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Full Length Research Article

SUB-ACUTE TOXICITY EFFECTS OF GARCINIA KOLA ON SERUM ELECTROLYTES, HAEMATOLOGICAL, VISCERAL ORGAN WEIGHTS AND HISTOPATHOLOGICAL PROFILES IN WISTAR RATS

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ABSTRACT
This study was undertaken to evaluate the toxicity effects of root extract of *G. kola* on serum electrolyte, haematological and histological parameters of rats. Rats of both sexes were randomized into groups and orally administered daily with determined doses of *G. kola* extract using distilled water as a control for 21 days. On the 22nd day, all the animals were sacrificed and dissected to collect blood and selected organs. The serum and whole blood were assayed for serum electrolytes and haematological parameters respectively while selected organs were examined for their weight and histopathological lesions. The extract of *G. kola* did not cause significant alteration in majority of the serum electrolytes and hematological indices. However, the extract significantly elevated the mean corpuscular heamoglobin concentration. On the other hand, the extract reduced mean corpuscular volume, haematocrit (150 and 600 mg/kg), mean platelet volume (150 and 600 mg/kg) and procalcitonin (150 mg/kg). In the vital organs, there were no significant lesions observed except at the highest dose. The root extract of *G. kola* is relatively safe in rats when repetitively administered orally in small doses for a prolonged period of time to ensure that its use is free of toxicity to humans.

Keywords: Toxicity, guttifereae, Traditional medicine, *Garcinia kola*, Biochemical, Haematological, Histopathalogical, Wistar albina rats.

INTRODUCTION

Plants have been used as sources of remedies for the treatment of many diseases by peoples from all continents, particularly in Africa, with its diverse culture and rich source of traditional medicines, since ancient times (Erhabor *et al*., 2015). Many African countries rely on folk medicine to meet their medical needs (Ouedraogo *et al*., 2007; Ojatula *et al*., 2023). And in the folkloric medicine of Ilaje people area of Ondo State, Nigeria; root of the botanical plant, *Garcinia kola* is used in the treatment of appendicitis. Because new drugs are rarely affordable in most West African countries, up to 80% of the population relies on medicinal plants for treatment (Hostettmann and Marson, 2002; Tugume and Nyakoojo, 2019). This is because plants contain abundant secondary metabolites (phytochemicals) with potential pharmacological activity against various diseases (Ojatula, 2019b). Therefore, the use of herbal medicines in management of several ailments continues to gain momentum in several communities due to their availability, affordability, perceived effectiveness and safety (Hostettmann and Marson, 2002). Their use in management of infectious diseases and cancer is even expected to increase due to increasing development of resistance to the available chemotherapeutic agents (Hostettmann and Marson, 2002).

Medicinal plants typically contain a variety of pharmacologically active compounds that can improve health either individually, additively, or synergistically (Azaizeh *et al*., 2005; Ojatula, 2022). The isolation of phytochemicals in plants that could become important drugs in modern medicine has fueled ongoing interest in the evaluation of natural products of plant origin as potential chemotherapeutic agents (Wintola *et al*., 2010; Ojatula and Nwanja, 2023). Plants produce bioactive compounds that serve as defense mechanisms against predators while also being potentially toxic in nature (Da Roch *et al*., 2002; Bents, 2004; Yeshi, 2022). With the increased interest in medicinal plants, there is a greater need for comprehensive scientific research into their efficacy and potential toxicity (Ashafa *et al*., 2010; Ojatula, 2020). The potential toxicity of herbal remedies remains a huge challenge that limits their use despite the general public belief that they are safe and devoid of potential toxicities (WHO Report on Traditional Medicine, 2019). The common toxicities are hepatotoxicity, nephrotoxicity, neurotoxicity, pulmonary toxicity, cardiac toxicity, adult respiratory distress syndrome, seizures, and acute eosinophilic pneumonia (Obakiro *et al*., 2021). The cause of toxicity may be due to presence of inherent toxic secondary metabolites, preparation procedure of the herbal product, variability in active and/or toxic ingredients due to growth conditions and soil chemistry, misidentification of herbs during harvesting, contamination by pathogenic fungi during storage and transport, and adulteration (Selamoglu, 2021). The study of medicinal plant toxicity is beneficial for the advancement of traditional medicine as well as the development of new therapeutic molecules (Fransworth, 1994; Ojatula, 2020). Concerns have recently been expressed about the lack of quality control and scientific evidence for the efficacy and safety of natural products of plant origin (Rousseaux and Scachter, 2003; Firenzuoli

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and Gori, 2007). Therefore, the World Health Organization (WHO) recommends that herbal remedies undergo rigorous scientific testing for both efficacy and safety so as to protect the public against exposure to poisonous phytochemicals.

