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EFFECTS OF *GARCINIA KOLA* ETHANOLIC EXTRACTS ON SEX HORMORNES IN STARVATION-INDUCED STRESS MALE WISTAR RATS MODEL

[1]Kianen Sekiita, [2]*Dr. Solomon M. Uvoh, [3]Dr. Emily Kiridi G. E. and [4]Bonnie K. Goodhope

[1]Department of Human Physiology School of Basic Medical Sciences, College of Health Sciences University of Benin, Benin CityEdo State, Nigeria.
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences University of PortHarcourt, Rivers State Nigeria.
[3]Department of Human Physiology, Faculty of Basic Medical Sciences College of Health Sciences Niger Delta University Ammasona Bayelsa State, Nigeria.
[4]Department of Chemistry, Faculty of Science University of Lagos, Lagos State, Nigeria.

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***Corresponding author:**

***Dr. Solomon M. Uvoh**

Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences University of Port Harcourt, Rivers State Nigeria.

ABSTRACT

Aim/Objective: This study investigated the Effects of Starvation-Induced Stress and ethanolic extracts of *Garcinia kola* on sex hormones in male wistar rat's models. **Materials/Methods:** forty male wistar rats, weighing between 120-200g were purchased and acclimatized for a period of 14 days. They were divided into 4 Groups; Groups A (control) was fed with standard diets and water only with no starvation while groups B, C and D were each treated with 1ml of G. kola extract at different concentration. The experiment for starvation induced stress lasted for 42 days while that for the ethanolic extract of *G. kola* took 28 days. Group B were fed with 15 hours SD with 9 hours of starvation and treated at 25mg/kg. Group C were fed with 6 hours SD with 18 hours of starvation and treated at 50mg/kg while Group D were fed with 12 hours and treated at 100mg/kg ethanolic extract of G-Kola respectively. The Layman and Tiet method was used to determine the various sex hormones. **Results:** The change in body weights was (control = 58.50g, test groups=40.50g, 56.50g) while those administered ethanolic extracts of *G. kola* at 25mg/kg(1.55ng/ml), 50mg/kg (0.98ng/ml) and 100mg/kg (0.96ng/ml) was observed to progressively decrease LH serum level compared with the control. Similar decrease was also observed in FSH and testosterone as well when compared with the control group respectively while FSH, LH and Testosterone remain unchanged ($p>0.05$) in starved rats administered SD and water only. **Conclusion:** The results of this study indicated a remarkable decrease in male reproductive sex hormones in starvation induced stressed male wistar rats administered prolonged G. *kola* ethanolic extracts.

KEYWORDS: Kola, Garcinia, Starvation, Weight, Stress.

INTRODUCTION

Stress is a state of mental and emotional strain resulting from demanding circumstances. It involves complicated immunological, biochemical mechanisms and the human body responds to it with a fight or flight reaction. Therefore, the cognitive, behavioural status of an

organism is remodelled following the lifelong self-adjustment and self-optimization processes to meet the ever-changing demand. Stress induced by physical or emotional challenges produces alterations in reproductive functions. For example, males may exhibit suppression of testosterone secretion, spermatogenesis, and libido (Collu *et al.*, 1984; Rabin, *et al.*, 1988). Prolonged starvation which includes deliberate lengthy fasting may also have some effects on the gonadotropic hormone level (Gonzales., 2004) and the testosterone level in fertile males, either via the hypothalamus-pituitary-testicular axis or by direct effect on the testis, and could therefore affect spermatogenesis. The quality of spermatozoa is a significant determinant of male fertility.

This is evident in countries where famine had prevailed where some links have been seen between prenatal famine and birth weight, reproductive performance, and age of menopause of the conceived child (Elias, 2005).

