

Original Article**Safety Evaluation of Aqueous Extract of *Garcinia Kola* Seeds in Male Wistar Rats**Musa Toyin Yakubu*¹, Ayodeji Luqman Quadri¹

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ABSTRACT

Background: *Garcinia kola* seed is consumed indiscriminately in Nigeria without recourse to its potential toxicity. Therefore, this study was aimed at assessing the toxicity of the aqueous extract of *G. kola* seeds on selected tissues of male rats.

Methods: Thirty male rats (215.00 ± 18.58 g) were assigned into four groups: A, B, C and D which received 0.5 ml of distilled water, 25, 50 and 100 mg/kg body weight of the extract respectively, once daily for 7 days. Biochemical indices of organ damage and toxicity were determined using standard methods.

Results: The extract significantly ($P < 0.05$) increased the testes-body weight ratio, activities of testicular alkaline phosphatase (ALP), heart, testes and serum gamma glutamyl transferase (GGT) activity, serum concentrations of uric acid, K⁺, creatinine and PO₄³⁻. The liver-body weight ratio, activities of kidney and serum ALP, liver, heart and serum alanine and aspartate aminotransferases (ALT and AST), serum and testicular acid phosphatase (ACP), concentrations of serum albumin, globulin, urea, Na⁺, HCO₃⁻, conjugated and total bilirubin were reduced. The heart- and kidney-body weight ratios and liver ALP were not significantly ($P > 0.05$) altered.

Conclusion: The treatment related alterations in the present study indicates that the aqueous extract of *G. kola* seeds at the doses of 25, 50 and 100 mg/kg body weight caused functional toxicity to the organs of the animals and thus not safe as an oral remedy.

Keywords: Functional toxicity, *Garcinia kola*, Guttifera, Organ dysfunction, Safety.

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INTRODUCTION

The use of plants for healing purposes is becoming increasingly popular, as it is believed that herbal medicines are beneficial, cheaper, more effective and natural in origin, hence free of side effects when compared with synthetic drugs [1, 2]. The medicinal value of these plants lies in some chemical substances (phytochemicals) that produce definite physiological effects on men and animals. Unfortunately, along with beneficial bioactive agents, plants also produce potentially toxic compounds. In addition, some of these phytochemicals such as alkaloids, saponins, elements etc. are also toxic. Contrary to the belief of a large proportion of the populace that anything natural is safe, many commonly used herbs such as *Bulbine natalensis*, *Echinodorus macrophyllus*, *Leonotis leonurus*, *Crateva adansoni* and *Solanum aculeastrum* can cause

acute and chronic toxic effects [1-5]. Therefore, short- and long-term toxicological investigations are required for evaluation and classification of herbal preparations based on safety data. Despite the widespread use of medicinal plants, few scientific studies are available on their safety and toxicity risks. One plant in which there is a dearth of information on its safety is *Garcinia kola*.

G. kola Heckel (Family - *Guttifera*) commonly called bitter kola, male kola and false kola (English), *Orogbo* (Yoruba), *Cida goro* (Hausa), *Aku ilu* or *Ugugolu* (Igbo), *Efiari* (Efik), and *Igoligo* (Idoma) is an evergreen, unbuttressed, heavily crowned dicotyledonous tree found in tropical forests of Sierra Leone, Angola and Nigeria. It is a medium sized tree, which grows up to about 13-15 m high. The seeds have been claimed to be consumed as stimulant [6], in the management of liver

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disorders and diarrhoea [7, 8], diabetes, bronchitis and throat infections [9, 10]. Although, its seeds have a bitter taste and possess some hepatoprotective and anti-atherogenic activities [11-13], the acclaimed aphrodisiac potential has been refuted with scientific evidence [14]. The seed extract contained saponins, flavonoids, steroids, cardiac glycosides, cardenolides and dienyloides [14]. It is also known to contain high concentrations of bioflavonoid compounds [15]. Its remarkable bioactivity has been ascribed principally to the presence of these antioxidant flavonoids [13, 16]. Although, there have been several reports on the pharmacological activities of various parts of *G. kola*, information is scanty on the safety or toxicity of the plant in male rats. Therefore, this study was carried out to investigate the safety of the aqueous extract of the seeds by determining certain biochemical parameters of toxicity and damage to the cellular systems of male rats.

