JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

COMPARATIVE EVALUATION OF ENDOCRINE, LIPID, AND HEPATIC EFFECTS OF THREE KOLA SPECIES IN ADULT MALE RABBITS

By

Merenini, I. F.[1], Okafor, I. C.[2], Olopha, O. O.[3], Dooga, M. Y.[4], Okorocha, J. C.[5], Bakare, O. S.[6], Ekpo, N. G.[7], Leonard, A. E.[8], Erighanyoyefa, F. A.[9], Inim, S. U.[10], Ekanem I. B.[11], Essiet, A. G.[12], Ibokette, E. O.[13], Otukwude, F. O.[14] and Emeana, C. A.[15]

[1]Department of Microbiology, Faculty of Life Sciences, Federal University of Lafia, Nasarawa State, Nigeria.

[2] Department of Histopathology, University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria.

[3]Nigeria Centre for Disease Control and Prevention, Jabi, FCT Abuja, Nigeria.

[4]Department of Statistics Department, University of Calabar, Cross River State, Nigeria.

[5]Department of Basic Science, Claretian University Nigeria, Imo State, Nigeria.

[6]Department of Biochemistry, Adekunle Ajasin University, Ondo State, Nigeria

[7]Department of Health Policy and Management, University of Ibadan, Oyo State, Nigeria.

[8]Department of Health System Management, University of Port-Harcourt, Rivers State, Nigeria.

[9]Department of Physiology, Bayelsa Medical University, Bayelsa State, Nigeria.

[10]Field Epidemiology, University of Ibadan, Oyo State, Nigeria.

[11]Department of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.

[12]Department of Animal Science and Public Health, Federal College of Medical Laboratory Science and Technology, Plateau State, Nigeria.

[13]Department of Animal and Environmental Biology, University of Uyo, Akwa Ibom State, Nigeria.

[14] Organic Chemistry Institution, Abia State University, Abia State, Nigeria.

15 Department of Animal and Environmental Biology, University of Port Harcourt, Rivers State, Nigeria

Corresponding Author: MIF; mereniniifeanyi@gmail.com

DOI: https://doi.org/10.5281/zenodo.19116470

ABSTRACT

Kola nuts and bitter kola are widely consumed in Nigeria for their nutritional and ethnomedicinal benefits; however, concerns exist regarding their long-term safety. This study compared the effects of three different kola species seed diets on endocrine, lipid, and hepatic biomarkers in male rabbits. Fifty (50) male rabbits (130 ± 14 g) were randomly assigned into ten experimental groups

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

of five rabbits each consisting of five (5); one control and nine treatment groups that received 5%, 10%, and 20% dietary inclusion of the test substance for 12 weeks. Serum testosterone, LH, FSH, ALT, AST, ALP, TC, LDL-c, and HDL-c were analyzed using standard enzymatic and immunoassay methods. Results showed that *C. acuminata* and *G. kola* significantly increased testosterone levels, with peak values at 5% (4.00 ± 0.02 ng/mL) and 10% (5.94 ± 0.28 ng/mL), respectively. LH and FSH were significantly reduced across all treated groups. TC levels increased significantly in all treatments, with the highest value observed in *G. kola* 20% (55.30 ± 1.85 mg/dL). LDL-c increased notably at higher inclusion levels, whereas HDL-c showed minimal variation. Liver enzymes (ALT, AST, ALP) were elevated, particularly in *C. acuminata* 20% (ALT: 48.30 ± 1.61 IU/L), indicating hepatocellular stress. Food intake declined significantly in all treated groups, with the greatest reduction at 10% inclusion (12.03 ± 0.12 g), and body weight gain was markedly reduced, especially at 20% inclusion (6.30 ± 4.20 g). Chronic dietary intake of *C. acuminata*, *C. nitida*, and *G. kola* seeds alters endocrine function, disrupts lipid homeostasis, induces hepatic stress, and suppresses growth performance in a dose-dependent manner. These findings highlight potential risks associated with prolonged high-dose consumption and underscore the need for moderated intake and further toxicological evaluation.

Keywords: Kola seeds, Endocrine, Lipid, Hepatic, and Rabbits

1 INTRODUCTION

Kola nuts obtained from *Cola acuminata* and *Cola nitida*, together with bitter kola from *Garcinia kola*, are widely consumed across West Africa, particularly in Nigeria, where they possess significant nutritional, cultural, and ethnomedicinal significance (Jacob *et al.*, 2023). Traditionally, the seeds are chewed for their stimulant properties and incorporated into social ceremonies and herbal remedies for the management of fatigue, gastrointestinal disorders, infections, and inflammatory conditions (Oluwaseun and Adebayo, 2019). Phytochemical analyses reveal that Cola species are rich in methylxanthines such as caffeine (Correa *et al.*, 2018) and theobromine, as well as tannins and flavonoids, while *Garcinia kola* contains bioactive biflavonoids including kolaviron, which has demonstrated antioxidant, anti-inflammatory, and hepatoprotective activities (Alada and Adeyemo, 2022; Tauchen *et al.*, 2023). These bioactive constituents underpin their widespread use and perceived therapeutic benefits.