The plant *Garcinia kola* Heckel (Family - *Guttifereae*) commonly known as bitter kola, male kola and false kola (English) is also known by various tribes in Nigeria as *Orogbo* (Yoruba), *Cida goro* (Hausa), *Aku ilu* or *Ugugolu* (Igbo), *Efiari* (Efik), and *Igoligo* (Idoma).It occurs naturally in Sierra Leone, Angola and Nigeria. It is an evergreen, unbuttressed, heavily crowned dicotyledonous plant found in moist forest as a medium sized tree which is about 13-15 m high. The plant has been acclaimed to be used in the management of liver disorders and diarrhoea (Iwu *et al.*, 1990; Blaide, 1991; Dogara *et al*., 2022), diabetes, bronchitis and throat infections (Tita *et al*., 2001; Dogara *et al*., 2022) and as an aphrodisiac (Erhabor *et al*., 2015). The root is traditionally used as cough remedy and as cure for appendicitis (Data unpublished). Although this plant is of a wide spread use in tropical Africa, and despite the sufficient evidence for its efficacy, there was little scientific evidence to support its safety with regards to its use in herbal medicines. Since there is widespread use of the root of this plant in preparation of herbal remedies for management for appendicitis and other chronic illnesses, it is necessary to evaluate the toxicity effects of this plant following prolonged repetitive administration. This study was therefore undertaken to evaluate the effect of aqueous root extract of *G. kola* on biochemical (electrolytes), haematological and histological parameters of Wistar albino rats following daily oral administration of the extract for 21 days.

MATERIALS AND METHODS

Plant Material

The plant under investigation (*G. kola* root) was collected from Sapele Village, South zone of Igboegunrin Community, Ilaje Local Area, Ondo State, Nigeria. The plant was authenticated by the herbarium Curator at the Botany Unit, Department of Biological Sciences, School of Science, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria.

Preparation of Crude Plant Extract

The roots were air dried, ground into a coarse powder form and soaked for 5 days in each of aqueous and methanol consecutively. The plant material was then shaken overnight on a shaker, then filtered, evaporated to dryness under reduced pressure in a rotatory evaporator and weighed.

Experimental Animals, Randomization, Dose Determination and Administration

The methods and processes involved in experimental animal's procurement, grouping and their dosing were designed and developed based on personal-conceptual interest on the research study. Thirty five male Wistar rats weighing 150 grams were procured from the Animal Holding Unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The rats were divided into four equal groups of rats (GR1 – GR4). Each group was kept in a plastic cage, freshly spread with saw wood to absorb urine and housed under standard conditions of temperature, and humidity with alternating 12 hours light/dark cycles. Commercial standard diet and water was supplied *ad libitum* throughout the experimental period (three weeks). Groups 2, 3, and 4 rats were orally dosed with 150, 300 and 600 mg/kg body weight (BW) aqueous plant extract, respectively, while rats of group 1 were orally dosed with distilled water (1 mL/kg) and acted as a control group. The extract doses were administered using special stomach tube with a smooth tip to protect the oral mucosa and esophagus from injury.

Blood Sampling and Processing

The method described and published by Ojatula (2019) was employed and adopted for this study. Observations of any toxic symptoms were under studied systematically daily up to the end of the experiment. And at the end of the experiment, experimental rats were decapitated and dissected to obtain blood (by means of cardiac puncture) and vital organs (liver, kidney, heart, and spleen) for determination of serum electrolytes level, haematological and histopathological analyses. The weights of the vital organs were measured using a digital analytical balance. From the obtained blood sample; two blood samples were obtained from each rat. One sample was collected in a tube containing potassium ethylene di-amine tetra acetate (anticoagulant) for hematological analysis and the other sample was collected in plain tube to obtain serum; blood was left for one hour to clot and the tube was centrifuged at 3000 rpm for 15 minutes and the harvested serum was used for biochemical analysis.

