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

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1. INTRODUCTION

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

is also implicated in the slow depletion of the natural defence system of the host against infections and imbalance of normal human gastrointestinal microflora (Cohen, 1992). The emergence of multi-drug resistant strains of microorganisms and the side effects of prolonged or uncontrolled oral drug intakes compounded the phlegm for synthetic antibiotics globally. Consequently, the attention of the science community is becoming more and more focused on a search for suitable organic and natural bioresources or their derivatives with inherent similar potential as veritable alternatives in infection therapy (Harbottle *et al*., 2006; Rafii *et al*., 2008). Bacteria have the genetic ability to transmit and rapidly acquire resistance to drugs commonly used as antibiotics than their slightly complex fungi counterparts (Gould, 1996). About 1,340 plants have been identified as good sources of antimicrobial compounds but only about 250,000 species have had their diverse bioactive compounds elucidated (Kim *et al*., 1995). The antimicrobial properties of these plants are mainly attributed to phytochemicals such as terpenes, essential oils, coumarines and flavonoids which are differentially concentrated in major parts of the plants (Vargas *et al*., 1999; Lis-Balchin *et al*., 1998; Mau *et al*., 2001; Eleyinmi *et al*., 2006). These phytochemicals show different solubility property in water and solvents of varying polarity which are fundamental extraction media in traditional practices (Calvo *et al*., 2006). Previous studies have attributed beneficial biologic activities of plant extracts such as antimicrobial, anti-inflammatory, immunomodulatory, spasmolytic and anti-insomnia to the nature of composite phytochemicals (Kamel, 2002; Mosaad *et al*., 2003; Calvo *et al*., 2006). This according to Adedegi *et al*. (2008) also accounted for their use as probiotics in animal production. Conversely, bioflavonoids is one of the potent phytochemicals observed in some plants that invoke antioxidant property responsible for amino acid oxidation impairment, exert antimicrobial action against some intestinal micro-organisms, promote intestinal absorption, stimulate enzyme secretion, increased feed palatability and intake, and consequently improve the immunologic status of grazing animals (Ralphs *et al*., 1995; Farmand *et al*., 2005).

Based on the premise that free radicals are generated during pathogenesis from chronic lead exposure, chelation by supplementation with antioxidants or displacement reaction therapies became suitable alternative treatments of some heavy metal poisoning (Gurer and Ercal, 2000; Flora *et al*., 2003; Dalia, 2010). Lead (Pb) has no physiologic role in the biological systems (Enagbonma *et al.*, 2015) 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, neurologic and behavioural dysfunction in both humans and animals (Gurer and Ercal, 2000; Suradkar *et al*., 2009). Generally, heavy metal toxin produced ligands or complexes that bind organic compounds with strong implication for biological molecules and oxidative biochemical enzymes functions (Nwokocha *et al*., 2011). Lead may be absorbed through the skin, inhaled or ingested and distributed in the blood, soft (liver, kidney, brain, lungs) and mineralizing tissues (bones and teeth) of living systems. The resultant toxicity is predicated on the interactions of Pb with essential metals (Ahamed and Siddiqui, 2007; Vesey, 2010) and oxidative stress due to the denudation of the binding sites

essentially required for the body minerals [Farmand *et al*., 2005; Liu *et al*., 2009). This may weakens resistance in biologic systems and hypothetical predisposed it to either secondary or opportunistic infections. Additionally, dietary supplements have been reported to play important roles 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 major tribes in Nigeria. The use of the plant is ancient with strong application in traditional medicine practice as tincture, concoction (decoction or infusion) or steam for the treatment of malaria, mouth odour, chest-cold, impotence, eye complications, bacterial infections and tuberculosis (Cohen, 1992). Additionally, the uncoated nuts or seeds are important masticatory in African hospitality traditions; a means of livelihood for many farming households (exportation, retail marketing, processing and storage); anticholinergic by pregnant women, flavouring agent of local beverages and probiotics in poultry businesses (Kanmegne and Omokolo, 2007; Adedegi *et al*., 2008; Babalola and Agbeja, 2010). Many Nigerians hitherto have sentimental attachment to bitter cola as an aphrodisiac, ornamental and multipurpose therapeutics. Like all other economic tree crops, the prevalence and spatial distribution of *G. cola* also faces imminent threats from deforestation and rising trend of firewood business in Nigeria. **N**umerous scientific reports involving bitter cola have focused more on their ethnomedicinal use rather than their non-therapeutic benefits (Nanyak *et al*., 2013; Adesuyi *et al*., 2012; Enemchukwu *et al*., 2015). Nottidge *et al*. (2008) and Ofoego *et al*. (2015) remarked that bitter cola has dosage and prolong exposure response related adverse effects underpinning the place of dosage precision and standardization in traditional therapy. Bitter cola is hypothetically implicated in a few ethnobotanical reports involving its use as antivenom, antitoxin and antipoison while there is paucity of information on their *in vivo* heavy metal abatement potential**.** This study therefore seeks to evaluate the potency of aqueous extract of *Garcinia kola* Heckel as antimicrobial against some pathogenic microorganisms and natural abatement of lead poisoning in Wistar rats.

