normal control	Tea extract		
		50 mg/kg	100 mg/kg	200 mg/kg
WBC (10^9 cells/L)	2.83 ± 0.18[a]	2.01 ± 0.19[b]	3.07 ± 0.90[a]	4.19 ± 0.92[c]
RBC (10^9 cells/L)	6.49 ± 0.47[a]	6.95 ± 0.18[a]	6.79 ± 0.29[a]	6.38 ± 0.88[a]
HGB (g/dl)	12.00 ± 0.72[a]	13.13 ± 0.38[a]	12.97 ± 0.35[a]	11.83 ± 1.62[a]
PCV (%)	36.97 ± 2.40[a]	39.43 ± 1.22[a]	38.10 ± 1.08[a]	35.47 ± 4.97[a]
MCV (fL)	56.97 ± 1.51[a]	56.77 ± 2.32[a]	56.10 ± 0.87[a]	55.60 ± 0.56[a]
MCH (pg)	18.50 ± 0.35[a]	18.93 ± 0.91[a]	19.10 ± 0.26[a]	18.60 ± 0.20[a]
MCHC (g/dl)	32.53 ± 1.35[a]	33.33 ± 0.45[a]	34.00 ± 0.10[a]	33.40 ± 0.10[a]
PLT (10^9 cells/L)	599.33 ± 122.73[a]	556.00 ± 76.60[a]	593.33 ± 102.65[a]	691.67 ± 115.52[a]

Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$).

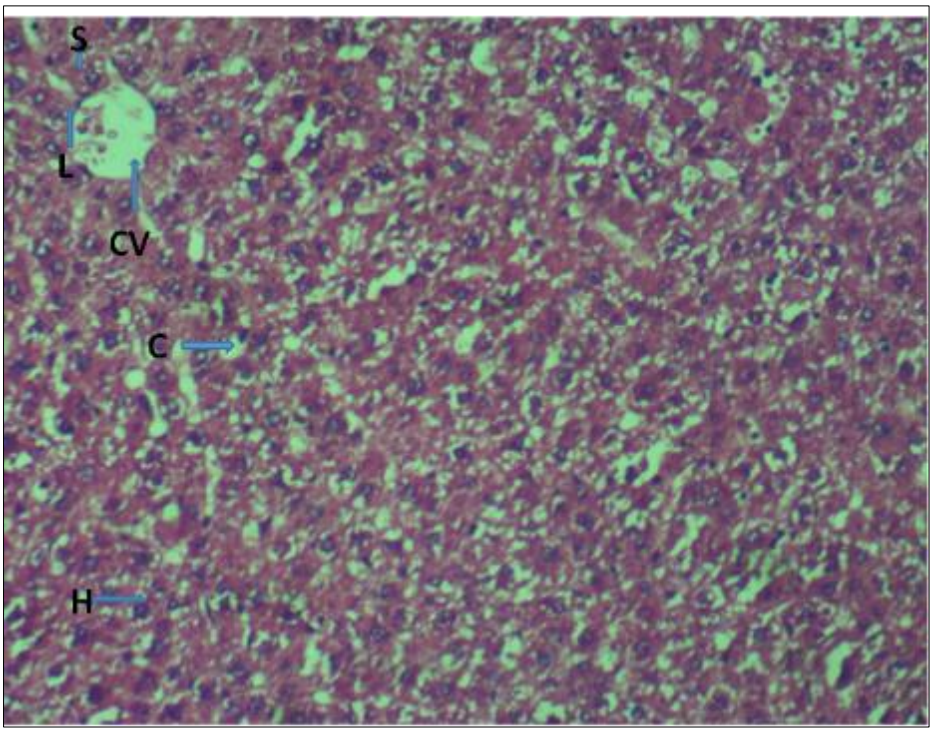

Figure 1 Liver section of normal control group animals showing normal central vein (CV), scanty stroma and cystic (C) spaces. The lamella (L) and sinusoidal (S) arrangement appears distorted. Also, some of the hepatocytes (H) appear unremarkable (x400), Stain: H and E