Determination of Serum Electrolytes Levels

Blood (2 mL) was collected by cardiac puncture from each rat into non-heparinized vacutainers using syringes. Blood was centrifuged at 3,000 rpm for 5 min to obtain serum which was assayed using an automated chemistry analyzer (HumaStar 200, China) for levels of different serum electrolytes parameters. Test kits for measurement of different parameters were purchased from Sigma-Aldrich and used according to the manufacturer's instructions.

Determination of Vital Organs Weights

At the termination of treatment, 21 days, vital organs (liver, heart, kidneys, and spleen) were harvested from scarified rats. They were washed with ice-cold saline solution (0.9 %w/v), blotted, and weighted. The weight of each organ was standardized to 100 g body weight of each animal.

Haematological Analysis

Blood (2 mL) was collected by cardiac puncture from each rat into heparinized vacutainers using syringes and analyzed using an analyzer (Sysmex 1000i) for hematological counts of different parameters. These included White blood cell (WBC), neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophil (EO), basophils (BASO), red blood cells (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PCH).

Histological Evaluation

The obtained vital organs (liver, heart, spleen, and kidney) were grossly examined for the observable histomorphological changes and the weight of each organ measured using a digital weighing scale. The isolated organs were fixed in 10 % (v/v) buffered formalin labeled bottles for 72 h. After fixation, the tissues were trimmed and loaded in cassettes for processing using an automated tissue processer (Leica 40, China). They were first dehydrated by placing them in tissue cassettes with graded alcohol

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concentration (70, 80, 90, and 96%, v/v) and then removed and placed into xylene solution baths to clear off the alcohol. They were then impregnated with molten wax and allowed to dry. The tissues were then sectioned by use of Rotary microtome (at 5 µm thickness), and then stained with hematoxylin and eosin (H & E). Slides were prepared and then examined using a research light microscope connected to computerized camera (Lieca LB2–image analyzer). Photomicrographs were captured and then examined for histological changes by two independent pathologists who were not aware of the biochemical and haematological data.

Statistical Analysis

Quantitative data was entered in Microsoft excel version 2013 and its means and standard error of mean calculated. The results were presented as means ± standard error of mean. Statistically significant differences were determined using one way analysis of variance (ANOVA) and/or Student's t-test followed by Dunnett's post hoc test using Graph Pad Prism version 5.01 (Graph Pad software, San Diego, California, United States). Differences were considered statistically significant at $p < 0.05$ as presented by Ali and Bhaskar (2016).

Ethical Approval

Ethical approval for the study (UNIBEN/PHYTOMED/23/081) was obtained from the UNIBEN Committee on Animal Use and Care. All investigations were conducted in accordance with the accepted principles for laboratory animal use and care (NRC, 2011).

RESULTS

Effect of *G. kola* Extract on Serum Electrolytes Levels

Extract administration at all doses did not significantly alter the concentration of sodium, potassium, chloride, and calcium ions except at 300 mg/kg where it elevated the potassium ions (Table 1). On the other hand, all doses significantly depressed the hydrogen ion concentration (lowered the pH).

Table 1: Mean levels of electrolytes after 21 days at different doses of *G. kola* aqueous root extract.

Mean concentration of electrolytes	Control (1 mL Distilled water)	150 mg/kg	300 mg/kg	600 mg/kg
K+ (mmol/L)	4.29 ± 0.03	4.8 ± 0.11	5.0 ± 0.27**	4.52 ± 0.05
Na+ (mmol/L)	144.3 ± 0.67	143.4 ± 0.37	144 ± 0.47	142.6 ± 0.39
Cl− (mmol/L)	104.6 ± 0.65	104.2 ± 0.65	106.2 ± 0.44	105.5 ± 0.8
ICa2+ (mmol/L)	0.56 ± 0.06	0.22 ± 0.07	0.51 ± 0.15	0.67 ± 007
TCa2+ (mmo/L)	1.06 ± 0.08	0.42 ± 0.15	0.99 ± 0.29	1.31 ± 0.14
pH	7.38 ± 0.17	7.018 ± 0.02[a]	7.01 ± 0.02[a]	6.97 ± 0.01[a]

KEYS: Data were expressed as mean ± SEM, n = 6
Intracellular calcium (ICa^{2+}), Total calcium (TCa^{2+}).
**Significant elevation of Potassium ions, [a]significant at $p < 0.05$.