2. Materials and Methods

2.1. *Preparation of Bitter Cola Powder and Extract*

Fresh *Garcinia kola* nuts or bitter cola were purchased from Sabo market, 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 were sorted by removing blemished and diseased nuts, air dried for two weeks, then divided into three treatment groups that include nuts with intact outer coat (A), nuts were uncoated or without the outer covering or coat (B), and pre-treated population of uncoated nuts that were 24 hrs water-soaked and 3-day air-dried (C). Each of these treatment

groups was then pulverized using a local manual milling machine and kept in air-tight glass jars until 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 (Enemchukwu *et al*., 2015).

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 vigorously whirled every 6 hrs interval for 3 mins with a mechanical shaker. The mixture was then filtered using No. 1 Whatmann filter paper (9cm) with the filtrate concentrated to a crude the form using a rotary evaporator after which this was stored in the refrigerator until used.

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

2.2. *Sterility Test of Extract*

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 respectively. These were incubated at 37^{o}C and 25^{o}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 qualitatively analysed for alkaloids, flavonoids, reducing sugars, saponins, steroids, tannins and terpenoids according to the procedure described by Trease and Evans (1983) and AOAC (1990). Colour change of the precipitates was used to determine the presence or absence of phytochemicals.

2.4. *Preparation of Agar Media*

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

2.5. *Preparation of Test Antimicrobial Drugs*

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

2.6. *In Vitro Antimicrobial Susceptibility Testing.*

Determination of the antifungal properties of aqueous *G. kola* extracts was done using the agar well diffusion method (Magaldi *et al*., 2004). The PDA medium was centrally inoculated with each of the target fungi (*Aspergilus niger*, *Aspergillus flavus*, *Candida albicans*) in triplicate. This protocol was equally followed for *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* from previously prepared stock culture (nutrient broth) using Nutrient Agar. Wells of about 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 about 0.1ml of the respective extracts. Positive control plates were inoculated with target fungal species and standard commercial antibiotics while the negative control ones have their wells filled with sterile distilled water as test drug (placebo). The PDA plates were then incubated at 25^{o}C for 72 hrs while the NA plates were incubated at 25^{o}C for 24 hrs and all were monitored daily for antimicrobial activity or susceptibility.

2.7. *Animal Selection and Care*

Twenty female Wistar rats having 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, fed daily with commercial rat diet and water *ad libitum* prior to the commencement of experiment. These cages were cleaned every other day, the experimental animals treated humanely and subjected to standard conditions of temperature and humidity with 12 hrs 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 (Vesey, 2010). Similarly, the handling protocol was subjected to the University Ethical Research Committee with clearance number UERC/ASN/2015/036.

2.8. *Preparation of Lead Acetate Solution and Administration*

5 g of lead acetate salt (Sigma-Aldrich, USA) was measured using a sensitive top loading weighing balance, dissolved in 1000 ml of distilled water according to Adikwu *et al* (2013). The solution was shaken thoroughly to ascertain complete dissolution out of which 5 mg/0.5 ml of the solution was administered daily to induce chronic lead toxicity in the esperimental rats.

Each of the rats was orally administered with 0.5 ml of 5 mg/kg body weight of lead acetate salt with a gavage daily for 28 days. The animals were allowed access to the supplemented feed and water *ad libitium* throughout the period of 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 previously soaked in water for 24 hours prior to a 3-day drying while Group E rats were fed with feed supplemented 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 after which the rats were sacrificed 24 hours after the last day of treatment.

2.10. *Animal Sacrifice and Organ Harvest*

The procedure used for the sacrifice of the rats was described by Yakubu *et al.* (2008). The animals were sacrificed under anaesthesia with diethyl-ether. The jugular vein was cut with sterile surgical blade and the blood quickly collected in sample bottles for 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^{o}C. The supernatant blood serum was collected into another sample bottle and then frozen until used. Another set of blood samples were collected in ethylenediaminetetra-acetic acid (EDTA) sample bottles for haematological assays.

