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Toxicological evaluation of anti-cough herbal tea made from aqueous extracts of *G. Kola, C. Citratus and B. pinnatum*

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Abstract

Background: Herbal formulations currently contribute significantly to medical remedies with varying effects on health indices. *Garcinia kola* seeds, *Cymbapogon citratus,* and *Bryophyllum pinnantum* leaves are among the widely used in herbal formulations.

Objectives: This study evaluated the toxicological effects of the ternary combination of these plant materials on some renal, hepatic, hematological and histological parameters of streptococci-infected white albino rats.

Materials and Methods: Samples of *Garcinia kola* seeds, *Cymbapogon citratus* leaves, and *Bryophyllum pinnantum* leaves were sourced locally and authenticated by a taxonomist. Aqueous extract of the ternary combination comprising of 40% *G. kola*, 30% *C. citratus*, and 30% *B. pinnantum* was prepared and administered on albino rats. Thirty five white albino rats grouped into 7 groups comprising of normal control, treatment control, disease control, and test group were used for the evaluation after a 14 days treatment on infected and uninfected rats. The rats were sacrificed, and samples collected. Some renal, hepatic, histological, and hematological indices were analyzed.

Results: Comparison of all hematological parameters (except for platelet count) between uninfected-untreated and unifected-treated with extract showed no significant ($p>0.05$) difference. Platelet count was significantly ($p<0.05$) raised in all groups treated with the extract. Comparison control and the extract-treated group showed no significant ($p>0.05$) difference in renal parameters indicating no adverse effects on renal function. However, comparison of parameters between control and the infected-treated group showed significantly elevated creatinine which alone may not be sufficient to support renal damage. Albumin, and total bilirubin were significantly ($p<0.05$) lower in the extract-treated group compared to the control group. In contrast, total protein and globulin were significantly ($p<0.05$) higher in the controls to the infected groups. ALT and AST were significantly ($p<0.05$) lower in the extract treated groups compared to the control group. Although comparison of ALP activity between uninfected-untreated and infected-treated group showed significant ($p<0.05$) elevation this alone may not be sufficient to support liver damage. The hematoxyline and eosine stained sections of the kidney and the liver tissues of the tea fed groups show normal kidney and liver histology. There was no significant change in the liver and kidney histology of the test and control groups. The extract did not cause adverse impact on these organs.

Conclusion: Ternary combination of 40% *G. kola*, 30% *C. citratus*, and 30% *B. pinnantum* can effectively be used in treatment of cough without significant adverse effects on renal, hepatic, hematological and histological parameters. The ternary combination rather demonstrated significant hepato-protective feature.

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Keyword: *Garcinia kola; Cymbapogon citratus; Bryophyllum pinnantum*; Toxicological; Renal; Hepatic; Hematological; And Histological Parameters.

1. Introduction

Herbal medicine has continued to be a substantial part of medical practice, howbeit some deleterious outcomes. The pharmacological properties of many herbal materials have long been determined, and their usefulness as remedies to certain pathological conditions established. There has been studies evaluating the strength and weaknesses of herbal medicine, with a view to improve its usefulness [1]. Some of the practices involve the administration of crude material and unregulated dosage, which could be deleterious to health [2]. The main objective of toxicological assessment of any herbal medicine will be to identify adverse effects and to determine limits of exposure level at which such effects occur. Primarily of importance are the nature and significance of the adverse effect, and in addition the exposure level where the effect is observed. Extracts of *Garcinia kola, Cymbapogon citratus, Bryophyllum pinnantum* have been previously reported to possess antimicrobial efficiency against some cough-causing bacteria [3]. Toxicological evaluation of these herbal plants is thus necessary to reveal some potential risks that may be associated with use of the herbs, and especially for possible use by human in the treatment of cough.

Klein [4] stated that medicinal plants have the potential to be harmful, postulating that it is dependent on the dose of substance consumed. However, because medicinal plants are natural, a great proportion would have to be consumed to cause harm when compared with orthodox drugs. Consumers use herbal medicine due to lack of satisfaction with conventional medicine, affordability, ease of access, and traditional beliefs. However there is a growing concern for health risk due to misbranded toxic ingredients, contaminants, adulterants and herb-drug interactions with co-administered drugs [5].

