Antimicrobial, biochemical, histopathological analysis and teratogenic investigations of *Garcinia kola* seed extract on gravid *wistar* rats pups

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ABSTRACT

Background: The use of herbal remedy for the treatment and management of several ailments in the 21st century is becoming a global norm. The World health organization asserted that over 60 % of the world population and most especially in the developing nations depend on herbal remedies for their healthcare needs.

Objective: The objective of the study is to evaluate the extract of *G. kola* seed for its antimicrobial activity on clinical isolates, biochemical contents and teratogenic assay on the pups of gravid *wistar* rats.

Methods: Phytochemical screening of the seeds of *G. kola* were determined by qualitative approach. Sensitivity test of clinical isolates of *Salmonella* spp, *Escherichia coli* and others were done by agar well diffusion method. The zones of growth inhibitions of the extracts were recorded and interpreted by CLSI standard. Acute toxicity of the extract evaluated in young albino rats. The gravid wistar rats in their gestational periods were administered with *G kola* seed extracts at different concentrations. The animals were sacrificed, liver and kidney tissues examined for possible histophathological changes and their pups investigated for possible teratogenic malformations using standard procedures.

Results: Phytochemical screening shows the presence of flavonoids, saponin, tannin and alkaloid. The crude extracts exhibited antimicrobial activity on the test isolates. The zone of growth inhibition diameter in millimeter of n-hexane fractions elicited varied values; *S. aureus* (29.5mm), *Salmonella* spp (33. 0), *E. coli* (31.5) in comparison with ciprofloxacin (40.0mm) , a standard antibiotic. Teratogenic evaluation of the pups from gravid *wistar* rats treated with the extract shows no malformations as well as the histopathological survey of the liver and kidney tissues of the mothers. The biochemical analysis did not indicate significant alterations in the liver and kidney functions biomarkers.

Conclusion: The extract of *G. kola* seed exhibited remarkable activities on selected isolates of bacteria and fungi tested and histological survey presented with no malformation.

Keywords: Antimicrobial, Histopathology, *Garcinia kola,* Teratogenic

Analyse antimicrobienne, biochimique, histopathologique et études tératogènes de l'extrait de graines de Garcinia kola sur ratons gravides de souche Wistar

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RÉSUMÉ

Contexte: Au XXIe siècle, le recours aux plantes médicinales pour le traitement et la prise en charge de nombreuses affections est devenu une norme mondiale. L'Organisation mondiale de la santé a affirmé que plus de 60 % de la population mondiale, et plus particulièrement dans les pays en développement, dépend des remèdes à base de plantes pour ses besoins en matière de soins de santé.

Objectif: L'objectif de l'étude est d'évaluer l'extrait de graines de G. kola pour son activité antimicrobienne sur des isolats cliniques, son contenu biochimique et son test tératogène sur les petits de rats gravides de souche Wistar.

Méthodes: Le criblage phytochimique des graines de G. kola a été déterminé par une approche qualitative. Le test de sensibilité des isolats cliniques de Salmonella spp, Escherichia coli et autres a été effectué par la méthode de diffusion en puits d'agar. Les zones d'inhibition de croissance des extraits ont été enregistrées et interprétées selon la norme CLSI. La toxicité aiguë de l'extrait a été évaluée chez de jeunes rats albinos. Les rates gravides de souche Wistar en période de gestation ont reçu des extraits de graines de G. kola à différentes concentrations. Les animaux ont été sacrifiés, les tissus hépatiques et rénaux ont été examinés afin de détecter d'éventuelles malformations histopathologiques et leurs petits ont été examinés afin de détecter d'éventuelles malformations tératogènes en utilisant des procédures standard.

Résultats: Le criblage phytochimique révèle la présence de flavonoïdes, de saponine, de tanin et d'alcaloïdes. Les extraits bruts ont montré une activité antimicrobienne sur les isolats testés. Le diamètre de la zone d'inhibition de croissance en millimètres des fractions de n-hexane a donné lieu à des valeurs variées : S. aureus (29,5 mm), Salmonella spp (33,0 mm), E. coli (31,5 mm) comparé à la ciprofloxacine (40,0 mm), un antibiotique standard. L'évaluation tératogène des ratons nés de rates gravides de souche Wistar traitées avec l'extrait n'a révélé aucune malformation, tout comme l'examen histopathologique des tissus hépatiques et rénaux des mères. L'analyse biochimique n'a pas révélé d'altération significative des biomarqueurs des fonctions hépatiques et rénales.

Conclusion: L'extrait de graines de G. kola a montré des activités remarquables sur des isolats sélectionnés de bactéries et de champignons testés et l'étude histologique n'a montré aucune malformation.

Mots clés: Antimicrobien, histopathologie, Garcinia kola, tératogène

INTRODUCTION

As the burden of urgency in the development of safer drugs for the treatment of man's ailments and infections surges, the need for accelerated scientific evaluations to validate the safety of the several African folklore in herbal remedies. This is has become very important as the World Health organization (WHO) asserted that the application of medicinal plants uses in health care system is on the rise globally,[1,2,3] with the developing countries in the lead.[3] The height of recognition accorded herbal remedy have been attributed to a number of positive perceptions derived thereon. Some of these perceptions include availability, affordability, accessibility, effectiveness, and non-synthetic background of the products, among others.[4] Others view it from the point of thought that plants are the most bio resources of many synthetic agents.[5]

However, in many developing nations including West African countries where there is much dependences on natural medicines as alternative to synthetic ones, there is poor documentations and little or no scientific evaluations to establish the claims of the traditional users of herbal preparations.[6,7]

Furthermore, it is estimated that about 15 % of congenital structural anomalies observed are due to adverse effects arising from intake of some natural products and the environments on pre-natal development.[8,9] This implies that 1 in every 250 newborns infants have structural defects due to environmental exposure to teratogenic indices.[10]

Also, it is believed that about 12-59 % of women in Africa use herbal products during pregnancy for a variety of purposes, including pregnancy related conditions (nausea, vomiting, constipation), to prepare for labor and to induce abortion,[10,11] and routinely administered to women during pregnancy and childbirth.[8,9,10] It has been further reported that there are re-emerging case due to high costs of prescription drugs in the maintenance of personal health and well-being, pre-natally and post-natally.[12]

Although herbs are "natural", not all herbs are safe to take during pregnancy. thus, as the pursuit for alternative medicines surges on in our societies today, several locally consumed natural products have been implicated in folklore to have remedies for preventive and curative remedies in both microbial and anti-teratogenic remedies but not scientifically established nor their safety in human organs.

