

SEX HORMONAL AND BIOCHEMICAL PROFILES OF CHRONIC CONSUMPTION OF KOLANUT SEEDS SUPPLEMENTED DIET ON EXPERIMENTAL RATS

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ABSTRACT

*This study investigated the sex hormonal and biochemical profiles of chronic consumption of kola nut seeds on fifty (50) experimental male rats that were randomly assigned to groups receiving diets containing 0%, 5%, 10%, and 20% kola nut inclusion for 12 weeks. For hormonal and biochemical analyses, blood samples were collected to analyze for follicle-stimulating hormone (FSH) luteinizing hormone (LH), testosterone, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) parameters were analyzed using standard ELISA methods. Data were expressed as mean $\pm$ SEM and analyzed using ANOVA at $p < 0.05$. Dietary inclusion of *Cola acuminata* and *Garcinia kola* significantly increased ($p < 0.05$) serum testosterone levels in all treated groups compared with control, with the highest elevations observed at 5–10% inclusion levels. In contrast, *Cola nitida* produced no significant change in testosterone across treatment groups. Despite these differences, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were significantly reduced ($p < 0.05$) in all kola nut treated groups relative to control, although partial recovery of gonadotropins was observed at 20% inclusion. Biochemically, total cholesterol levels increased significantly ($p < 0.05$) in all treatment groups across the three kola species. *Cola acuminata* caused a significant increase in LDL-c at 5% inclusion but significant reductions at higher doses, while HDL-c changes were not significant. *Cola nitida* showed significant increases in total cholesterol only, with no significant changes in LDL-c or HDL-c. *Garcinia kola* produced dose-dependent, significant increases in both total cholesterol and LDL-c, with no significant reductions in HDL-c. This study recommends moderate consumption (5–10%), and further studies on long-term safety and mechanisms are recommended.*

Keywords: Sex Hormones, Biochemical, Kola Nut, Experimental Diet and Wister Rat.

INTRODUCTION

Kola nuts, derived mainly from *Cola nitida*, *Cola acuminata*, and *Garcinia kola*, are widely consumed across West Africa due to their stimulant properties and strong cultural, social, and ceremonial significance. Traditionally,

kola nuts are chewed to combat fatigue, enhance alertness, suppress hunger, and are commonly believed to improve male sexual performance and fertility (Adebayo *et al.*, 2018). Their widespread use cuts across socioeconomic and age groups, particularly

among adult men in Nigeria and other parts of West Africa (Akinwale and Olatunji, 2020).

Phytochemical studies have demonstrated that kola nuts contain several bioactive constituents, including caffeine, theobromine, flavonoids, tannins, alkaloids, saponins, and phenolic compounds, many of which are capable of influencing metabolic, neurological, and endocrine pathways (Adegboye *et al.*, 2016; Odebiyi and Sofowora, 2017). Caffeine and theobromine, the major methylxanthines present in kola nuts, exert stimulatory effects on the central nervous system and have been shown to influence hormonal secretion through modulation of the hypothalamic–pituitary axis (Nehlig, 2018). Although these compounds possess antioxidant and anti-inflammatory properties, concerns persist regarding their long-term physiological and endocrine effects when consumed chronically (Ojo *et al.*, 2019).

In the testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) play critical roles in spermatogenesis, libido, and reproductive competence. Alterations in these hormones are often associated with impaired fertility and reduced sperm quality (Ramasamy *et al.*, 2015). Experimental investigations into kola nut consumption have yielded inconsistent findings. Some studies report that *Garcinia kola* seed extracts enhance testosterone production and sperm parameters without significantly altering gonadotropin levels (Sewani-Rusike *et al.*, 2016; Yakubu and Quadri, 2012). Conversely, other studies indicate that prolonged administration of *Cola nitida* extracts results in reduced testosterone, suppressed LH secretion, impaired spermatogenesis, and testicular histopathological changes in male rats (Umoh *et al.*, 2014; Adedara *et al.*, 2015; Ezeja *et al.*, 2015). These conflicting outcomes underscore the need for a more comprehensive evaluation of the endocrine effects of chronic kola nut seed consumption.