Effect of *G. kola* Extract on Haematological Parameters

The extract did not have a significant effect ($p > 0.05$) on the white blood cells and its differentials at all doses (Table 2). However, it significantly ($p < 0.05$) altered some red blood differentials (HCT, MCV, MCHC, and RDW-CV) and Platelet differentials (MPV and PCT). At all doses the extract significantly reduced the MCV and HCT while increased the MCHC. MPV was significantly reduced at 150 and 300 mg/kg of extract while PCT at 150 mg/kg only.

Table 2: Mean levels of hematological parameters after 21 days at different doses of the extract.

Haematological parameters	Control (1 mL Distilled water)	150 mg/kg	300 mg/kg	600 mg/kg
WBC (10*3/UL)	12.3 ± 1.37	8.30 ± 1.62	9.45 ± 1.17	10.52 ± 1.01
NEUT (10*3/uL)	1.58 ± 0.28	1.03 ± 0.16	1.19 ± 0.17	0.795 ± 0.10
LYMPH (10*3/uL)	9.73 ± 1.17	6.37 ± 1.34	7.35 ± 0.96	8.67 ± 0.88
MONO (10*3/uL)	0.56 ± 0.11	0.78 ± 0.18	0.70 ± 0.10	0.90 ± 0.08
EO (10*3/uL)	0.19 ± 0.05	0.11 ± 0.03	0.20 ± 0.05	0.14 ± 0.03
BASO (10*3/uL)	0.26 ± 0.05	0.18 ± 0.03	0.49 ± 0.32	0.15 ± 0.03
RBC (10*6/UI)	7.01 ± 0.31	7.30 ± 0.43	6.83 ± 1.24	7.25 ± 0.25
HGB (g/dL)	13.47 ± 0.22	13.17 ± 0.69	14.53 ± 0.46	13.28 ± 0.44
HCT (%)	52.83 ± 1.32	45.12 ± 2.09[a]	48.1 ± 1.55	44.93 ± 1.61[a]
MCV (FL)	69.92 ± 3.45	62.05 ± 1.08[a]	58.92 ± 0.29[a]	61.95 ± 0.47[a]
MCH (pg)	19.38 ± 0.87	18.08 ± 0.14	17.82 ± 0.05	18.33 ± 0.18
MCHC (g/dl)	27.78 ± 0.22	29.12 ± 0.28[a]	30.23 ± 0.14[a]	29.60 ± 0.28[a]
RDW-SD (fL)	35.23 ± 4.13	33.25 ± 0.89	33.88 ± 1.02	36.65 ± 2.51
RDW-CV (%)	14.68 ± 0.69	16.65 ± 0.80	18.35 ± 0.81[a]	17.48 ± 1.18
PLT (10*3/uL)	774.50 ± 28.42	544.00 ± 79.23	636.70 ± 64.24	663.30 ± 77.53
PDW (fL)	8.57 ± 0.15	7.83 ± 0.29	7.90 ± 0.14	8.10 ± 0.28
MPV (fL)	8.15 ± 0.11	7.53 ± 018[a]	7.38 ± 0.11[a]	7.57 ± 0.19
P-LCR (%)	10.98 ± 0.76	8.38 ± 1.16	7.28 ± 0.67	8.82 ± 1.25
PCT (%)	0.63 ± 0.02	0.41 ± 0.06[a]	0.47 ± 0.05	0.50 ± 0.05

KEYS: Data were expressed as mean ± SEM, n = 6
[a]significant at $p < 0.05$.

Effect of *G. kola* Extract on the Weight of Vital Organs after 21 days

The extract did not significantly alter the weight of the various organs as compared to the control group except for the liver and kidney which were significantly reduced ($p < 0.05$) at 600 mg/kg dose of the extract (Table 3).

Table 3: Mean weight of vital organs after 21 days at different doses of the extract.

