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Full Length Article

In vitro Antimicrobial and *in vivo* Lead Acetate Poison Abatement Study of *Garcinia kola* Heckel

Omorefosa Osarenkhoe Osemwegie [a,*], Charles Obiora Nwonuma [a], Abimbola Peter Oluyori [b], Praise Ocheanya Abraham [a], Abraham Abayomi Akanbi [a], Deborah Oluyemisi Opaleke [c], Omokolade Oluwaseyi Alejolowo [a]

[a] *Department of Biological Sciences, College of Science and Engineering, Landmark University, Omu Aran, Kwara State, Nigeria*
[b] *Department of Chemistry, College of Science and Engineering, Landmark University, Omu Aran, Kwara State, Nigeria*
[c] *Department of Home Economics and Food Science, Faculty of Agricultural Science, University of Ilorin, Ilorin, Kwara State, Nigeria*

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Abstract

The *in vitro* antimicrobial and *in vivo* heavy metal abatement properties of aqueous extracts of *Garcinia kola* Heckel (bitter cola) were investigated using opportunistic pathogens and Wistar rats as experimental models. A marked inhibitory activity against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* was recorded at 100 mg/ml of the crude relative to ketoconozole and fluconazole drugs. Similarly, different concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml) of the crude extracts of bitter cola inhibited species of *Escherichia coli* and *Pseudomonas aeruginosa* almost as effectively as the control drug of streptomycine used. Chronic lead acetate poisoned wistar rats in groups B, C, D, E exposed to *G. kola* supplemented feed and water *ad libitum* showed variable decrease in the serum alkaline phosphatase level while aspartate and alanine aminotransferases level reduces in C and D groups compared to the negative control group. The kidney biomarkers; serum creatinine and urea concentrations were not significantly different at $P \leq 0.05$ for rat groups C, D, E when compared with the positive and negative control groups respectively. Mild infiltration and cell distortion were observed in the liver and kidney sections of the rats exposed to uncoated bitter cola supplemented feed while suggesting an overriding effect from the nut coats. The study reaffirms the medicinal potential of coated and uncoated bitter cola to act as abatement of lead toxicity and alternative antimicrobial. Furthermore, *G. kola* could be a double-edged drug for the spontaneous amelioration of lead toxicity and secondary infections due to lead poisoning.
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Keywords: *Garcinia kola*; Antimicrobial; Lead abatement; Histopathology; Opportunistic pathogens

1. Introduction

The prevalent use or misuse of synthetic antibiotics is one of the major cause of antimicrobial resistance (AMR) that may be linked to biological phenomena, such as mutations, membrane permeability or efflux dynamics, and physio-chemical modifications of the target

* Corresponding author.
E-mail address: osemwegie.omorefosa@landmarkuniversity.edu.ng (O.O. Osemwegie).
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microbe. AMR is also implicated in the slow depletion of the natural defence system of the host against infections and an imbalance of the normal human gastrointestinal microflora [1]. The emergence of multi-drug resistant strains of microorganisms and the side effects of prolonged or uncontrolled oral drug intake compounds the phlegm for synthetic antibiotics globally. Consequently, the attention of the science community is becoming more and more focused on the search for suitable organic and natural bioresources or their derivatives with inherent similar potentials as veritable alternatives in infection therapy [2,3]. Bacteria have the genetic ability to transmit and rapidly acquire resistance to drugs commonly used as antibiotics compared to their slightly complex fungi counterparts [4]. Approximately 1,340,000 plants are identified as good sources of antimicrobial compounds, but only approximately 250,000 species have had their diverse bioactive compounds elucidated [5]. The antimicrobial properties of these plants are mainly attributed to phytochemicals, such as terpenes, essential oils, coumarines and flavonoids, which are differentially concentrated in the major parts of the plants [6–9]. These phytochemicals show different solubility properties in water and solvents of varying polarities, which are the fundamental extraction media in traditional practices [10]. Previous studies have attributed the beneficial biologic activities of plant extracts, such as antimicrobial, anti-inflammatory, immunomodulatory, spasmolytic and anti-insomnia, to the nature of the composite phytochemicals [10–12]. This, according to Adedegi et al. [13], also accounts for their use as probiotics in animal production. Conversely, bioflavonoids are one of the potent phytochemicals observed in some plants that invoke antioxidant properties that are responsible for amino acid oxidation impairment, exert an antimicrobial action against some intestinal micro-organisms, promote intestinal absorption, stimulate enzyme secretion, increase feed palatability and intake, and, consequently, improve the immunological status of grazing animals [14,15].

Based on the premise that free radicals are generated during the pathogenesis due to chronic lead exposure, chelation by the supplementation with antioxidants or displacement reaction therapies are suitable alternative treatments for some heavy metal poisoning [16–18]. Lead (Pb) has no physiological role in biological systems [19] and is one of the most commonly implicated heavy metals in the Nigerian environment, especially in oil polluted, mining and urban areas. The paradigm shift in the economic mainstay of crude oil to radical natural mineral mining activities in Nigeria has led to increased incidents of lead poisoning, morbidity and mortality, resulting from saturnism. Lead is a non-reactive metal in its natural state that induces a broad range of biochemical, physiological, neurological and behavioural dysfunctions in both humans and animals [16,20]. Generally, heavy metal toxins produce ligands or complexes that bind organic compounds with a strong implication on biological molecules and oxidative biochemical enzyme functions [21]. Lead can be absorbed through the skin, inhaled or ingested and is then distributed in the blood, soft (liver, kidney, brain, and lungs) and mineralizing tissues (bones and teeth) of living systems. The resultant toxicity is predicated based on the interactions of Pb with essential metals [22,23] and oxidative stress due to the denudation of the binding sites that are essentially required for the body minerals [15,24]. This weakens resistance in biologic systems and, hypothetically, predisposes it to either secondary or opportunistic infections. Additionally, dietary supplements play an important role in the amelioration of Cd and Pb toxicity by facilitating their excretion from biological systems.