Despite the extensive consumption of kola nuts and persistent traditional beliefs regarding their aphrodisiac and fertility-enhancing

properties, there is need for clear scientific consensus on their long-term effects on male sex hormones. Available experimental evidence remains limited and contradictory, with some studies suggesting androgenic stimulation and others indicating endocrine disruption characterized by reduced testosterone and gonadotropin levels (Adedara *et al.*, 2015; Sewani-Rusike *et al.*, 2016). Differences in kola nut species, extraction methods, dosage, duration of exposure, and experimental models further complicate interpretation of existing findings (Adebayo *et al.*, 2018; Ojo *et al.*, 2019). Consequently, a significant research gap exists concerning the effects of chronic consumption of kola nut seeds on the male hormonal profile, necessitating further controlled experimental investigation.

Addressing this problem is important from both public health and scientific perspectives. Kola nuts are frequently consumed by men in Nigeria and other West African countries, often without awareness of potential endocrine or reproductive risks (Akinwale and Olatunji, 2020). If chronic kola nut consumption adversely affects testosterone, LH, or FSH levels, it may contribute to declining male fertility, reduced sexual function, and broader reproductive health challenges (Ramasamy *et al.*, 2015; Adedara *et al.*, 2015). Moreover, scientific evaluation of traditional claims surrounding kola nut consumption is necessary to bridge the gap between ethnomedicine and evidence-based health practices. Establishing reliable data on the endocrine effects of kola nut intake will support informed consumption, public health education, and regulatory decision-making, while contributing to the growing body of research on dietary phytochemicals and endocrine modulation (Nehlig, 2018; Ojo *et al.*, 2019).

This study is justified by the need to address the existing knowledge gap and reconcile conflicting findings in the literature regarding the reproductive endocrine effects of chronic kola nut consumption. Most previous studies have focused on short-term exposure or the administration of isolated extracts rather than

prolonged consumption of whole seeds, which more closely reflects human use patterns (Ezeja *et al.*, 2015; Adebayo *et al.*, 2018). Conflicting evidence demonstrating both hormonal stimulation and suppression highlights the necessity for well-controlled experimental studies that assess chronic exposure under standardized conditions and provide comprehensive hormonal profiling (Adedara *et al.*, 2015; Sewani-Rusike *et al.*, 2016). Given the cultural relevance and widespread consumption of kola nuts in West Africa, generating robust empirical evidence on their long-term hormonal effects is essential. Findings from this study will contribute to reproductive toxicology, endocrine physiology, and public health awareness, while providing a scientific basis for informed consumption and future research.

MATERIALS AND METHODS

Study Design

The study employed a controlled laboratory experimental design to investigate the effects of chronic dietary consumption of kola nut seeds on sex hormonal profiles in male Wistar Rats.

Collection and Preparation of Plant

Materials

Fresh kola nut seeds (*Cola nitida*, *Cola acuminata*) and bitter kola (*Garcinia kola*) were purchased from Okon Oyom Market, Akpabuyo Local Government Area, Cross River State. Botanical identification and authentication were carried out by a botanist in the Department of Botany, University of Calabar. The seeds were peeled, washed, sliced, oven-dried at 60 °C for 12 hours, and ground into fine powder using a manual blender.

Experimental Design and Acclimatization

Fifty (50) male Wistar rats weighing 120–150 g were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Calabar. The animals were housed individually in metabolic cages and acclimatized for two weeks under standard

laboratory conditions (27 ± 2 °C). All experimental procedures complied with institutional ethical guidelines for animal experimentation. Animals were randomly divided into ten groups (n = 5 per group), consisting of a control group fed standard chow and test groups receiving varying concentrations of *Cola acuminata*, *Cola nitida*, or *Garcinia kola* incorporated into their diets.

Diet Formulation and Feeding Regimen

Powdered kola nut seeds were incorporated into standard rat chow at concentrations of 5%, 10%, and 20%. The mixtures were homogenized with water, pelletized using a syringe, and oven-dried at 60 °C. Rats were fed according to their assigned dietary groups for a period of 12 weeks, with daily monitoring of feed intake and weekly measurement of body weight.