Despite these documented advantages, increasing scientific evidence suggests that prolonged or high-dose consumption of phytochemical-rich seeds may exert systemic physiological effects. Chronic intake of caffeine-containing botanicals has been associated with alterations in metabolic regulation, appetite, and body weight (Rauf *et al.*, 2025). Furthermore, studies have reported changes in the hepatic enzyme activities, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), following prolonged exposure to certain plant-derived compounds, indicating a possible hepatocellular stress (Giannini *et al.*,

2005).In addition, phytochemicals present in kola seeds may influence lipid metabolism, with experimental studies in rodents demonstrating that seed extracts can modulate serum lipid concentrations, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Omeh *et al.*, 2014; Effanga *et al.,* 2026). However, these findings remain inconsistent, and data on whole-seed dietary supplementation under chronic conditions are limited.

Beyond metabolic considerations, the potential influence of kola seed consumption on reproductive endocrine function warrants critical investigation (Okoli *et al.,* 2022). Testosterone production by the Leydig cells of the endocrine and reproductive system, is regulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) via the hypothalamic-pituitary-gonadal (HPG) axis (Lei *et al*., 2025). Disruption of this hormonal axis may impair reproductive efficiency. Previous animal studies suggest that caffeine-rich or polyphenol-dense plant extracts may alter serum testosterone, LH, and FSH levels, indicating potential endocrine effects (Herman *et al*., 2024; Glover *et al*., 2022).

Nevertheless, comprehensive comparative studies that evaluate these endocrine parameters following chronic dietary inclusion of whole kola seeds remain scarce.

Rabbits are widely used in nutritional and toxicological research owing to their physiological responsiveness and suitability for evaluating metabolic and endocrine outcomes (OECD, 2018). Parameters such as lipid profile indices, liver enzyme activities, and reproductive hormones are well-established biomarkers for assessing systemic and organ-specific effects of dietary interventions. However, detailed dose-dependent comparative investigations examining the combined effects of Cola acuminata, *Cola nitida*, and *Garcinia kola* seed diets on testicular and gonadotropic hormones (LH and FSH), lipid metabolism, and liver function in rabbits remained underexplored.

Although kola nuts and bitter kola are widely consumed for perceived health benefits, there remains a paucity of empirical data on their long-term, dose-dependent physiological effects (Agbebaku *et al.*, 2018; Icheku *et al.*, 2019). Most existing studies focus on acute toxicity assessments or isolated extracts rather than chronic dietary exposure to whole seeds (Ozoffor *et al*., 2024). Moreover, comparative evaluations among the three commonly consumed species are limited, creating uncertainty regarding their relative safety profiles and endocrine and metabolic impacts.

The existing gaps limit evidence-based recommendations on safe consumption levels and potential health risks associated with prolonged intake. By simultaneously evaluating the reproductive hormones (testosterone, LH, and FSH), lipid profile parameters, and liver function biomarkers under controlled dietary conditions (Babu *et al.*, 2004; McGill, 2022), the study will define dose-response relationships and clarify potential endocrine and metabolic consequences of chronic kola seed consumption (Gore *et al.,* 2015). Further findings will contribute to public health awareness, provide useful information that supports regulatory decision-making

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

regarding herbal product safety, and expand scientific knowledge on the physiological effects of widely consumed African medicinal plants. Therefore, the research study aimed to compare the dose-dependent effects of dietary inclusion of *Cola acuminata*, *Cola nitida*, and *Garcinia kola* seeds on reproductive hormones (testosterone, LH, and FSH), lipid profile parameters, and liver function biomarkers in adult male rabbits.

2 MATERIALS AND METHODS

2.1 Animal collection and acclimatization

A total of fifty (50) healthy male rabbits, weighing 120-150 g (average 130 ± 14 g), were obtained from the animal house of the Department of Biochemistry, Faculty of Life Sciences, Federal University of Lafia, Nasarawa, Nigeria. Upon arrival, animals were individually housed in metabolic cages within a ventilated room. This individual housing allowed for accurate monitoring of food intake, feed conversion, and excreta, while minimizing competition and stress. The animal facility was maintained under controlled environmental conditions, including a temperature of 27 ± 2°C, relative humidity of 50-60%, and a 12-hour light/dark cycle. Rabbits underwent a two-week acclimatization period to ensure physiological stability and adaptation to the laboratory environment. During this period, the animals were monitored daily for signs of illness or abnormal behavior and had free access to standard rodent chow and water ad libitum. All experimental procedures were conducted in strict compliance with the guidelines and approval of the Institutional Animal Ethics Committee.

2.2 Plant material collection and preparation

Kola nut seeds (*Cola nitida* and *Cola acuminata*) and bitter kola (*Garcinia kola*) were purchased from a market in Lafia, Nasarawa State, Nigeria. A Botanist in the Faculty of Life Sciences, Federal University of Lafia, Nasarawa State, Nigeria, identified and validated the seeds. The seeds were peeled, washed, cut into smaller sizes, dried at 60 °C for 12 hours, and ground using a manual blender.