G. kola locally called 'akiilu','ogorigo', 'orogbo' 'ogorigo/ ogoligo" in various Nigerian languages,[10,11,12] is claimed to have diverse medicinal uses without much scientific proof, thus the necessity for the study however. *Garcinia kola* is regarded as a wonder plant because every part of the plant (bark, leaves, root and wood) has been found to be of medicinal importance. The medicinal importance of bitter kola is based mainly on the phytochemical components of the plant. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties.[13,14]

The present study describes the antimicrobial sensitivity pattern of the *G. kola* seeds and the biochemical analysis of the blood as well as the histopathological assay of the livers and kidneys of gravid wistar rats. It also, evaluates the teratogenic status of the pups of their mothers.

MATERIALS AND METHODS

Plant materials

The seeds of *G. kola* were collected from a local market in Sagamu, Ogun state, Nigeria. The seed was identified at the Pharmacognosy department, Olabisi Onabanjo University, Sagamu campus, Sagamu. The bark was removed, caught in smaller pieces, grounded to powder and dried in a laboratory hot air oven (MRC LTD, Germany). about 500 g of the powder was extracted in a soxhlet apparatus using ethanol solvent for 24 hours. The extract was filtered and concentrated in a rotary evaporator (N4FR205D, 2010), and dried at low heat rate using water bath.

Phytochemical screening

Qualitative phytochemical screening was carried out on the powdered seed of *G. kola* using standard procedures in line with standard procedures[15] to test for the presence of flavonoid, tannins, saponins, alkaloids, anthraquinone, cardiac glycosides and cyanogenic glycosides.

Organisms:

Test organisms were all clinical isolates obtained from the laboratory of Medical Microbiology and Parasitology department of Olabisi Onabanjo University teaching hospital (OOUTH) and were confirmed biochemically and reconfirmed with analytical profile index (api). Api20 staph was used for Staphylococcus species, Api20 used for other Gram-positive bacteria and Api20E for Gram negative bacteria, while api-Candida was used for *Candida* spp.

Preparation of extracts and media:
Agar well diffusion method was used. Initial concentration of 400mg/ml was prepared and diluted serially to 200, 100, 50, 25, 12.5 and 6.25, as well as 5µg ciprofloxacin and fluconazole discs 5µg (standard antibacterial and antifungal agents, respectively. Both agar - Mueller Hinton agar (MHA), sabrourd dextrose agar (SDA) for Aspergilus and chromatic candida, 042723501, Liofilchem Sr. Italy were prepared according to the manufacturers' instructions.

Preparation of inoculums
Overnight cultures of bacteria and fungi were obtained by sub-culturing from agar slant. The culture was made by streaking the slant with a sterile wire loop inoculating unto a single strength peptone water for bacteria and single strength sabrourd dextrose broth for fungi in test tubes and were incubated for 24 hours at 37^{o}C for bacteria and 48 hours at 27^{o}C for fungi. Turbidity of bacteria and fungi were adjusted to 0.5 % McFarland standard 2 Gram positive, 5 Gram negative and 2 fungi were prepared in line with CLSI standard.[16]

Determination of antimicrobial sensitivity
The inoculum was plated unto the agar plates by agar well method. *Candida albicans* was studied with chromatic candida plates 042723501, Liofilchem Sr., Italy. Ciprofloxacin and fluconazole discs were used as standard agents, and incubated at 37^{o}C for 24 hours in an incubator in line with CLSI standard.[17]

Determination of the activity on the bacterial seeded plates
Colonies Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus feacalis and *Salmonella* spp. were taken from 24-hour culture on slanted nutrient agar, and suspended in sterile peptone water overnight. Optical density was in consonance with 0.5 % McFarland standard (10^{8}cfu/ml and the setup was allowed to stabilized for about 15 minutes. A 6 mm cork borer was afterward used to make wells on the different agar plates containing various microorganisms into each well while the reconstituted seed extracts at concentrated 400, 200, 100, 50, 25 12.5 and 6.25 mg/ml. The extracts were allowed to diffuse into the agar for 1hr before they were incubated at 37^{o}C for 24hrs. 5µg of ciprofloxacin/fluconazole was used as the (positive) control. The diameter of the zone of inhibition for each of the wells was measured to the nearest millimeters (mm) after the incubation period.[18,19] This was measured edge to across the zone of inhibition over the centre of the disc.[16] These results were recorded. The minimum inhibition concentration (MIC) of the extracts that inhibit the visible growth of each of the organism was determined using the agar dilution method in line standard procedure.[18]

Determination of the activity on the fungi seeded plates
Colonies of *Candida albicans* and *Aspergillus niger* were grown in Sabouraud dextrose broth at 27^{o}C for 48hrs. Turbidity was adjusted to optical density of 1:100 by dilution with Sabouraud dextrose broth. A moist swab was used to obtain fungi suspension and spread evenly on previously prepared dry plates of Sabouraud dextrose broth and left for about 15 minutes. The wells were bored and extracts introduced into each well and allowed to pre-diffuse up to an hour and incubated at room temperature for 48 hrs, using 1µg of fluconazole as the standard agent and ethanol as negative control. The zones of inhibition were measured after 48 hours. The minimum fungicidal concentration (MFC) of the extracts that inhibit visible growth of each of the organism was determined by using the agar dilution method.

Animal handling
A total of forty-one adult Wistar rat *(Rattus novergicus)* weighing approximately 175 g were obtained from the animal house of Olabisi Onabanjo University, Sagamu campus, reweighed and housed in well ventilated animal cages and acclimatized for 7 days prior the study. They were offered pure clean water and standard pelletized rat feed from Apple and Pears, Sagamu, Ogun state.

Ethical approval
Ethical approval for use of animal in the research was obtained from the Ministry of Agriculture and Animal Husbandry, Benin city, Edo state, Nigeria.

Determination of acute lethal dose (LD_{50})
Lorke model was used to determine the lethal dose of the extract.[22] The model consists of two phases comprising of 18 animal of 180 g average. The first phase which is of lower concentration is between 10 - 1200 mg/kg/body weight doses respectively while the second phase is made up of the larger doses from 1600 - 5000 mg/kg/body weight doses respectively. The experiment was carried out for two consecutive days starting with the lower doses and after confirming that the treated animals survived, then the second phase was carried out. At each occasion, the animals were being observed for any manifestation of toxicity for at least 48 hours.