MATERIALS AND METHODS

Plant Material and Authentication

G. kola seeds were obtained from a market (Agor Market) in Ilorin, Nigeria and were authenticated at the Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria. A voucher specimen (FHI 10847) was deposited at the herbarium of the Institute.

Preparation of Aqueous Extract of G. kola Seeds

The seeds were peeled, cut into pieces and oven-dried at 40 °C to a constant weight using Uniscope SM9053 Laboratory Oven, (Surgifriend Medicals, Essex, England). The dried pieces were pulverized with an electric blender (Crownstar Blender CS- 242B, Trident (H.K) Ltd, China. Three hundred grams of the powder were extracted in 1000 ml of distilled water for 48 h at room temperature. This was then filtered using Whatman No. 1 filter paper. The filtrate was concentrated on a steam bath to give 16.42 g of brown slurry residue, which corresponded to 5.47% of the starting material. This residue was reconstituted in distilled water to give the required doses of 25, 50 and 100 mg/kg body weight used in this study. The doses were as used in the previous aphrodisiac study [9].

Assay Kits

The assay kits for creatinine, urea, uric-acid, sodium, potassium, phosphorus, albumin, bilirubin, ACP, ALP, GGT, ALT and AST were obtained from Randox Laboratories Limited, County Antrim, United Kingdom. All other reagents used were of analytical grade obtained from BDH Chemicals Ltd, Poole, England.

Experimental Animals

Thirty male Wistar rats with an average weight of 215.00 ± 18.58 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean aluminium cages placed in well-ventilated conditions (temperature 23 ± 2°C; photoperiod: 12 h natural light and 12 h dark; humidity: 45-50%) and were allowed free access to rat pellets (Bendel Feeds and Flour Mill Ltd., Ewu, Nigeria) and tap water throughout the experimental period.

Ethical Consideration

The animals were handled according to the guidelines on the Care and Use of experimental animals of the Ethical Committee of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria and the guidelines of the National Institute for Health, USA [17].

Animal Grouping and Extract Administration

The animals were assigned into four groups (A, B, C and D) of six rats each. Animals in group A (control) received orally 0.5 ml of distilled water, while those in groups B, C and D were treated like the control except that they received the same volume corresponding to 25, 50 and 100 mg/kg body weight of the extract. The distilled water and extract were administered once daily for 7 days.

Preparation of Serum and Tissue Homogenates

The procedure described by Yakubu and Akanji [18] was used for the preparation of serum and tissue homogenates. Exactly 24 hours after the 7 days treatment period, the animals were weighed separately and anaesthetized in a jar containing cotton wool soaked in diethyl ether fume. When the animals became

unconscious, the neck areas were quickly cleared of fur and skin to expose the jugular veins. The jugular veins were slightly displaced from the neck region (to prevent contamination of the blood with interstitial fluid) and then cut with a sharp sterile blade. The rats were held head down and were allowed to bleed into a clean, dry centrifuge tube. The blood samples were left for 10 min at room temperature to clot. The tubes containing the blood samples were centrifuged at $503\text{ g} \times 10\text{ min}$ using Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, England). The resulting sera were aspirated into clean, dry, sample bottles using a Pasteur pipette.

The rats were thereafter quickly dissected and the organs (liver, kidney, heart and testes) were transferred into ice-cold 0.25 M sucrose solution. The organs were cleaned of fatty attachments, blotted with clean blotting paper, weighed, cut with clean sterile blades and homogenized in ice-cold 0.25 M Sucrose solution (1:5w/v). The homogenates were kept frozen overnight to ensure maximum release of the cellular enzymes of the organs before being used for various biochemical assays.

Determination of Biochemical Parameters

The determination of the levels of biomolecules were carried out using standard methods as described for protein, sodium, potassium, chloride, inorganic phosphorus, globulin, uric acid, urea, creatinine, total and conjugated bilirubin, albumin, activities of ALP, ACP, GGT, ALT and AST [19-28]. The formula described by Yakubu et al. [29] was used to compute the organ-body weight ratio of the animals.