2.12. *Determination of Biochemical Parameters*

Alkaline phosphatase (EC 3.1.3.1) activities was determined following the procedures of Wright *et al*. (1972a and b) while the Biuret method by Gornall *et al*. (1949) was used to estimate the total serum protein concentration. The serum urea concentration was estimated using diacetylmonoxime assay (Veniamin and Varkirtzi-Lemonias, 1970) while the serum creatinine concentration was determined according to Jaffe's reaction (Cook, 1975). The activities of aspartate and alanine aminotransferases (AST and ALT respectively) were investigated using the method by Reitman and Frankel (1957).

2.13. *Haematological Assays*

The haemoglobin (HGb) concentration, hematocrit (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 histological examination were prepared according to Adeyemi and Akanji (2012). The representative portions of the liver and kidney were fixed in 10% buffered formalin (pH 7.4) for 12 hrs and embedded in paraffin. The paraffin embedded tissues were cut into 5 μm sections using a microtome. The tissue sections were deparaffinised and stained with haematoxylin and eosin solution. The stained sections were viewed under the light microscope and captured using Bresser DSC-W35 (Meade instruments Berlin, Germany)

2.15 *Data Analysis*

Data were analysed using one way ANOVA. The Duncan post-hoc mean comparison test was used to estimate the significant differences between variables. The analysed data were

presented as mean of four replicates ± standard error of mean (SEM). *P*-values less than *0.05* were considered statistically significant. All 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 insignificant level of alkaloids and terpenoids with mild presence of saponin, steroids and tannins. Flavonoid and reducing sugars were however prominent in the analysed extract (Table 1).

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

The zone of inhibition (25 mm) recorded at 100 mg/ml concentration of the aqueous extract on *A. niger* showed stronger antimicrobial performance compared to those recorded for the standard antiobiotics (Fig. 1). Varying but reduced inhibitory performance was observed at 25 mg/ml and 50 mg/ml concentrations of the extract on *A. flavus* compared to the standard drugs. While inhibitory activity against *A. flavus* reduces with 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 lower concentration (25 mg/ml) of the extracts than at 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, MCH and MCHC plunged in all the rat treatment groups, the most significant reduction was recorded in rat fed with feed supplemented with pre-treated uncoated and coated bitter kola respectively. This is however contrary to PLT activity of the pre-treated bitter cola extract which had a high value compared to all other treatment groups and the control groups (Table 2).

3.4. *Biochemical Assay*

The protein concentration in rat groups exposed to *G. kola* supplemented feed showed significant increase ($P \leq 0.05$) which is comparatively higher than the result obtained from the negative control group (Fig. 3). All the groups given the supplemented feed experienced serum alanine aminotransferase and serum aspartate aminotransferase diminution compared to the positive control group that had rats administered with lead acetate solution and non-supplemented rat feed (Fig. 4 and Fig. 5). The serum alkaline phosphatase enzyme activity also marginally improved in groups C, D and E than the positive control group (B) but lower than values obtained in negative control group (Fig.2). An increase was observed in serum urea concentration of all the groups given supplemented feed above the negative and positive control groups while serum creatinine concentration is higher in the positive control group compared to all the other groups which are relatively different (Fig. 3).

3.5. *Histopathology*

The histopathological micrograph showed mild infiltration of the liver hepatocytes and mononuclear cells in treatment groups B-E compared to the negative control group (Figure 4). Similarly, the kidney histo-preparation showed insignificant levels or variation of cell distortion in the different treatment groups except B, C and D as indicated by the glomerular and mild interstitial mononuclear cells' integrity (Figure 5).

4. Discussion

While *G. kola* tree is widely distributed in many parts of Nigeria, it constitutes a paramount enterprise for agricultural settlements and a common therapeutics in folk medical practice. There is however a few body of knowledge on other uses of this plant relating to ornamental, nutritional, construction and biotechnological values (Babalola and Agbeja, 2010; Kanmgne *et al*., 2010; Dike and Nnamdi, 2012). Study of the medicinal and traditional applications, pharmacopeia, biogeography, and phytochemical of *G. kola* are rife and involves mostly the uncoated fruits (bitter cola) rather than the coated ones (Eleyinmi *et al*., 2006; Adesuyi *et al*., 2012; Enemchukwu *et al*., 2015). This therefore forms the premise underlying this study.