Garcinia kola Heckel, which is colloquially known as bitter cola, is an important economic and prevalent crop in Nigeria that belongs to the plant family Guttiferae. It has different dialectic names (Orogbo-Yorubas, Miji-goro-Hausas and Akiilu-Igbos) among the major tribes in Nigeria. The use of the plant is ancient with a strong application in traditional medicine practice as a tincture, concoction (decoction or infusion) or steam for the treatment of malaria, mouth odour, chest-cold, impotence, eye complications, bacterial infections and tuberculosis [1]. Additionally, the uncoated nuts or seeds are an important masticatory in African hospitality traditions, are a means of livelihood for many farming households (exportation, retail marketing, processing and storage), function as an anticholinergic for pregnant women, are flavouring agent in local beverages and are probiotics in the poultry businesses [25,13,26]. Many Nigerians, hitherto, have a sentimental attachment to bitter cola as an aphrodisiac and as an ornamental and multipurpose therapeutic. Like all other economic tree crops, the prevalence and spatial distribution of *G. cola* also faces imminent threats from deforestation and the rising trend of the firewood business in Nigeria. Numerous scientific reports, involving bitter cola, have focused more on their ethnomedicinal use rather than their non-therapeutic benefits [27–29]. Nottidge et al. [30] and Ofoego et al. [31] remarked that bitter cola has dosage and prolong exposure responses that are related to adverse effects underpinning their place of dosage precision and standardization in traditional therapy. Bitter cola is hypothetically implicated in a few ethnobotanical reports involving its use as an antivenom, antitoxin

and antipoison, while there is a paucity of information on their *in vivo* heavy metal abatement potential. This study, therefore, seeked to evaluate the potency of aqueous extract of *Garcinia kola* Heckel as an antimicrobial against some pathogenic microorganisms and a natural abatement of lead poisoning in Wistar rats.

2. Materials and Methods

2.1. Preparation of the Bitter Cola Powder and Extract

Fresh *Garcinia kola* nuts or bitter cola were purchased from the Sabo market in Kaduna State, Nigeria. The nuts were authenticated by the Herbarium Service Unit (HSU), Department of Plant Biology, University of Ilorin, Kwara State, Nigeria using herbarium voucher number F.H.I. 10847.

The bitter cola was sorted by removing the blemished and diseased nuts, was air dried for two weeks and was then divided into three treatment groups that include the nuts with an intact outer coat (A), the nuts that were uncoated or without the outer covering or coat (B), and a pre-treated population of uncoated nuts that were water-soaked for 24 hrs and air-dried for 3-day air-dried (C). Each of these treatment groups was then pulverized using a local manual milling machine and kept in airtight glass jars until each was used.

Each group of pulverized bitter cola was thereafter used to supplement commercial rat feeds at the ratio of 12 g/0.002 g feed to bitter cola powder. These preparations were fed to the rats daily for a period of 28 days [29].

Similarly, 250 g of each pulverized treatment group of the bitter cola was measured and soaked in 500 ml distilled water for 48 hrs and was vigorously whirled every 6 hrs for 3 mins with a mechanical shaker. The mixture was then filtered using No. 1 Whatmann filter paper (9 cm), with the filtrate concentrated to a crude form using a rotary evaporator, and after which this was stored in the refrigerator until it was used.

Extracts at concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml were prepared from the crude preparation of each of the bitter cola treatment group and were used for investigating the antimicrobial properties.

2.2. Extract Sterility Test

The aqueous extract was tested to ensure sterility before being used for the experiment. One millilitre (1 ml) of the extract was aseptically inoculated onto streptomycin treated nutrient agar (NA) and chloramphenicol-treated potato dextrose agar (PDA) media. These were incubated at 37 °C and 25 °C for 24 hrs and 72 hrs, respectively, and observed daily for signs of microbial growth.

2.3. Phytochemical Analysis

The aqueous extract of the pulverized uncoated bitter cola was selected as the standard extract and was qualitatively analysed for alkaloids, flavonoids, reducing sugars, saponins, steroids, tannins and terpenoids according to the procedure described by Trease and Evans [32] and AOAC [33]. Colour changes in the precipitates were used to determine the presence or absence of phytochemicals.

2.4. Preparation of the Agar Media

A digital top loading weighing balance (model) was used to weigh 14 g of commercial PDA and NA powder into Pyrex conical flasks containing 500 ml of distilled water. The flasks were later manually swirled and plunged with cotton wool stoppers wrapped in foil paper, and after which they were autoclaved for 15 mins at 121 °C. The media were allowed to slowly cool to room temperature, aseptically treated with chloramphenicol and streptomycin, poured into previously dry-heat sterilized glass petri dishes and, thereafter, allowed to set in preparation for the inoculation.

2.5. Preparation of the Test Antimicrobial Drugs

The standardization of the commercial synthetic antibiotics (fluconazole, ketoconazole, streptomycin, and tetracycline) was done by dissolving 250 mg of each of the test drugs in 50 ml of distilled water (5 mg/ml).

2.6. In Vitro Antimicrobial Susceptibility Testing

The determination of the antifungal properties of the aqueous *G. kola* extracts was done using the agar well diffusion method [34]. The PDA medium was centrally inoculated with each of the target fungi (*Aspergilus niger*, *Aspergillus flavus*, and *Candida albicans*) in triplicate. This protocol was equally followed for *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* from previously prepared stock cultures (nutrient broth) using Nutrient Agar. Wells, of approximately 7 mm, were bored at the four cardinal positions of the inoculated plates using a sterile cork borer, and the agar plugs were aseptically removed using sterile forceps. These wells (4 per

plate) were later filled with approximately 0.1 ml of the respective extracts. The positive control plates were inoculated with the target fungal species and the standard commercial antibiotics, while the negative control wells were filled with sterile distilled water as the test drug (placebo). The PDA plates were then incubated at 25 °C for 72 hrs, while the NA plates were incubated at 25 °C for 24 hrs. All the plates were monitored daily for antimicrobial activity or susceptibility.