Garcinia kola, which is an angiospermae belonging to the family of Guttiferae is commonly known as bitter cola. *G. kola* seed, on chewing, has a bitter styptic and resin-like taste, seemingly similar to that of raw coffee, followed by a slight agreeable taste. It is widely referred to as bitter kola in English name, orogbo in Yoruba, Aku ilu in Igbo and Namijin goro in Hausa. Bitter cola is a highly valued constituent in ethno medicine of Africa because of its numerous and varied uses which are medicinal and social; thus, making the plant an essential component in folk medicine. Medicinal seeds such as *G. kola* are believed to be an important source of modern chemical substances with potential curative benefits (Buba *et al*., 2016). *G. kola* seed is known to contain certain nutritional components such as moisture, ash (minerals), crude fat, crude fibre and crude protein (total nitrogen) and Carbohydrate (nitrogen free extract) (Onyeike and Osuji, 2003). These nutritional components could be analysed by a chemical method known as the proximate analysis. These nutritive components are known to be complemented with certain antinutrients such as Tannin, Oxalate, Phytate and Trypsin inhibitor. *G. kola* seed extracts are also known for their non-nutritive phytochemical composition. These include Phenol, Saponin, Alkaloids, Flavonoids and Glycosides (AOAC, 1990).

Undue starvation has been consciously induced by many for checking weights, fitness, appetite and hence the resulting consequences (Seriki *et al*., 2015). In addition, starvation is a common component of religious fasting, implications of which are not known to those engaging in them. There seems to be a link between diets and starvation in respect to various body systems especially reproduction. There is a trending need to inquire about the possible beneficial and deleterious effects that ethanolic extracts of *G. kola* exert on reproductive parameters. Extracts of *Garcinia Cola* have been reported for its use in African traditional medicine for the cure of illnesses like laryngitis, liver diseases, cough, hoarseness of voice, diabetes and other related complications (Adedara & Farombi, 2015), but there is a paucity of information from literatures on the effect of Cola Extracts on reproductive parameters in male wistar rats (Oyedeji *et al*., 2020)

***Garcinia kola* (Bitter Cola) seeds**

MATERIALS AND METHODS

The materials used include simple weighing scale, wooden cage, wistar rats (40), standard diets (normal laboratory Shaw), water, *Garcinia kola* (bitter kola).

A. 1ml of serum testosterone calibrates containing serum references for testosterone at concentrations of (a) 0.1 (b) 0.5 (c) 1.0 (d) 2.5, (e) 5.0 (f) and (g) 12.0 all in ng/ml.

B. Mouse monoclonal anti-α-FSH antibody coated micropiece with 96 wells.

C. Mouse monoclonal anti-α-LH antibody coated microplate with 96 wells.

D. Follicle stimulating Hormone and luteinizing Hormone reference standards.

E. Enzyme and conjugate reagent

F. One 7.0ml steroid conjugate buffer.

G. 6ml of one testosterone biotin reagent

H. Streptavidin coated plate contains 96 wells coated with 1.0µg/ml streptavidin.
I. 20ml of one wash solution concentrate.
J. One substrate A – 7ml and contains tetramethylbenzidine (TMB) in buffer.
K. One stops solution of 8ml and contains a strong acid (1NHCL). All these materials come with the testosterone kit and were stored at a temperature of 2.8^{o}C.

Other materials which will be provided in the laboratory are:
Pipette capable of delivering 10µl, 50µl and 100µl with a precision of at least 1.5%
Dispenses for repetitive delivery of 0.100ml and 0.350ml with precision of 1.5%
Adjustable volume (200-1000µl) dispenses for conjugate
Microplate washer
Microplate reader with 450mm and 650mm wavelength absorbance capability
Absorbent paper
Microplate cover
Vacuum aspirator
Times
METHODS USED TO DETERMINE THE HORMONES: FSH-Layman, LH- Layman, and Testosterone- Tiet method

SERUM HORMONAL ANALYSIS
While the animals were being pinned in a supine position on the dissecting board prior to dissection, 5ml of blood sample were collected from each rat through cardiac punctures and was temporarily stored in a plain centrifuge tube and labelled. After some time, the plain centrifuge tube containing the blood samples were centrifuged at 3000rev/min for fifteen (15) minutes using a centrifuging machine; then the sera were separated from the cells and stored in a sample bottle labelled and frozen at 4^{o}C before being used for hormonal assays. The stored sera were analysed for testosterone, follicle stimulating hormone (FSH) and luteinizing hormones (LH) following the outline of Uotila, *et al*., (1981); with the hormonal kit supplied by Monobind Inc. Lake Forest, CA 92630. USA with product code: 3725-300; for testosterone test system and Biocheck, Inc 323 Vintage Park Dr. Foster city, CA 94404; for FSH and LH.