The various concentrations of the crude extract showed different degree of antimicrobial activity against target pathogenic microorganisms with *E. coli*, *P. aeruginosa* and *A. niger* proving to be more susceptible. Inhibition 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 (Di Carlo *et al.,* 1999; Montoro *et al.,* 2005; Dike and Nnamdi, 20012). Zehra *et al*. (2012) and Dah-Nouvlessounon *et al*. (2015) hinged the antimicrobial property of the extract of *G. kola* fruits on the interactive effect of constituent secondary metabolites (Naczk & Shahidi, 2004). The susceptibility pattern of target clinical microbes used in this study showed direct proportionality to increase extract concentration and this was corroborated by Adegboye *et al*. (2008), and Nwankwo and

Sadique (2011). Furthermore, some target opportunistic microbes like *C. albicans*, *P. mirabilis* and *S. aureus* were mildly to insignificantly vulnerable at lower concentrations (25 mg/ml and 20 mg/ml) of the extracts compared to the standard antibiotics. While further study may be required to fully understand the reason for the susceptibility variations and linearity pattern observed between extract and their concentrations, it may logically be assumed to be caused by any one or a combination of factors. These may include the source of target pathogenic isolates, the isolates preservation protocol used during this investigation, the nature of the genetic or acquired microbial resistance mechanism(s), the counteracting or synergistic effect of bioactive constituents of the extract, ambient conditions and target microbes' membrane functionality or biologic nature (Zahra *et al*., 2012). This finding underscores the growing global concerns for the emerging trend of rapidly changing susceptibility pattern of microbes to different clinical treatment drugs and microbicides. It further attracts research interests to more studies on the underlying mechanisms of drug resistance development by many opportunistic microbes. It further supports a vigorous search for safer microbial treatment drugs with strong potential for minimal to zero threat to the health of biological systems due to prolonged exposure.

The toxicity effect of lead predisposes biologic systems to opportunistic infections in addition to other clinical symptoms (Farmand *et al*., 2005). This is why a study into screening for natural resources with a double edged advantage of toxicity abatement and infection therapy is paramount for medicine as well as humanity.

Gradual improvement in the haematological profile especially the WBC status of lead acetate poisoned rats treated with bitter cola supplemented feed underscores the protective influence of bitter cola in the erythropoietin and immunological processes. This validates it traditional usage as antianaemic medicine (Falke and Zwennis, 1990). While this observation is supported by Dzierzak and Philipsen (2013), it may hypothetically suggests that the bitter cola supplemented feed promotes the synthesis of serum proteins and erythropoiesis which are responsible for the homoestatic wellbeing of the liver (Ikpesu *et al*., 2014). The biochemical mechanism responsible for this is not yet understood. It is noted that the coated bitter cola supplemented feed proved more effective at improving haematological profile and lead toxicity abatement relative to what was observed for the other rat treatment groups fed with uncoated bitter cola supplemented feed. Further study is therefore necessary to isolate, identify and characterize the potent abatement principles and haematological modifier 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 the low mortality result obtained for all the poisoned groups when compared to the negative control group **(**Falke and Zwennis, 1990**)**.

Biochemical assay of the liver recorded an increase in the ALT, AST and serum creatinine concentration of the positive control group contrary 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 hepato-protective ability. The mechanism of protection of the liver against the damaging effect of chronic lead acetate poison by bitter cola is still unclear. However, it may be attributed to the physiochemical processes of chelation and membrane biochemistry (Batra *et al.*, 1998; Agada and Braide, 2009; Dike and Nnamdi**,**

2012**)**. Rat groups exposed to lead acetate poison and fed with either non-supplemented feed or bitter cola supplemented feed were observed to have decreased levels of alkaline phosphatase activity which is higher in the negative group. Though, the low level of ALP activity is uncommon in many biological systems, it linearly influences dephosphorylation, glutamyltransferase activity and physiologic mineral balance in living systems (Golub and Boesze-Battaglia, 2007). The concentration variability of ALP activity and its implication according to Orimo (2010) 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. High serum protein and urea concentrations in all poisoned rat groups agrees with Dalia (2010) and may have increased above the concentration values of the negative groups due to 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 (1998) and/or increase HCT as validated by the haematological results obtained in this study. Ghorbe *et al.* (2001) observed that oral administration of lead acetate result in significant increase of 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, subclinical impairment of the kidney could also not be ignored as a consequence of increase blood urea and creatinin level (Sands and Layton, 2009).