Preparation of extract

Garcinia cola seed was purchased in Sabo market located in Sagamu, Ogun state, the bark was removed, caught in smaller pieces, grounded to powder and dried in a laboratory hot air oven (MRC LTD). About 250 g of the powder was extracted in soxhlet apparatus using ethanol for 24 hours. The extract was filtered and concentrated in a rotary evaporator (N4FR205D, 2010).

Determination of the oestrous cycle and mating the animals.

Oestrous cycle was determined through vaginal smearing process in line with,[17] by establishing morphological changes in ovarian cortex, the uterus and the vagina1[7] through pro-oestrus, oestrus, met-oestrus and di-oestrus.[17] A matured male rat of similar age range was introduced to each group made up of four fertile females for seven days. The males were removed from each group as soon as pregnancy was established via microscopic analysis.

Administration of *G. kola*

The pregnant wistar rats were grouped into 5 consisting of 5 pregnant rats in each. Groups 1,2,3 were given 200 mg/ml/kg for the period of 7days (1st trimester), group 2 for 14 days (second trimester), group 3 for 21 days (third trimester), while group 4 received multivitamin and the 5th group was given pure natural water as the negative control. The extract was administered once a day for the given period of time with the aid of an oral cannula.

Tissue processing for light microscopy

The animals were anaesthetized, dissected and the kidneys and liver were removed, weighed with a digital weighing balance and immediately fixed in 10 % neutral formalin.

Dehydration

Tissues were dehydrated in ascending grades of alcohol, and were passed through two changes each of 50 %, 70 %, 80 %, 95 % and absolute alcohol at intervals of an hour between each change.[9,22]

Clearing and embedding

Dehydrated tissues were cleared in two changes of xylene for 30 mins and an hour respectively to render the tissue translucent and free of alcohol for impregnation and infiltration in molten paraffin wax and paraffin infiltrated tissues were finally buried (embedded) into molten paraffin wax and allowed to cool.

Microtomy

Microscopy was carried out rotary microtome, the tissues were trimmed to expose the tissue surfaces. Serial sections were taken at 5 microns, floated on a warm water bath of 45^{o}C temperature, to spread the tissues and neaten the folds before pre-mounting on a slide warmer to melt excess wax.

Staining

Tissues were first taken to xylene to remove the remaining wax and leave it translucent to react with the stain and dyes. They were stained using Ehrlich haematoxylin and eosin techniques as described below, while others were specially stained.

Photomicrography

Photomicrographs of histological sections of the lungs, liver, kidney and spleen, were taken with scope image 9.0 exe camera, coupled to a system in line with standard procedures.[19]

Anthropometric analysis

Adult female albino rats were weighed during acclimatization, mating period and extract administration as well as litters sizes.

Fetal measurements

Crown rump length, dead circumference, femur length and humeral length.

Statistical analysis

Descriptive and inferential statistics were carried out as follows: data were analyzed using EXCEL 2007; the data were expressed as mean ± standard errors of mean (Mean ± SEM) mean values were compared using one - way analysis of variance (ANOVA). P values less than 0.05 (P>0.05) were taken to be statistically significant. All graphs were drawn with EXCEL 2007.

RESULTS

Qualitative phytochemical screening of *G. kola* seed

The results of the phytochemical screening are shown in Table 1.

Table 1: Physicochemical properties of the extract

Bioactive compound	Test	Result
Alkaloid	free alkaloid	+
Anthraquinone	free anthraquinone	+
Saponin	(i) froting test (iii) emulsion test	+ +
Tannins	ferric test	+
Cardiac glycoside	keller-killani test	-

Key: + represents positive result and – represents absence.

Antimicrobial activity of the extracts

The zones of inhibition of the various concentrations of the extracts are shown in Table 2a while the comparisons are shown in Tables 2b and 2c.

Table 2a: Antimicrobial activity of the methanol extract of *G. kola* seed extract

	Extract (mg/ml)							cipro/ Fluc (µg)
Concentration	400	200	100	50	25	12.5	6.25	5
Organisms	Zone of inhibition (mm)							
K. pneumonia	24.3	19.5	17.0	11.0	-	-	-	290
p. *aeruginosa*	18.5	12.5	09.5	-	-	-	-	27.5
S. *aureus*	22.5	19.5	15.9	10.5	-	-	-	27.0
E. *coli*	26.0	23.0	17.5	12.5	12.5	09.4	-	32.0
P. vulgaris	24.0	19.0	10.0	-	-	-	-	27.0
S. *typhi*	29.0	27.0	17.5	10.9	-	-	-	33.0
E. faecalis	19.5	17.5	-	-	-	-	-	27.0
C. *albicans*	26.0	24.9	17.5	-	-	-	-	32.7
A. terreus	19.5	14.7	-	-	-	-	-	29.7

Key: *K. pneumonae = Klebsiella pneumoniae, E. coli = Escherichia coli, S. typhii =Salmonella typhii, P.= aeruginosa =Pseudomonas aeruginosa, S. aureus = Staphylococcus aureus, E. faecalis= Enterococcus feacalis, P. vulgaris = Proteus vulgaris, A. terreus = Aspergilus terreus, C. albicans = Candida albicans*

Table 2b. Statistical comparison of the antimicrobial activity of the *G. kola* seed extract by zones of inhibition for organisms

Organism	Mean (mm)	Std. Deviation
K. *pneumonia*	12.9444	11.43537
P. *aeruginosa*	10.8000	10.74756
S. *aureus*	9.3333	12.20400
E. *coli*	10.3333	11.37706
P. *vulgaris*	10.6556	13.34608
S. *typhii*	11.4444	12.08678
E. *feacalis*	11.3000	12.67665
C. *albicans*	14.6889	12.37926

F ratio = 0.0173, p value= 0.990

Table 2c. Statistical comparison of the standard drugs with the *G. kola* seed extract

Preparation	Mean (mm)	Std. Deviation
400 mg/ml extract	23.2556	3.55497
200 mg/ml extract	19.7333	4.65645
100 mg/ml extract	11.6556	7.31558
50 mg/ml extract	4.9889	5.94042
25 mg/ml extract	1.3889	4.16667
12.5 mg/ml extract	1.0444	3.13333
6.25 mg/ml extract	0. 0000	0.0000
5 µg cipro/Fluc	29.4333	2.54313

F ratio = 59.952, p value = 0.000

Acute toxicity study results

The results of acute toxicity study on the extract are shown in Table 3

Table 3. Acute toxicity study (Ld_{50}) of the *G. kola* extract in the wistar albino rats

Concentration (mg/ml)	No treated	No of living	No of dead	Total
1st phase				
10	3	3	0	3
100	3	3	0	3
500	3	3	0	3
1000	3	3	0	3
1200	3	3	0	3
2nd Phase				
1600	1	1	0	1
2700	1	1	0	1
5000	1	1	0	1

Effect of the extract on pregnancy and body organs

The effects of the extract on pregnancy and body organs are shown in Tables 4-6 and Plates 1-10. There was a significant increase in the kidney weight of treated Garcinia kola GR 3 (0.144 ± 0.009) compared to control (0.138 ± 0.004).