Herbal teas may consist of mono or poly-herbal materials that are brewed as decoction or infusion and drank for their therapeutic benefits [6]. *Garcinia kola* seeds*, Cymbapogon citratus* leaves*,* and *Bryophyllum pinnantum* leaves are plant materials used in different ways for different medicinal purposes. The phytochemical properties of these plants include alkaloids, phenols, saponines, tannins, and flavonoids. They have been widely used in the formulation of various medicinal preparations due to their effectiveness against certain health problem as a result of their antimicrobial, anti cancerous and anti-inflammatory properties apart from nutritional benefits, however their effectiveness has not been fully explored, especially in their various combinations for different ailments. This study thus hopes to explore the safety in their beneficial uses in the treatment of cough.

The spectrum of pharmacological and toxicological effects of herbs may include confusing laboratory result, allergic reactions, genotoxicity, carcinogenicity, tetratogenicity, organ damage and even fatality. Determination of acute and sub acute toxicity properties of medicinal plants are essential to guide herbal medicine practitioners and researchers [7].

Plants are valuable source of food and medicine for prevention of illness and maintenance of human health. Plants produce secondary metabolites that are not directly associated with plant development but play an essential role in plant protection and adaptation to the environment [8]. These serve as eminent source of new therapeutic agent providing alleviation to human ailments and good health. These preventative and protective properties are related to their strong antioxidative, antimutagenic, and anticarcinogenic potentials. These compounds are divided into three families: nitrogeneous compounds (alkaloids), phenolics also known as polyphenols, and terpenoids [9].

Alkaloids constitute an important structurally diversified compounds having nitrogen atom in the heterocyclic ring and are derived from amino acid [10]. Alkaloids are unique lead compounds in medicine. They have basic properties in which they are water soluble under acidic condition and lipid soluble under neural and basic conditions. They are important therapeutic agents due to their efficacy in preventing the onset of different diseases by scavenging free radicals or binding with catalysts of oxidative reactions, such as some metal ions.

Phenolic compounds also known as polyphenols include flavonoids, tannic acid, eligitannin and tannins. Tannins are classified as hydrolysable or condensed. They posses reactive oxygen species reduction characteristics and play a role in homeostasis. These activities are associated with their diverse roles as anti inflammatory, antiaging, and antipoliferative health benefits [7]. Flavonoids are the commonest phenolic constituents having fifteen compounds, generally distributed throughout the plants kingdom. They are associated with a large range of health benefits arising from their bioactive properties, such as inflammatory, anti cancer, antiaging, cardio-protective, neuroprotective, immunomodulatory, antidiabetic, antibacterial, antiparasitic, and antiviral properties [11]. Some flavonoids have been

reported to have more anti bacterial function with gram positive species being more sensitive to flavonones than their negative counterpart. Phenolic compounds have antifungal and anti-microbial effects [12].

Terpenoids also referred to as isoprenoids are the most abundant secondary metabolites found in plant. They form a unique defense system against biotic and abiotic stresses [13]. They also partake in induced defense system responses of plants against herbivores, insects and microbial pathogens [14]. Examples of terpenoid include carotenoids, sterols, gibberellins, chlorophylls and plastoquinines [15].

Saponins are glycosides occurring widely in form of triterpenoids. They are surface active in nature as they contain hydrophilic sugar moieties covalently attached to a triterpene backbone or hydrophobic steroid [16]. Saponins are associated with defense mechanism in plants due to their anti microbial, antifungal, antiparasitic, and mollusicidal effects [17].

The increasing interest in herbal medicine has led to a growing emphasis on the pharmacological and toxicological properties of medicinal plants. This is crucial for ensuring the safety and efficacy of these natural remedies, and the understanding their mechanisms of action. Also by understanding their biological effects and potential toxicity, we can harness the therapeutic potentials of plants while minimizing risks to human health.