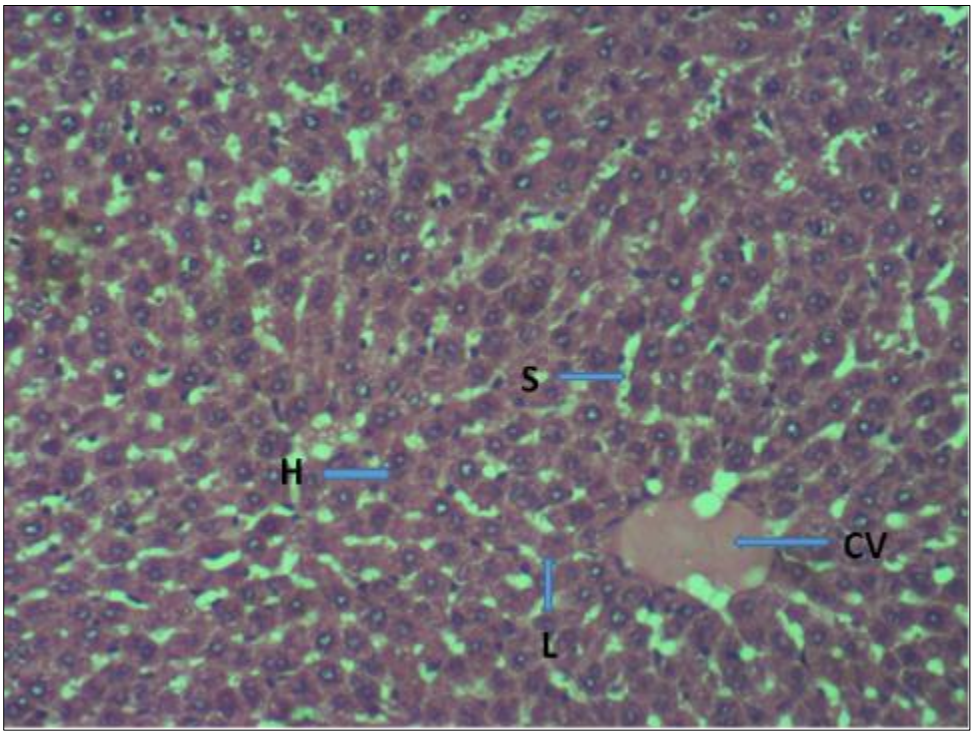

Figure 2 Liver section of treatment group animals exposed to 50 mg/kg b. wt. showing normal tissue architecture. The central vein (CV), the lamella (L), sinusoids (S) and hepatocytes (H) all appear normal (x400), Stain: H and E

