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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

Effanga, B. O.[1*], Bassey, S. O.[1*], James, O. O.[2], Nta, E. E.[2*]

[1]*Department of Human Nutrition and Dietetics, Faculty of Allied Medical Sciences, University of Calabar, Cross River, Nigeria*
[2]*Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Cross River, Nigeria*

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***CorrespondingAuthor:** Effanga, B. O.

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Abstract **Review Article**

Widely consumed kola nuts and bitter kola in Nigeria have nutritional and medicinal benefits. However, long-term consumption causes chronic liver and hepatic malfunctioning. This study investigated the 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. Fifty (50) male rats (135 ± 15g) were randomly assigned into ten groups; one control and nine treatments (n = 5) receiving 5%, 10%, and 20% dietary inclusion of each seed for 12 weeks. Testosterone, LH and FSH, and ALT, AST, and ALP and TC, LDL-c, and HDL-c were determined using standard procedures. Data were expressed as mean ± SEM and analyzed by One-way ANOVA, with significance set at $p < 0.05$. Results revealed that treatment with KA and GK significantly increased serum testosterone levels ($p < 0.05$), with the highest values observed at 5% (KA) and 10% (GK) inclusion levels. Conversely, LH and FSH levels were significantly reduced in all treated groups compared with control. Lipid profile analysis revealed significant elevations in TC across all treatment groups, particularly with GK at 20% inclusion, while LDL-c and HDL-c showed variable trends. Chronic kola seed consumption also elevated liver enzyme activities (ALT, AST, ALP), indicating possible hepatocellular stress. Food intake decreased significantly ($p < 0.05$) in all treatment groups, with the greatest reduction at 10% inclusion, and correspondingly, body weight gain was significantly lower compared with control, with the 20% inclusion group exhibiting the lowest gain. Chronic dietary intake of *C. acuminata*, *C. nitida*, and *G. kola* seeds elevates testosterone, suppresses LH and FSH, disrupts lipid balance, induces hepatic stress, and reduces food intake and weight gain, highlighting potential risks of prolonged whole seed consumption and the need for moderated intake.

Keywords: Kola seeds, Lipid profile, Hepatic enzymes, Body weight and Wister Rat.

1. Introduction

Kola nuts derived from *Cola acuminata*and *Cola nitida*, as well as bitter kola (*Garcinia kola*), are widely consumed in West Africa, particularly in Nigeria, where they hold nutritional, cultural, and ethnomedicinal importance (Eze*et al.,* 2020; Ajayi*et al.,* 2021). These seeds are traditionally chewed as stimulants and are used in social ceremonies and herbal medicine for the management of fatigue, digestive disorders, infections, and inflammatory conditions (Oluwaseun and Adebayo, 2019).

Phytochemical investigations reveal that *Cola* species contain significant quantities of caffeine, theobromine, theophylline, tannins, flavonoids, and phenolic compounds, which contribute to their stimulant and antioxidant properties (Iwu*et al.*, 2019; Olaniyan*et al.,* 2022). Similarly, *Garcinia kola* seeds are rich in biflavonoids such as kolaviron, known for potent antioxidant, anti-inflammatory, and hepatoprotective effects (Adaramoye and Farombi, 2018; Nwankwo*et al.*, 2021).

Despite these reported benefits, evidence suggests that chronic or high-dose consumption may produce adverse physiological effects. Studies have indicated that prolonged intake of caffeine-rich kola preparations may influence body weight, appetite regulation, and metabolic parameters (Owolabi*et al.,* 2020). Additionally, excessive intake of phytochemical-rich botanicals has been associated with alterations in liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), which are biomarkers of hepatocellular integrity (Yakubu*et al.,* 2019).

Animal models, particularly male Wistar rats, are widely used in toxicological and nutritional studies due to their physiological similarity to humans. Parameters such as growth performance, and hepatic enzyme biomarkers provide reliable indicators of systemic and organ-specific toxicity (OECD, 2018). However, comprehensive dose-dependent evaluations comparing *Cola acuminata*, *Cola nitida*, and *Garcinia kola* under chronic dietary conditions remain limited.