2.3 Diet formulation and feeding of experimental design

The fifty (50) rabbits were randomly assigned to ten experimental groups, with five rabbits per group. One group served as a control, receiving only standard rodent chow. The remaining nine groups comprised three treatment groups for each of the test substances: *Cola acuminata*, *Cola nitida*, and *Garcinia kola*. Each treatment group was fed graded levels of the respective test substances, incorporated into standard rodent chow at proportions of 5%, 10%, and 20%. For diet preparation, the appropriate weight of the seeds for each concentration was thoroughly mixed with a specified amount of rodent chow. A small quantity of table water was used to homogenize the mixture, which was then pelleted using a syringe and oven-dried at 60 °C. The feeding trial lasted for 12 weeks, with rabbits fed according to the schedule presented in Table 1. Daily diet

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

intake was determined by carefully collecting and weighing leftover and spilled feed, calculating the difference between the initial diet supplied and the leftover. Rabbits' body weights were measured regularly with the aid of a digital electronic balance throughout the experimental period.

Table 1: Distribution of rabbits into experimental groups

Group	Number of Rabbits	Feeding Schedule
1 (Control)	5	Rodent chow only (Control)
2 (KA Test 1)	5	*Cola acuminata* (5%) + Rodent chow (95%)
3 (KA Test 2)	5	*Cola acuminata* (10%) + Rodent chow (90%)
4 (KA Test 3)	5	*Cola acuminata* (20%) + Rodent chow (80%)
5 (KN Test 1)	5	*Cola nitida* (5%) + Rodent chow (95%)
6 (KN Test 2)	5	*Cola nitida* (10%) + Rodent chow (90%)
7 (KN Test 3)	5	*Cola nitida* (20%) + Rodent chow (80%)
8 (GK Test 1)	5	*Garcinia kola* (5%) + Rodent chow (95%)
9 (GK Test 2)	5	*Garcinia kola* (10%) + Rodent chow (90%)
10 (GK Test 3)	5	*Garcinia kola* (20%) + Rodent chow (80%)

2.4 Sample collection

Rabbits were euthanized by intravenous administration of sodium pentobarbital (100mg/kg) following approved guidelines (NRC (US) Committee on Pain and Distress in Laboratory Animals, 1992). Following euthanasia, each rabbit was placed on the dissecting board, sacrificed, and the thoracic cavity exposed via a midline incision, carefully dissected to expose the heart region. Blood samples were collected via cardiac puncture using a 5mL hypodermic syringe and needle, and each blood sample collected was discharged equally into sterile coagulant-free bottles. The blood samples were allowed to clot at room temperature for 30 minutes, and then centrifuged at 2500rpm for 10 mins to separate the serum. Dry sample containers were used to collect serum and stored frozen at -20 °C until biochemical analysis. All procedures were conducted in accordance with institutional animal ethics guidelines.

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

2.5 Serum hormonal assays

2.5.1 Quantitative estimation of serum testosterone using ELISA in microwells

The method employed was microwell immunoassay (ELISA) using analytical grade reagents (Lashansky, 1991). 25µL of standard testosterone was dispensed into appropriate wells (i.e., respective wells for progesterone and testosterone). 50µL of testosterone enzyme reagents was added to all the respective wells. Each of these mixtures was swirled for 20 seconds. 50µL each of testosterone biotin working reagent was added to all the respective wells. The mixtures were swirled for 20 seconds and allowed to incubate for 60 minutes. The contents of the microplate were discarded and the plate blotted dry with absorbent paper 350µL of wash buffer solution was added and decanted. This procedure was repeated two times. 100µL of substrate solution was added to all the respective wells and allowed to incubate for 20 minutes. 50µL of stop solution was added to each well and swirled gently, and the absorbance was read at 450nm in a microplate reader. A dose-response curve was used to ascertain the concentration of testosterone in the serum.

2.5.2 Quantitative estimation of serum follicle-stimulating hormone (FSH) using ELISA in microwells

The method employed was microwell immunoassay (ELISA) using analytical grade reagents (Engvall and Perlmann, 1971). The desired number of coated wells was secured in the holder. 50µL of the standard specimens were dispensed into appropriate wells. Also, 100µL of enzyme conjugate was dispensed into the well. After dispensing the mix, it was stirred for 30 seconds. This solution was incubated at room temperature for 60 minutes. The incubation mixture was removed by decantation. 350µL of wash buffer solution was added and decanted twice. 100µL of working substrate was added to all the wells. This mixture was incubated for 15 minutes. 50µL of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450nm in a microplate reader.

2.5.3 Quantitative estimation of serum luteinizing hormone (LH) using ELISA in microwells.

Serum luteinizing hormones (LH) concentrations were determined using a solid-phase enzyme immunoassay based on the sandwich principle (Kosasa, 1981). The assay was performed using a commercially available LH ELISA Kit (Invitrogen™ Human LH ELISA Kit)). Microwells pre-coated with LH antibodies were secured in a plate holder. A total of 50µL of the standards, specimens, and controls was dispensed into the appropriate wells. This was preceded by 100µL of enzyme conjugate reagent added to each well. The plate was gently mixed for 30 seconds and then incubated at room temperature for 60 minutes. After incubation, the contents were discarded, and the wells were washed by adding 350µL of wash buffer solution, which was further aspirated or decanted twice. The washing step was repeated twice. 100µL of freshly prepared substrate was then added to each well, and the plate containing the mixture was incubated for 15 minutes at room temperature in the dark. The enzymatic reaction was terminated

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

by adding 50µL of stop solution to each well and gently mixing for 20 seconds. The absorbance in each well was determined at 450nm using a microplate reader. A dose-response curve was used to ascertain the concentrations of LH in the serum sample.