GROUPING AND ADMINISTRATION OF EXTRACT TO EXPERIMENTAL ANIMALS
Forty (40) adult male wistar rats, each within the confined weights of 120-200g were purchased and acclimatized for a period of fourteen (14) days. They were divided into four (4) groups (A, B, C, D of ten (10) rats per group. Groups A serve as the control while B, C and D were fed with standard diets (SD and treated with ethanolic extract of *G. kola* at 25, 50, and 100mg/kg body wt. respectively.

Figure 1: Lab experimental male wistar rat sample used for this study.

Starvation Induced Stress with Standard Diet
The methodology of the starvation induced stress was in line with the work performed by Iranloye *et al*., 2013.

Ethanolic Extract of *G. kola*
Ethanolic extract of *G. kola* preparation were in accordance with Atsukwei *et al*., 2015.

Plant Materials: Fresh specimens of cola were bought from Benin boundary market and authenticated in the taxonomy unit of the department of Plant Biology and Biotechnology, Uniben, Benin City.

Experimental Design
- Group A (Control 1): 24 hours SD and water ad libitum No Starvation
- Group B: ……15 hours SD and water hours' starvation daily/25mg kg^{-1} body weight of *G. kola* Extracts
- Group C: ……6 hours SD and water 18 hours' starvation daily/50mg kg^{-1} body weight of *G. kola* Extracts
- GROUP D: ……. 8 hours SD and water, 12hours starvation daily/100mg kg^{-1} body weight of *G. kola* Extract

Bioactive Constituents of *Garcinia kola* Leaf Extracts (Anadebe *et al.*, 2021)

Table Bioactive constituents of BKL extract.

Active compounds	Qualitative	Quantitative/mg100 g^{-1}
Flavonoids	++	763
Alkaloids	++	1355
Phenolics	+++	124
Phytates	++	73
Saponins	++	80
Tannins	++	940
Cardiac glycosides	+	213

SAMPLE COLLECTION AND ANALYSIS

Five rats from each group were sacrificed. Each of the rats was then anesthetized in a desiccator containing cotton wool soaked with chloroform, laid in a supine position, on a dissecting board and with limbs fastened to the board with dissecting pins under this condition 5ml of blood were collected by cardiac puncture for hormonal analysis.

WEIGHT MEASUREMENT

The body weights of each rat were measured at the beginning and at the end of the experiment using a simple weighing scale. Changes in their weight differences were determined by subtracting the initial weights from the final weights.

STATISTICAL ANALYSIS

SPSS version 23.0 was used.
Data were analysed using (ANOVA) followed by;
Dunnett post Hoc for multiple comparisons.
Values were considered significant at 95% confidence interval ($P<0.05$).

RESULTS

The results are presented as Mean ± SEM

Table 1: Comparing the mean values of weight of test group with Control.

Parameters	Control	9 Hours Starvation	18 Hours Starvation
Initial body weight (g)	145.0 ± 1.78	134.0 ± 3.85	140.0 ± 6.81
Final body weight (g)	203.5 ± 5.32	174.5 ± 7.26	196.5 ± 9.29
Body weight change (g)	58.50 ± 7.01	40.50 ± 4.11	56.50 ± 6.65

Table 2: Hormonal assay markers mean values compared with standard diet.