2.7. Animal Selection and Care

Twenty female Wistar rats, with an average weight of 126 g, were procured from the Experimental Animal Unit of the Department of Biological Sciences, Landmark University, Omu-Aran, Nigeria. The animals were acclimatized for 7 days in wire-meshed cages and were fed daily with a commercial rat diet and water *ad libitum* prior to the commencement of the experiment. The cages were cleaned every other day. The experimental animals were treated humanely and were subjected to standard conditions of temperature and humidity with 12 hr light/dark cycles. The handling of the experimental rats followed prescribed international guidelines by the National Research Council on the care and use of laboratory animals [23]. Similarly, the handling protocol was subjected to the University Ethical Research Committee with the clearance number UERC/ASN/2015/036.

2.8. Preparation and Administration of the Lead Acetate Solution

Five grams of lead acetate salt (Sigma-Aldrich, USA) was measured, using a sensitive top loading weighing balance and was dissolved in 1000 ml of distilled water according to Adikwu et al [35]. The solution was shaken thoroughly to ascertain complete dissolution and, out of which, 5 mg/0.5 ml of the solution was administered daily to induce chronic lead toxicity in the experimental rats.

Each of the rats was orally administered with 0.5 ml of 5 mg/kg body weight of the lead acetate salt by a gavage daily for 28 days. The animals were allowed access to the supplemented feed and water *ad libitium* throughout the period of the lead acetate administration.

2.9. Animal Treatment and Groupings

Twenty female Wistar rats were used in this experiment and were randomly grouped into A, B, C, D, and E. The negative group (A) was administered 0.5 ml of distilled water and unsupplemented feed, while the rats in all the other groups (B-E) were administered 0.5 ml of 5 mg/kg body weight of lead acetate. Group B (positive control) was given commercial rat feed non-supplemented with *G. kola,* while Group C was fed with feed supplemented with uncoated milled *G. kola*. Group D received feed supplemented with uncoated milled bitter cola that was previously soaked in water for 24 hours prior to a 3-day drying, while the Group E rats were given with feed that was supplemented with coated milled bitter cola. The rats in all the groups were allowed access to water *ad libitum,* and the experiment ran for the duration of 28 days, and after which the rats were sacrificed 24 hours after the last day of treatment.

2.10. Animal Sacrifice and Organ Harvesting

The procedure used for the sacrifice of the rats was described by Yakubu et al. [36]. The animals were sacrificed under anaesthesia with diethyl-ether. The jugular vein was cut with a sterile surgical blade, and the blood was quickly collected in the sample bottles for the serum biochemical assays. The lower abdominal region was surgically opened to remove the liver and kidney for histological assays.

2.11. Blood and Tissue Sample preparation

The blood samples were collected in transparent vial bottles, allowed to clot for 10 minutes and spun in a refrigerated centrifuge (Cryofuge LG 25M) for 15 mins at 5000 g and a temperature of 4 °C. The supernatant blood serum was collected into another sample bottle and was then frozen until it was used. Another set of blood samples was collected in ethylenediaminetetra-acetic acid (EDTA) sample bottles for the haematological assays.

2.12. Determination of the Biochemical Parameters

The alkaline phosphatase (EC 3.1.3.1) activities were determined following the procedures described Wright et al. [37,38], while the Biuret method by Gornall et al. Gornall et al., 1949 was used to estimate the total serum protein concentration. The serum urea concentration was estimated using a diacetylmonoxime assay [39], while the serum creatinine concentration was determined according to Jaffe's reaction [40]. The activities of aspartate and alanine aminotransferases (AST and ALT, respectively) were investigated using the method described by Reitman and Frankel [41].

2.13. Haematological Assays

The haemoglobin (HGb) concentration, haematocrit (HCT), RBC count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count and platelet count (PLT) were estimated using an automated haematological analyser (SYSMEX-KX21).

2.14. Histological Assay

The tissues for the histological examination were prepared according to Adeyemi and Akanji [42]. The representative portions of the liver and kidney were fixed in 10% buffered formalin (pH 7.4) for 12 hrs and were embedded in paraffin. The paraffin embedded tissues were cut into 5 μm sections using a microtome. The tissue sections were deparaffinised and stained with a haematoxylin and eosin solution. The stained sections were viewed under the light microscope and were captured using Bresser DSC-W35 (Meade instruments Berlin, Germany)

2.15. Data Analysis

The data were analysed using a one-way ANOVA. The Duncan post hoc mean comparison test was used to estimate the significant differences between the variables. The analysed data are presented as the mean of four replicates ± the standard error of the mean (SEM). *P*-values less than *0.05* were considered statistically significant. All the statistical analyses were done using the Statistical Package for Social Science (IBM SPSS Statistics 19).

3. Results

3.1. Phytochemical Profile

The aqueous extract of the uncoated bitter cola showed an insignificant level of alkaloids and terpenoids with a mild presence of saponin, steroids and tannins. Flavonoids and reducing sugars were, however, prominent in the analysed extract (Table 1).

Table 1
Phytochemical profile of the aqueous extract of uncoated *G. kola*.

s/n	Phytochemicals	Results
1	Saponins	+
2	Flavonoid	++
3	Alkaloids	–
4	Reducing sugars	++
5	Terpenoids	–
6	Steroids	+
7	Tannins	+

(++) heavily present; (+) mildly present; (–) absent.