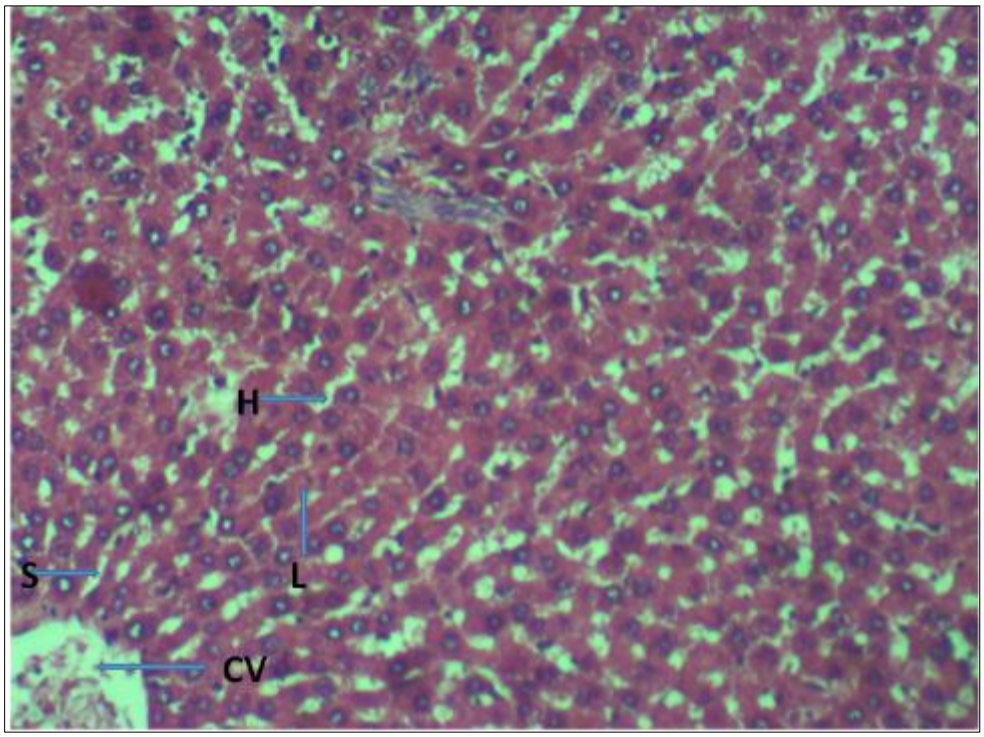

Figure 3 Liver section of treatment group animals exposed to 100 mg/kg b. wt. showing normal tissue architecture. The central vein (CV), the lamella (L), sinusoids (S) and hepatocytes (H) all appeared normal (x400), Stain: H and E

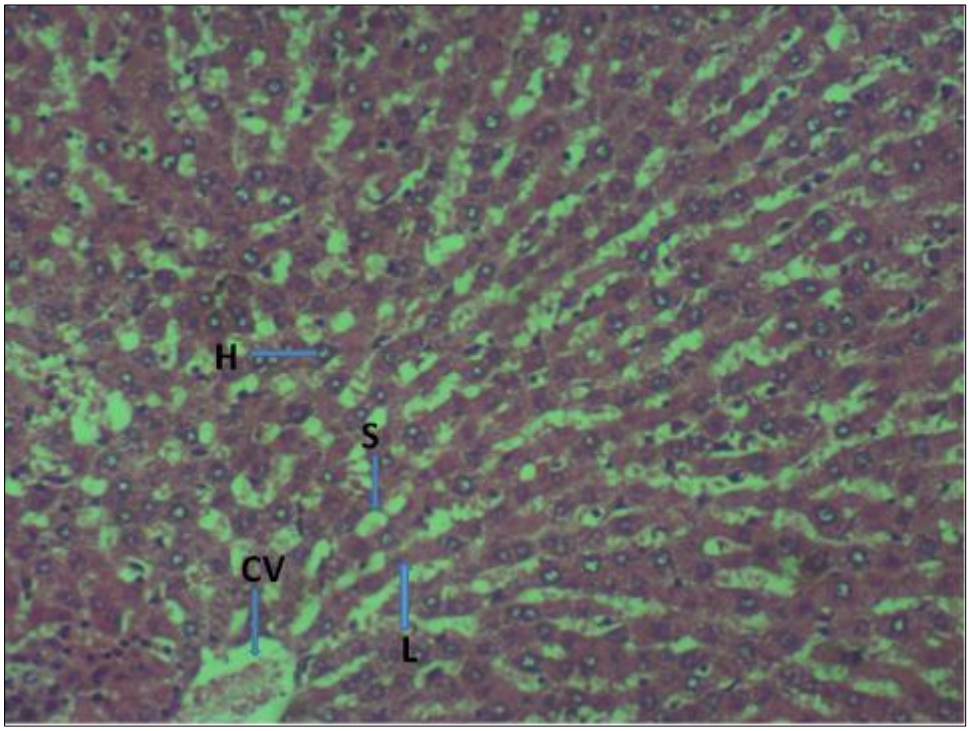

Figure 4 Liver section of treatment group animals exposed to 200 mg/kg b. wt. of tea extract showing scanty stroma with dilated sinusoids (S) and hepatocytes (H) with halo. The central vein (CV) and the lamella (L) appear unremarkable (x400), Stain: H and E