Parameters	Control	9 Hours Starvation	18 Hours Starvation	12Hours Starvation
Luteinizing hormone (ng/ml)	2.323 ± 0.166	2.150 ± 0.27	2.200 ± 0.26	2.180± 0.22
Follicle stimulating hormone (ng/ml)	2.953 ± 0.64	2.830 ± 0.25	3.255 ± 0.22	2.960± 0.24
Testosterone level (ng/ml)	1.665 ± 0.45	1.275 ± 0.13	1.525 ± 0.41	1.32± 0.12

ˣ$P < 0.05$ indicates significant different.

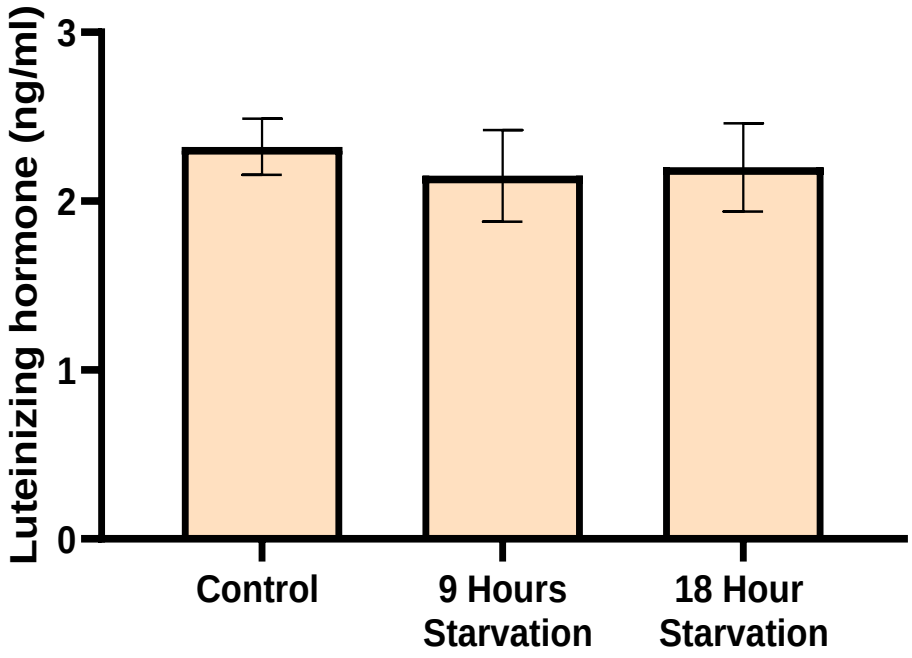

Figure 2: Hours of starvation effect on luteinizing hormone on standard diets.
There were no significant differences in 9 hours and 18 hours' starvation group compared with control.