2. Methods

2.1. Plant materials collection and preparation

Fresh healthy leaves of *B. pinnatum* and *C. citratus* and seeds of *G. kola* used for the study were sourced from Owerri, Nigeria and authenticated by a plant taxonomist, Mr. Francis Iwueze of the Department of Forestry and Wildlife Technology, Federal University of Technology Owerri (FUTO), Imo State and given voucher numbers FUTO/FWT/ERB/2021/59, FUTO/FWT/ERB/2022/66, and FUTO/FWT/ERB/2023/102 respectively. The samples were washed under running tap water, air-dried at room temperature, and pulverized into fine particulate forms using industrial-grade grinding machine. The ternary (40% *G. kola*, 30% *C. citrates* and 30% *B. pinnatum*) combinations was prepared, and subsequently stored in labeled compact containers at room temperature. Aqueous extraction of the plant samples followed standardized preparation method [18]. Four hundred and fifty grams of the ground plant sample was dissolved in distilled water in a 2.5 L volumetric flask and the solution made up to mark and subjected to boiling for a duration of 30 minutes, followed by decanting, filtration, and freeze-drying.

2.2. Experimental animal handling and grouping

Healthy 35 adult male albino rats of Wister strain weighing 150 to 200g was used for the study. The animals was purchased from Animal friend Farm, Owerri, and kept five per cage using standard laboratory metal animal cage, for 14 days. They were maintained under standard environmental conditions (12hr light and 12hr dark cycle uniform temperature of 28± 5°C). All animals had free access to food (Vital feed finisher pellets, Ibadan) and water. All investigations involving the animals were conducted in accordance with the accepted principles for animal care and use according to the ethical guidelines for the use of animal in research [19].

Table 1 Table of animal grouping and treatment

Groups	Number of rats	Treatment for 14 days
1: normal control	5	Water *ad libitum*
2: Test group A	5	1 ml tea extract/kg body weight
3: Test groups B	5	2 ml tea extract/kg body weight
4: Test groups C	5	Infected with a cough causing organism (untreated)
5: Test groups AB	5	Infected with a cough causing organism and treated with the standard drug
6: Test groups AC	5	Infected with a cough causing organism and treated with 1 ml Tea extract/kg
7 :Test groups BC	5	Infected with a cough causing organism and treated with 2 ml Tea extract/kg

2.3. Administration of the extract

The 'test' compound [was prepared using 30 % *Bryophyllum pinnatum, 30 % Cymbopogan Citratus, 40 % G. Kola)*. One ml equivalent to 0.265 %/g body weight; 2ml equivalent 0.526 %/g body weight. Ciprofloxacin 0.45mg/g body weight was used as the standard drug for treatment. The treatment was administered as shown above in the animal grouping.

2.4. Animal sacrifice and sera preparation

All the experimental animals were sacrificed 24 hours after the last administration of the extracts. Blood samples for sera preparation were collected by cardiac puncture into sterile plain bottles for clinical chemistry analysis, and EDTA bottles for hematological analysis. Sera were obtained from the blood by centrifugation using a bench top centrifuge (MSE) at 3000g for 10 minutes.

2.5. Laboratory analysis

The hematological analysis (full blood count) was done using a whole blood hematology analyzer (automated) method [20]. Liver function parameters comprising of Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phospatase (ALP), total protein (TP), albumin and bilirubin (Bil) were done by their respective spectrophotometric methods [21-25]

The electrolytes were assayed using ion selective electrode potentiometric method [26] using SFRI electrolytes analyzer (model: ISE 4000, France).

Urea was estimated using the urease Berthelot method [27]. While creatinine estimation was done using the Jaffe's reaction method [28].

2.6. Histological analysis of liver and kidney tissues

Two samples of liver and kidney per group was randomly selected and dissected out and immediately fixed in 10% formal saline for 7 days for histopathological analysis. They were then dehydrated in graded ethanol, treated in acetone and cleared in xylene, then infiltrated with and embedded in paraffin wax. Each was sectioned at 5µm and was stained with hematoxylin and eosin [29]. The sections were subsequently viewed and their photomicrographs were taken.

2.7. Statistical analysis

Data obtained from the study were presented as mean ± standard deviation, and were analyzed using one-way analysis of variance (ANOVA) and Turkey post-HOC test with the aid of GraphPad Prism Version 5.0. Statistical level of significance was taken at $p<0.05$.