Table 4 Mean weight of adult wistar rat during pregnancy and period of administration

Groups	Initial mean weight (g)	1st trimester (g)	2nd trimester (g)	3rd trimester (g)
Group 1	186.60±3.79	205.26±3.94	238.72±4.35	266.03±6.04
Group 2	174.80±8.13	191.11±10.33	215.23±11.82	240.91±9.99
Group 3	202.10±1.82	206.17±6.84	230.04±5.19	246.37±7.72
Group 4	189.50±3.65	201.60±4.39	201.60±4.39	201.60±4.39

Key:

Mean weight of rats during the various trimester (n =5)

Group 1 - 1st trimester, Group 2 - 2nd trimester, Group 3 - 3rd trimester, Group 4 - control

Table 5: Characteristics of the foetuses

	Group				
Parameters	**1**	**2**	**3**	**4**	**Control**
litter size(n)	19	17	9	6	**21**
Hc (cm)	3.90±0.07	3.93±0.06	3.85±0.08	3.96±0.08	4.02±0.05
crl(cm)	5.11±0.17	5.30±0.17	5.69±0.18	5.70.±0.29	5.44±0.13
fl(cm)	0.76±0.02	0.74±0.02	0.73±0.03	0.93±0.03	0.76±0.02
hl(cm)	0.74±0.01	0.72±0.02	0.64±0.02	0.68±0.03	0.69±0.01
Malformation	–	–	–	–	–
Resorption	–	–	–	–	–
Still birth	–	–	–	–	–
Death after delivery	–	–	–	–	–
feotal weight(g)	5.34±0.11	5.30±0.14	5.04±0.19	6.42±0.17	5.12±0.11

Key: hc: head circumference, crl: crown rump length, fl: femur length, hl: humeral length , minus(-) : absent, plus(+): present; group 1- 1st trimester, group 2 -2nd trimester, group 3 -3rd trimester

Table 6: Mean weight of organs

. Groups	**Liver (g)**	**Kidney (g)**
Group 1	0.940±0.004	0.132±0.002
Group 2	0.932±0.006	0.132±0.004
Group 3	0.936±0.006	0.144±0.009
Group 4	0.938±0.005	0.136±0.004
Group 5	0.946±0.002	0.138±0.004

Data expressed as Mean±SEM. P>0.05

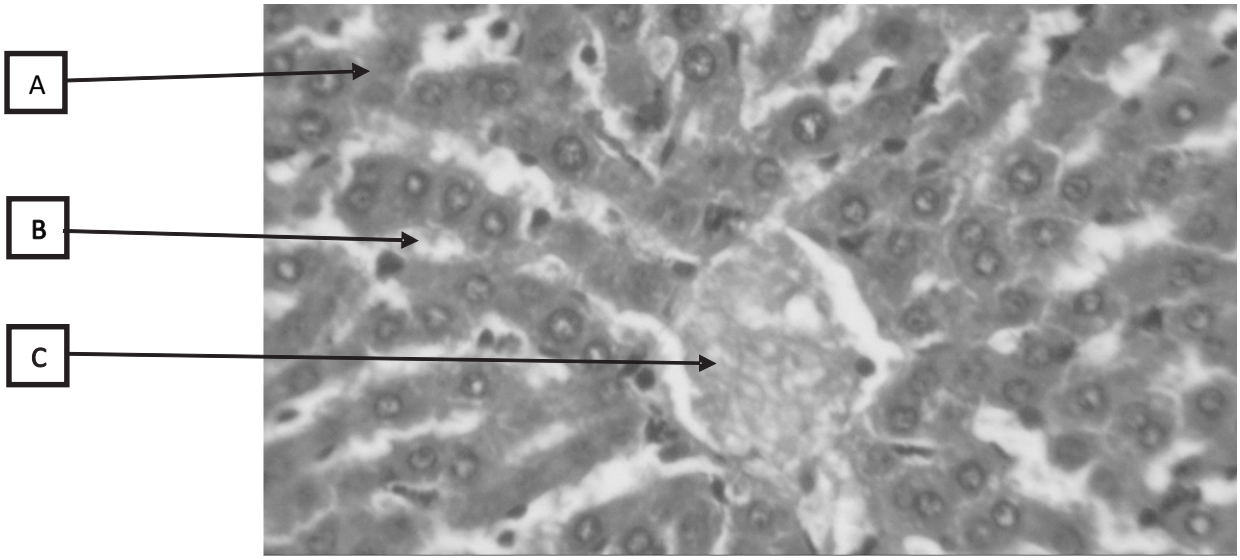

Plate 1 Photomicrograph of control animal liver.

Key: A: hepatocytes, B: sinusoids and C: central vein (H&E x 400)