3.2. In Vitro Antimicrobial effect of the aqueous extract of G. kola

The zone of inhibition (25 mm), recorded at a concentration of 100 mg/ml of the aqueous extract, on *A. niger* showed a stronger antimicrobial performance compared to those that were recorded for the standard antibiotics (Fig. 1). A varying, but reduced, inhibitory performance was observed at concentrations of 25 mg/ml and 50 mg/ml of the extract on *A. flavus* compared to the standard drugs. While the inhibitory activity against *A. flavus* reduced with the concentration strength, *C. albicans* was noted to be invulnerable to the different concentrations of the extract compared to its response to the standard test drugs. Similarly, the different concentrations of the aqueous extract inhibited the growth of *E. coli* and *P. aeruginosa* as effectively as streptomycin and tetracycline, with *P. aeruginosa* appearing to be most susceptible to the extract compared to the standard drugs (Fig. 1). *Proteus mirabilis* and *Staphylococcus aureus,* however, showed less susceptibility at the lower concentration (25 mg/ml) of the extracts than at the higher concentrations (50 mg/ml and 100 mg/ml) compared to the marked zone of inhibition recorded for the standard test drugs.

3.3. Haematological screening

A significant increase in the WBC, RBC and HCT was noted in the experimental rat groups fed with *G. kola* supplemented rat feed. Although, the MCH and MCHC plunged in all the rat treatment groups, the most significant reduction was recorded in rats that were fed with the feed that was supplemented with the pre-treated uncoated and coated bitter kola. This is, however, contrary to the PLT activity of the pre-treated bitter cola extract, which had a high value compared to all the other treatment groups and the control groups (Table 2).

3.4. Biochemical Assay

The protein concentration in the rat groups exposed to *G. kola* supplemented feed showed a significant increase ($P \leq 0.05$), which was comparatively higher than the result obtained from the negative control group (Fig. 3). All the groups given the supplemented feed experienced

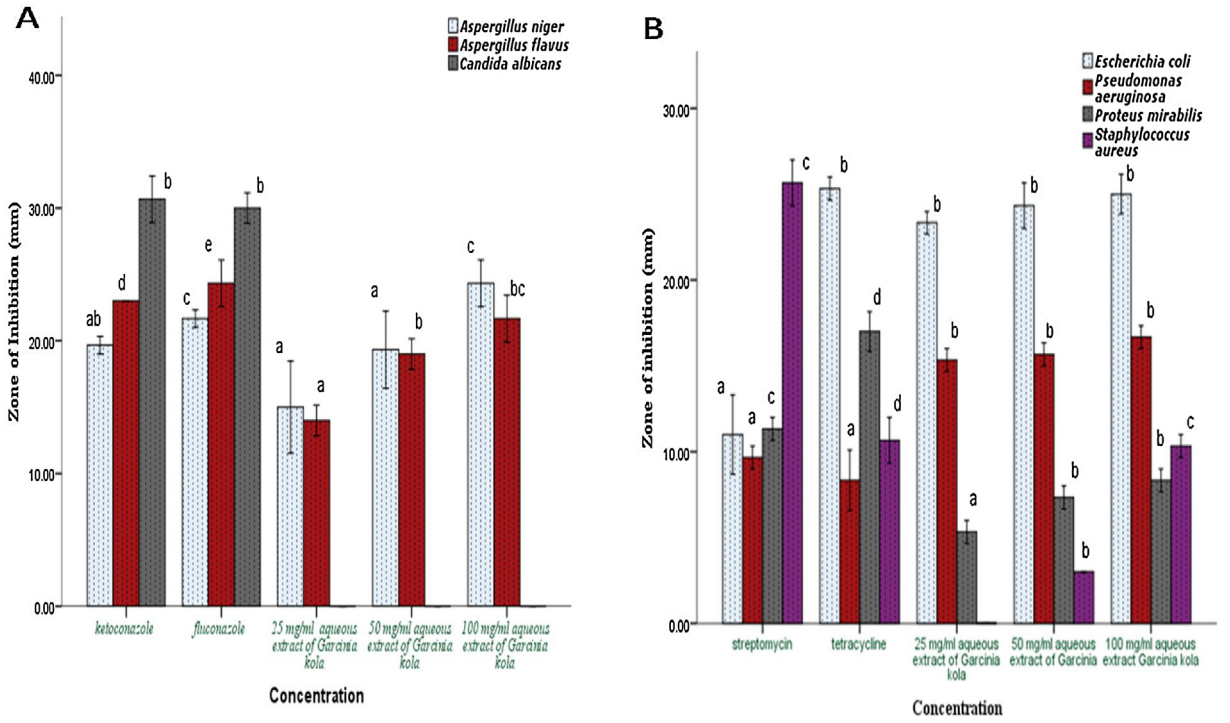


Fig. 1. The inhibitory activity of different concentrations of aqueous extract of *G. kola* and conventional test drugs against opportunistic pathogens of fungal and bacterial origin. Each value is represented as the mean of three replicates ± SEM. A dissimilar letter or combination of letters represents a significant difference relative to the control at $p \leq 0.05$.

Table 2
Haematological Parameters of the different animal group treatments.

Haematological Parameters	A	B	C	D	E
WBC	6.60 ± 0.32^{a}	7.40 ± 0.40^{ab}	8.77 ± 1.33^{abc}	11.40 ± 1.14^{bc}	11.77 ± 0.88^{c}
RBC	4.23 ± 0.30^{a}	6.20 ± 0.11^{b}	6.66 ± 0.17^{b}	5.92 ± 0.51^{b}	6.04 ± 0.13^{b}
HGB	11.30 ± 0.61^{a}	10.70 ± 0.21^{a}	11.63 ± 0.62^{a}	10.00 ± 0.58^{a}	10.06 ± 0.06^{a}
HCT	22.40 ± 1.75^{a}	37.40 ± 0.72^{b}	40.17 ± 2.72^{b}	28.23 ± 5.91^{ab}	37.23 ± 0.39^{b}
MCH	30.27 ± 4.78^{a}	17.23 ± 0.12^{b}	17.43 ± 0.52^{b}	18.10 ± 1.07^{b}	16.47 ± 0.52^{b}
MCHC	42.43 ± 1.47^{a}	28.50 ± 0.29^{b}	29.03 ± 0.82^{b}	27.56 ± 1.44^{b}	27.33 ± 0.35^{b}
MCV	63.77 ± 4.36^{a}	60.43 ± 0.27^{a}	60.17 ± 2.89^{a}	57.90 ± 1.50^{a}	61.83 ± 2.0^{a}
PLT	76.86 ± 10.18^{ab}	74.27 ± 3.41^{ab}	51.93 ± 6.04^{a}	91.03 ± 6.69^{b}	49.46 ± 1.19^{a}