Research focusing on the therapeutic applications of bitter cola and all other medicinal plants in traditional and self health care practices is baised against possible side effects of prolong use (Babalola and Agbeja, 2010). Nanyak *et al*. (2013) and Ofoego *et al*. (2015) reported a structural distortion effect from the prolonged mastication of bitter cola on the liver and gonads respectively. This observation agrees with the histopathology results of the liver and kidneys cells obtained from this study. Contrary to earlier studies suggesting a controvertibly damaging repercussion of 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 (Ahamed and Siddiqui, 2007; Suradkar *et al.,* 2009). Gurer and Ercal (2000) were explicit on the oxidative stress effect of lead poisoning on vital organs of a biological system. The positive control group of rats that were fed with supplemented feed showed slight distortion of liver and kidney cells compared to the negative control. These infiltrations which align with the biochemical assays are clearly visible in tissues from all the treatment groups except the groups fed with coated and pre-treated, uncoated bitter cola whose tissues showed no valid degenerative property. The pre-treatment of the uncoated bitter cola may have attenuated the potential impact of bioactive constituents while the counteractive effect of inherent chemical composition of the coat mitigates infiltration of the kidney and liver. One can therefore assume that bitter cola feed supplementation treatments have a strong potential as alternative detoxifier of lead acetate poison under precise dosage regime.

There is no doubt that the results from this study proves the potential of aqueous extracts of bitter cola as medicinal and contributes additional information on its capacity to act as *in vivo* abatement for heavy metal toxicity and a virile alternative antimicrobial. Additionally, the results incontrovertibly lend credence to the need for 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 has proved that it's prolong administration and ingestion is not without harmful effect. 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 Nigerian 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 emerging controversies in scientific literature on conflicting histopathological findings from medicinal plants experiments involving animal models.

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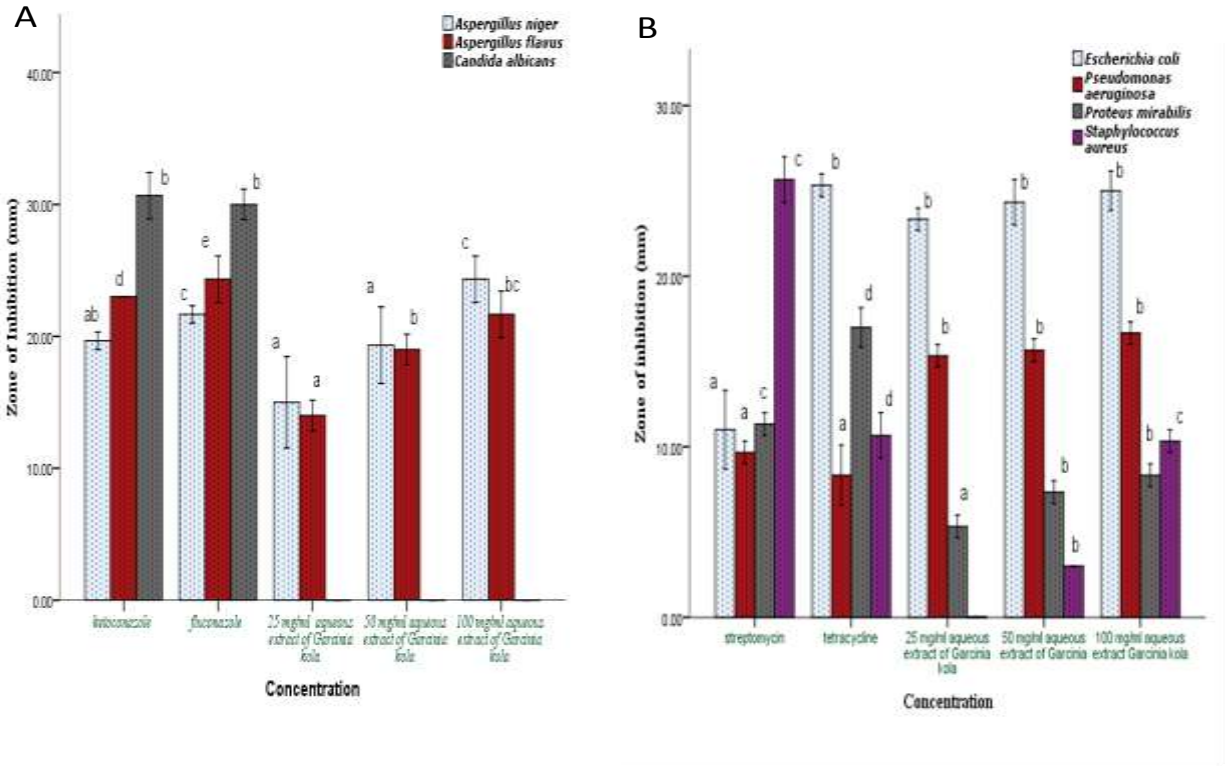

Fig 1: The inhibitory activity of different concentrations of aqueous extract of *G. kola* and conventional test drugs against opportunistic pathogens of fungal and bacterial origins. Each value is represented as mean of three replicates ± SEM. Desimilar letter or combination of letters represent statistical difference relative to control at $p \leq 0.05$.