3. Results

3.1. Kidney function parameters

Comparison of renal function parameters between uninfected-untreated and uninfected-treated with extract in table 2 showed no significant ($p>0.05$) difference in renal parameters. Similarly comparison of renal function parameters between uninfected-untreated and uninfected-treated with extract showed no significant difference indicating that infection had no adverse effects on renal function. However, comparison of parameters between uninfected-untreated and infected-treated showed significantly elevated creatinine which alone may not be sufficient to support renal damage

3.2. Liver function parameters

Table 3 show the comparison of liver function parameters among the test groups. Albumin, ALT, AST and total bilirubin were significantly ($p<0.05$) lower in the extract treated group, and in the infected group compared to the uninfected-untreated control group. In contrast, total protein and globulin were significantly ($p<0.05$) higher in the control and in the infected untreated groups compared to the extract-treated group. ALP activity was significantly ($p<0.05$) higher in the extract-treated group, which alone may not be sufficient to support liver damage.

3.3. Hematological profile

Results of the hematological profile are shown in table 4. Comparison of all haematological parameters (except for platelet count) between uninfected-untreated and unifected-treated with extract showed no significant ($p>0.05$) difference. Platelet count was significantly ($p<0.05$) raised in all groups treated with the extract. Hemoglobin and

packed cell volume were significantly ($p<0.05$) lower in the infected and untreated group compared to all other groups; probably indicating the impact of infection in the pathogenesis of aneamia.

Table 2 Kidney function and electrolyte profile of albino rats administered tea infusion made from ternary combination of *G. kola, C. citratus* and *B. pinnatum* extracts.

Parameter	Uninfected and Untreated	Uninfected + 1ml Tea	Uninfected + 2 ml Tea	Infected and Untreated	Infected + STD drug	Infected + 1ml Tea	Infected + 2 ml Tea
Urea (mmol/L)	2.31 ± 0.04^{a}	2.25 ± 0.21^{ab}	2.44 ± 0.18^{a}	1.62 ± 0.48^{b}	2.11 ± 0.45^{ab}	2.55 ± 0.31^{a}	1.80 ± 0.10^{b}
Creatinine (mmol/L)	132.00 ± 10.39^{ab}	123.25 ± 14.29^{b}	145.50 ± 9.00^{abc}	144.50 ± 32.14^{abc}	141.00 ± 14.70^{abc}	180.75 ± 17.15^{c}	167.75 ± 22.85^{ac}
Sodium (mmol/L)	134.00 ± 0.82^{ab}	132.75 ± 2.50^{b}	133.75 ± 0.50^{ab}	134.00 ± 2.16^{ab}	134.25 ± 1.26^{ab}	137.00 ± 0.82^{a}	133.50 ± 2.08^{ab}
Potassium (mmol/L)	3.90 ± 0.19^{ac}	2.88 ± 0.15^{b}	4.29 ± 0.08^{a}	2.97 ± 0.19^{bd}	2.95 ± 0.13^{bd}	4.22 ± 0.39^{a}	3.40 ± 0.28^{cd}
Chloride (mmol/L)	102.50 ± 1.29^{ab}	102.50 ± 1.00^{ab}	103.25 ± 0.50^{a}	102.25 ± 0.50^{ab}	102.25 ± 0.96^{ab}	104.00 ± 0.82^{a}	101.25 ± 0.50^{b}
Calcium (mmol/L)	1.68 ± 0.04^{a}	1.82 ± 0.03^{b}	1.79 ± 0.03^{b}	1.91 ± 0.02^{c}	1.87 ± 0.01^{bc}	1.69 ± 0.04^{a}	1.83 ± 0.04^{b}
pH value	8.04 ± 0.05^{a}	8.05 ± 0.06^{a}	8.04 ± 0.01^{a}	8.13 ± 0.02^{a}	8.10 ± 0.03^{a}	8.03 ± 0.01^{a}	8.12 ± 0.09^{a}

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

Table 3 Liver function profile of albino rats administered tea infusion made from ternary combination of *G. kola, C. citratus* and *B. pinnatum* extracts.