Statistical Analysis

The data were expressed as mean \pm SEM of six replicates and were analyzed for statistical

significance using Duncan Multiple Range Test and Tukey's post-hoc. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The extract significantly ($P < 0.05$) increased the testes-body weight ratio of the animals whereas the liver-body weight ratio was reduced. In contrast, the kidney- and heart-body weight ratios were not significantly ($P > 0.05$) altered (Table 1).

All the doses of the extract increased the testicular ALP activity whereas the enzyme activity in the kidney and serum decreased significantly. The liver ALP activity was not significantly altered (Table 2).

The GGT activity in the testes, heart and serum of the animals increased significantly whereas there was no generalized pattern of effect on the liver enzyme. For instance, the liver GGT activity at 25 and 50 mg/kg body weight doses of the extract decreased significantly whereas the 100 mg/kg body weight dose increased the activity of the enzyme by 1.65 fold over that of the control value (Table 3). The doses of the extract significantly decreased the activities of both ALT and AST in the liver, heart and serum of the animals (Table 4). This pattern of decrease was also extended to testicular and serum ACP activities of the animals (Table 5). By the end of the exposure period, those treated with 100 mg/kg body weight of the extract had 63.25% reduction in testicular ACP activity and 40.58% reduction in serum ACP (Table 5).

All the doses of the extract reduced the serum concentrations of albumin, globulin, Na^+ , HCO_3^- , total and conjugated bilirubin, whereas serum uric acid, urea, creatinine, K^+ and PO_4^{3-} increased (Table 6).

Table 1. Effects of Aqueous Extract of *G. kola* Seeds on Some Organ-body Weight Ratio of Male Rats.

Organ-body weight ratio (%)	Extract (mg/kg body weight)			
	Control	25	50	100
Kidney-body weight ratio	0.60 \pm 0.06 ^a	0.60 \pm 0.06 ^a	0.59 \pm 0.03 ^a	0.60 \pm 0.04 ^a
Liver-body weight ratio	3.14 \pm 0.16 ^a	2.73 \pm 0.11 ^b	2.49 \pm 0.12 ^c	2.49 \pm 0.10 ^c
Heart-body weight ratio	0.26 \pm 0.01 ^a	0.28 \pm 0.03 ^a	0.28 \pm 0.03 ^a	0.28 \pm 0.01 ^a
Testes-body weight ratio	0.97 \pm 0.04 ^a	1.29 \pm 0.10 ^b	1.25 \pm 0.02 ^b	1.25 \pm 0.10 ^b

n=6 \pm SEM;

Values with superscripts, b and c, different from that of the control, a, for each organ-body weight ratio are significantly different ($P < 0.05$)

Table 2. Alkaline phosphatase activity of selected tissues and serum of male rats administered aqueous extract of *G. kola* seeds.

Doses (mg/kg body weight)	Alkaline Phosphatase Activity (nmol/min/mg protein)			
	Liver	Kidney	Testes	Serum
Control	4.63±0.18 ^a	56.00±4.57 ^a	14.86±0.32 ^a	2.93±0.13 ^a
25	4.73±0.33 ^a	16.05±0.53 ^b	23.96±1.57 ^b	1.73±0.22 ^b
50	4.87±0.41 ^a	34.68±2.16 ^c	29.00±1.00 ^c	1.99±0.16 ^b
100	4.79±0.60 ^a	36.92±1.66 ^d	23.78±0.51 ^b	1.52±0.16 ^b

n=6 ± SEM;

Values with superscripts, b, c and d different from that of the control, a, for each organ are significantly different ($P<0.05$)**Table 3.** Gamma glutamyl transferase activity of selected tissues and serum of male rats administered aqueous extract of *G. kola* seeds.

Doses (mg/kg body weight)	Gamma Glutamyl Transferase Activity (U/L)			
	Liver	Heart	Testes	Serum
Control	155.50±5.60 ^a	16.00±2.38 ^a	11.00±1.10 ^a	9.00±1.86 ^a
25	72.30±0.33 ^b	28.50±2.55 ^b	17.00±2.48 ^b	20.00±2.38 ^b
50	108.50±3.31 ^c	28.50±1.83 ^c	17.00±1.05 ^b	16.50±1.83 ^c
100	255.50±3.02 ^d	26.50±1.64 ^b	19.00±0.79 ^b	18.00±0.49 ^b

n=6 ± SEM;

Values with superscripts, b, c and d different from that of the control, a, for each organ are significantly different ($P<0.05$)**Table 4.** Alanine aminotransferase and aspartate aminotransferase activities of selected tissues and serum of male rats administered aqueous extract of *G. kola* seeds.

