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Full Length Research Paper

THE EFFECTIVENESS OF PHYTOPHARMACA OF EXTRACTS *GARCINIA KOLA*(HECKEL) ROOT ON CHANGES INBODY WEIGHT, SERUM BIOCHEMICAL PARAMETERS AND HISTOLOGICAL PROFILES OF WISTAR RATS

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ABSTRACT
The effect of solvents extracts of *G. kola*administered to rats at 50, 250 and 500 mg/kg for two weeks was investigated on body weight and serum biochemical parameters using standard analytical-biochemical methods. The extracts at all doses investigated proved that only the 250 mg/kg of the aqueous and methanolroot extracts were able to reduce rat's body weights. Rats given the methanol extract had no significant differences in the values of AST, total protein, cholesterol, total bilirubin, and urea whereas had significantly increased ALP activity, and albumin content (at all doses used) and direct bilirubin (at 250 mg/ kg), compared to control ($p<0.05$). Rats dosed with the aqueousextract showed higher AST activity but comparable ALT and ALP activities to that of control. Compared to control values total protein and D.Bwere significantly lower in the three treated groups, T.B and albumin were significantly lower at the 250 and 500 dose levels ($p<0.05$). Cholesterol was significantly low in the 250 dose group and no significant difference was observed for urea levels. In the vital organ, there were no lesions or evidence of hepatotoxicity. The little alterations in some parameters indicated that the root extracts of *G. kola* possess slight toxicity. Therefore, crude extracts of rootshould be taken with caution for safety.

Keywords: *Garcinia kola*,Biochemical parameters, Wistar rats, Body weight, Toxicity

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.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, 2007; Tugume and Nyakoojo, 2019).

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 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 and Gori, 2007). As a result, screening plants for toxicity and ensuring their safety is a critical ethical issue.

The plant *Garcinia kola* Heckel (Family - *Guttifera*) 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 widespread use in tropical Africa yet there is dearth or little available literature on the scientific evaluation of its root's toxicological effects. This work, therefore, investigates the effects of root extracts on some biochemical parameters and body weight of Wistar rats.

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 Extracts

The roots were air dried, ground into a coarse powder form and soaked for 5 days in each of aqueousand 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 and Dosing

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-fivemale 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 seven equal groups of rats (GR1 – GR7). 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 (two weeks).

Group 1, 2, and 3 rats were orally dosed with 50, 250 and 500 mg/kg body weight (BW) aqueousplant extract, respectively, while rats of groups 5, 6 and 7 were dosed similarly but with the methanol plant extract. Rats of groups 4 were orally dosed with distilled water 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.

Monitoring of Body Weights

Animals were individually weighed at the beginning and at the end of the experiment to record changes in body weight as described and reported by Ghasemi*et al*. (2021).

Blood Sampling and Processing

The method described and published by Ojatula (2019) was employed and adopted for this study, and at the end of the experiment, experimental rats were decapitated, and 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.

Biochemical Analyses

The methods described and published by Yan *et al*. (2023) was employed and adopted for this study. In the study, serum samples were analyzed for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and for the concentrations of total protein, albumin, cholesterol, total and direct bilirubin, and urea using the Roche Diagnostic Hitachi 902 Analyzer**.**

Histological Studies

Fixed excised tissues were sectioned with microtome (5 lm thickness), fixed in slides, and stained using Harry's Haematoxilin and Eosin staining procedures. The slides were viewed under light microscope and photomicrographs were taken.

Statistical Analysis

The collected data will be analyzed and expressed as means ± SD of six replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $p < 0.05$ as presented by Ali and Bhaskar (2016).

RESULTS

Effect of *G. kola*Extracts on Body Weight of Wistar Rats

Generally, there was no significant difference in body weight between the control and test groups of rats dosed with various concentrations of the aqueousor methanol extracts. However, rats given the 250 mg/ kg BW dose of both extracts had

the lowest weight gains. Moreover, the results showed that both the 500 mg/ kg body weight dose of the aqueous and methanol extracts did not exhibit significant reduction in body weight lost when compared to control and the other test groups (Table 1 and Table 2). The result of this study agrees with the work of Konate (2011), where he tested the toxicity of aqueous and acetone extracts of *Cienfuegosia digitata* on mice and rats andrecorded wide insignificant variations in weights of rats with no other detectable clinical signs.

Table 1. Effect of different concentrations of *G. kola* aqueous root extract on rats body weights.