Although kola nuts and bitter kola are extensively consumed in Nigeria for their perceived health benefits, there is insufficient scientific evidence regarding their long-term dose-dependent physiological effects. Existing studies primarily focus on acute toxicity or isolated extracts, with limited attention to chronic dietary supplementation and its impact on growth performance and hepatic biomarkers (Yakubu*et al.,* 2019; Owolabi*et al.,* 2020).

Furthermore, comparative analyses among *Cola acuminata*, *Cola nitida*, and *Garcinia kola* are scarce, leaving uncertainty regarding their relative safety profiles. Given their high caffeine and polyphenol content, prolonged intake may potentially disrupt metabolic homeostasis or induce subclinical hepatotoxicity. Therefore, a systematic dose-response study is necessary to establish safety margins and clarify whether chronic consumption confers beneficial or adverse physiological effects.

Kola nuts (*Cola acuminata*, *Cola nitida*) and bitter kola (*Garcinia kola*) are widely consumed in Nigeria for cultural, dietary, and medicinal purposes (Ajayi*et al.,* 2021; Nwankwo*et al.,* 2021). Despite their extensive traditional use, scientific evidence regarding the effects of chronic consumption and safe dosage levels remains limited (Owolabi*et al.,* 2020). Therefore, systematic evaluation of their physiological and toxicological effects is necessary.

Assessment of growth performance provides insight into the metabolic and nutritional impacts of supplementation (OECD, 2018). In addition, liver enzyme biomarkers; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) which are critical indices for detecting hepatocellular damage and functional impairment (Yakubu*et al.,* 2019).

This study is significant because it establishes dose–response relationships, clarifies potential health benefits or risks, and provides scientific data to guide safe consumption practices and regulatory decisions. By integrating traditional knowledge with controlled experimental evidence, the study contributes to public health awareness and advances research on African medicinal plants.

The aim of this study was to investigate the dose-dependent effects of dietary supplementation with kola nuts (*Cola acuminata*and *Cola nitida*) and bitter kola (*Garcinia kola*) on growth performance and hepatic enzyme biomarkers (ALT, AST, and ALP) in male Wistar rats.

2.0. Materials and Methods

2.1 Laboratory Design / Experimental Layout

A total of fifty (50) healthy male Wistar rats, with an average weight of 135 ± 15g, were obtained from the university animal house, and individually housed in metabolic cages. Individual housing allowed accurate monitoring of food intake, feed conversion, and excreta, minimizing competition and stress among animals. The animal facility was maintained under strict environmental conditions, including a temperature of 27 ± 2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle to mimic standard laboratory conditions and reduce environmental stress. To ensure physiological stability and adaptation to the laboratory environment, the rats underwent a two-week acclimatization period before the feeding trial commenced. During this period, animals were monitored daily for signs of illness or abnormal behavior, and standard rat chow and water were provided ad libitum. This careful housing and acclimatization protocol ensured that all rats started the experiment under uniform conditions, thereby improving the reliability and reproducibility of subsequent experimental observations.

2.2. Collection and identification of plant material

Kola nut seeds (*Cola nitida*and *Cola acuminata*) and bitter kola (*Garcinia kol a*) were purchased from OkonOyom market in Akpabuyo local Government Area of Cross River State. A Botanist in the Department of Botany, University of Calabar, Calabar identified and validated the seeds. The seeds were peeled, washed, cut into smaller sizes and dried at 60^{o}C for 12 hours, and ground using manual blender.

2.3. Collection of experimental animals

In this study, 50 male rats of the Wistar strain weighing 120-150g were obtained from the healthy stock of the animal house and taken to the animal house of Biochemistry department both in the Faculty of Basic Medical Sciences, University of Calabar. They were distributed into individual metabolic cages and allowed two weeks of acclimatization, under room temperature (27 ± 2^{o}C) before the commencement of the experimental feeding procedures. All the experimental procedures were done following the experimental guidelines of Institutional Animal Ethics Committee.

2.4. Diet formulation and feeding of experimental rats

Cola acuminata, Cola nitida and Garcinia kola seeds were incorporated in the rats' diet in proportion of 5%, 10% and 20% respectively. The appropriate weight of the seeds in each case was thoroughly mixed with specified amount of the rat chow; a little quantity of table water was used to homogenize the mixture and made into pellets using syringe then oven dried at 60^{o}C. The rats were fed according to the schedule in Table 1. Furthermore, leftover and spilled diet were carefully collected and weighed in order to determine the rats' diet intake i.e. the difference between initial diet supplied and leftover.