2.6 Liver enzymes assay

2.6.1 Estimation of alanine aminotransferase (ALT) activity

Alanine aminotransferase concentration of the sera samples was determined by Randox kit according to Reitman and Frankel (1957). The principle behind this method is the formation of pyruvate and glutamate by the transfer of an amino group from L-alanine to α-Ketoglutarate by ALT.

2.6.2 Estimation of Aspartate Aminotransferase (AST) activity

The AST concentration in the samples was estimated by the Randox kit method of Reitman and Frankel (1957). This is based upon the catalytic transfer of the amino group from L-aspartate to α-Ketoglutarate with oxaloacetate and glutamate as the new moieties formed.

The activity of AST was determined by monitoring the concentration of oxaloacetate hydrazone formed at 546nm from aspartate and 2,4-dinitrophenyl hydrazine.

2.6.3 Estimation of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was determined based on the estimation of the rate of hydrolysis of phosphate esters using the kit method of Tietz (1995). The absorbance of P-Nitrophenol (formed by the hydrolysis of P-Nitrophenol phosphate) at 405nm is proportional to the activity of ALP.

2.7 Lipid enzymes assay

Total cholesterol (TC): This was estimated by using the Randox assay kit (CHOD-PAP method) based on NCEP (2001). Estimation of high-density lipoprotein (HDL) - Cholesterol level.

Serum High-Density Lipoprotein Cholesterol: This was estimated by precipitating chylomicrons, VLDL and LDL with phosphotungstate and magnesium reagent, as described by Bowman and Wolf (1962).

Estimation of serum low-density lipoprotein (LDL) - Cholesterol level: This was estimated as described by Friedewald *et al.,* (1972).

2.8 Statistical analysis

Results were presented as means ± S.E and statistically analyzed using one-way analysis of variance (ANOVA) with SPSS Windows software programme, while Student's *t*-test was used for pair-wise comparison and differences were considered to be significant at $P<0.05$.

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

3.0. RESULTS

3.1. Hormonal Profile of Experimental Animals

The effect of dietary administration of *Cola acuminata* (KA), *Cola nitida* (KN), and *Garcinia kola* (GK) on serum reproductive hormones is presented in Table 2.

In the KA-treated groups, serum testosterone levels were significantly increased ($p < 0.05$) at 5%, 10%, and 20% inclusion levels when compared with the control group (3.43 ± 0.06 ng/mL). The highest testosterone level was observed in the 5% group (4.00 ± 0.02 ng/mL), although no significant difference was observed among the treated groups. In contrast, serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were significantly decreased ($p < 0.05$) in all KA-treated groups relative to control (LH: 0.61 ± 0.05 ng/mL; FSH: 0.89 ± 0.02 ng/mL). The 20% group showed significantly higher LH and FSH levels compared with the 5% and 10% groups, though still significantly lower than control.

For KN-treated animals, testosterone levels showed a non-significant decrease at 5% inclusion (2.85 ± 1.22 ng/mL) and non-significant increases at 10% and 20% inclusion levels when compared with control. However, LH and FSH levels were significantly reduced ($p < 0.05$) across all treated groups relative to control. No significant differences were observed among the KN-treated groups for LH. FSH levels showed slight variations but remained significantly lower than control.

In the GK-treated groups, serum testosterone levels were significantly elevated ($p < 0.05$) at all inclusion levels compared with control, with the highest value recorded at 10% inclusion (5.94 ± 0.28 ng/mL). Conversely, LH and FSH levels were significantly decreased ($p < 0.05$) across all GK-treated groups relative to control. Although minor variations existed among treated groups, they remained significantly lower than control values.

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

Table 2: Effect of KA, KN and GK Seed Diets on Serum FSH, LH and Testosterone Levels in Male Rabbits

Group	FSH (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)
Control	0.89±0.02	0.61±0.05	3.43±0.06
KA 5%	0.28±0.01*	0.12±0.01*	4.00±0.02*
KA 10%	0.34±0.02*[a]	0.18±0.00*	3.92±0.17*
KA 20%	0.40±0.01*[a,b]	0.68±0.03*[a,b]	3.81±0.06*
KN 5%	0.32±0.03*	0.12±0.01*	2.85±1.22
KN 10%	0.40±0.05*	0.18±0.02*	4.14±0.24
KN 20%	0.26±0.03*	0.18±0.01*	4.12±0.32
GK 5%	0.35±0.05*	0.26±0.00*	4.77±0.20*
GK 10%	0.37±0.03*	0.15±0.02*	5.94±0.28*[a]
GK 20%	0.41±0.01*	0.30±0.12*	4.73±0.20*[b]

Note: Values expressed as Mean ± SE (n = 3); Significantly different from control ($p < 0.05$) a = significantly different from 5% group ($p < 0.05$); b = significantly different from 10% group ($p < 0.05$)

3.2. Lipid Profile

The effects of KA, KN, and GK seed diets on serum total cholesterol (TC), LDL-c, and HDL-c are presented in Table 3.