Parameter	Control	50mg/kg	250mg/kg	500mg/kg
Initial weight	151.37^{a}±4.48	151.43^{a}±4.71	151.30^{a}±4.13	151.41^{a}±4.25
Final weight	152.70^{a}±0.1	150.67^{a}±0.1	142.21^{b}±0.2	144.32^{ab}±0.3

Means with the same superscript across the row for each parameter are not significantly different ($p<0.05$)

Table 2. Effect of different concentrations of *G. kola* methanol root extract on rats body weights.

Parameter	Control	50mg/kg	250mg/kg	500mg/kg
Initial weight	150.45^{a}±4.43	150.40^{a}±4.82	150.24^{a}±4.76	150.14^{a}±2.53
Final weight	152.40^{a}±0.2	147.23^{ab}±0.01	143.02^{b}±0.01	139.00^{b}±0.01

Means with the same superscript across the row for each parameter are not significantly different ($p<0.05$)

Biochemical Findings

Following repeated administration, the results revealed a consistent, yet mostly significant reduction changes in the values of biochemical indices, in both extracts administered rats (Table 3 and Table 4). It should be noted however, that AST and ALT werenot significantly higher than the control in all tested doses of the aqueous extract administered rats, and significantly lower in AST of the methanol extract administered group of rats. Also, our results showed a consistently reduction in all the biochemical parameter testedacross tested doses following repeated doses. In general, experimental rats of both administered extracts, appeared to show more reduced sensitivity to the effects of the extract on biochemical indices when compared with the control group of rats. The non-significantly higher values of AST and ALT in the aqueous extract administered group of rats, and Urea in the methanol extract administered group of rats when compared to control pointed to possible reversibility of toxic effects in a manner similar to acute test. Moreover, the observed consistent reduction in the values of the other biochemical parameters of the extracts administered group of rats, signalling potential for sustaining non-accumulative toxic effects on the body system and organ, the liver.

Table 3. Biochemical levels in rats orally dosed with different doses of *G. kola* root aqueous extract.

Parameter	Control	50mg/kg	250mg/kg	500mg/kg
AST U/L	156.67^{a}±7.80	168.33^{a}±10.2	160.67^{a}±9.20	158.69^{a}±8.20
ALT U/L	24.83^{a}±2.0	25.33^{a}±1.2	29.83^{a}±1.3	27.43^{a}±0.3
ALP U/L	265.33^{a}±10.6	302.83^{a}±15.9	260.33^{a}±16.6	242.43^{a}±16.3
T.P g/L	8.20^{a}±1.1	6.08^{b}±.001	5.80^{b}±.002	3.60^{b}±.002
Alb. g/L	5.17^{a}±.80	3.48^{b}±.31	3.58^{b}±.32	3.54^{b}±.32
Chol. mg/dL	73.5^{a}±4.3	81.83^{ab}±5.2	72.83^{b}±4.6	61.83^{b}±3.6
T.B mg/dL	0.60^{a}±.06	0.15^{b}±.03	0.178^{b}±.01	0.158^{b}±.02
D.B mg/dL	0.122^{a}±005	0.04^{a}±.001	0.095^{a}±.001	0.091^{a}±.001
Urea mg/dL	52.43^{a}±3.2	31.0^{b}±1.2	40.17^{b}±4.0	42.15^{b}±2.0

Means with the same superscript across the row for each parameter are not significantly different ($p<0.05$).

Table 4. Biochemical levels in rats orally dosed with different doses of *G. kola* root methanol extract.

Parameter	Control	50mg/kg	250mg/kg	500mg/kg
AST U/L	152.65^{a}±16.0	211.63^{a}±00.1	152.81^{b}±12.0	142.67^{c}±402
ALT U/L	26.83^{a} ±1.3	31.67^{a} ± 3.1	32.33^{a} ±1.0	25.17^{a}±6.1
ALP U/L	285.33^{a}±30.1	282.17^{a}±30.5	222.00^{a}±30.5	248.50^{a}±30.5
T.P g/L	8.20^{a}±1.0	7.40^{b}±1.0	5.90^{c}±2.1	5.55^{c}±2.1
Alb. g/L	5.05^{a}±1.0	4.73^{a}±1.0	3.60^{b}±2.2	3.87^{b}±2.5
Chol. mg/dL	93.50^{a}±8.5	84.83^{ab}±2	69.33^{b}±6.1	90.33^{ab}±7.1
T.B mg/dL	0.60^{a}±.01	0.53^{a}±.01	0.092^{b}±.002	0.072^{b}±.002
D.B mg/dL	0.122^{a}±.001	0.0767^{b}±006	0.050^{cb}±.001	0.117^{c}±.002
Urea mg/dL	52.83^{a}±5.3	59.33^{a}±1.2	59.17^{a}±1.6	41.50^{a}±6.2

Means with the same superscript across the row for each parameter are not significantly different ($p<0.05$).