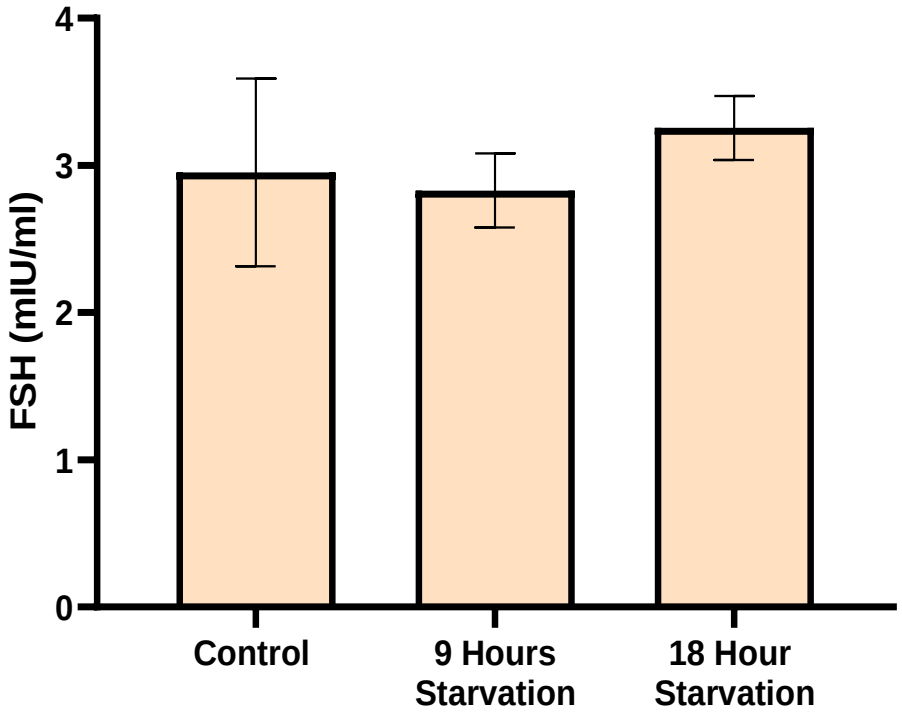

Figure 3: Effect of hours of starvation on follicle stimulating hormone on standard diets.
There were no significant differences in 9 hours and 18 hours' starvation group when compared with control.

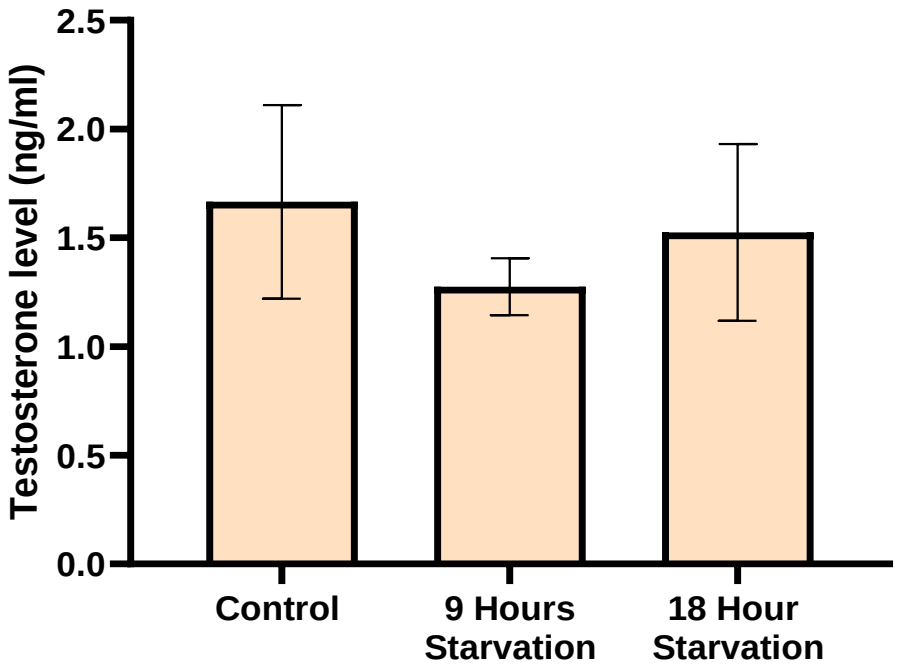

Figure 4: Effect of hours of starvation on testosterone level of Wistar rats fed with standard diets.
There were no significant differences in 9 hours and 18 hours' starvation group when compared with control.