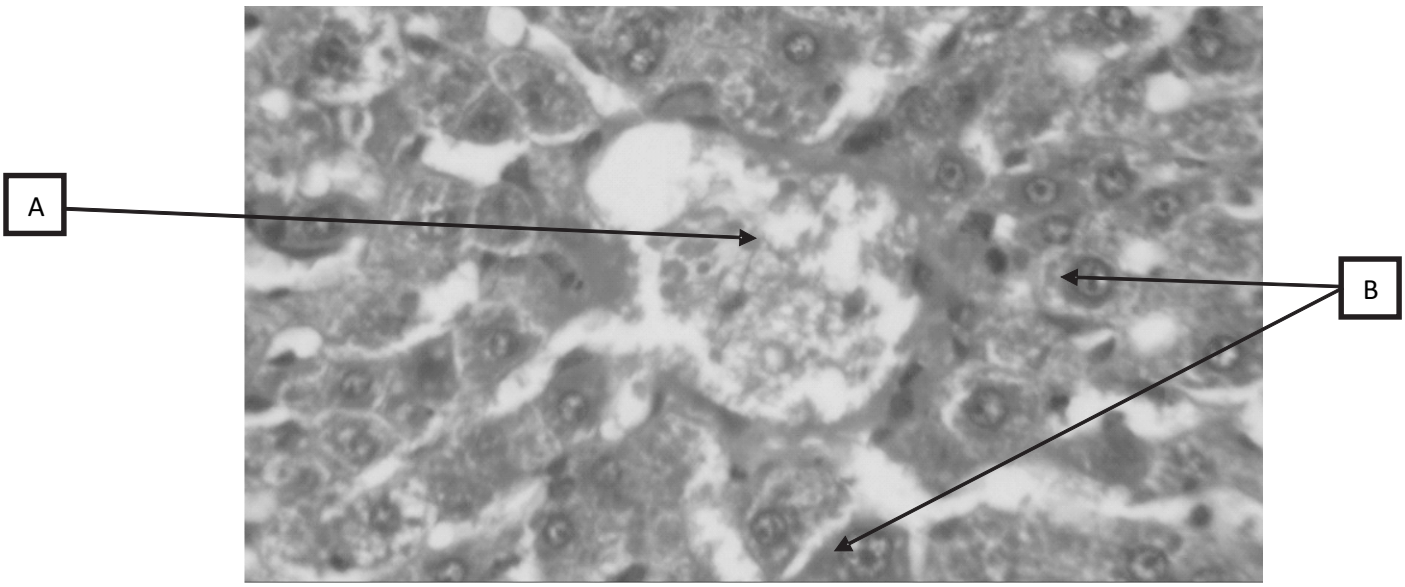

Plate 2 Photomicrograph of liver of rat given Multivitamin for 3 weeks.

Key: A: active vascular congestion, B: prominent hepatocyte nucleolus (H&E x 400)

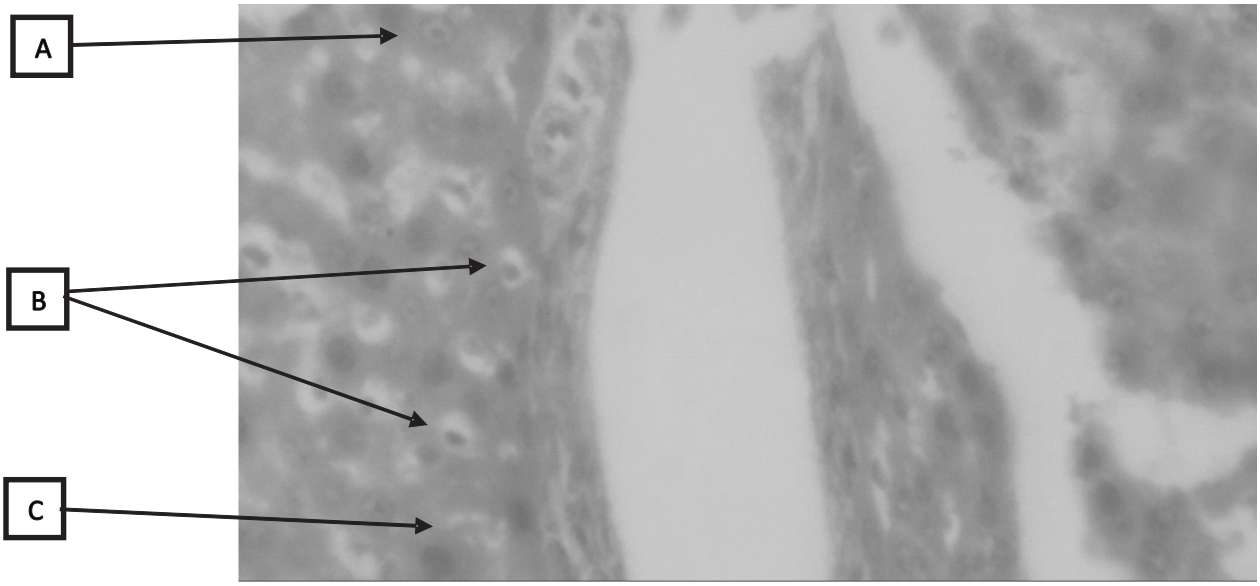

Plate 3 Photomicrograph of liver of rat given 200mg/ml of G. *Kola* for the first week

Key: A: prominent nucleolus, B: kupffer cell activation and C: active sinusoidal congestion (H&E x 400)

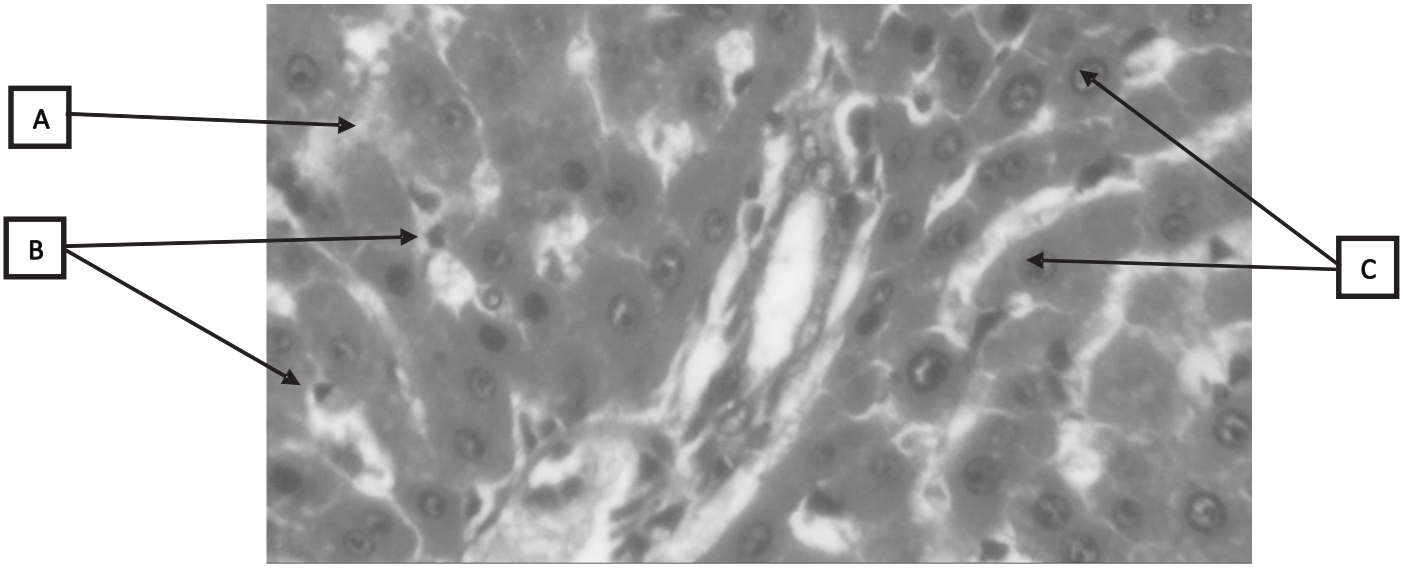

Plate 4 Photomicrograph of liver of rat given 200mg/ml G *Kola* for the 2nd week.