Analyses of Repeated Doses of the Extracts on Histology of the LiverTissues of Experimental Rats

Plate 1 showed histological sections of livers of experimental ratsadministered *G. kola* root aqueousextract. In the negative control group (Plate 1(a)), normal hepatocytes with preserved cytoplasm and nucleus were observed. On assessment of the effect of the test substance, i.e., the extract, on the cellular architecture of the liver of the extract administered rats, the same characteristics were observed in the liver of the animalstreated with EI (Plate 1(b - d), without pyknotic nuclei, vacuolized cells, hepatocellular degeneration, and necrosis. This indicatedenhanced hepatic modulating effect of phytoextract of the studied plant at all dose levels used in this study.

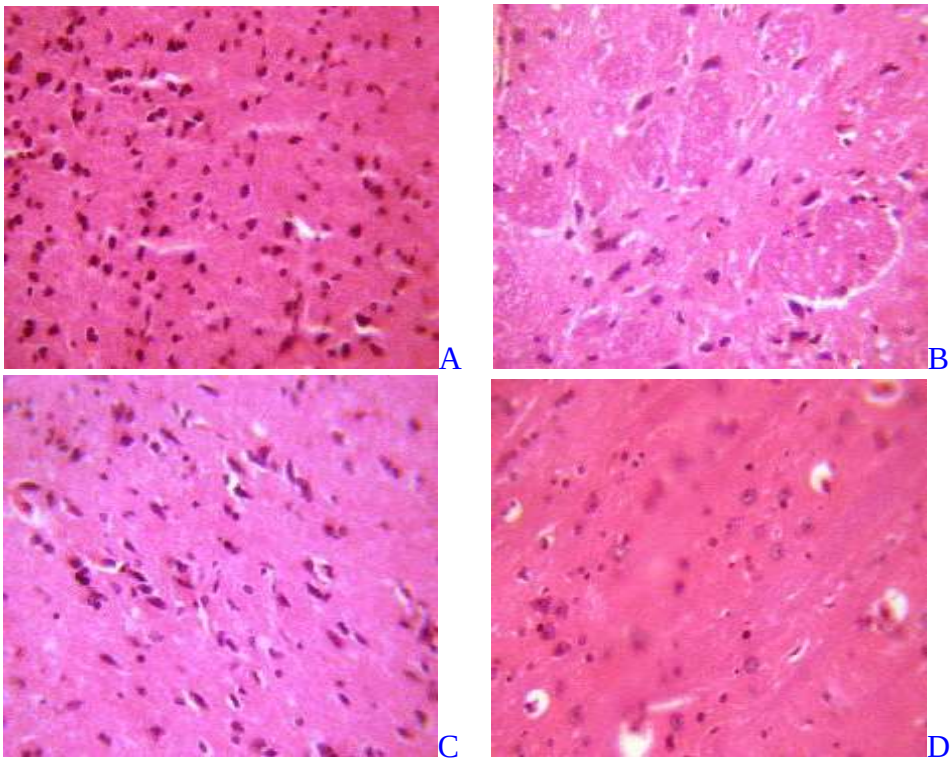


Plate 1: Histological profiles of the rat's liver administered *G. kola* root aqueous extract(hematoxylin and eosin stain): (A) Control rat liver, (B) Rat liver treatedwith 50 mg/kg body weight, (C) Rat liver treated with 250 mg/kg body weight, (D) Rat liver treated with 500 mg/kg body weight, magnification 20x. Bar: 10 μm. The extract administered group (B-D) are showing normal hepatocytes with preserved cytoplasm and nucleus when compared with the control.

Plate 2 showed histological sections of the livers of experimental ratsadministered *G. kola* root methanol extract.The livers of animals in the control group (Plate 2(a)) and those treated with 50, 250 and 500 mg/kg body weight phytoextract of the studied plant EI (Plate 2(b - d) presented well-preserved cellular cyto-architecture, without hepatic tubules characterized by lesionsand loss of the hepatic borders, which are suggestive of non-intense vascularization effect of the studiedphytoextract at the dose levels used in the study.