Dose of extract (mg/kg)	Kidney	Liver	Heart	Spleen
Control (1 mL Distilled water)	1.15 ± 0.06	5.97 ± 0.30	0.63 ± 0.02	0.93 ± 0.09
150	1.03 ± 0.04	5.80 ± 0.30	0.65 ± 0.03	0.90 ± 0.08
300	1.25 ± 0.06	5.80 ± 0.20	0.67 ± 0.03	0.65 ± 0.04
600	0.80 ± 0.03[a]	4.80 ± 0.18[a]	0.55 ± 0.04	0.82 ± 0.07

KEYS: Data were expressed as mean ± SEM, n = 6
[a]significant at $p < 0.05$

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Effect of Repeated Doses of *G. kola* Extract on Histopathology of the Liver, Kidney, Heart, and Spleen

No significant organ lesions were associated with administration of the extract in the heart, liver, kidney and spleen (Figures 1A–D). However, at the 600 mg/kg dose, the extract caused mild to moderate multifocal parenchymal hepatocytes necrosis (Figure 1A), periportal mononuclear inflammatory cell infiltration and focal interstitial nephritis (Figure 1C). In all the treatment groups when compared with the control, the spleen exhibited nonspecific immune-stimulation as indicated by mild to moderate diffuse lymphoid hyperplasia within the white pulp (Figure 1D). A summary of the organ specific findings is indicated in Table 4.

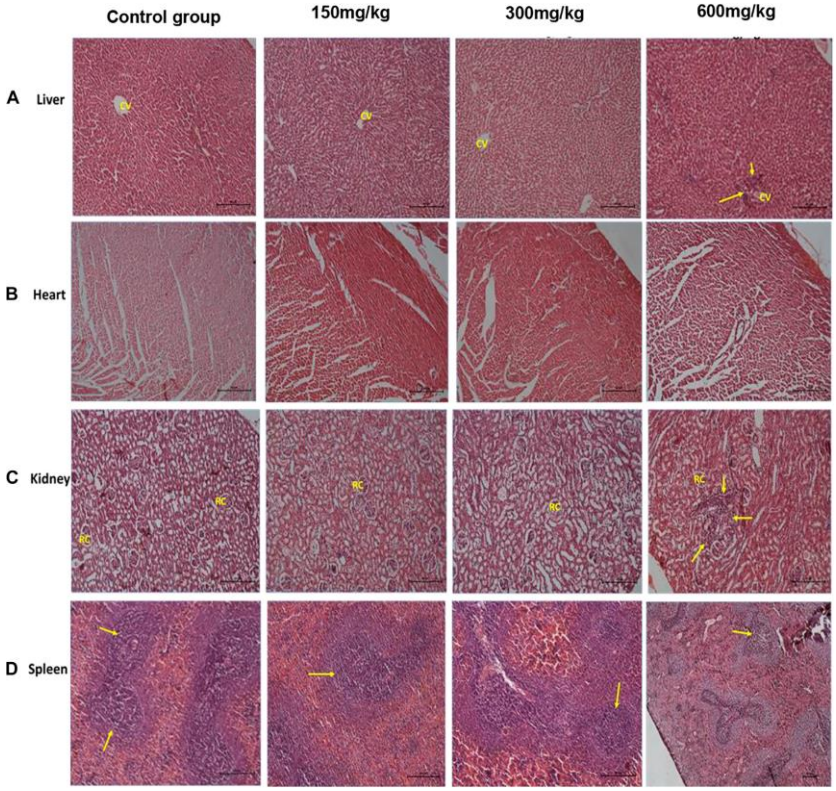

Plate 1: Hematoxylin and eosin stain rat organ sections. Panel (A) shows normal liver architecture at lower doses with clear central vain, at 600 mg/kg, cellular infiltration is seen (arrow heads). Panel (B) shows normal heart section with clear cardiac fibers. Panel (C) shows normal kidney architecture at lower doses with clear renal capsules, at 600 mg/kg focal interstitial nephritis is seen (arrow heads). Panel (D) shows spleen white pulp hyperplasia due to non-specific immune-stimulation at all levels (arrow heads). Each photomicrograph enlargement is 100 µm

Table 4: Summary of organ specific histology.