In KA-treated rabbits, TC levels were significantly increased ($p < 0.05$) at all inclusion levels compared with control (26.95 ± 0.35 mg/dL). The highest TC value was recorded at 5% inclusion (50.40 ± 1.21 mg/dL). LDL-c significantly increased at 5% inclusion but significantly decreased at 10% and 20% inclusion compared with control. HDL-c showed no significant improvement across treatment groups.

For KN-treated animals, TC levels significantly increased ($p < 0.05$) at 5% and 10% inclusion levels compared with control, while the 20% group showed a non-significant increase. LDL-c levels did not show significant variation relative to control. HDL-c levels also showed no significant differences.

In GK-treated rabbits, TC levels were significantly elevated ($p < 0.05$) at all inclusion levels, with the highest concentration observed at 20% inclusion (55.30 ± 1.85 mg/dL). LDL-c levels were

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

significantly increased at 20% inclusion compared with control and other groups. HDL-c levels showed slight non-significant reductions.

Table 3: Effect of KA, KN and GK on Serum Lipid Profile

Group	TC (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)
Control	26.95±0.35	17.50±0.92	16.80±0.00
KA 5%	50.40±1.21*	26.60±1.40*	17.50±0.70
KA 10%	35.00±2.80*[a]	16.10±2.80[a]	14.00±0.35[a]
KA 20%	32.90±0.70*[a]	14.35±0.92*	15.40±1.75
KN 5%	44.10±1.21*	20.59±0.92	17.50±0.70
KN 10%	41.30±7.00*	17.20±1.26	16.80±1.21
KN 20%	38.50±2.80	17.50±2.52	18.41±0.35
GK 5%	39.90±1.21*	18.55±0.70	15.05±0.70
GK 10%	41.30±2.80*	19.60±2.13	15.75±0.00
GK 20%	55.30±1.85*[a,b]	25.90±1.52*[a,b]	16.18±2.73

Note: Values expressed as Mean ± SEM (n = 3); Significantly different from control ($p < 0.05$) a = significantly different from 5% group ($p < 0.05$); b = significantly different from 10% group ($p < 0.05$)

3.3. Liver Enzyme Activities

As presented in Table 4; the control values were ALT (13.30 ± 0.70 IU/L), AST (11.20 ± 0.70 IU/L), and ALP (39.09 ± 2.07 IU/L). All KA-treated groups showed significant elevations. The highest ALT was observed at 20% inclusion (48.30 ± 1.61 IU/L). KN-treated groups showed significant increases in ALT and AST, while ALP remained non-significant. GK-treated groups showed significant increases in ALT and AST at 5% inclusion, and ALP significantly increased at 20% inclusion.

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

Table 4: Effect of KA, KN and GK Seed Diets on Serum ALT, AST and ALP Activities

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	13.30±0.70	11.20±0.70	39.09±2.07
KA 5%	27.65±0.92*	71.05±0.70*	66.71±0.70*
KA 10%	17.85±0.00*[a]	45.50±17.15*	44.91±2.38a
KA 20%	48.30±1.61*[ab]	43.75±5.43*	56.46±2.91*[ab]
KN 5%	23.10±1.61*	57.05±6.68*	33.08±0.95
KN 10%	40.95±0.61*[a]	17.50±0.70[a]	31.89±2.56
KN 20%	18.55±0.70*[ab]	51.45±3.15*[b]	36.72±3.50
GK 5%	30.10±6.71*	60.90±5.55*	35.63±4.06
GK 10%	21.70±0.35	51.10±0.92*	44.34±2.15[a]
GK 20%	17.85±0.00[a]	29.75±1.40*[ab]	64.23±0.55*[ab]

Note: Values expressed as Mean ± SEM (n = 3); Significantly different from control ($p < 0.05$) a = significantly different from 5% group ($p < 0.05$); b = significantly different from 10% group ($p < 0.05$)

3.4. Food Intake

The control group recorded a mean food intake of 17.84 ± 0.18 g. The 5% group recorded 12.82 ± 0.13 g, significantly lower ($p < 0.05$) than control. The 10% group recorded 12.03 ± 0.12 g, significantly lower than control and 5%. The 20% group recorded 12.27 ± 0.07 g, significantly lower than control and different from 5%, but not from 10%. The greatest reduction was observed at 10% inclusion.

3.5. Body Weight Changes

All treated groups showed significantly lower weight gain compared with control ($p <$ 0.05). The 20% inclusion produced the lowest weight gain.

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

Table 5: Effect of KA, KN and GK Seed Diets on Body Weight Changes

Group	Initial (g)	Final (g)	Weight Gain (g)
Control	133.35±4.58	194.25±1.66	60.90±3.15
5%	141.75±2.35	180.39±2.52*	38.64±1.17*
10%	134.40±3.93	155.40±4.87*[a]	21.00±2.88*[a]
20%	138.60±3.93	144.90±3.93*[ab]	6.30±4.20*[ab]

Note: Values expressed as Mean ± SEM (n = 3); Significantly different from control ($p < 0.05$) a = significantly different from 5% group ($p < 0.05$); b = significantly different from 10% group ($p < 0.05$)

4.0. DISCUSSION

The findings demonstrate biological effects among the three species, suggesting differences in phytochemical composition, endocrine modulation, and dose-dependent metabolic responses.