The feeding of the experimental rats with diets lasted for 12weeks. Within this period, the rats' body weights (g) were measured with the aid of a digital electronic balance.

2.5 Experimental Setup

A total of fifty (50) Wistar rats were randomly assigned into ten experimental groups of five rats each, comprising a control group fed with rat chow only and nine treatment groups administered graded dietary inclusions (5%, 10%, and 20%) of *Cola acuminata*, *Cola nitida*, and *Garcinia kola* mixed with standard rat chow.

Table 1: Distribution of rats into experimental groups

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

2.6. Sample collection

Rats were anesthetized by exposing them in a desiccators containing cotton wool soaked with chloroform for about three minutes. After that the rats were placed on the dissecting board sacrificed, and carefully dissected to expose the heart region. Blood samples were collected using 5ml hypodermic syringe and needle and each blood sample collected were discharged equally into an EDTA bottle. Blood samples were allowed to clot and centrifuged at 2500rpm for 10mins. Dry sample containers were used to collect serum and stored frozen for biochemical analysis.

2.7. Serum hormonal assays

2.7.1 Quantitative estimation of serum testosterone using ELIZA using microwells

The method employed was microwell immunoassay (ELIZA) 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 respectively swirled for 20 secs. 50µl each of testosterone biotin working reagent was added to all the respective wells. The mixtures were swirled for 20secs, and allowed to incubate for 60mins. 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 20mins. 50µl of stop solution was added to each well and swirled gently, 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.

2.7.2 Quantitative estimation of serum follicle stimulating hormone (FSH) using ELIZA using microwells

The method employed was microwell immunoassay (ELIZA) using analytical grade reagents (Odel,

1981). The desire numbers of coated wells were 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 of the, the mix up was stirred for 30sec. this solution was incubated at room temperature for 60mins. The incubation mixture was removed by decantation. 350µl of wash buffer solution was added and decanted for two times. 100µl of working substrate was added to all the wells. This mixture was incubated for 15mins. 50µl of stop solution was added to each well and gently mixed for 15-20secs. The absorbance in each well was read at 450nm in a microplate reader.

2.7.3 Quantitative estimation of serum luteinizing hormone (FSH) using ELIZA using microwells.

The microwell PSA EIA was the solid phase enzyme immunoassay used. It was based on the sandwich principle (Kosasa, 1981). The desired numbers of coated wells were secured in the holder. 50µl of the standard specimens and control were added into appropriate wells. 100µl of enzyme conjugate reagent was added to each well. The setup was thoroughly mixed for 30 secs. The solution was incubated for 60mins. The content of the incubation was decanted. 350µl of wash buffer solution was added and decanted for two times. 100µl of working substrate was added to all the wells. This mixture was incubated for 15mins. 50µl of stop solution was added to each well and gently mixed for 20 secs. The absorbance in each well was read at 450nm in a microplate reader. A dose response curve was used to ascertain the concentration of the LH in the serum.

2.8. Liver enzymes assay

2.8.1 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.

2.8.2 Estimation of Aspartate Aminotransferase (AST) activity

The AST concentration in the samples was estimated by Randox kit method of Reitman and Frankel (1957). This is based upon the catalytic transfer of 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.

2.8.3 Estimation of alkaline phosphate (ALP) activity

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

2.9. Lipid enzymes assay

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

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

Estimation of serum low density lipoprotein (LDL) – Cholesterol level: Thiswas estimated as described by Friedwalds*et al.*, (1972).

2.10 Statistical analysis

Results were presented as means ± S.E.M and statistically analyzed using one-way analysis of

variance (ANOVA) with SPPS window software programme, while student *t*-test was used for pair-wise comparison and differences were considered to be significant at $P<0.05$.

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 (3.27 ± 0.06 ng/mL). The highest testosterone level was observed in the 5% group (3.81 ± 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: 0.58 ± 0.05 ng/mL; FSH: 0.85 ± 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 (2.71 ± 1.16 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 (5.66 ± 0.27 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.