A: active sinusoidal congestion, B: kupffer cell activation and C: a prominent hepatocyte nucleolus (H&E x 400)

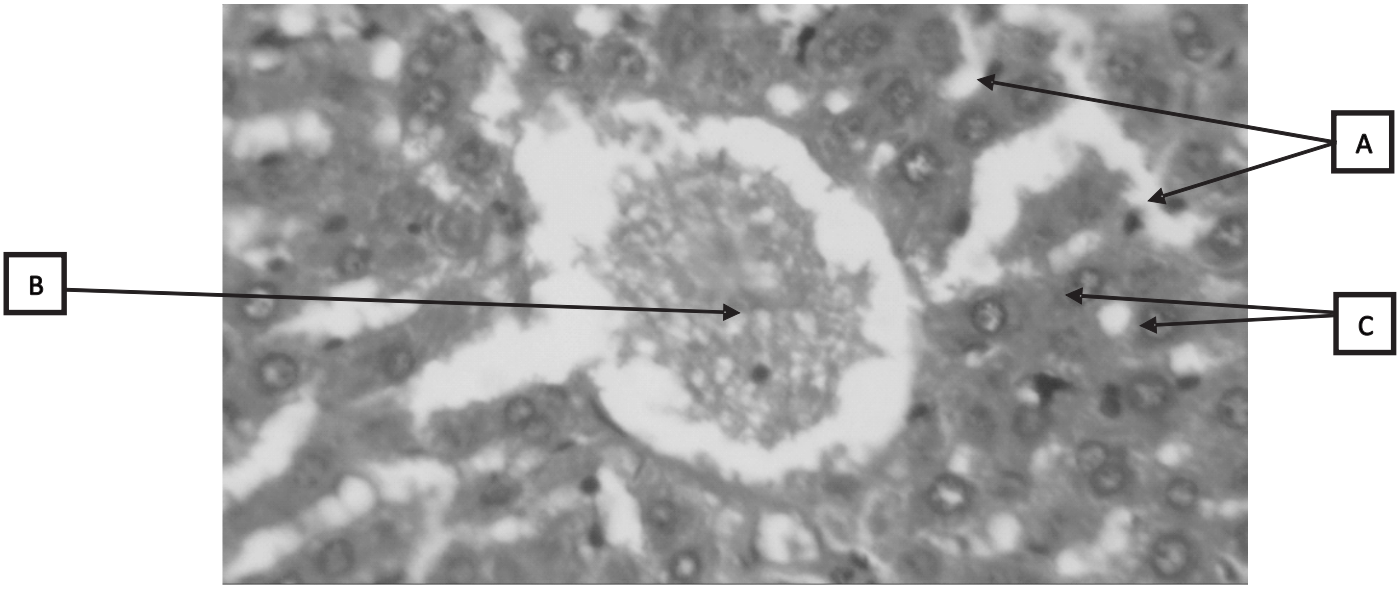

Plate 5 Photomicrograph of liver of rat given 200mg/kg G. *kola* for the 3rd week.

A: mobilization Of kupffer cells, B: active vascular congestion and C: numerous inconspicuous nucleoli (H&E x 400)

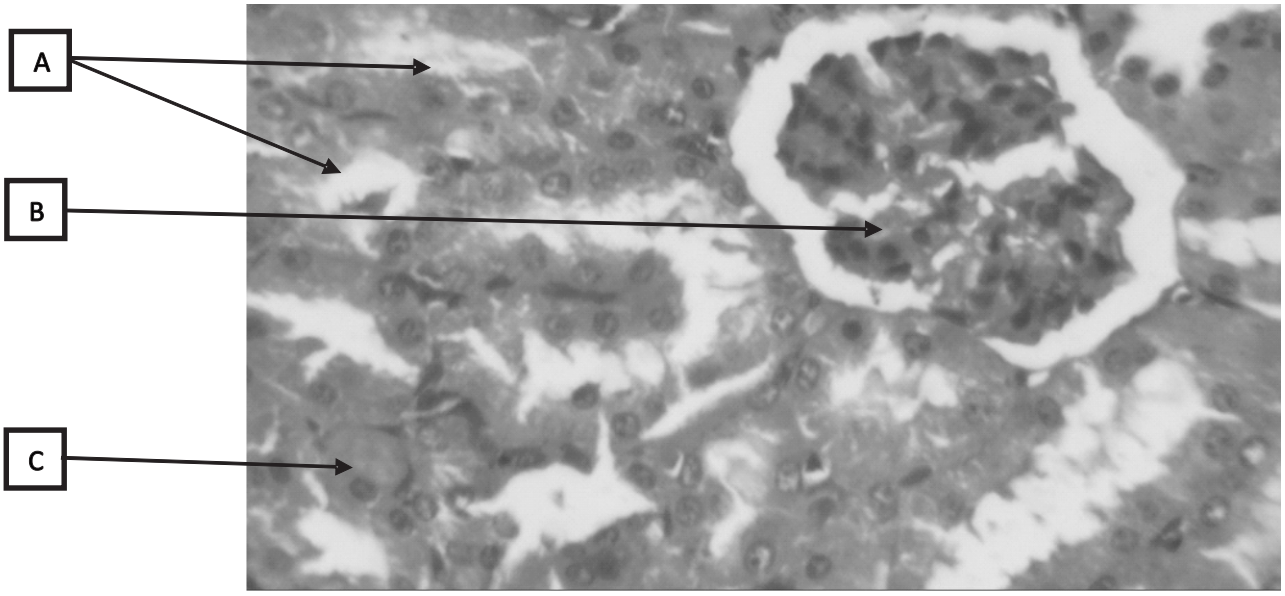

Plate 6 Photomicrograph of control Kidney.