Experimental protocol

Fifty male Wistar rats were used for the study and randomly divided into ten experimental groups, with five rats in each group. Group 1 served as the control and was fed rat chow only. Groups 2, 3, and 4 received diets containing *Cola acuminata* at 5%, 10%, and 20% inclusion levels, respectively, mixed with standard rat chow. Groups 5, 6, and 7 were fed *Cola nitida* at 5%, 10%, and 20% inclusion levels, respectively. Groups 8, 9, and 10 received *Garcinia kola* at 5%, 10%, and 20% inclusion levels, respectively. All other proportions of the diets consisted of standard rat chow.

Sample Collection

At the end of the feeding period, rats were anesthetized using chloroform vapor and sacrificed. Blood samples were collected via cardiac puncture into EDTA bottles, allowed to clot, centrifuged at 2500 rpm for 10 minutes, and serum was stored frozen for biochemical analysis.

Biochemical and Hormonal Assays

Serum testosterone, FSH, and LH concentrations were determined using

microwell enzyme-linked immunosorbent assay (ELISA) techniques. Lipid profile parameters (total cholesterol, HDL, LDL) were analyzed using enzymatic colorimetric methods.

Statistical Analysis

Data obtained were expressed as mean $\pm$ standard error of mean (SEM). Statistical analysis was performed using One-Way analysis of variance (ANOVA) with SPSS software. Student's *t*-test was used for pairwise comparisons, and differences were considered statistically significant at $p < 0.05$.

RESULTS

Hormonal Profile of Experimental Rats

The effect of *Cola acuminata* diet on serum testosterone levels significantly increased ($p < 0.05$) in all test groups (5%, 10%, and 20%) compared to the control, with the highest increase at 5% and 10% inclusion. Serum LH and FSH concentrations significantly decreased ($p < 0.05$) in all treatment groups

relative to control. The 20% inclusion group showed relatively higher LH and FSH levels compared to 5% and 10%, though still lower than control.

The effect of *Cola nitida* diet on the testosterone levels showed a non-significant decrease at 5% and non-significant increases at 10% and 20% compared to control. LH and FSH levels significantly decreased ($p < 0.05$) across all treatment groups. No significant variation in LH among the test diets, while FSH showed a slight recovery at higher inclusion levels.

The effect of *Garcinia kola* diet on the testosterone concentrations significantly increased ($p < 0.05$) in all test groups, with the 10% inclusion producing the highest elevation. LH and FSH levels significantly decreased ($p < 0.05$) across all treated groups compared to control. The 20% group showed slightly higher gonadotropin levels than 5% and 10%, though still below control values.

Table 1: Effects of Kola Nut Diets on Serum Levels in Experimental Rats

Plant	Treatment	FSH (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)
<i>Cola acuminata</i> (KA)	Control	0.85 $\pm$ 0.02	0.58 $\pm$ 0.05	3.27 $\pm$ 0.06
	5%	0.27 $\pm$ 0.01*	0.11 $\pm$ 0.01*	3.81 $\pm$ 0.02*
	10%	0.32 $\pm$ 0.02* ^a	0.17 $\pm$ 0.00*	3.73 $\pm$ 0.16*
	20%	0.38 $\pm$ 0.01* ^a	0.65 $\pm$ 0.03 ^b	3.63 $\pm$ 0.06*
<i>Cola nitida</i> (KN)	Control	0.85 $\pm$ 0.02	0.58 $\pm$ 0.05	3.27 $\pm$ 0.06
	5%	0.30 $\pm$ 0.03*	0.67 $\pm$ 0.05	2.71 $\pm$ 1.16
	10%	0.38 $\pm$ 0.05*	0.17 $\pm$ 0.02*	3.94 $\pm$ 0.23
	20%	0.25 $\pm$ 0.03* ^b	0.17 $\pm$ 0.01* ^a	3.92 $\pm$ 0.30
<i>Garcinia kola</i> (GK)	Control	0.85 $\pm$ 0.02	0.58 $\pm$ 0.05	3.27 $\pm$ 0.06
	5%	0.33 $\pm$ 0.05*	0.25 $\pm$ 0.00*	4.54 $\pm$ 0.19*
	10%	0.35 $\pm$ 0.03*	0.14 $\pm$ 0.02*	5.66 $\pm$ 0.27* ^a
	20%	0.39 $\pm$ 0.01*	0.29 $\pm$ 0.11*	4.50 $\pm$ 0.19* ^b