The most pronounced androgenic effect was observed in the GK 10% group. However, all treated groups showed significant reductions in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both products of gonadotropic cells, which suggested negative feedback inhibition at the hypothalamic-pituitary-gonadal axis. Increased testosterone with suppressed gonadotropins may reflect enhanced peripheral steroidogenesis or direct pituitary modulation. Previous studies have demonstrated that caffeine-containing botanicals can influence the endocrine, signaling pathways, and steroid hormone synthesis (Iwu *et al.,* 1999; Li *et al.*, 2025). In addition, biflavonoids such as kolaviron from *Garcinia kola* have been reported to modulate oxidative stress and reproductive function (Tauchen *et al.,* 2023). Experimental evidence also suggests that phytochemicals in kola nuts may alter reproductive hormone dynamics in rodents (Obidike *et al.,* 2011; Eze *et al.,* 2020). The comparatively weaker endocrine response in KN may be attributed to quantitative or qualitative differences in methylxanthine and flavonoid composition between *C. acuminata* and *C. nitida* (Ajayi *et al*., 2021; Monteiro *et al.,* 2016).

In the lipid profile assessment, KA and GK produced more marked hypercholesterolemic effects than KN. TC increased significantly in all KA and GK groups, with GK at 20% inclusion showing the highest elevation. LDL-cholesterol followed a similar pattern, particularly at higher inclusion levels, while HDL-cholesterol remained largely unchanged or slightly reduced. These findings indicate a potential atherogenic tendency associated with chronic high-dose supplementation. Earlier studies have reported alterations in lipid metabolism following prolonged caffeine-rich botanical intake (Nyadanu *et*

JOURNAL OF BIOSCIENCES AND ENVIRONMENTAL STUDIES (JBES)
VOL. 1., NO. 1, MARCH 2026 || ISSN: 0189-1456
https://wordastrajournals.com/index.php/jbes/index

A Publication of Word Astra Publishers
Southern Europe
admin@wordastrajournals.com; wordastrajournals@gmail.com

al., 2020). Although kolaviron has demonstrated lipid-lowering effects under certain experimental conditions (Iwu *et al*., 2019), whole-seed consumption may exert different metabolic outcomes due to synergistic phytochemical interactions. Additionally, tannins and phenolic compounds present in kola species may influence hepatic lipid biosynthesis and transport (Iwu *et al*., 2019; Nyadanu *et al.,* 2020). Therefore, while these botanicals are traditionally considered beneficial, excessive intake may compromise lipid homeostasis.

Liver enzyme activities further differentiated the physiological impacts of the three seed species. KA supplementation resulted in the most pronounced elevation of ALT, AST, and ALP, indicating potential hepatocellular stress (Eze *et al.,* 2020). KN significantly increased ALT and AST but had minimal influence on ALP, whereas GK produced moderate enzyme elevations, particularly at higher inclusion levels. Elevated transaminases are widely accepted biomarkers of hepatic membrane permeability and cellular injury (Tamber *et al.,* 2023). While *Garcinia kola* has been described as hepatoprotective in certain toxicological models (Farombi, 2011; Ogwu *et al.,* 2024), chronic high-dose dietary exposure may overwhelm hepatic detoxification mechanisms. The relatively greater enzyme perturbation observed in KA groups may be linked to higher caffeine concentration and associated metabolic demand on hepatic cytochrome systems (Crew, 2014; Eze *et al*., 2020).

Food intake and body weight responses were consistent across all treated groups. Significant reductions in feed consumption were observed at all inclusion levels, with the 10% group showing the greatest suppression. Correspondingly, treated rabbits exhibited significantly lower weight gain compared with controls, and the 20% inclusion groups demonstrated the least weight increase. These findings align with documented anorectic and thermogenic effects of caffeine and related methylxanthines present in kola nuts (Owolabi *et al.,* 2020; Ajayi *et al.,* 2021). Appetite suppression likely contributed directly to reduced caloric intake and subsequent lower body mass accumulation. The uniformity of this effect across KA, KN, and GK suggests a shared stimulant mechanism despite differences in secondary metabolic alterations.

Comparatively, GK exhibited the strongest androgenic response and marked lipid elevation at higher doses. KA showed the most pronounced hepatic enzyme disruption and significant cholesterol increases, whereas KN demonstrated relatively milder endocrine and lipid alterations but still significantly reduced gonadotropins and body weight. Collectively, these results indicate that although these botanicals are culturally significant and widely consumed in West Africa, chronic high-dose intake may alter endocrine regulation, lipid balance, and hepatic integrity.

5.2. CONCLUSION

Chronic dietary supplementation with *Cola acuminata*, *Cola nitida*, and *Garcinia kola* produced distinct but overlapping physiological alterations in male rabbits, which are characterized by increased

testosterone with suppressed gonadotropins, elevated total cholesterol, variable LDL-c changes, hepatic enzyme perturbations, reduced food intake, and diminished weight gain. These findings indicate that although these seeds are widely consumed for cultural and ethnomedicinal purposes, prolonged high-dose intake may compromise endocrine balance, lipid homeostasis, and liver functionality. It is therefore recommended that consumption be moderated, particularly at high doses, and that public health awareness be strengthened regarding potential long-term risks. Further long-term studies involving larger sample sizes, histopathological assessments, and mechanistic investigations are necessary to establish safe dietary thresholds and clarify species-specific toxicity profiles.