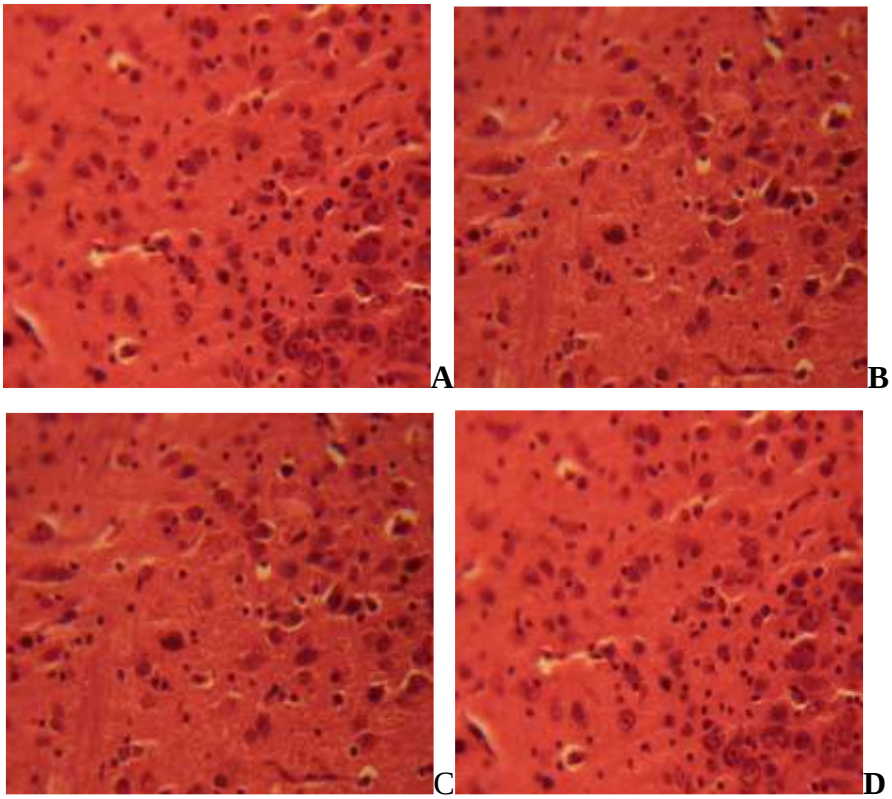


Plate 2: Histological profiles of the rat's liver administered *G. kola* root methanol extract(hematoxylin and eosin stain): (A) Control rat liver, (B) Rat liver treatedwith 50 mg/kg body weight, (C) Rat liver treated with 250 mg/kg body weight, (D) Rat liver treated with 500 mg/kg body weight, magnification 20x. Bar: 10 μm. The extract administered group (B-D) are showing normal hepatocytes with preserved cytoplasm and nucleus when compared with the control.

DISCUSSION AND CONCLUSION

In repeated daily oral doses for 14 days, there were no significant differences in body weight between the control and test groups of rats dosed with various concentrations of the aqueous or methanol extractsover time except for the dose of 250 mg/kg of the aqueous and methanol root extracts which were able to reduce rat's body weights. In the latter groups, it is plausible that the extracts were in optimum concentration to stimulate the conversion of nutrients into body tissues; this agrees with the study of Obakiro *et al*. (2021), who worked on sub-acute toxicity effects of methanol stem-bark extract of *Entada abyssinica*on biochemical, haematological and histopathological parameters in wistar albino rats.

Aspartate amino transferase (AST) and alanine amino transferase(ALT) are considered useful markers for liver damage (Kaneko and Cornelius, 1985; Bush, 1991; Mumoli and Cosimi, 2006; Ojiako and Nwanjo, 2006; Afolayan and Yakubu, 2009); and they are non-specific enzyme whoseactivity/concentration in serum could be due to injury to various vital organs in the body (Akanmu *et al*., 2020). The non-elevated levels of AST and ALT levels in the serum of treatment groups of rats to indicate non- hepatotoxic effects of the studied medicinal plant. And this agrees

with the work of Mohammed *et al*.(2010) who reported observed decreased levels of ALT and AST in rats receiving methanol and butanol fraction of *Buchholzia coricea* seeds; to which streptozotocin was added. Similarly, the aforementioned results on AST and ALT as observed in this study, also agrees with the work of Nwanjo (2007) who considered and reported insignificant increases in AST and ALT levels in serum of experimental rats, having administered *Phyllanthus nisruri* leaf extract in his research study; to indicate non- hepatotoxic effects of any studied natural product or substances of plant origin.