The data ± SEM with similar superscripts are not significantly different, while the data with a combination of superscripts are significantly different at $p \leq 0.05$.

serum alanine aminotransferase and serum aspartate aminotransferase diminution compared to the positive control group, in which the rats were administered the lead acetate solution and the non-supplemented rat feed (Figs. 4 and 5). The serum alkaline phosphatase enzyme activity also marginally improved in groups C, D and E compared to the positive control group (B) but was lower than the values obtained in the negative control group (Fig. 2). An increase was observed in serum urea concentration of all the groups given the supplemented feed, which was above the negative and positive control groups, while the serum creatinine concentration was higher in the positive control group compared to all the other groups, which were relatively different (Fig. 3).

3.5. Histopathology

The histopathological micrograph showed a mild infiltration of the liver hepatocytes and mononuclear cells in treatment groups B-E compared to the negative control group (Fig. 4). Similarly, the kidney histo-preparation showed insignificant levels or a variation of cell distortions in the different treatment groups, except

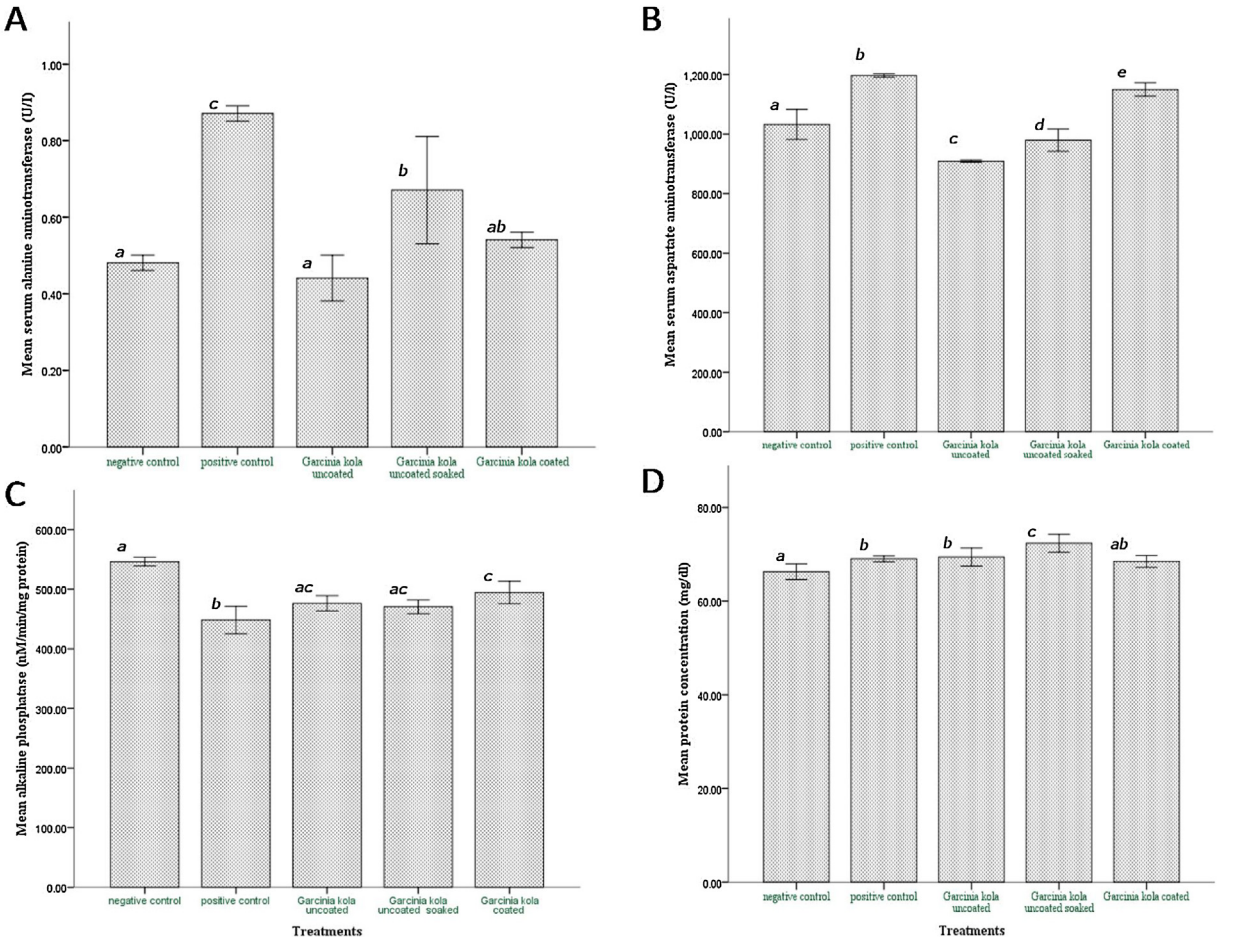


Fig. 2. The effect of *Garcinia kola* (bitter kola) supplemented feed on ALT (A), AST (B), ALP (C) and serum protein levels in Wistar rats poisoned with a lead acetate solution. Each value represents the mean of three replicates ± SEM. A dissimilar letter or combination of letters represents a significant difference relative to the control at $P \leq 0.05$.

for B, C and D, as indicated by the glomerular and mild interstitial mononuclear cell integrity (Fig. 5).

4. Discussion

While *G. kola* tree is widely distributed in many parts of Nigeria, it constitutes a paramount enterprise for agricultural settlements and is a common therapeutic in folk medical practice. There is, however, a limited body of knowledge on the other uses of this plant related to it ornamental, nutritional, construction and biotechnological value [26,43]. The study of the medicinal and traditional applications, pharmacopeia, biogeography, and phytochemical of *G. kola* are rife and mostly involve the uncoated fruits (bitter cola) rather than the coated ones [9,28,29]. This, therefore, forms the premise underlying this study.