Parameter	Uninfected and Untreated	Uninfected + 1ml Tea	Uninfected + 2 ml Tea	Infected and Untreated	Infected + STD drug	Infected + 1ml Tea	Infected + 2 ml Tea
Total protein (g/L)	58.73 ± 2.18^{a}	63.75 ± 1.16^{bc}	61.48 ± 2.02^{ab}	63.45 ± 2.67^{bd}	67.48 ± 1.63^{cd}	66.13 ± 1.67^{cd}	64.85 ± 0.87^{bd}
Albumin (g/L)	42.60 ± 2.29^{ac}	35.68 ± 2.99^{b}	36.75 ± 1.34^{b}	37.63 ± 1.33^{b}	39.25 ± 1.56^{ab}	44.60 ± 1.39^{c}	38.53 ± 3.06^{ab}
Globulin (g/L)	16.13 ± 1.30^{a}	28.08 ± 4.07^{b}	24.73 ± 1.13^{bc}	25.83 ± 1.91^{bc}	28.23 ± 1.11^{b}	21.53 ± 0.74^{c}	26.33 ± 2.90^{bc}
Albumin/Globulin ratio	2.66 ± 0.31^{a}	1.30 ± 0.29^{b}	1.49 ± 0.07^{b}	1.46 ± 0.10^{b}	1.39 ± 0.09^{b}	2.07 ± 0.09^{c}	1.48 ± 0.26^{b}
Total bilirubin (umol/L)	45.47 ± 5.31^{a}	22.27 ± 4.95^{b}	25.45 ± 1.25^{b}	25.00 ± 1.76^{b}	23.82 ± 1.54^{b}	35.46 ± 1.54^{c}	25.39 ± 4.48^{b}
ALP (IU/L)	601.25 ± 39.85^{ac}	721.50 ± 19.02^{b}	549.75 ± 18.73^{a}	730.00 ± 51.40^{b}	649.75 ± 47.13^{bc}	670.00 ± 44.95^{bc}	722.25 ± 33.26^{b}
ALT (IU/L)	50.75 ± 0.96^{a}	29.00 ± 0.82^{b}	38.25 ± 0.96^{c}	34.00 ± 0.82^{d}	30.50 ± 1.73^{b}	48.50 ± 0.58^{a}	30.75 ± 0.96^{b}
AST (IU/L)	75.00 ± 0.82^{a}	63.25 ± 1.26^{b}	59.25 ± 0.50^{c}	80.50 ± 0.58^{d}	66.50 ± 1.29^{e}	83.50 ± 1.29^{f}	75.25 ± 0.50^{a}

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

Table 4 Haematological profile of albino rats administered tea infusion made from ternary combination of *G. kola, C. citratus* and *B. pinnatum* extracts.

Parameter	Uninfected and Untreated	Uninfected + 1ml Tea	Uninfected + 2 ml Tea	Infected and Untreated	Infected + STD drug	Infected + 1ml Tea	Infected + 2 ml Tea
WBC (K/µl)	8.13 ± 1.38^{a}	10.15 ± 1.21^{ab}	8.03 ± 1.28^{a}	9.05 ± 1.30^{a}	8.73 ± 1.25^{a}	12.28 ± 1.19^{b}	10.53 ± 1.27^{ab}
HB (g/dl)	12.13 ± 0.53^{a}	12.55 ± 0.68^{a}	12.15 ± 0.42^{a}	10.42 ± 0.88^{b}	12.75 ± 0.47^{a}	12.90 ± 0.42^{a}	12.80 ± 0.83^{a}
RBC (million/ml)	6.91 ± 0.33^{ab}	7.48 ± 0.59^{a}	7.33 ± 0.28^{a}	6.10 ± 0.64^{b}	7.14 ± 0.37^{ab}	7.58 ± 0.55^{a}	7.48 ± 0.43^{a}
HCT (%)	36.03 ± 1.98^{a}	38.55 ± 2.89^{a}	37.05 ± 0.80^{a}	30.55 ± 1.87^{b}	37.73 ± 1.54^{a}	38.40 ± 1.36^{a}	37.10 ± 2.47^{a}
MCV (fl)	52.18 ± 0.71^{a}	51.70 ± 3.36^{a}	50.70 ± 2.61^{a}	50.35 ± 2.08^{a}	52.93 ± 0.80^{a}	50.83 ± 2.02^{a}	49.65 ± 1.44^{a}
MCH (g/dl)	17.48 ± 0.33^{ab}	16.75 ± 0.50^{a}	16.58 ± 0.62^{a}	17.08 ± 0.32^{ab}	17.85 ± 0.37^{b}	17.03 ± 0.68^{ab}	17.05 ± 0.31^{ab}
MCHC (g/dl)	33.63 ± 0.51^{a}	32.58 ± 1.48^{a}	32.75 ± 1.19^{a}	34.03 ± 0.79^{a}	33.73 ± 0.25^{a}	33.55 ± 0.21^{a}	34.48 ± 0.56^{a}
Platelet count (/µl)	447.00 ± 14.79^{a}	515.25 ± 24.54^{b}	529.25 ± 60.40^{b}	421.75 ± 78.89^{a}	485.75 ± 77.31^{c}	487.25 ± 16.13^{c}	527.25 ± 18.08^{bc}