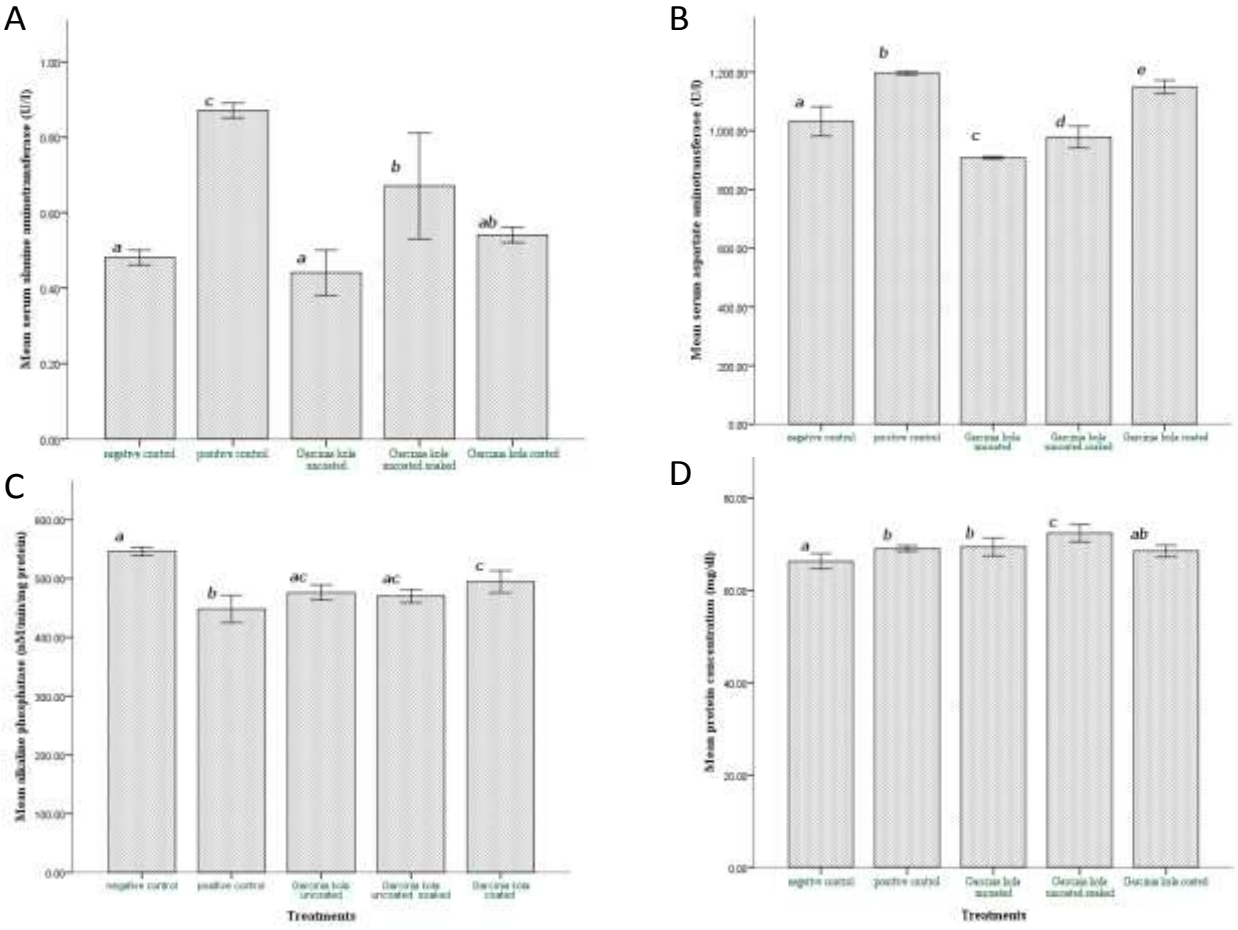

Figure 2: The effect of *Garcinia kola* (bitter kola) supplemented feed on ALT (A), AST (B), ALP (C) and serum protein levels in Wistar rats poisoned with lead acetate solution. Each value is represented as mean of three replicates ± SEM. Desimilar letter or combination of letters represent statistical difference relative to control at $P \leq 0.05$