Serum or plasma protein is a very complex mixture of proteins, and their determination is clinically valuable and reflects the internal status of an individual, which is essential for growth and replacement of worn-out tissues. And the rate of cell divisions is determined chiefly by the rate of protein synthesis (Baxter *et al*.,1987). Increased significant values of plasma protein may indicate liver damage and reduction in hepatic functions (Naganna, 1989; Tietz, 1994; Yakubu *et al*., 2007). However, subject to the results of this study, the studied plantexhibited a significant decreased ($p < 0.05$) changes in total protein (T. P) contentsin all rats dosed with aqueous and methanol extracts compared to control.The result of this study agrees with the findings of Atere and Ajoa (2009), who worked onrats administered with crude alkaloidal fraction from *Gnestis ferruginea* roots and reported a decreasing trend in serum total protein levels. Total protein and albumin at decreased levels are considered as an indication of diminished synthetic functioning of the liver, whichmay be due to reduction in protein intake from the intestine (Rolls, 2000). The observed results in this study, agrees with the study reported by Dhanotiya (2004).

Elevated levels of cholesterol increase the risk for coronary heart disease (Ojatula, 2019). Cholesterol is measured to help assess the risk status and to follow the progress of treatment to lower serum cholesterol concentrations, and the desirable cholesterol levels are considered to be those found at a decreased level of concentration (Ojatula, 2020).Sequel to the findings of this study, the extract at various concentrations showed a decreased ($p < 0.05$)values of cholesterol (Chol.), total bilirubin (T. B) and direct bilirubin (D. B)when compared to control. The decreased values observed on the aforementioned biochemical indices conforms and agrees with the findings of Ojatula (2020), who worked on preliminary assessment of acute and 14-days repeated dose oral toxicity of *Pausinystalia yohimbe*methanol root extract on experimental rats. The decreased values observed could probably be due to presence of bioactive agents in the extracts that interact with enzyme's action.

The non-elevated levels of urea in the treatment group indicate a non-disturbance in the nitrogen metabolism which could probably be due to presence of phytochemicals in the extracts that interact with enzymes or calm the nitrogen metabolism. Additionally, it could as well be due to the extract interfering with renal excretion of uric acid as observed from some histopathology on the Kidney. Therefore, there is a likely benefit of hypouricemia and inhibition of gout development in patients who chronically use herbal remedies that contain this medicinal plant.Urea concentrations showed no significant change in aqueousgroups of rats, while a significant decrease was observed ($p < 0.05$) in the methanol rat groups (Table 4). The depicted results, agrees with the previous work of Ashafa and Olunu (2011), in which Wistar rats were administered leaf extracts of *Marinda lucida* and the

resultsshowed no significant differences in urea concentrations when compared to control.

Histological findings revealedthat the decreased values observed in the study could have been probably due to the non-toxic effectsof the phytoconstituents of the studied plant at various studied concentrations which interferes with the cellular nature of the liver tissuesof experimental rats. Also, the tolerance effectiveness observed in the cellular architecture of the liver of the extract administered rats could indicatesenhanced hepatic modulating effect of phytoextract of the studied plant at all dose levels used in the study. The aforementioned findings, agrees with the previous work carried out by Ojatula *et al*. (2023), where they embarked on *In-vitro* evaluation of potential antioxidant properties of *Eleusine indica* and *in-vivo* visceral organ protective effect of higher-dose of the phytoextract in experimental rats, and it was reported in their results that compounds present in the root of *Eleusine indica* (EI) possesses the potentialof minimizing any deleterious effects associated with higher doses of the studied plant and showed that the goosegrass could protect animals from damage caused by toxic agent of plant origin.

Equally importantly, the changes and variations seen in the biochemical parameters in treated rat groups for both aqueousand methanol extracts may be attributed to the difference in extractable ingredients with different solvents (Haborne, 1973). It seems that the aqueoushas more positive extractability for components; due to the extraction medium polarity and the ratio of solute to solventwhich would influence blood biochemical parameters. The alterations observed in some of the parameters reported in this study may indicate that the root extracts of *G. kola*possess mild toxicity affecting liver function. Therefore, crude extracts of rootof the botanical plant may not be completely safe as oral remedies, and further safety investigations on folkloric use of the phytopharmaca of the studied plant is hereby recommended.

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