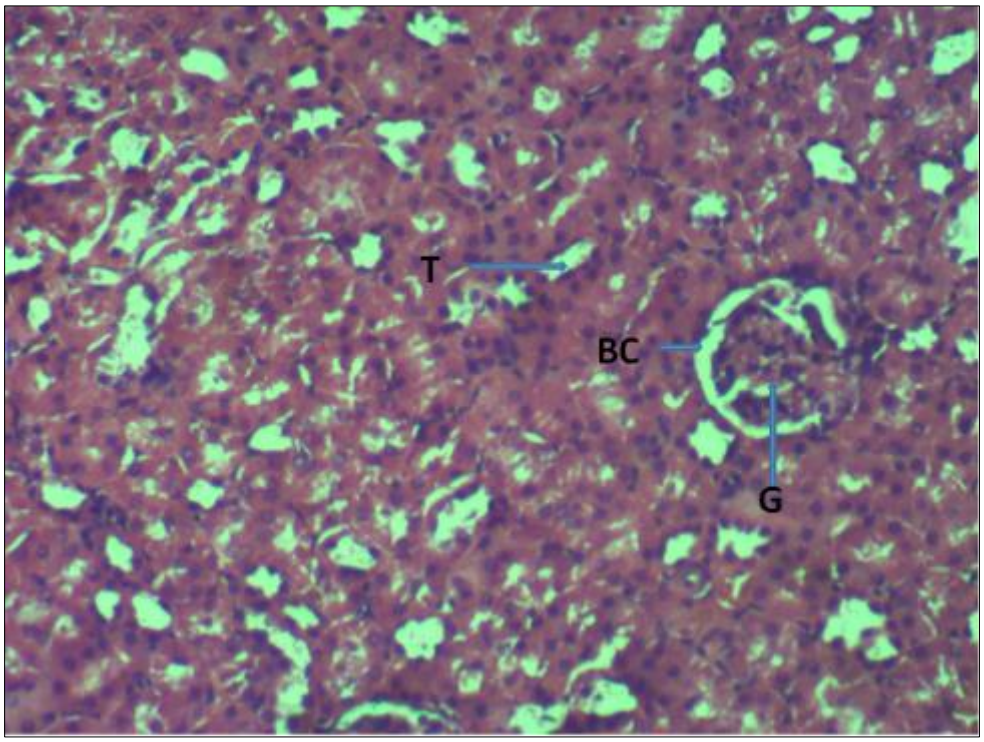

Figure 5 Kidney section of the normal control group animals showing normal tissue architecture. The glomeruli (G), the Bowman's capsule (BC) and tubules (T) appear unremarkable (x400), Stain: H and E