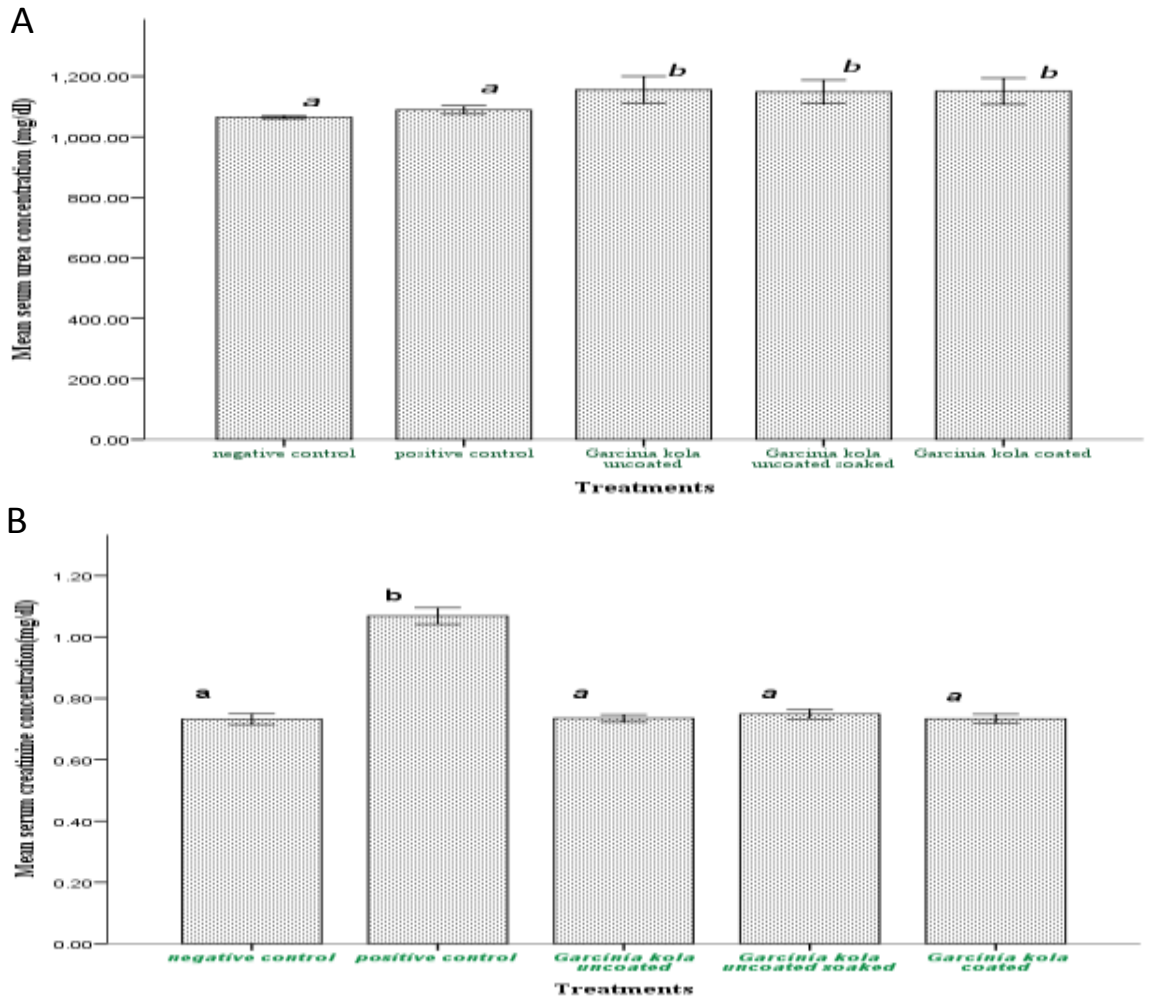

Figure 3: The effect of *Garcinia kola* (bitter kola) supplemented feed on serum urea (A) and creatinine concentration in Wistar rats poisoned with lead acetate solution. Each value is represented as mean of three replicates ± SEM. Desimilar letter or combination of letters represent statistical difference relative to control at $P \leq 0.05$