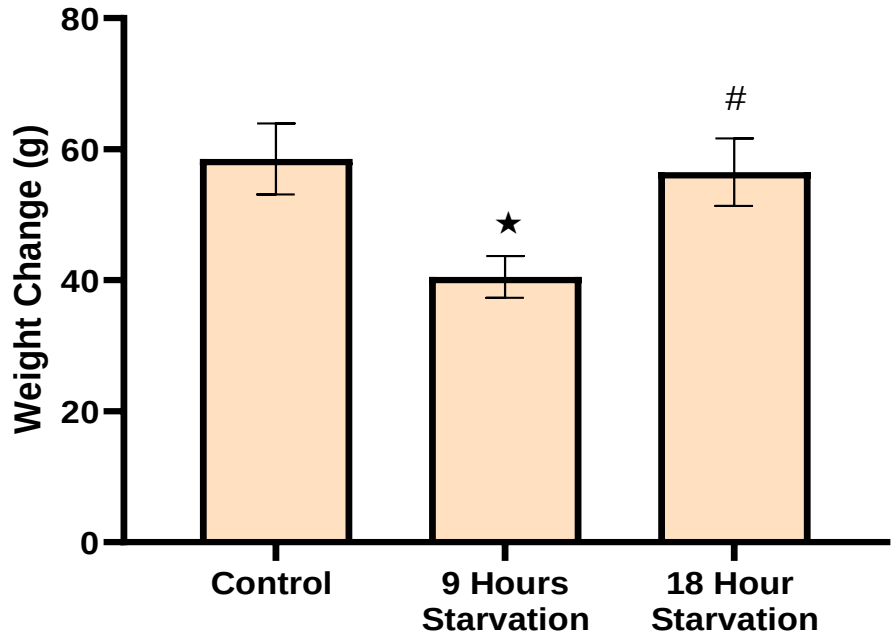

Figure 5: Effect of hours of starvation on the weight change on standard diets.
There was a significant decrease in 9 hours starvation compared with control and 18 hours starvation group, but there was no significant difference in 18 hours starvation compared with control.
*** = Significant**

Table 3: Comparing the mean values of different hormonal assays administered with ethanolic extract of *G. kola* at different doses.

Parameters	Control	25mg/kg	50mg/kg	100mg/kg
Luteinizing hormone	2.323 ± 0.166	1.550 ± 0.249	0.9850 ± 0.11	0.9625 ± 0.151*
Follicle stimulating hormone	2.950 ± 0.636	1.800 ± 0.27	1.130 ± 0.16	1.198 ± 0.34*
Testosterone level	1.665 ± 0.45	1.043 ± 0.18	0.6050 ± 0.14	0.4550 ± 0.12*

* P < 0.05 indicates significant different.

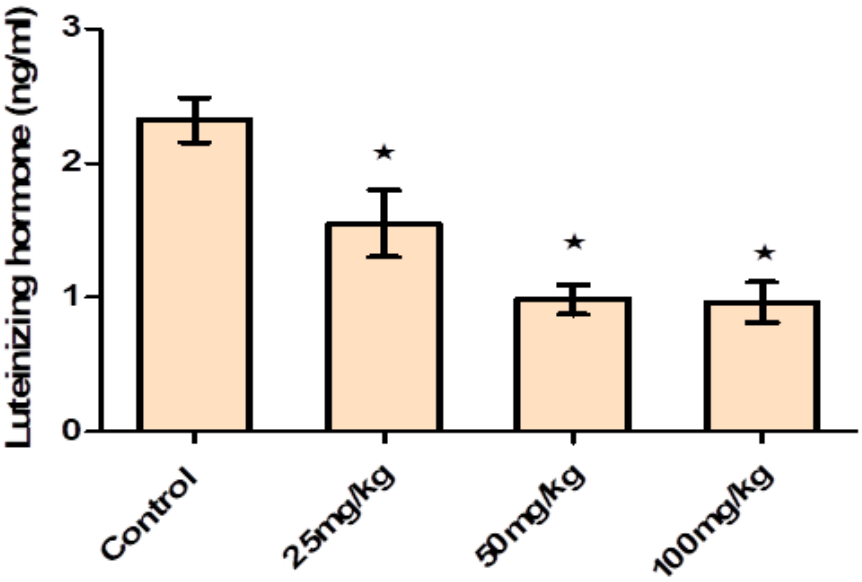

Figure 6: Effect of *G. kola* ethanolic extract on luteinizing hormone.