A: tubules, B: glomerulus and C: interstitial space (H&E x 400)

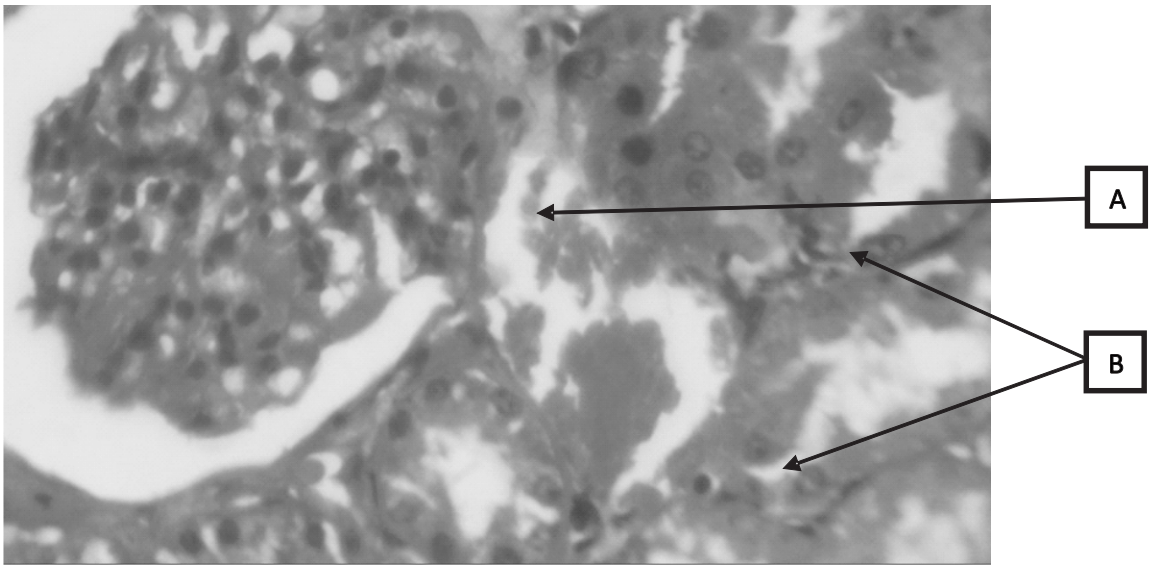

Plate 7 Photomicrograph of kidney of rat given multivitamin for 3 weeks.

A: active interstitial congestion and B: inconspicuous tubular nucleoli (H&E x 400)

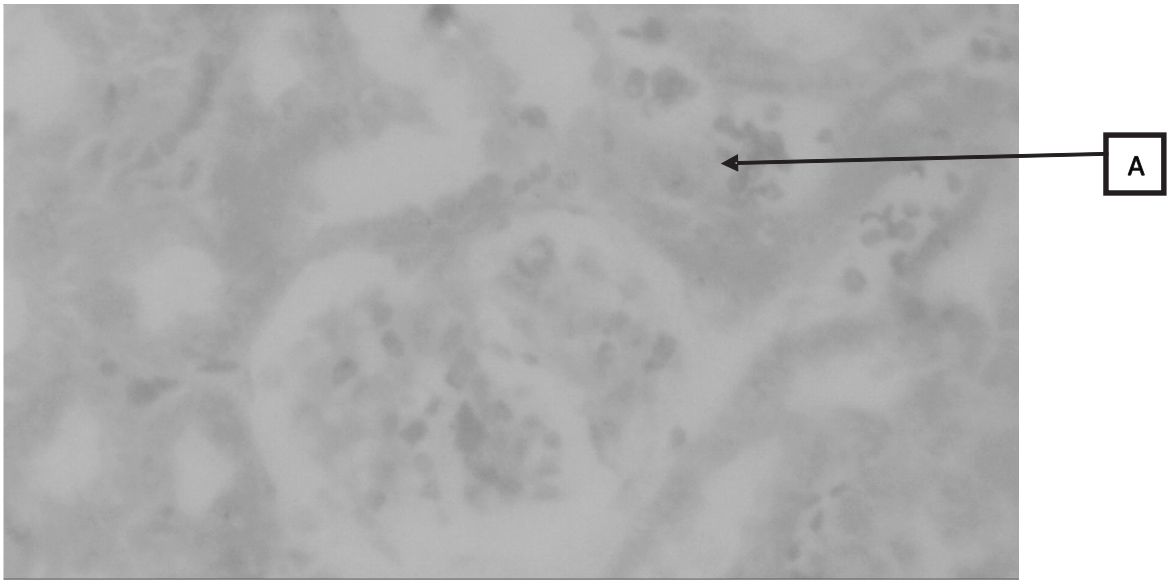

Plate 8 Photomicrograph of kidney of rat given 200mg/ml G*. Kola* for the first week.

A: active interstitial congestion (H&E x 400)

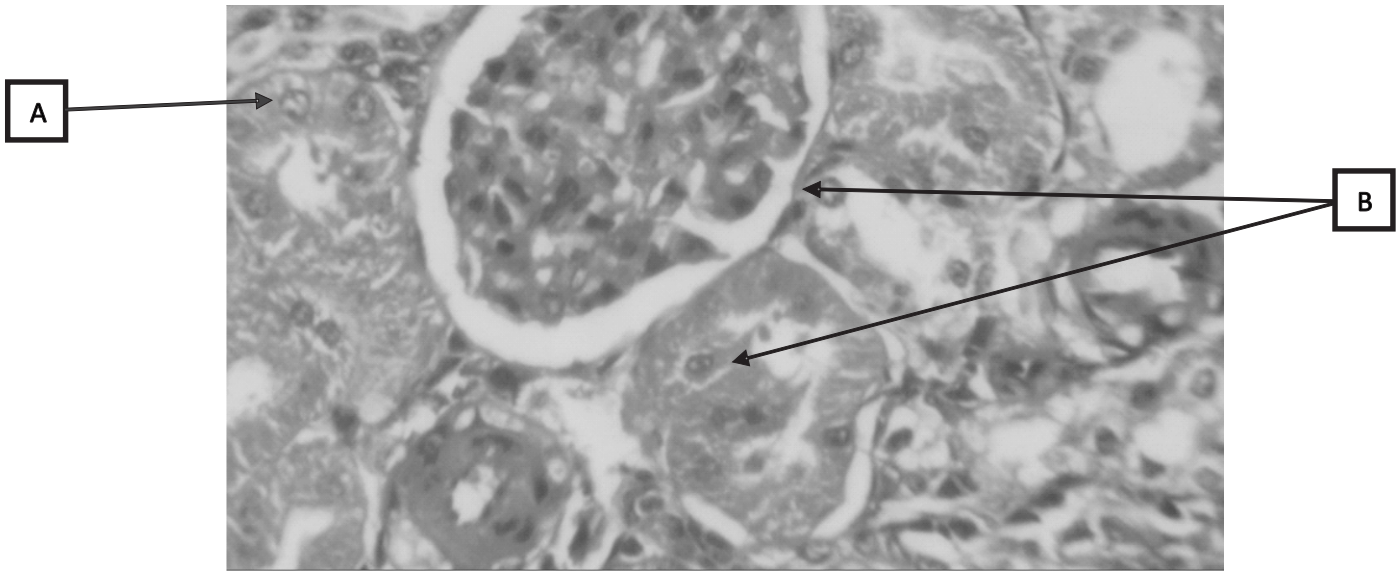

Plate 9 Photomicrograph of kidney of rat given 200mg/ml G. *Kola* second week.