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

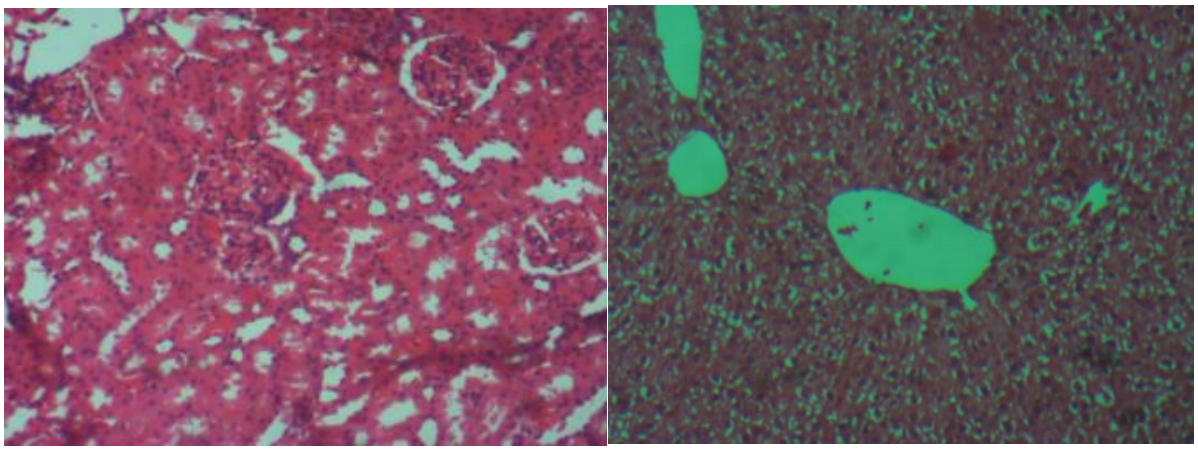

Figure 1 Hematoxyline and eosine stained sections of the kidney (left) and the liver (right) of study group 1 (Normal:Uninfected and Untreated) showing a usual kidney and liver histology.

Section show glomeruli (G) having tuft of capillaries, with outer and inner epithelium. Also seen are the proximal convoluted tubule (PCT) and distal convoluted tubules (DCT). The PCT is bordered by simple layer of cuboidal cells which are absent in the DCT. Sections of the liver show hepatic cells(H) radiating outward from the central vein (CV). The hepatocytes architecture are intact, and are organized in lobes consisting of the bile ducts (BD), hepatic artery and vein