Doses (mg/kg body weight)	Enzyme activities (U/L)					
	Alanine Aminotransferase			Aspartate Aminotransferase		
	Liver	Heart	Serum	Liver	Heart	Serum
Control	496.00±7.10 ^a	96.00±4.38 ^a	19.50±2.74 ^a	370.00±5.95 ^a	120.00±7.89 ^a	67.00±3.29 ^a
25	390.00±5.48 ^b	75.00±5.00 ^b	7.00±1.10 ^b	145.00±5.48 ^b	105.00±2.50 ^b	42.50±6.02 ^b
50	120.50±4.78 ^c	62.50±8.22 ^b	14.50±2.55 ^c	267.50±8.22 ^c	75.00±5.48 ^c	59.50±4.93 ^c
100	250.00±5.91 ^d	57.50±2.74 ^c	17.50±2.74 ^d	100.00±6.00 ^d	90.00±3.95 ^d	48.67±3.08 ^b

n=6 ± SEM;

Values with superscripts, b, c and d different from that of the control, a, for each organ are significantly different ($P<0.05$)**Table 5.** Testicular and serum acid phosphatase activity of male rats administered aqueous extract of *Garcinia kola* seeds.

Doses (mg/kg body weight)	Enzyme activity (nmol/min/mg protein)	
	Testes	Serum
Control	34.88±1.92 ^a	4.14±0.17 ^a
25	23.13±1.31 ^b	1.02±0.10 ^b
50	25.07±1.74 ^b	3.36±0.21 ^c
100	12.82±1.54 ^a	2.46±0.74 ^d

n=6 ± SEM;

Values with superscripts, b, c and d different from that of the control, a, for each organ are significantly different ($P<0.05$)

Table 6. Liver and Kidney function indices of male rats administered aqueous extract of *G. kola* Seeds.

Serum Parameters	Doses (mg/kg body weight)			
	Control	25	50	100
Albumin (g/L)	20.33±0.40 ^a	17.00±0.10 ^b	15.00±0.10 ^c	16.50±0.55 ^c
Globulin (g/L)	24.00±0.55 ^a	19.33±0.25 ^b	20.50±0.27 ^b	15.00±0.79 ^c
Total bilirubin (g/L)	1.75±0.05 ^a	1.40±0.11 ^b	0.95±0.05 ^c	0.72±0.11 ^d
Conjugated bilirubin (g/L)	0.25±0.01 ^a	0.13±0.00 ^b	0.20±0.00 ^c	0.22±0.01 ^d
Urea (g/L)	2.00±0.05 ^a	1.47±0.09 ^b	1.95±0.06 ^a	1.80±0.04 ^c
Uric acid (g/L)	0.012±0.002 ^a	0.016±0.001 ^b	0.017±0.002 ^b	0.017±0.001 ^b
Creatinine (g/L)	23.00±0.38 ^a	31.00±0.48 ^b	34.00±0.20 ^c	42.00±0.30 ^d
Na ⁺ (mmol/L)	64.00±1.10 ^a	59.00±1.10 ^b	53.50±1.64 ^c	54.00±1.10 ^c
K ⁺ (mmol/L)	11.00±0.90 ^a	17.00±0.90 ^b	18.50±0.55 ^b	17.50±0.20 ^b
HCO ₃ ⁻ (mmol/L)	11.00±0.89 ^a	8.50±0.55 ^b	9.50±0.55 ^b	8.50±0.55 ^b
PO ₄ ³⁻ (mmol/L)	0.56±0.01 ^a	0.61±0.02 ^b	0.64±0.02 ^c	0.79±0.01 ^d

n=6 ± SEM; Values with superscripts, b, c and d different from that of the control, a, for each parameter are significantly different ($P<0.05$)

DISCUSSION

For several centuries, medicinal plants and herbal remedies have continued to enrich the health care needs of animals and humans. However, medicinal plants, in spite of being popularly claimed as naturally safe need to be authenticated by scientifically validated tests for toxicological properties before being introduced for widespread pharmacological use [30].