The various concentrations of the crude extract showed different degrees of antimicrobial activity against the target pathogenic microorganisms, with *E. coli*, *P. aeruginosa* and *A. niger* proving to be more susceptible. The inhibition of the activity at higher concentrations of the extract was almost as effective as those of the standard antibiotics. This result concurs with previous studies on bitter cola and may be qualitatively attributed to the prevalence of flavonoids in the extract relative to other assayed phytochemicals [43–45]. Zehra et al. [46] and Dah-Nouvlessounon et al. [47] hinged the antimicrobial property of the extract of *G. kola* fruits on the interactive effect of constituent secondary metabolites [48]. The susceptibility pattern of the target clinical microbes used in this study showed a direct proportionality to the increased extract concentration, and this was corroborated by Adegboye et al. [49], and Nwankwo and Sadique [50]. Furthermore, some of the target oppor-

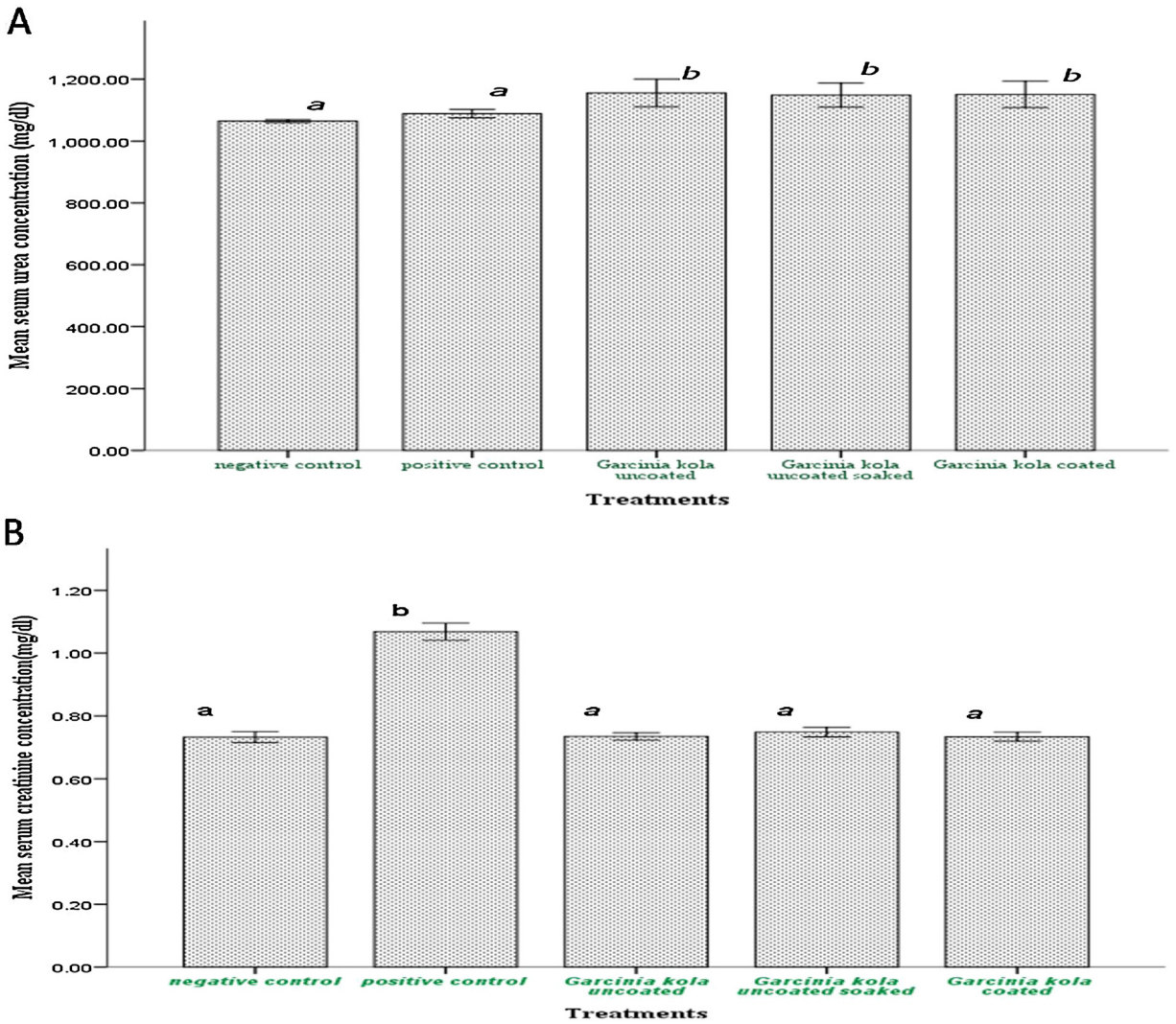


Fig. 3. The effect of *Garcinia kola* (bitter kola) supplemented feed on serum urea (A) and creatinine concentrations in Wistar rats poisoned with a lead acetate solution. Each value is represented as the mean of three replicates ± SEM. A dissimilar letter or combination of letters represents a significant difference relative to the control at $P \leq 0.05$.

tunistic microbes, such as *C. albicans*, *P. mirabilis* and *S. aureus,* were mildly to insignificantly vulnerable at lower concentrations (25 mg/ml and 20 mg/ml) of the extract compared to the standard antibiotics. While further study may be required to fully understand the reason for the susceptibility variations and the linearity pattern observed between the extract and their concentrations, they might logically be assumed to be caused by any one or a combination of factors, which might include the source of the target pathogenic isolates, the isolates preservation protocol used during the investigation, the nature of the genetic or acquired microbial resistance mechanism(s), the counteracting or synergistic effect of the bioactive constituents of the extract, the ambient conditions and the target microbes' membrane functionality or biological nature [46]. This finding underscores the growing global concerns for the emerging trend of the rapidly changing susceptibility patterns of microbes to different clinical treatment drugs and microbicides. It further attracts research interest to more studies on the underlying mechanisms of drug resistance development by many opportunistic microbes. In addition, it further supports a vigorous search for safer microbial treatment drugs with a strong potential for minimal to zero threat to the health of biological systems due to prolonged exposure.