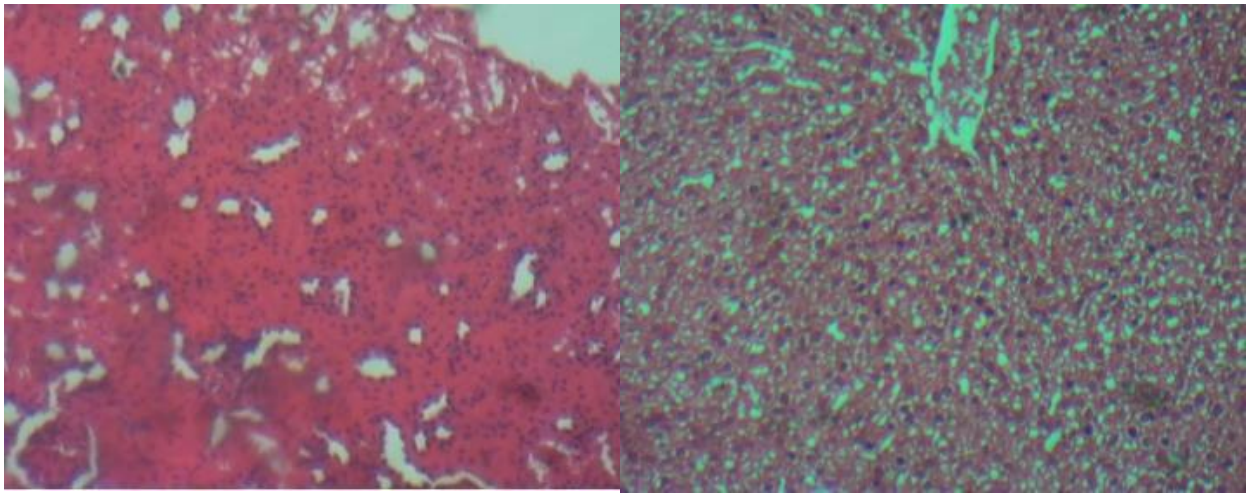

Figure 2 Hematoxyline and stained eosine sections of the kidney (left) and the liver (right) of study group 2 (extract-treated) showing a normal kidney and liver histology.

The kidney section show glomeruli (G) having tuft of capillaries, with outer and inner epithelium. Also seen are the proximal convoluted tubule (PCT), and distal convoluted tubules (DCT). The PCT is bordered by simple layer of cuboidal cells which are absent in the DCT. Sections of the liver show hepatic cells (H) radiating outward from the central vein (CV). The hepatocytes architecture intact and are organized in lobes consisting of the bile ducts (BD), hepatic artery and vein

3.4. Histological analysis of liver and kidney.

Results of the liver and kidney histological analysis are as shown in figures1 and 2. All the study groups showed normal liver architecture, liner parenchyma with a distinct central lobular vein and hepatocytes. Histology of the kidney showed normal parenchyma with distinct glomerulus and urinary space. The intact cellular integrity in the treatment groups is comparable to the control.

4. Discussion

The kidney is responsible for filtration, secretion and reabsorption, as a result plays a crucial role in the removal of toxic substances from the system. The toxicological evaluation of a substance in animal model was to ascertain the possible risk to human life. The effect of the investigated herbal treatment on sodium, potassium, urea, creatinine, and chloride are seen in tables 2. The herbal treatment increased urea concentration in the infected group treated with 1ml of tea extract compared to the infected and untreated. However these values were not statistical ($p>0.05$) increased when urea was compared with the control group (uninfected and untreated). Similarly, creatinine concentration did not significantly vary across all the groups compared to normal control with exception of the infected and treated group. This finding is indicative of the influence of infection on renal function.

Similar trend was observed for serum electrolyte profile result where potassium concentrations were seen to be significantly ($p>0.05$) increased in infected groups treated with 1ml and 2ml extract in a dose dependent manner. However, sodium, chloride concentrations and ph level were not significantly altered following the administration of poly herbal tea extract. The above findings preclude nephrotoxicity.

The liver is one of the prime target organs of any form of toxicity. This is because liver plays a critical role in the biotransformatiom of chemical substances and facilitates their elimination from the body [30]. In this study, assessment of liver damage was determined by serum activities of ALT, AST and ALP. The ALT, ALP, and AST activities did not significantly ($p>0.05$) increase following the administration of the aqueous extract to the study groups. This precludes the incidence of hepatoxicity, and rather suggestive of hepao-protective characteristics of the tea; having caused a significant reduction in the activities of the enzymes in a dose dependent manner. *B.pinnatum, C.citratus and Garcinia Kola* contain many phytochemicals such as alkaloids, triterpenes, glycosides , bufadienolides, with pharmacological actions which have been confirmed by previous studies to be hepatoprotective [31-32].