Note: Values are expressed as Mean $\pm$ SEM. '*' indicates significant difference from control ($p < 0.05$). Superscripts a, b indicate significant differences among treatment groups within the same plant. FSH - Follicle-Stimulating Hormone and LH - Luteinizing Hormone.

Biochemical changes of the experimental animals

The effect of *Cola acuminata* (KA) seed diet on lipid profile of male rats revealed that the total cholesterol (TC) levels increased significantly ($p < 0.05$) in all KA-treated groups compared with the control. Among the test diets, the 20% inclusion group showed a significantly lower TC level than the 10% group, but was not significantly different from the 5% group. Low-density lipoprotein cholesterol (LDL-c) increased significantly ($p < 0.05$) in the 5% KA group, while significant reductions were observed in the 10% and 20% groups compared with the control. The 20% group differed significantly from the 10% group but not from the 5% group. High-density lipoprotein cholesterol (HDL-c) showed a non-significant increase in the 5% group and non-significant decreases in the 10% and 20% groups compared with the control. No significant differences were observed among the test diets, except between the 20% and 10% groups.

For *Cola nitida* (KN) seed diet on lipid profile of male rats revealed that Total cholesterol

levels were significantly higher ($p < 0.05$) in all KN-treated groups compared with the control, with no significant differences among the 5%, 10%, and 20% diets. LDL-c levels showed non-significant changes across all test diets compared with the control, and no significant differences were observed among the treatment groups. HDL-c levels exhibited non-significant increases in the 5% and 20% groups, while no change was observed in the 10% group. Differences among test diets were not significant.

The *Garcinia kola* (GK) seed diet on lipid profile of male rats revealed that the Total cholesterol increased significantly ($p < 0.05$) in all GK-treated groups compared with the control. The 20% diet produced a significantly higher TC level than the 5% and 10% diets. LDL-c levels showed non-significant increases in all test diets relative to the control; however, the 20% group had significantly higher LDL-c compared with the 5% and 10% groups. HDL-c levels decreased non-significantly in all GK-treated groups compared with the control, with no significant differences among the test diets.

Table 2: TC, LDL-c and HDL-c Comparative Changes of Experimental Animals Fed with KA, KN and GK Test Diets

Plant	Diet Group	TC (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)
Reference Range	—	10–54	7–27	>35
<i>Cola acuminata</i> (KA)	Control	25.67 ± 0.33	16.67 ± 0.88	16.00 ± 0.00
	5%	48.00 ± 1.15*	25.33 ± 1.33*	16.67 ± 0.67
	10%	33.33 ± 2.67* ^a	15.33 ± 2.67 ^a	13.33 ± 0.33 ^a
	20%	31.33 ± 0.67* ^a	13.67 ± 0.88*	14.67 ± 1.67
<i>Cola nitida</i> (KN)	Control	25.67 ± 0.33	16.67 ± 0.88	16.00 ± 0.00
	5%	42.01 ± 1.15*	19.61 ± 0.88	16.67 ± 0.67
	10%	39.35 ± 6.67*	16.38 ± 1.20	16.00 ± 1.15
	20%	36.61 ± 2.67	16.67 ± 2.40	17.53 ± 0.33
<i>Garcinia kola</i> (GK)	Control	25.67 ± 0.33	16.67 ± 0.88	16.00 ± 0.00
	5%	38.01 ± 1.15*	17.67 ± 0.67	14.33 ± 0.67
	10%	39.33 ± 2.67*	18.61 ± 2.03	15.00 ± 0.00
	20%	52.67 ± 1.76* ^a	24.64 ± 1.45* ^b	15.41 ± 2.60

Values are expressed as mean ± SEM (n = 3). Significantly different from control at $p < 0.05$

^a Significantly different from KA 5% at $p < 0.05$, ^b Significantly different from KA 10% at $p < 0.05$. TC– Total Cholesterol, LDL-c – Low-Density Lipoprotein Cholesterol, HDL-c – High-Density Lipoprotein Cholesterol.