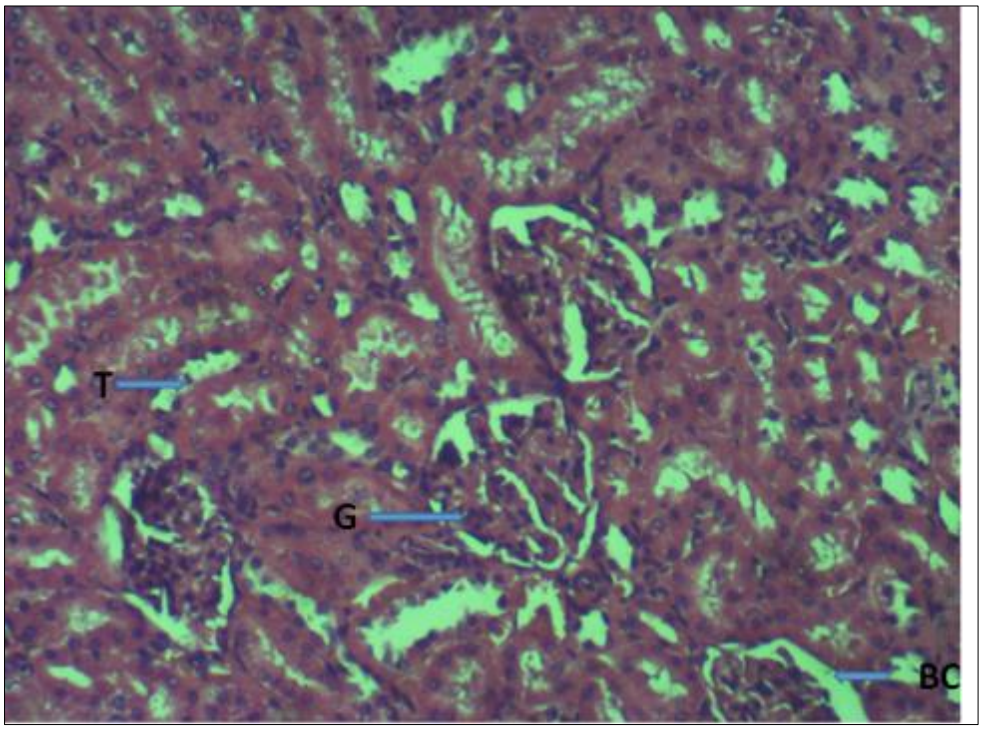

Figure 6 Kidney section of treatment group animals exposed to 50 mg/kg b. wt. of tea extract showing normal tissue architecture. The glomeruli (G), Bowman's capsule (BC) and the tubules (T) all appear unremarkable (x400), Stain: H and E

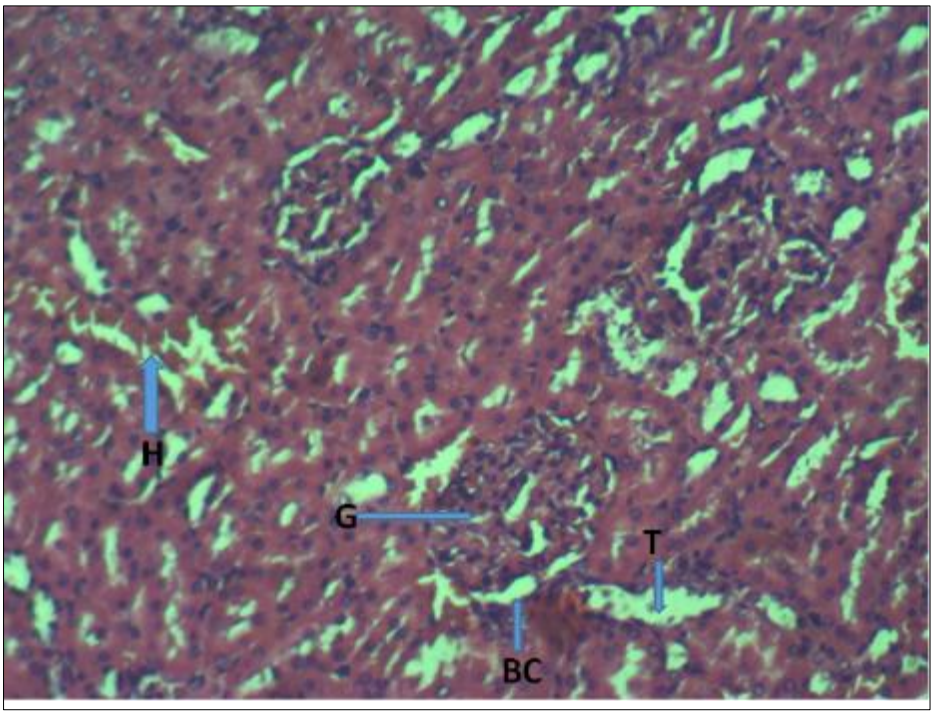

Figure 7 Kidney section of treatment group animals exposed to 100 mg/kg b. wt. of tea extract showing glomeruli (G) which appear closely adherent to the Bowman's capsule (BC). Some of the tubules (T) are enlarged and there is a focus of haemorrhage (H) (x400), Stain: H and E