REFERENCES

Adewale, F. O., Adebayo, A. F., and Olaleye, M. T. (2022). Kolaviron ameliorates carbon tetrachloride-induced hepatotoxicity in Wistar rats. *Journal of Biochemical Toxicology, 37*(2), e22987.

Agbebaku, E. E. O., Okunade, A. F., Awodumila, D. J. Fagbami, O. O., Akande, M. A., Adeleke, S. A. and Edereno, K. U. 2018. "Empirical Study and Preference on Cola Garcinia (Bitter kola) with Cola Malvaceae (kola-nuts) Consumption in Nigeria", International Journal of Development Research, *8*(11), 24414-24416.

Ajayi, I. A., Oderinde, R. A., and Taiwo, V. O. (2021). Phytochemical composition and biological activities of *Cola* species: A review. *African Journal of Traditional, Complementary and Alternative Medicines, 18*(2), 45–58.

Alada, A. R. A., and Adeyemo, D. O. (2023). Effects of kola flavonoids on testicular steroidogenesis in male rabbits. *Journal of Endocrine Research, 15*(1), 45–53.

Babu, S. R., Sadhnani, M. D., Swarna, M., Padmavathi, P. and Reddy, P. P. (2004). Evaluation of FSH, LH and testosterone levels in different subgroups of infertile males. *Indian J Clin Biochem.*, *19*(1), 45-9.

Bowman, R. E., and Wolf, C. R. (1962). *A rapid and specific ultramicro method for total serum cholesterol*. *Clinical Chemistry, 8*(3), 302–309.

Correa, M., SanMiguel, N., López-Cruz, L., Carratalá-Ros, C., Olivares-García, R. and Salamone, J. D. (2018). Caffeine Modulates Food Intake Depending on the Context That Gives Access to Food: Comparison With Dopamine Depletion. *Front Psychiatry*, *9*, 411.

Crews C. (2014). Natural Toxicants: Alkaloids [M]//MOTARJEMI Y. Encyclopedia of Food Safety. Waltham; *Academic Press*, 251-60.

Effanga, B. O., Bassey, S. O., James, O. O., and Nta, E. E. (2026). Dose-dependent effects of *Cola acuminata, Cola nitida* and *Garcinia kola* seed supplementation on serum reproductive hormones, lipid profiles and hepatic health in male Wistar rats. *ISA Journal of Medical Sciences (ISAJMS)*, *3*(2), 11-23.

Engvall, E. and Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA): Quantitative assay of immunoglobulin G. *Immunochemistry, 8*(9), 871–874. https://doi.org/10.1016/0019-2791(71)90454-X

Eze, M. O., Okeke, G. C., and Umoh, I. J. (2023). Chronic bitter kola seed feeding induces hepatic stress in male rabbits. *Toxicology Reports, 10*, 1234–1242.

Farombi, E. O. (2011). Chapter 26 - Bitter Kola (Garcinia kola) Seeds and Hepatoprotection [M]//PREEDY V R, WATSON R R, PATEL V B. Nuts and Seeds in Health and Disease Prevention. San Diego; Academic Press. 2011: 221-8.

Friedewald, W. T., Levy, R. I., and Fredrickson, D. S. (1972). d *Clinical Chemistry, 18*(6), 499–502.

Giannini, E. G., Testa, R and Savarino, V. (2005). Liver enzyme alteration: a guide for clinicians. *CMAJ*, *172*(3), 367-79.

Glover, F. E., Caudle, W. M., Del Giudice, F., Belladelli, F., Mulloy, E., Lawal, E. and Eisenberg, M. L. (2022). The association between caffeine intake and testosterone: NHANES 2013-2014. *Nutr J.*, *21*(1), 33.

Gore A. C., Chappell V. A., Fenton S. E., Flaws J. A., Nadal A., Prins G. S., Toppari J., Zoeller R. T. (2015). EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews*, *36*(6), E1-E150.

Herman, A. P., Tomczyk, M., Wójcik, M., Bochenek, J., Antushevich, H., Herman, A., Wiechetek, W., Szczepkowska, A., Marciniak, E., and Tomaszewska-Zaremba, D. (2024). Effect of Caffeine on the Inflammatory-Dependent Changes in the GnRH/LH Secretion in a Female Sheep Model. *International Journal of Molecular Sciences*, *25*(5), 2663.

Icheku, V., Onianwah F. and Nwulia A. (2018). A descriptive cross-sectional study on various uses and outcomes of *Garcinia kola* among people of Oshimili North in the Delta State of Nigeria. *Ayu.*, *39*(3), 132-138.

Iwu, M. M., Duncan, A. R., and Okunji, C. O. (1999). New antimicrobials of plant origin. In J. Janick (Ed.), *Perspectives on new crops and new uses* (pp. 457–462). ASHS Press.

Jacob, D. E., Nelson, I. U. and Izah, S. C. (2023). *Cola accuminata*: Phytochemical Constituents, Nutritional Characteristics, Scientific Validated Pharmacological Properties, Ethnomedicinal Uses, Safety Considerations, and Commercial Values. In: Izah, S.C., Ogwu, M.C., Akram, M. (eds) Herbal Medicine Phytochemistry. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-031-21973-3_59-1

Kosasa, T. S. (1981). Measurement of human luteinizing hormone. *Journal of Reproductive Medicine*, **26**, 201–206.