A: inconspicuous tubular nucleoli and B: vesicular nuclei (H&E x 400)

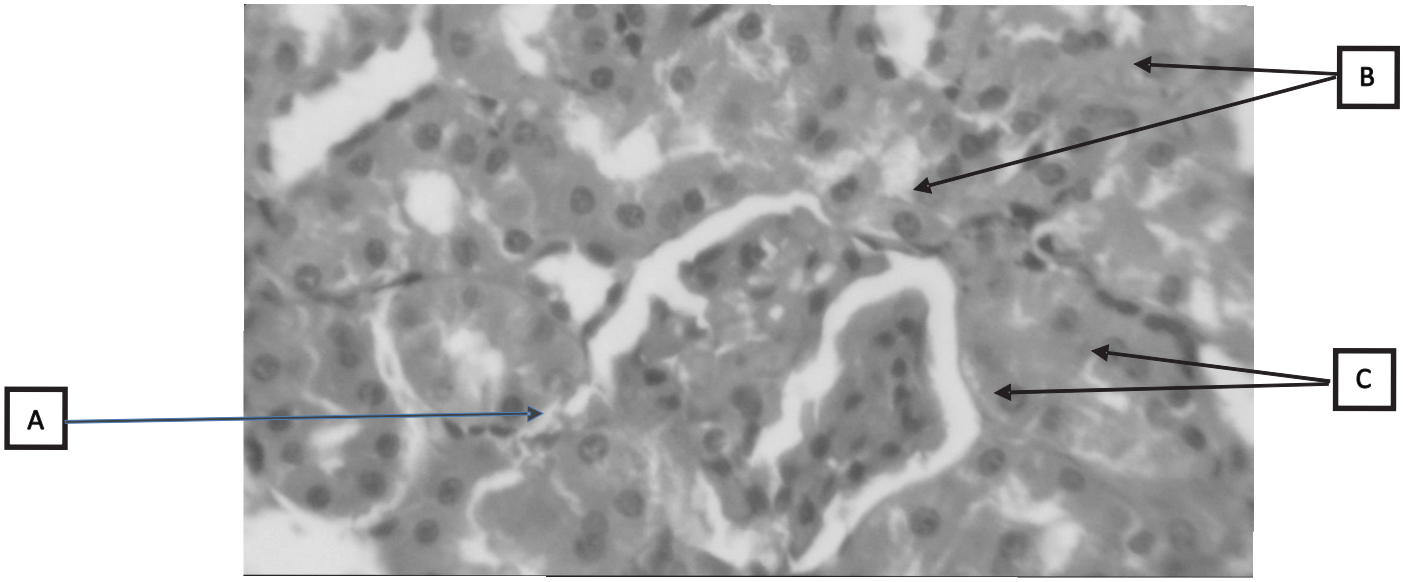

Plate 10 Photomicrograph of kidney of rat given 200mg/ml G. *Kola* third week.

A: active interstitial congestion, B: hyperchromatic nuclei and C: inconspicuous nucleoli (H&E x 400)

DISCUSSION

The investigation of the *Garcinia kola* reveals that phytochemical evaluation shows the presence of alkaloids, tannins, and other compounds, (Tables 1). This is in consonance with earlier studies by other scientists that asserted that there is a relationship between the presence of phytochemicals and antimicrobial activities of medicinal plants. [18]

The antimicrobial inhibition assay suggests that the seed extract demonstrated inhibitory activity against all tested organisms. Gram positive bacteria, Gram negative bacteria as well as fungi were all inhibited at varying zones of inhibition. The nature of the inhibitory action of the plant on the studied organisms were both broad and dose dependent (Table 3). The investigation is in agreement with Iwu,11 who asserted on the antibacterial activity of crude extract of the leaf and stem bark of *G. kola.* There is also, a strong potency relationship between the extract of seed and the standard drugs (ciprofloxacin/fluconazole) (Tables 2a).

On the other hand, the acute toxicity study (LD50) presented the safety dose of >5000mg/ml in laboratory rats. Also, the gravid rats administered with Garcinia kola extractat at 200mg/m/kg body weight strenght from the 1st trimester through 3rd trimester showd increase wight gain whn compared with the control (266.03±6.04, 247.69 ± 3.02), respectively, in comparison with the control group. This result disagrees with some earlier studies [19,20] who reported that there was significant decrease in the body weight of fetus. Also, the crown rump length which serves as indicator for gestational ageing, there was an increase in CRL for group 4 compared to the control. And same was seen in group 3, however, there was no significant increase in the crown rump lengt of the fetuse. The reason for the variation in weight is attributable to enviromental conditions.

Futhermore, the histopathological investigation focused on possible teratogenic effects on the livers and kidneys of all the pups at both second and third trimesters. The study shows that while the control animal took natural water, (plates 1 & 2), a took multivitamins - mimicing normal multivitam suplementation in pregnancy (plates 6 & 7), while the other groups investigated for possible terratogenicity (plates 3, 4, 5 8, 9 & 10) took Garcinia extract.

There were no architectural changes suggestive of abnormalies at all when compared with the control group and the groups that took multivitamin supplementation. This finding is suggestive of preliminary safety of *Garcinia kola* seed in pregnancy and also it is in agreement with an earlier work by some scholars that suggest the safety of natural products in pregnancy. [20]

CONCLUSION

The study indicates that the plant has broad spectra of activities against both bacteria and fungi. The study further upholds the claim of folkclore on the safety of consumption of bitter kola seed in pregnancy. The positive histopathological and terratogenic results further stenghtened the call and trust for herbal remedy in our health care systems. This study has hieghtened the need for continous sceening of the African forests flora for possible drug candidate to combat the menace of drug failure.

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