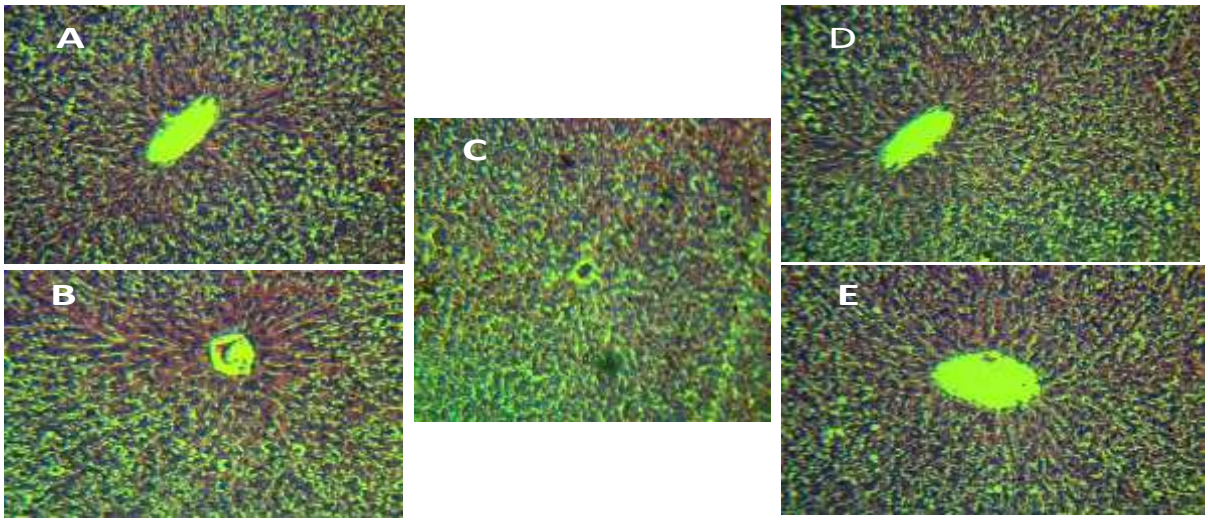

Figure 4: Photomicrographs (X100 H &E) of rat's liver: negative control (A) - rats administered with distilled water showed normal morphological architecture of the surrounding hepatocytes; positive control (B) - rats administered with lead acetate only normal morphological architecture of the surrounding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

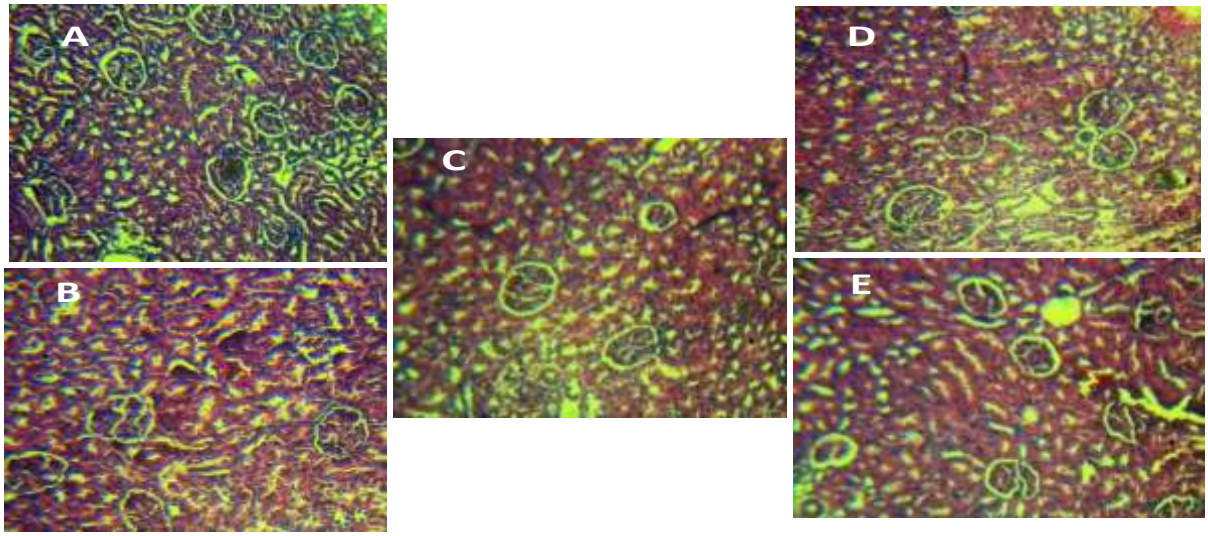

Figure 5: Photomicrographs (X100 H &E) of rat's kidney: negative control (A) rats administered with distilled water showed normal renal histo architecture of the glomerular, surrounding tubules; positive control (B) rats administered with lead acetate exhibited mild nephritic cell infiltration; Group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

Table 1: Phytochemical profile of 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

Table 2: Haematological Parameters 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}

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