Lei, T., Yang, Y. and Yang, W. X. (2025). Luteinizing Hormone Regulates Testosterone Production, Leydig Cell Proliferation, Differentiation, and Circadian Rhythm During Spermatogenesis. *Int J Mol Sci.*, *26*(8), 3548.

Li, S., Yuan, Z., Zhao, Y. and Wang, N. (2025). Associations of caffeine and caffeine metabolites with sex hormones among 6-19-year-old children and adolescents. *Sci Rep.*, *15*(1):23052.

McGill, M. R. (2022). Biomarkers of Liver Injury Due to Toxic Agents: Progress, Current Applications, and Emerging Directions [M]//PATEL V B, PREEDY V R, RAJENDRAM R. Biomarkers in Toxicology. Cham; *Springer International Publishing*, 1-20.

Monteiro, J. P., Alves, M. G., Oliveira P. F., Silva B. M. (2016). Structure-Bioactivity Relationships of Methylxanthines: Trying to Make Sense of All the Promises and the Drawbacks. *Molecules*, *21*(8), 974.

NCEP (National Cholesterol Education Program). (2001). *Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)*. NIH Publication.

NRC (National Research Council) (US) (1992). Committee on Pain and Distress in Laboratory Animals. *Recognition and Alleviation of Pain and Distress in Laboratory Animals. Washington (DC)*: National Academies Press (US); 1992. 7, Euthanasia. Available from: https://www.ncbi.nlm.nih.gov/books/NBK235440/

Nyadanu, D., Lowor, S. T., Akpertey, A., Tchokponhoué, D. A., Pobee, P., Dogbatse, J. A., Okyere, D., Amon-Armah, F. and Brako-Marfo, M. (2020). Genetic variability of bioactive compounds and selection for nutraceutical quality in kola [Cola nitida (Vent) Schott. and Endl.]. *PLoS One*, *15*(12), e0242972.

Obidike, I. R., Aka, L. O. and Ezema, W. S. (2011) Effects of caffeine extract from kola nut on body weight, hematology, sperm reserve and serum enzyme activities in albino rats. *Comp Clin Pathol., 20*, 625–630.

OECD (Organisation for Economic Co-operation and Development). (2018). *OECD guideline for the testing of chemicals: Repeated dose 90-day oral toxicity study in rodents (Test No. 408).* OECD Publishing.

Ogwu, M.C., Ogwu, H.I., Osawaru, M.E., Izah, S.C. (2024). *Garcinia kola* Heckel. (Clusiaceae): An Overview of the Cultural, Medicinal, and Dietary Significance for Sustainability. In: Izah, S.C., Ogwu, M.C., Akram, M. (eds) Herbal Medicine Phytochemistry. *Reference Series in Phytochemistry. Springer, Cham.*, 273-301

Okoli, C. A., Ifeanyi, O. P., and Ugochukwu, C. O. (2022). Reproductive endocrine effects of *Cola nitida* seed extracts in Wistar rats. *Journal of Experimental Animal Sciences, 10*(4), 112–123.

Oluwaseun, A., and Adebayo, S. (2019). Traditional uses and pharmacological properties of kola nut in West Africa. *Journal of Herbal Medicine, 17*, 100–109.

Omeh, Y. N., S. O. Onoja, M. I. Ezeja, W. C. Uchendu, E. Okorie, and Raymond, M. (2014). Quantitative Phytochemical, Proximate Analysis and Hypolipidemic Effect of Garcinia Kola. *Journal of Advances in Medicine and Medical Research, 4*(36), 5770-78.

Owolabi, J. O., Omogbai, E. K. I., and Falodun, A. (2020). Effects of caffeine-containing kola nut preparations on metabolic parameters in experimental animals. *Journal of Ethnopharmacology, 249*, 112–118.

Ozoffor, C. H., Onyemelukwe, N. F., and Ogbuagu, E. (2024). Proximate analysis and pathological studies of extracts of *Cola acuminata* and *Cola nitida* in visceral organs of albino rabbits. *International Journal of Current Microbiology and Applied Sciences, 13*(4), 15–25.

Rauf, M. Q., Sharma, L, Essiet, E., Elhassan, O., Fahim, R., Irem-Oko, F. and Sreedharan, J. (2025). Caffeine Consumption Patterns, Health Impacts, and Media Influence: A Narrative Review. *Cureus*, *17*(6), e86215.

Reitman, S., and Frankel, S. (1957). *A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic-pyruvic transaminases*. *American Journal of Clinical Pathology, 28*(1), 56–63.

Tamber, S. S., Bansal P., Sharma S., Singh R. B. and Sharma R. (2023). Biomarkers

of liver diseases. *Mol Biol Rep.*, *50*(9), 715-7823.

Tauchen, J., Frankova, A., Manourova, A., Valterova, I., Lojka, B. and Leuner, O. (2023). *Garcinia kola*: a critical review on chemistry and pharmacology of an important West African medicinal plant. Phytochem Rev., 1-47.

Tietz, N. W. (1995). *Clinical guide to laboratory tests* (3rd ed.). W.B. Saunders Company.