Similar to the outcome from the enzyme studies, bilirubin which is also a good index of assessment of liver damage, was significantly ($p<0.05$) reduced in the extract administered groups. This further support the hepato-protective characteristics of the plant extract. Reduction in serum bilirubin concentration is a pointer to a non-toxic effect of the aqueous extracts on liver tissue and may be an indication of a possible regenerative effect on hepatocytes and increasing potential of bilirubin transport to the liver for conjugation [33].

Furthermore, assessment of effect on liver function showed that serum total protein concentration was seen to be elevated in a dose dependent manner when the infected group treated with the aqueous extract is compared with infected and untreated group. This may indicates that the liver function of protein synthesis is enhanced by the extract. While as protein synthesis can be stimulated in response to both endogenous and exogenous stimuli [34], it is likely that the phytochemical composition of the tea could induce protein synthesis. Furthermore, hormones such as insulin are known to stimulate protein synthesis [35]. And certain phytochemicals possess insulin-like properties [36].

Results of the hematological profile are shown in table 4. Comparison of all hematological parameters (except for platelet count) between uninfected-untreated and uninfected-treated with extract showed no significant difference. Platelet count was significantly raised in all groups treated with the extract. Green leafy vegetables, milk, papaya, pomegranate, as well as many other natural plant substances have been shown to contain substances that increase platelet production [37]. Similarly, substances contained in the ternary mixture of the tea have been shown to favor platelet production. Platelets abnormalities can be observed in the pathogenesis of certain disease or as a complication of therapy [38]. This positive effect of the tea on platelets will thus offer a beneficial role in the management of such disorder; including cough. Also, the red cell count was significantly higher in all groups treated with the ternary extract than the untreated groups. This further support the hematenic property of the plants and its possible utility in the prevention of anemia during cough treatment. This is similar to findings from recent studies [39].

There were no significant difference in the hemoglobin concentration, packed cell volume, MCV, MCH, MCHC, of the extract treated and the untreated groups. However, hemoglobin and packed cell volume were significantly lower in the infected and untreated group compared to all other groups; probably indicating the impact of infection in the pathogenesis of anemia.

There was no significant difference in the WBC count of the uninfected-untreated group compared with the infected - untreated group. However there were significant increases in WBC counts in the extract treated groups compared to the groups devoid of the extract. The mechanism of the WBC cell induction is not well understood. Although this could possibly be an immunologic activation by certain compounds in the extract that enhance WBC actions, promoting phagocytosis in the antimicrobial function of the plant extract [40]. This result is in consonant with the findings of Ale et al. [41] where the extract of *Cymbopogan citratus* on alloxan induced rat lead to elevated level WBC in a dose dependent manner.

The result of the present study showed that the aqueous extracts of the ternary mixture of *Cymbopogan citratus*, *Bryophyllium pinnatum*, and seeds of *Garcinia kola* did not change the histology of the liver and kidney tissue which were illustrated by the normal architectures of the kidney and liver. The absence of pathological changes in the histological evaluation of liver and kidney in this study is a reflection of the normal hepatic and renal metabolism, and relates to the safety of the poly herb, which is linked to their abundant antioxidant and scavenging properties.

5. Conclusion

The ternary tea extracts combination significantly affected hematologic parameters by improving platelet and red blood cell counts. There was also evidence of white blood cell induction by the extract. Furthermore, the aqueous extract of the ternary mixture of *Cymbopogan citratus*, *Bryophyllium pinnatum* and seeds of *Garcinia kola* did not demonstrate significant toxicity to the kidney and liver, as seen from the renal and liver function studies, suggesting the likely safety of the usage of the extract. The histological sections of the liver and kidney tissues showed normal architectures also collaborating the normal liver and kidney function tests obtained from the study. The study has provided evidence for the rationale use of these plants seed and leaf aqueous extract in treatment of bacterial-induced cough, due to its nutritional, phenolic, and phytochemical composition, antimicrobial property, and positive hematenic effects.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Authors' contributions

This work was carried out in collaboration with all authors. Authors CUI, NAO, CNP, FNU and JEO designed the study, wrote the protocol, and performed the statistical analysis and interpretation of study data. Authors NAO and JEO did the literature searches, while NAO wrote the first draft of the manuscript and incorporated all corrections from co-authors. Author CUI, CNP and FNU critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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This study was self-sponsored.

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