Organ	Control (1 mL/kg Distilled water)	150 mg/kg	300 mg/kg	600 mg/kg
Liver	No significant lesions.	No significant lesions.	No significant lesions.	Mild to moderate multifocal parenchymal hepatocytes necrosis and periportal mononuclear inflammatory cells infiltration.
Heart	No significant lesions.	No significant lesion.	No significant lesion.	No significant lesions.
Kidney	No significant lesions.	No significant lesion.	Non-significant lesion.	Focal interstitial nephritis.
Spleen	No significant lesions.	Mild to moderate diffuse lymphoid hyperplasia in the follicles (nonspecific Immune-stimulation).	Mild to moderate diffuse follicular lymphoid hyperplasia (non-specific Immune-stimulation).	Mild to moderate diffuse follicular lymphoid hyperplasia (non-specific immune-stimulation).

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DISCUSSION

Phytotherapy is increasingly becoming important in the treatment of numerous diseases and disorders due to many side effects of surgery and synthetic drugs, thus phytotherapy based on product derived naturally from plant has emerged as an alternative treatment for human ailments (Allkanjari and Vitalone, 2015; Ojatula *et al*., 2020).

Electrolytes (sodium, potassium, chloride and bicarbonate) balance in the blood is a good indicator of how well the organs, especially, the kidneys and heart are functioning. Knowing which electrolytes are out of balance can help in the determination of a course of treatment. And changes in the level of serum electrolytes are known to have serious determinant effect on health implications of animals, human being inclusive (Ojatula, 2020). Sodium is regulated by the kidneys and adrenal glands. Sodium is the major cation of extracellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. Too much sodium (hypernatremia) or too little sodium (hyponatremia) can cause cells to malfunction, and extremes in the blood sodium levels (too much or too little) can be fatal. Potassium is the principal cation of the intracellular fluid. It is also an important constituent of the extracellular fluid due to its influence on muscle activity. Elevated potassium levels (hyperkalemia) are often associated with renal failure, dehydration shock or adrenal insufficiency. Decreased potassium levels (hypokalemia) are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex (Tietz, 1976). Chloride is important in the maintenance of the cation/anion balance between intra and extracellular fluids. This electrolyte is essential to the control of proper hydration, osmotic pressure, and acid/base equilibrium. Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in Addisonian crisis. Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction (Tietz, 1976). The low serum chloride concentration observed in the treated groups may be associated with metabolic alkalosis which could be due to volume loss from gastric content as gastric fluid is rich in chloride (CL-). The result of this study is in line with previous study reported by Garkuwa *et al*. (2021). A total calcium levels is often measured as part of routine health screening and abnormal result is an indicator of an underlying problem often associated with kidney disease (Kim *et al*., 2011). The calcium concentration of the treated animal in this study following the administration of the plant extract, showed reduction in calcium concentration in treated groups. Bicarbonate buffer system is the most important amongst blood buffers when the pH of the blood is considered (Kendrick *et al*., 2017); and also the serum or plasma bicarbonate content is a significant indicator of electrolyte dispersion and anion deficit. Alteration of bicarbonate and CO_2 dissolved in plasma are characteristics of acid-base imbalance, which may be due to renal tubular acidosis, hyperkalemic acidosis, renal failure or keto acidosis (Garkuwa *et al*., 2021). Thus any reduction in blood pH implies a reduction in serum bicarbonates. From the results, variations in different electrolyte concentrations were observed among the groups. The concentrations of serum electrolytes measured in this study reduced non-significantly in all groups administered different concentrations of the extract, except potassium in group 3 (administered low dose: 300 mg/kg bw of root extract), where sodium and chloride reduced non-significantly compared with normal control (Table 1). Though these alterations were not statistically significant in almost all the groups administered the various extract, but it is possible that its physiological effects could be adverse. This result shows that the extract could alter the functions of the organs associated with the electrolytes, especially the kidney. Serum electrolyte stability in the blood is known to be a good indicator of effective functioning of the kidneys, heart and other vital organs. Imo and Uhegbu (2015b) reported that significant alteration in the concentration of these body electrolytes is indicative of poor organ functions or organ impairment. However, most of the results from the study showed no significant alteration of serum electrolytes in all groups, except the pH when compared with the control, indicating that the administered extract did not alter the body haemostasis and physiology of experimental treated rats. The results of this study agrees with the findings of Onyeabo *et al*. (2021), where they worked on *Curcuma longa* extract to improves serum electrolytes and hormone profile of dihydrotestosterone - estradiol valerate induced benign prostatic hyperplasia male rats, and they reported that some serum electrolytes of extract treated rats showed significant ($p<0.05$) decreased following administration of plant extract, while others recorded no ($p>0.05$) difference in concentrations when compared with the control.