Organ-body weight ratio is an indication of organ swelling, atrophy or hypertrophy [31]. The increase in the testes-body weight ratio of the animals by the extract of *G. kola* seeds suggests cellular hypertrophy of the organ of the animals. It is also an indication that the increase in the absolute weight of the testes was not commensurate with total growth rate of the animals. This was reinforced by the increase in testicular enzymes (ALP and GGT) investigated in the present study. In contrast, the reduction in the liver-body weight ratio of the animals might be attributed to liver atrophy as evident by decrease in the secretory and synthetic constituents of the liver in the present study. It is however not clear why the animals did not manifest any significant alteration in the kidney- and heart-body weight ratio despite the disturbance in the milieu of the cells of these organs. The presence of alkaloids in *G. kola* seeds might have been responsible for the reduced liver-body weight ratio [32].

A number of tissue enzymes are valuable tools as diagnostic agents. These enzymes have gained wide acceptance as 'markers' of organ damage, cellular damage, activation or inhibition

processes and are still being employed in many studies. Testicular ALP and GGT have been reported to play important roles in the normal physiology of the testes in animals. For instance, while ALP have been implicated to play a role in the intercellular and intracellular transportation of metabolites during steroidogenesis and in the secretion of gonadotropins, GGT is a useful 'marker' of Sertoli cell maturation where it carries spermatozoa into the testicular tubules [33, 34]. The increase in ALP activity of the testes of the animals by *G. kola* seeds may suggest enhanced steroidogenesis and mobilization of carbohydrate and lipid metabolites needed by the spermatozoa in the seminal fluid while the elevated GGT might enhance the function of the Sertoli cells. All these will be clinically beneficial to the animals. The absence of an effect on ALP activity in the liver of animals treated with *G. kola* seeds suggested that the plasma membrane was not compromised. The reduction in the activity of ALP in both the kidneys and sera of the animals is an indication of *in situ* inactivation of the enzyme molecules. Furthermore, the differences in the pattern of effects on the organs of animals by *G. kola* seeds in the present study may be due to differences in the drug-metabolizing enzymes of the tissues.

The increased activity of GGT in the hearts of the animals suggests an increase in functional activity of the organ, which results in *de novo* synthesis of the enzyme. This increase, notwithstanding, was not sufficient to manifest a corresponding increase in the heart-body weight ratio. Similarly, the increase in serum GGT

could have been due to contributions from other organs not investigated in the present study.

Aminotransferases (ALT and AST) are useful indicators of liver cytolysis. The reduction in the aminotransferases of the liver, heart and serum could be attributed to suppression of further synthesis of these enzymes. This may also explain the similar trend of reduction in the testicular and serum ACP of the animals. All these would adversely affect the normal functioning capacity of the organs and therefore harm the animals. Alkaloid fraction from *Cnestis ferruginea* reduced the activity of liver ALT and AST [32]. Therefore, the reduction in the activity of the aminotransferases in the liver and heart could be due to the alkaloidal content of *G. kola* seeds.

Albumin and globulin are useful indicators of synthetic function of the liver while bilirubin can be used to assess the excretory function of this organ. The reduction in the levels of both albumin and bilirubin could be attributed to reduced albumin and bilirubin synthesis, which might suggest hepatocellular injury [35]. The diminished/impaired function of the liver by *G. kola* seeds in the present study is also corroborated by the reduced levels of total and conjugated bilirubin. Furthermore, the reduced Na^+ and HCO_3^- suggest decreased renal tubular reabsorption [36]. Toxicity of the aqueous extract of *G. kola* seeds on the kidney was further evidenced by the elevations in the levels of serum uric acid and urea which suggest dysfunction at the level of the glomerulus affecting glomerular filtration whereas the raised level of K^+ and PO_4^{2-} indicated tubular damage since the ions are normally reabsorbed at the distal tubules of the kidney. Again, the increase in the levels of creatinine is a consequence of abnormality in the normal function of the kidney at the glomerulus.

CONCLUSION

The treatment related alterations in the biochemical indices in this study suggested that *G. kola* seed extract could hamper the normal function of some organs and thus toxic to the liver, kidney, heart and testes of the animals. Therefore, the aqueous extract of *G. kola* seed at doses of 25, 50 and 100 mg/kg body weight would cause functional toxicity to the animals, and thus not safe when used as an oral remedy.

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