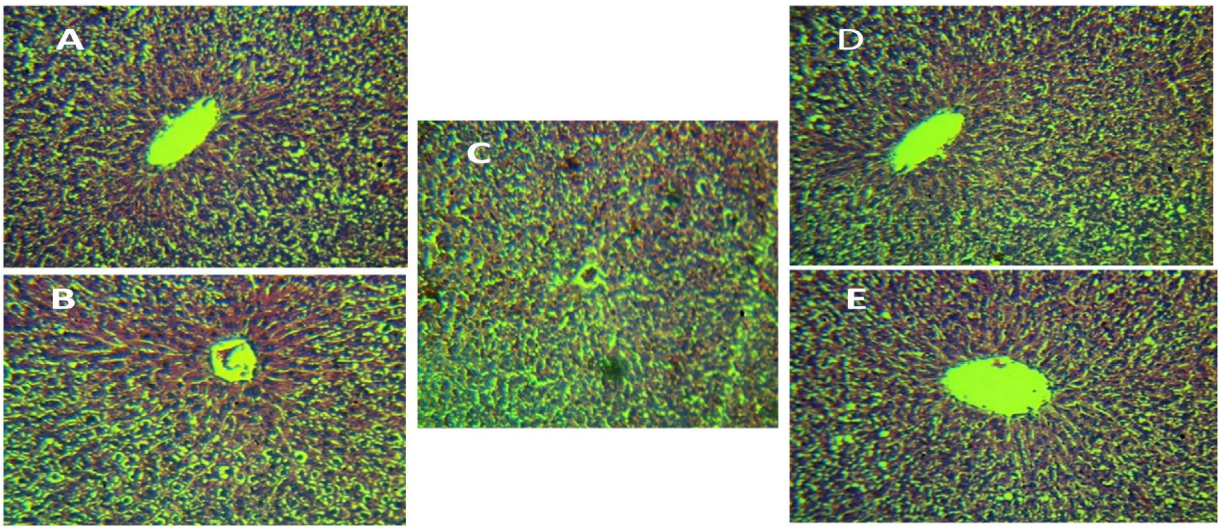


Fig. 4. Photomicrographs (X100 H &E) of rat liver: negative control (A) – the rats administered distilled water showed a normal morphological architecture of the surrounding hepatocytes; positive control (B) – the rats administered lead acetate only showed a normal morphological architecture of the surrounding hepatocytes; Group (C) – the rats administered lead acetate and fed the uncoated *G. kola* supplemented feed had a mild infiltration of the hepatocytes and mononuclear cells; Group (D) – the rats administered lead acetate and fed the rat feed supplemented with the pre-treated uncoated *G.* kola also showed hepatocyte and mononuclear cell infiltration; Group (E) – the rats administered lead acetate and fed the coated *G. kola* supplemented feed showed a normal morphological architecture of the surrounding hepatocytes.

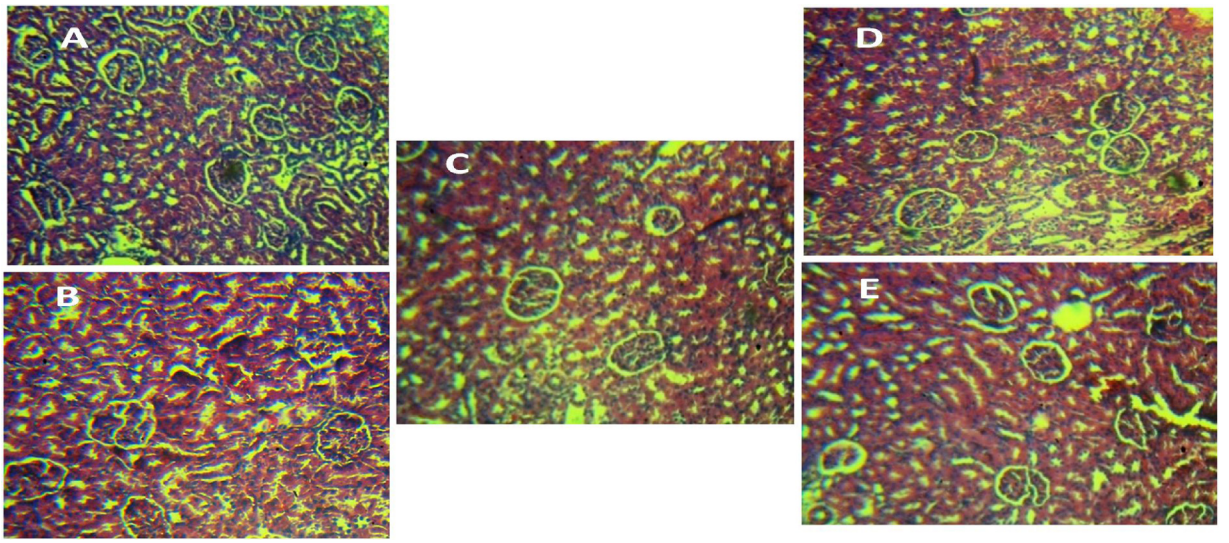


Fig. 5. Photomicrographs (X100 H &E) of rat kidney: negative control (A) rats administered with distilled water showed a normal renal histo architecture of the glomerular, surrounding tubules; positive control (B) rats administered with lead acetate exhibited a mild nephritic cell infiltration; Group (C) – the rats administered with lead acetate and fed the uncoated *G. kola* supplemented rat feed had normal glomerular integrity but interstitial cell infiltration compared with the negative control group; Group (D) – the rats administered with lead acetate and fed the pre-treated uncoated supplemented rat feed also showed a normal architecture of the glomerular/bowman capsules and a mild interstitial cell infiltration; Group (E) – the rats administered with lead acetate and fed the coated *G. kola* supplemented rat feed showed an insignificant level of nephritic cell infiltration.