DISCUSSION

The present study's findings on the hormonal and lipid profile effects of *Cola acuminata*, *Cola nitida*, and *Garcinia kola* diets in rats contribute valuable insights into the biochemical impacts of these kola nut species, which have been traditionally used for their stimulant and aphrodisiac properties. The significant increase in serum testosterone observed with *Cola acuminata* and *Garcinia kola* diets, particularly at 5% and 10% inclusion levels, supports their reputed role in enhancing male reproductive function. This aligns with studies from Ademiluyi (2018) demonstration that *Cola acuminata* and *Garcinia kola* extracts possess antioxidant properties and inhibit enzymes linked to erectile dysfunction, such as arginase and acetylcholinesterase, which may facilitate increased nitric oxide availability and improved penile blood flow, thereby enhancing testosterone synthesis and sexual function. However, the concurrent significant decrease in LH and FSH across all treatment groups contrasts with Mohamed *et al.* (2024), who reported that *Garcinia kola* increased both testosterone and gonadotropins in diabetic rats, suggesting that the hormonal response may be influenced by physiological or pathological context, dosage, or duration of exposure. The slight recovery of gonadotropin levels at higher inclusion rates in this study may indicate a dose-dependent modulation of the hypothalamic-pituitary-gonadal axis, warranting further mechanistic exploration.

In contrast, *Cola nitida*'s effect on testosterone was non-significant, with a slight decrease at 5% and increases at higher doses, while LH and FSH consistently decreased. This pattern is partially supported by Sanusi *et al.* (2024), who reviewed *Cola nitida*'s bioactive compounds caffeine, alkaloids, tannins, and flavonoids and noted its complex influence on endocrine function, including potential stimulatory effects on metabolism but possible adverse effects with chronic use. Tonbra *et al.* (2023) further reported that chronic *Cola nitida* consumption in human's elevated liver and kidney biomarkers without significantly

altering reproductive hormones, highlighting potential hepato-renal toxicity risks despite traditional use as a stimulant and aphrodisiac. These findings underscore the importance of considering duration and dosage in evaluating *Cola nitida*'s safety and endocrine effects.

Regarding lipid metabolism, the study found significant increases in total cholesterol (TC) across all kola nut diets, with variable effects on LDL-c and HDL-c. *Cola acuminata* increased LDL-c at 5% but reduced it at higher doses, while *Garcinia kola* showed a dose-dependent increase in both TC and LDL-c, and *Cola nitida* caused elevated TC without significant LDL-c or HDL-c changes. These results partially diverge from Mohamed *et al.* (2024), who observed that *Garcinia kola* and *Cola acuminata* extracts reduced serum total cholesterol in Wistar rats, suggesting that whole diet inclusion may have different metabolic effects than extract administration. Similarly, Salawu *et al.* (2021) demonstrated that aqueous extracts of *Cola acuminata* ameliorated hyperlipidemia induced by a high-fat diet, reducing atherogenic indices, which contrasts with the cholesterol elevations seen here. This discrepancy may be due to differences in preparation (whole seed powder versus extracts), dosage, or experimental models.

The protective potential of *Cola nitida* on lipid-induced organ damage is supported by Oni *et al.* (2024a, 2024b), who showed that kola nut extracts improved liver and pancreatic biomarkers in lipid-induced Wistar rats, reducing oxidative stress and fat deposition, and preserving pancreatic β -cell function. These studies suggest that bioactive compounds such as caffeine, flavonoids, and theobromine in *Cola nitida* may exert hepatoprotective and metabolic regulatory effects, which could mitigate some adverse lipid profile changes. However, the current study's finding of increased total cholesterol with *Cola nitida* diet indicates that these protective effects may not fully translate to lipid profile normalization, possibly due to differences in dosage, duration, or the form of kola nut administered.