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

Group	FSH (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)
Control	0.85±0.02	0.58±0.05	3.27±0.06
KA 5%	0.27±0.01*	0.11±0.01*	3.81±0.02*
KA 10%	0.32±0.02*[a]	0.17±0.00*	3.73±0.16*
KA 20%	0.38±0.01*[a,b]	0.65±0.03*[a,b]	3.63±0.06*
KN 5%	0.30±0.03*	0.11±0.01*	2.71±1.16
KN 10%	0.38±0.05*	0.17±0.02*	3.94±0.23
KN 20%	0.25±0.03*	0.17±0.01*	3.92±0.30

Group	FSH (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)
GK 5%	0.33±0.05*	0.25±0.00*	4.54±0.19*
GK 10%	0.35±0.03*	0.14±0.02*	5.66±0.27*[a]
GK 20%	0.39±0.01*	0.29±0.11*	4.50±0.19*[b]

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 ($p < 0.05$).

3.2. Lipid Profile

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

In KA-treated rats, TC levels were significantly increased ($p < 0.05$) at all inclusion levels compared with control (25.67 ± 0.33 mg/dL). The highest TC value was recorded at 5% inclusion (48.00 ± 1.15 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, with slight non-significant decreases observed at higher inclusion levels.

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 across treated groups.

In GK-treated rats, TC levels were significantly elevated ($p < 0.05$) at all inclusion levels, with the highest concentration observed at 20% inclusion (52.67 ± 1.76 mg/dL). LDL-c levels were significantly increased at 20% inclusion compared with control and other treatment groups. HDL-c levels showed slight non-significant reductions across treatment groups.

These findings indicate that chronic consumption of kola species, particularly GK at higher inclusion levels, may predispose to hypercholesterolemia.

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	25.67±0.33	16.67±0.88	16.00±0.00
KA 5%	48.00±1.15*	25.33±1.33*	16.67±0.67
KA 10%	33.33±2.67*,a	15.33±2.67a	13.33±0.33a
KA 20%	31.33±0.67*[a]	13.67±0.88*	14.67±1.67
KN 5%	42.00±1.15*	19.61±0.88	16.67±0.67
KN 10%	39.33±6.67*	16.38±1.20	16.00±1.15

Group	TC (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)
KN 20%	36.67±2.67	16.67±2.40	17.53±0.33
GK 5%	38.00±1.15*	17.67±0.67	14.33±0.67
GK 10%	39.33±2.67*	18.67±2.03	15.00±0.00
GK 20%	52.67±1.76*[a,b]	24.67±1.45*[a,b]	15.41±2.60

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

The effects of dietary administration of KA, KN, and GK on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are presented in Table 4. In KA-treated rats, ALT, AST, and ALP activities were significantly elevated ($p < 0.05$) at all inclusion levels compared with control (ALT: 12.67 ± 0.67 IU/L; AST: 10.67 ± 0.67 IU/L; ALP: 37.23 ± 1.97 IU/L). The highest ALT activity was recorded at 20% inclusion (46.00 ± 1.53 IU/L). Although enzyme activities varied among treated groups, all remained significantly higher than control.

For KN-treated groups, ALT and AST activities were significantly increased ($p < 0.05$) at all inclusion levels relative to control. However, ALP activity showed no significant differences compared with control.

In GK-treated animals, ALT and AST activities were significantly elevated ($p < 0.05$) at 5% inclusion, while moderate increases were observed at higher inclusion levels. ALP activity was significantly increased at 20% inclusion compared with control.

The elevation of these hepatic enzymes suggests possible hepatocellular stress or enzyme induction associated with chronic kola consumption.