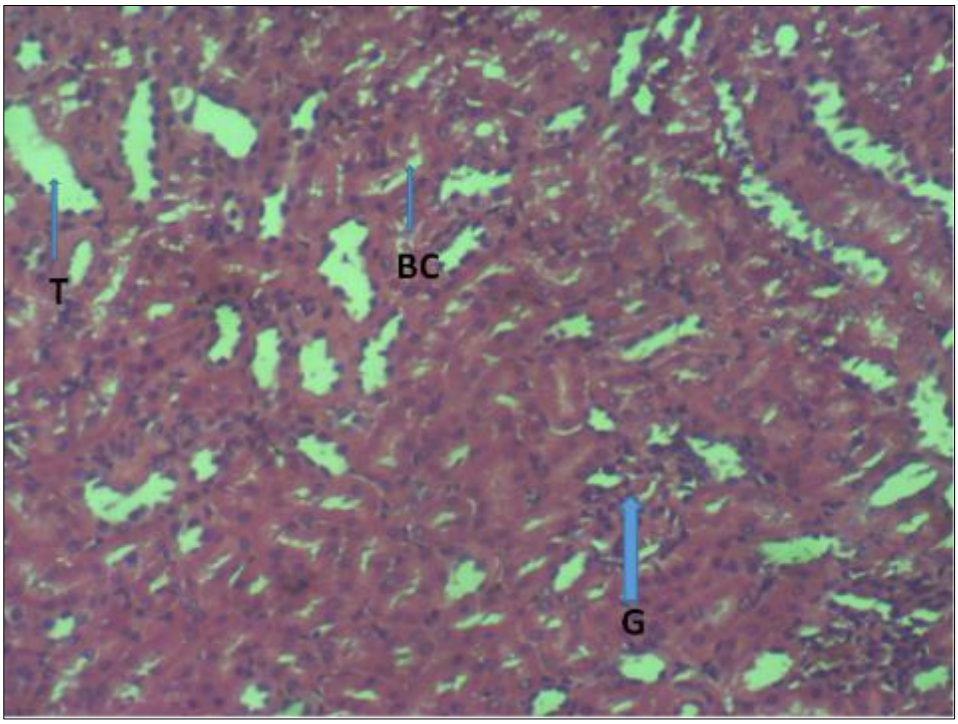

Figure 8 Kidney section of treatment group animals exposed to 200 mg/kg b. wt. of tea extract showing glomeruli (G) which appear closely adherent to the Bowman's capsule (BC). Some of the tubules (T) are enlarged (x400), Stain: H and E

4. Discussion

Aspartate and alanine aminotransferases (AST and ALT) are known indicators of liver function integrity. High activities of AST, and ALT as well as alkaline phosphatase (ALP) in serum are usually indicative of disease and necrosis in the liver of animals. The results of the present study indicates that the antimalarial tea extract has a significant positive impact on the liver enzymes (ALT, AST, ALP), suggesting potential hepatoprotective properties. This aligns with findings from previous studies that have documented the hepatoprotective effects of various medicinal plants which has been variously associated with their phytochemical compositions mainly their antioxidant properties (Igwe *et al.,* 2021; El-Kashef *et al*., 2021; Igwe *et al.,* 2022). The observed reduction in liver enzymes suggests that the tea extract may help in stabilizing hepatocyte membranes, thereby reducing leakage of these enzymes into the bloodstream. This mechanism is consistent with the known effects of plant-based antioxidants in preventing liver damage (Sreelatha *et al*., 2009; Igwe *et al.,* 2022). The lack of significant changes in total bilirubin, total protein, and albumin concentrations corroborated the earlier observation that the tea extract does not adversely affect liver function at the tested doses, indicating a good safety profile. These observed effects could be attributed to the antioxidant compounds present in the tea extract, which help in scavenging free radicals and protecting liver cells from oxidative damage (Ayoka *et al.,* 2023). Synthetic antimalarial drugs, such as chloroquine, have been associated with hepatotoxicity, leading to elevated liver enzymes (Ajayi *et al*., 2008). The findings from this study suggest that the tea extract could be a safer alternative with fewer, if any, hepatotoxic effects.

Results of the kidney function and electrolyte profile suggest that the antimalarial tea extract generally does not affect the kidney and electrolyte function parameters of animals especially at low doses. The data suggests that low doses (50 mg/kg and 100 mg/kg) of the extract did not disrupt kidney function or electrolyte balance, whereas higher doses (200 mg/kg) may pose a risk of nephrotoxicity given the observed significant increase in urea concentration. This information is crucial for determination of safe and effective dosages. The decrease in creatinine levels at the highest treatment dose could be due to enhanced clearance or reduced muscle mass, which needs further investigation. The observed changes in sodium ion concentrations, though not significant might be linked to the extract's diuretic effect, which could be beneficial in managing fluid balance in malaria patients but requires careful monitoring. Similar findings have been observed with other plant extracts known for their diuretic effects (Abdel-Rahman *et al*., 2011). The stability in the concentrations of potassium, chloride, and bicarbonate ions across the treatment groups suggests that the overall electrolyte balance in the animals is maintained to a fairly good extent by the extracts' administration. This indicates that the tea extract does not disrupt electrolyte homeostasis, which is a positive sign compared to synthetic antimalarials that can cause electrolyte imbalances (Adewoye *et al*., 2010; Uzoho *et al.,* 2020).