The observed decrease in gonadotropins (LH and FSH) despite increased testosterone in *Cola acuminata* and *Garcinia kola* groups may reflect negative feedback regulation within the hypothalamic-pituitary-gonadal axis, where elevated testosterone suppresses gonadotropin release. This mechanism is consistent with endocrine physiology but contrasts with some reports of increased gonadotropins following kola nut extract administration, suggesting that whole seed consumption might exert more complex or biphasic effects on reproductive hormones. The non-significant testosterone changes with *Cola nitida*, coupled with decreased gonadotropins, may indicate a less potent or different mode of action on the reproductive axis, possibly influenced by its unique phytochemical profile.

The lipid profile alterations, particularly the increase in total cholesterol, raise concerns about the cardiovascular implications of chronic kola nut consumption. While some studies highlight the antioxidant and lipid-lowering potential of kola nut extracts, the elevation of cholesterol observed here suggests that whole seed diets may contribute to dyslipidemia, especially at higher inclusion levels. This is important given the widespread traditional use of kola nuts and their caffeine content, which can influence lipid metabolism and cardiovascular risk factors. The differential effects on LDL-c and HDL-c among the three kola species further emphasize the need for species-specific evaluations and careful consideration of dosage and preparation methods.

In summary, the study corroborates the aphrodisiac and testosterone-enhancing effects of *Cola acuminata* and *Garcinia kola*, while highlighting a complex interplay with gonadotropins that may depend on dose and physiological context. *Cola nitida* exhibits more variable hormonal effects and potential hepato-renal risks with chronic use. The lipid profile findings suggest that whole seed consumption of these kola nuts may elevate total cholesterol, contrasting with some extract-based studies that report lipid-lowering effects. These discrepancies underscore the

importance of distinguishing between whole seed and extract forms, as well as considering dosage, duration, and health status in interpreting kola nut effects. Future research should focus on elucidating the molecular mechanisms underlying these hormonal and lipid changes, assessing long-term safety, and exploring the therapeutic potential of standardized kola nut preparations for reproductive and metabolic health.

CONCLUSION

This study demonstrates that dietary inclusion of *Cola acuminata*, *Cola nitida*, and *Garcinia kola* seeds produces distinct and dose-dependent effects on hormonal and lipid profiles in experimental rats. *Cola acuminata* and *Garcinia kola* significantly enhanced serum testosterone levels, particularly at 5% and 10% inclusion rates, supporting their traditional use as aphrodisiacs. However, these increases were accompanied by significant reductions in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), suggesting negative feedback regulation of the hypothalamic-pituitary-gonadal axis. In contrast, *Cola nitida* produced non-significant changes in testosterone levels while consistently suppressing gonadotropins, indicating a weaker or different endocrine modulatory effect. Biochemically, all kola nut diets elevated total cholesterol levels, with *Garcinia kola* showing dose-dependent increases in both total cholesterol and LDL-c, raising concerns about potential cardiovascular risks. Although *Cola acuminata* reduced LDL-c at higher inclusion levels and *Cola nitida* showed minimal effects on LDL-c and HDL-c, the overall lipid profile alterations suggest that whole-seed consumption may predispose to dyslipidemia.

Recommendations

Based on these findings, moderate consumption of kola nuts, particularly at lower inclusion levels (5–10%), is advisable to balance potential reproductive benefits with metabolic safety. Prolonged or high-dose intake, especially of *Garcinia kola*, should be approached with caution due to its lipid-

elevating effects. Future research should focus on long-term toxicity and mechanistic studies to clarify the pathways involved in hormonal modulation and lipid metabolism, with particular emphasis on differentiating the effects of whole-seed diets from standardized extracts. Additionally, controlled human studies are necessary to validate these animal findings and inform evidence-based dietary or therapeutic recommendations.

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