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	12.67±0.67	10.67±0.67	37.23±1.97
KA 5%	26.33±0.88*	67.67±0.67*	63.53±0.67*
KA 10%	17.00±0.00*[a]	43.33±16.33*	42.77±2.27a
KA 20%	46.00±1.53*[ab]	41.67±5.17*	53.77±2.77*[ab]
KN 5%	22.00±1.53*	54.33±6.36*	31.50±0.90
KN 10%	39.00±0.58*a	16.67±0.67a	30.37±2.44

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
KN 20%	17.67±0.67*[ab]	49.00±3.00*[b]	34.97±3.33
GK 5%	28.67±6.39*	58.00±5.29*	33.93±3.87
GK 10%	20.67±0.33	48.67±0.88*	42.23±2.05[a]
GK 20%	17.00±0.00[a]	28.33±1.33*[ab]	61.17±0.52*[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 mean food intake of the experimental animals is presented as follows: the control group recorded a mean food intake of 16.99 ± 0.17 g. In comparison, the 5% inclusion group showed a significantly lower ($p < 0.05$) mean food intake of 12.21 ± 0.12 g relative to the control. Similarly, the 10% inclusion group recorded a mean food intake of 11.46 ± 0.11 g, which was significantly lower ($p < 0.05$) than the control group and also significantly lower than the 5% group. The 20% inclusion group had a mean food intake of 11.69 ± 0.07 g, which was significantly reduced ($p < 0.05$) compared with the control and significantly different from the 5% group, but not significantly different from the 10% group. Overall, food intake decreased significantly in all treated groups compared with the control, with the greatest reduction observed in the 10% inclusion group.

3.5. Body Weight Changes

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

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

Group	Initial (g)	Final (g)	Weight Gain (g)
Control	127.00±4.36	185.00±1.58	58.00±3.00
5%	135.00±2.24	171.80±2.40*	36.80±1.11*
10%	128.00±3.74	148.00±4.64*[a]	20.00±2.74*[a]
20%	132.00±3.74	138.00±3.74*[ab]	6.00±4.00*[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 present study revealed that *Cola acuminata* (KA) and *Garcinia kola* (GK) seed diets significantly elevated serum testosterone while suppressing LH and FSH. Such endocrine modulation suggests interference with the hypothalamic–pituitary–gonadal (HPG) axis. Several rodent studies align with this pattern of hormonal disruption. For instance, Odo*et al.* (2020) reported that chronic administration of *Cola acuminata* extract in male rats significantly altered reproductive hormones and testicular histology, suggesting impaired endocrine regulation. Similarly, Okoli*et al.* (2022) observed reduced testosterone and suppressed gonadotropins in rats fed *Cola nitida* extract, indicating pituitary–gonadal dysregulation.

In contrast, some studies indicate that moderate doses of kola bioactives may transiently stimulate androgen production. Iwuanyanwu*et al.* (2019) found that low-dose *Garcinia kola* seed supplementation elevated testosterone without commensurate increases in LH, suggesting direct testicular effects independent of pituitary signaling. Alada and Adeyemo (2023) reported that kola flavonoids influence steroidogenic enzymes in Leydig cells, potentially enhancing conversion of cholesterol to testosterone. These divergent findings suggest that dose, preparation (whole seed vs. extract), and duration play critical roles in determining hormonal outcomes in rodent models.

Overall, the consistent suppression of LH and FSH across KA, KN, and GK groups supports the notion that chronic kola consumption disrupts gonadotropin release, possibly through negative feedback by elevated testosterone or direct pituitary effects. Such disruptions are clinically relevant, as they mirror patterns seen in endocrine toxicology (Gore *et al.*, 2022), where exogenous phytochemicals trigger feedback inhibition of pituitary signals.

The present study's observation of elevated total cholesterol (TC) and LDL-cholesterol (LDL-c), particularly at higher GK inclusion levels, suggests a pro-atherogenic impact of chronic kola diets in healthy rats. This finding contrasts with a substantial body of rodent research showing hypolipidemic effects of kola extracts in pathological contexts. For example, Salawu*et al.* (2020) demonstrated that *Cola acuminata* extract significantly lowered TC and LDL-c in high-fat diet–induced hyperlipidemia in rats, while increasing HDL-cholesterol, highlighting potential therapeutic effects under metabolic stress. Similarly, Ukpabi*et al.* (2019) reported that aqueous *Garcinia kola* extract lowered TC and improved lipid ratios in diabetic rodents.

However, Musa *et al.* (2021) found that whole seed inclusion of *Cola nitida* at high dietary levels led to mild hypercholesterolemia in rats, reinforcing the present results that unprocessed seed consumption may differ significantly from purified extract effects. Moreover, Sonibare*et al.* (2023) concluded that *Garcinia kola* seeds possess complex lipid modulatory effects, reducing triglycerides but altering LDL and HDL balance depending on dose and dietary context.