The haematologic profile results indicate that the antimalarial tea extract, at various doses, does not significantly disrupt the primary haematologic parameters, except for WBC count, which showed a notable increase at the highest dose (200 mg/kg). The observed increase in WBC may indicate an immune response or inflammatory reaction to the presence of the extract. Previous studies on medicinal plant extracts with similar properties have shown that certain compounds can stimulate the immune system, potentially leading to leukocytosis (Nworu *et al.*, 2011). This response might be beneficial in combating malaria, as a heightened immune response could aid in parasite clearance. The stability of RBC, HGB, PCV, MCV, MCH, and MCHC values across all doses aligns with findings from other studies where plant extracts did not adversely affect erythropoiesis or red blood cell integrity (Oluyemi *et al*., 2010; Uzoho *et al.,* 2020). This is crucial for antimalarial therapy, as maintaining healthy red blood cell counts, integrity and function are essential to avoid anaemia, a common complication of malaria, and aid in patient's stability and recovery. The stable platelet counts observed suggest that the tea extract does not induce thrombocytopenia, a condition characterized by low platelet counts and a known side effect of some antimalarial drugs (White, 2013). This further supports the safety profile of the tea extract regarding haematologic parameters.

The histopathological findings suggest that doses up to 100 mg/kg are relatively safe for both liver and kidney, with 50 mg/kg showing no observed adverse effects. Higher doses (200 mg/kg) show early signs of organ stress, warranting careful dose optimization to avoid potential toxicity. Previous studies on herbal extracts like those of *Chromolaena odorata* and *Setaria megaphylla* have shown hepatoprotective effects at certain doses but hepatotoxicity at higher doses (Nworu *et al*., 2011; Oluyemi *et al*., 2010). The observations in this study align with these findings, indicating that moderate doses are safe, while higher doses may induce mild liver stress. The adherence of glomeruli to Bowman's capsule and tubular enlargement observed at 200 mg/kg dose administration in this study support the notion of dose-dependent nephrotoxicity. The mild liver and kidney alterations at the highest dose indicate the crucial need to sensitively balance the antimalarial efficacy with the potential for organ toxicity. Thus, lower doses should be preferred for chronic use to minimize adverse effects.

5. Conclusion

The findings of this study demonstrated that the antimalarial tea extract possesses potential hepatoprotective properties at moderate doses, as evidenced by the significant reduction in liver enzymes (ALT, AST, and ALP) without adverse effects on bilirubin, total protein, or albumin levels. This suggests that the tea extract may help stabilize hepatocyte membranes, likely due to its antioxidant properties, thereby reducing the risk of liver damage. However, at higher doses (200 mg/kg), early signs of organ stress, particularly in the liver and kidneys, were observed, indicating a dose-dependent risk of toxicity.

The kidney function and electrolyte profile analysis further revealed that while lower doses of the tea extract do not significantly disrupt renal function or electrolyte balance, higher doses may pose a nephrotoxic risk, necessitating careful dose optimization. The observed increase in WBC counts at the highest dose suggests a potential immune-modulatory effect, which may contribute to its antimalarial efficacy but also warrants caution due to the risk of an inflammatory response.

Histopathological findings support the biochemical data, indicating that doses up to 100 mg/kg are relatively safe for liver and kidney tissues' integrity, with no significant adverse effects observed between 50 and 100 mg/kg. However, higher doses exhibited early signs of organ stress, highlighting the importance of balancing therapeutic efficacy with safety. These results suggest that while the antimalarial tea extract shows promise as a safer alternative to synthetic antimalarials, careful consideration of dosing is crucial to avoid potential hepatotoxic and nephrotoxic effects. Further studies are recommended to elucidate the long-term safety and efficacy of the tea extract in managing malaria, particularly at varying dosage levels.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All experimental procedures involving animals were conducted in accordance with the ethical standards and guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Federal University of Technology Owerri (FUTO). Efforts were made to minimize the number of animals used and to reduce their suffering throughout the experimental period.

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