The blood indices (white blood cells, red blood cells, platelets and their differentials) serve as an indicator of physiological and pathological status of the body and significant changes imply that the administered chemical is either protective or toxic to the haemopoietic tissue. Findings from our study report non-significant effects on most of the important blood indices by the aqueous extract of *G. kola*. The major functions of WBCs and its differential are to provide immunity and defend the body against invasion by pathogens or toxins. Therefore, the non-significant difference in WBC count and its differentials between the treatment and control groups suggested that the administered doses did not interfere with the differentiation of haemopoietic stem cells into these parameters. The significant effect on red blood cell differentials indicated that the extract affected the process of erythropoiesis probably by the phytochemicals interfering with the secretion and/or activity of erythropoietin (Zaruwa *et al*., 2016; Ojatula, 2019). Diminished levels of mean platelet volume and procalcitonin could probably be due to presence of toxic phytochemicals that interfere with the functioning of thrombopoietin or cause inflammation of the bowel (Mwale *et al*., 2014). The significant lowering of the pH indicated the potential of the extract to cause acidosis probably by stimulating the secretion of hydrogen ions into blood and/or inhibiting their renal excretion.

In repeated daily oral doses for 21 days, there were no significant differences in vital organ weight between the control and test groups of rats dosed with various concentrations of the aqueous extract of *G. kola* root over time except for the dose of 600 mg/kg which was able to significantly reduce rat's kidney and liver weights. On this regards, it is plausible that the extract was in optimum concentration to stimulate the conversion of nutrients into organ tissues, and as well, could have been probably due to the interference of the phytoconstituents of the studied plant at various studied concentrations with the cellular nature of the vital organ tissues of experimental rats; this results agrees with the study of Obakiro *et al*. (2021), who worked on sub-acute toxicity effects of

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methanol stem-bark extract of *Entada abyssinica* on biochemical, haematological and histopathological parameters in wistar albino rats.

The low extract dose levels (150 mg/kg, 300 mg/kg) exhibited no significant effect on the histomorphology and gross anatomy of the vital organs. However, at a high dose (600 mg/kg, the extract exhibited a significant decrease in the weight of the liver and kidney in comparison with the control. Histopathological assessment further revealed mild to moderate multifocal parenchymal hepatocytes necrosis and periportal mononuclear inflammatory cells infiltration as well as focal interstitial nephritis. These findings are indicative of infectious or inflammatory lesions that was not beyond tolerance limit of extract treated rats; although we could not ascertain or propose their actual causes (Singh *et al*., 2013). These results are in agreement with those reported from aqueous root extract of *Entada Africana* (a related species) which also did not show significant effect on many biochemical and hematological parameters (Tibiri *et al*., 2007). The later finding indicates the hepatoprotective effects of the extract while the former its risk of hyperlipidemia.

Phytochemical analysis of the aqueous extract of *G. kola* revealed presence of alkaloids, tannins, triterpenes, flavonoids, saponins, steroid glycosides and phenols as dominant secondary metabolites (Harborne, 1973; Ukaoma *et al*., 2013). Majority of these compounds were tannins and gallic acid derivatives. These chemical compounds have been reported to possess cytotoxicity, antioxidant and antimicrobial activities (Ukaoma *et al*., 2013; Sobeh *et al*., 2020). Therefore these phytochemicals have good pharmacological potential and elicit no-toxicity within possible therapeutic doses (Dzoyem *et al*., 2017). These *in vitro* findings as reported by Sobeh *et al*. (2020) resonate with our *in vivo* findings which report that the aqueous root extract of *G. kola* is relatively non-toxic on many biochemical, haematological and histological indices.

Conclusion

Phytotherapy is increasingly becoming important in the treatment of numerous diseases and disorders globally; and has emerged as an alternative treatment for human ailments, animal inclusive. And sequel to the findings of this study, the root extract of *G. kola* is relatively safe in rats when repetitively administered orally in small doses for a prolonged period of time. We therefore, recommend further and more chronic toxicity studies in animal and clinical trials on herbal remedies containing this plant to ensure that its use is free of potential toxicity to humans.

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