Mechanistically, kola nut phytochemicals (e.g., methylxanthines, polyphenols) may interact with hepatic lipid metabolism in ways that differ in healthy versus disease states, possibly influencing cholesterol biosynthesis and lipoprotein assembly (Ajani *et al.*, 2024). Thus, while kola extracts have demonstrated lipid-lowering benefits in hyperlipidemic models, whole seed diets in normal rodents may predispose to dyslipidemia.

Elevated hepatic enzyme levels (ALT, AST, ALP) in KA, KN, and GK groups suggest hepatocellular stress or injury. ALT and AST elevations are canonical indicators of hepatic damage and are widely used in rodent toxicity studies. Ozoffo*et al.* (2024) documented hepatocellular degeneration and inflammatory infiltrates in rats administered *Cola acuminata* and *Cola nitida* extracts, corroborating the present findings. Similarly, Eze*et al.* (2023) found that prolonged *Garcinia kola* seed feeding resulted in elevated transaminases and hepatocyte vacuolation in rats, indicating toxic stress at high dietary inclusion.

Conversely, hepatoprotective effects of *Garcinia kola* extracts have been reported in chemically induced models. For instance, Ismail *et al.* (2024) demonstrated that aqueous *Garcinia kola* extract attenuated indomethacin-induced hepatotoxicity in rats, lowering ALT and AST levels. Adewale*et al.* (2022) also noted that kolaviron (a biflavonoid from *Garcinia kola*) reduced oxidative stress biomarkers and normalized liver enzymes in carbon tetrachloride-exposed rodents. These protective outcomes likely reflect the antioxidant properties of isolated flavonoids, which may not translate when whole seeds are chronically consumed.

The elevation of ALP at higher dietary inclusion levels may reflect cholestatic stress or enzyme induction secondary to prolonged phytochemical exposure. Such changes have been observed in other rodent nutritional toxicology studies where plant alkaloids exerted hepatic stress (Hassan *et al.*, 2023). Collectively, these findings suggest that chronic kola seed consumption can compromise hepatic function, despite beneficial effects reported with concentrated extracts in pathological models.

All kola-fed groups exhibited significantly reduced food intake compared with control animals, with the greatest reductions at moderate to high inclusion levels. This appetite–suppressant effect likely stems from the high methylxanthine (caffeine, theobromine) and tannin content of kola seeds, compounds known to influence appetite regulation and gastric motility in rodents (Cheikh*et al.*, 2024). Iwuanyanwu*et al.* (2019) similarly reported decreased feed intake and weight gain in rats administered *Garcinia kola* seeds. Sonibare*et al.* (2023) also noted that bitter kola supplementation reduced voluntary feed intake in rats, contributing to lower body weight gains.

Reduced weight gain in the present study correlates with decreased caloric intake, but may also reflect altered endocrine signals affecting metabolism; for example, elevated testosterone is associated with increased basal metabolic rate and energy expenditure (Smith & Walker, 2023). These combined effects can blunt weight gain despite diet availability.

While appetite suppression and weight reduction may be considered beneficial in obesity models, in healthy organisms they may signal nutritional stress and impaired growth, consistent with findings by Musa *et al.* (2021) in rats fed high kola diets.

5.2. Conclusion

Chronic dietary inclusion of *Cola acuminata*, *Cola nitida*, and *Garcinia kola* seeds in healthy rats modulates endocrine function, elevating testosterone while suppressing LH and FSH, disrupts lipid homeostasis by increasing total and LDL cholesterol, induces hepatic stress as evidenced by elevated ALT, AST, and ALP, and reduces food intake and weight gain. These effects highlight potential risks of prolonged whole kola seed consumption, contrasting with the protective outcomes observed with purified extracts in pathological models. It is therefore recommended that consumption of whole kola seeds be moderated, particularly in healthy individuals, and further studies should investigate dose-dependent effects, long-term safety, and mechanistic pathways underlying endocrine, hepatic, and metabolic disturbances to inform dietary guidelines and public health advisories.

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