The toxicity effect of lead predisposes biological systems to opportunistic infections in addition to other clinical symptoms [15]. This is why a study into screening for natural resources with the double-edged advantage of toxicity abatement and infection therapy is paramount for medicine as well as for humanity.

A gradual improvement in the haematological profile, especially the WBC status, of the lead acetate poisoned rats treated with bitter cola supplemented feed

underscores the protective influence of bitter cola in erythropoietin and immunological processes. This validates it traditional use as an antianaemic medicine [51]. Although this observation is supported by Dzierzak and Pilipsen [52], it may, hypothetically, suggest that the bitter cola supplemented feed promotes the synthesis of serum proteins and erythropoiesis, which are responsible for the homeostatic wellbeing of the liver [53]. The biochemical mechanism responsible for this is not yet understood. It is noted that the coated bitter cola supplemented feed was more effective at improving the haematological profile and lead toxicity abatement relative to what was observed for the other rat treatment groups fed with the uncoated bitter cola supplemented feed. Further study is, therefore, necessary to isolate, identify and characterize the potent abatement principles and haematological modifiers in the coat of bitter cola. The potency of the coated bitter cola fruits, as observed in this study, may not be unconnected with the bioactive constituent(s) in the coat. The natural and slow acclimatization of the rats to chronic non-lethal poisoning by lead acetate may have accounted for by the low mortality result obtained for all the poisoned groups when compared to the negative control group [51].

The biochemical assay of the liver recorded an increase in the ALT, AST and serum creatinine concentration of the positive control group in contrast to the variably decreasing concentration noted in the other rat groups. This confirms the abatement property of the test plant on lead acetate poisoning and its putative hepatoprotective ability. The mechanism of the protection of the liver against the damaging effects of chronic lead acetate poison by bitter cola is still unclear. However, it may be attributed to the physiochemical processes of chelation and the membrane biochemistry [54,55,43]. The rat groups exposed to lead acetate poison and fed with either the non-supplemented feed or the bitter cola supplemented feed had decreased levels of alkaline phosphatase activity, which was higher in the negative group. Although, the low level of ALP activity is uncommon in many biological systems, and it linearly influences the dephosphorylation, glutamyltransferase activity and physiologic mineral balance in living systems [56]. The concentration variability of the ALP activity and its implications are presently unclear and may potentially qualify as a bioindicator of dysfunctionality in organs (liver, bile duct, kidney and bones) or hypomineralization in hard tissues. The high serum protein and urea concentrations in all the poisoned rat groups agreed with Dalia [18] and might have increased above the concentration values of the negative groups due to the lead acetate toxicity effect. This, hypothetically, influenced the renal activities of the poisoned rats, suggesting a mild renal dysfunction of the kidney in concordance with Cameron and Greger [57] and/or an increased HCT, as validated by the haematological results obtained in this study. Ghorbe et al. [58] observed that an oral administration of lead acetate resulted in a significant increase in blood urea and serum creatinine, which are indicative of uremia. Although, there might be evidence of mild polyuria due to the abatement effect of bitter cola, and the subclinical impairment of the kidney also could not be ignored as a consequence of the increased blood urea and creatinin levels.

Research focusing on the therapeutic applications of bitter cola and all other medicinal plants in traditional and self-health care practices is biased against the possible side effects of prolong use [26]. Nanyak et al. [27] and Ofoego et al. [31] reported a structural distortion effect from the prolonged mastication of bitter cola on the liver and gonads. This observation agrees with the histopathology results of the liver and kidneys cells obtained from this study. Contrary to earlier studies, suggesting the controvertibly damaging repercussion of the prolong use of bitter cola, further investigation is required to clearly establish the resultant histopathological effect of lead acetate on both the liver and the kidney tissues and possibly support the regulation of its use [22,20]. Gurer and Ercal [16] were explicit on the oxidative stress effect of lead poisoning to the vital organs of a biological system. The positive control group of rats that were fed the supplemented feed showed a slight distortion in the liver and kidney cells compared to the negative control. These infiltrations, which align with the biochemical assays, were clearly visible in the tissues from all the treatment groups except for the groups fed with coated and pre-treated, uncoated bitter cola whose tissues showed no valid degenerative property. The pretreatment of the uncoated bitter cola may have attenuated the potential impact of the bioactive constituents, while the counteractive effect of the inherent chemical composition of the coat mitigated the infiltration of the kidney and liver. One can, therefore, assume that bitter cola feed supplementation treatments have a strong potential as an alternative detoxifier of lead acetate poison under a precise dosage regime.

There is no doubt that the results from this study prove the potential of the aqueous extracts of bitter cola as medicinal and contribute additional information on its capacity to act as an *in vivo* abatement for heavy metal toxicity and a virile alternative antimicrobial. Additionally, the results incontrovertibly lend credence to the need for the dosage standardization and prescription management of traditional preparations in

rural health care practices while also hinting against self-herbal medication and abuse. Since bitter cola ranks as one of the most traditionally used therapeutic plants in Nigeria, this study proved that it's prolonged administration and ingestion is not without harmful effects. The logical exploitation of natural resources as safer and relatively potent alternatives to synthetic pharmaceutics is hinged on the inconsequence of their therapeutic usage. This refutes the hitherto belief of the locals in Nigeria that medicinal plants or nature-derived therapeutic products lack any side effects from prolong use and indiscriminate dosage administration. Therefore, further concrete investigation involving the careful screening of diverse medicinal plants and their putative tissue degeneration effect is important to reconcile the emerging controversies in the scientific literature on the conflicting histopathological findings from medicinal plant experiments involving animal models.

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