There were significant decreases in 25mg/kg, 50mg/kg and 100mg/kg when compared with control

*** = Significant**

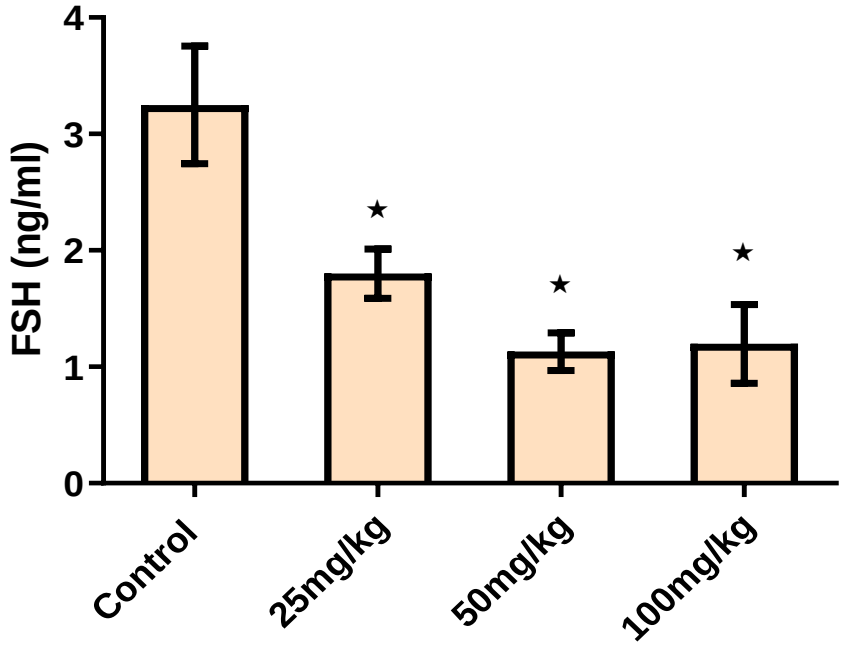

Figure 7: Effect of *G. cola* ethanolic extract on the follicle stimulating hormone of Wistar rats.

A significant decrease was observed in the 25mg/kg, 50mg/kg and 100mg/kg when compared with control.

*** = significant**

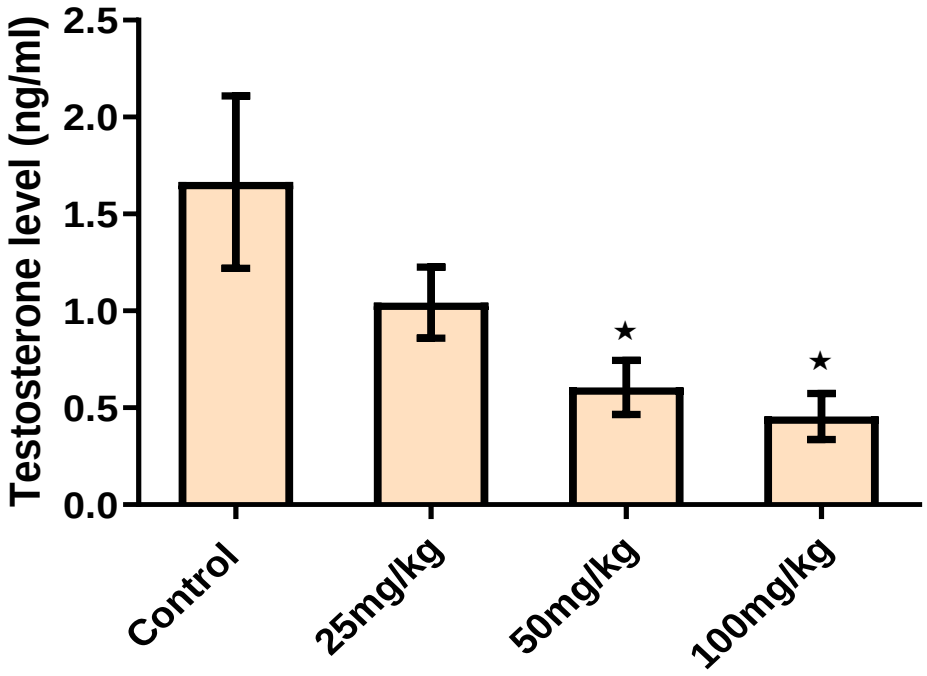

Figure 8: Effect of *G. kola* extract on testosterone.

There were significant decreases at 50mg/kg and 100mg/kg compared with control, though; there was no significant observation in 25mg/kg compared with control.

*** = Significant**

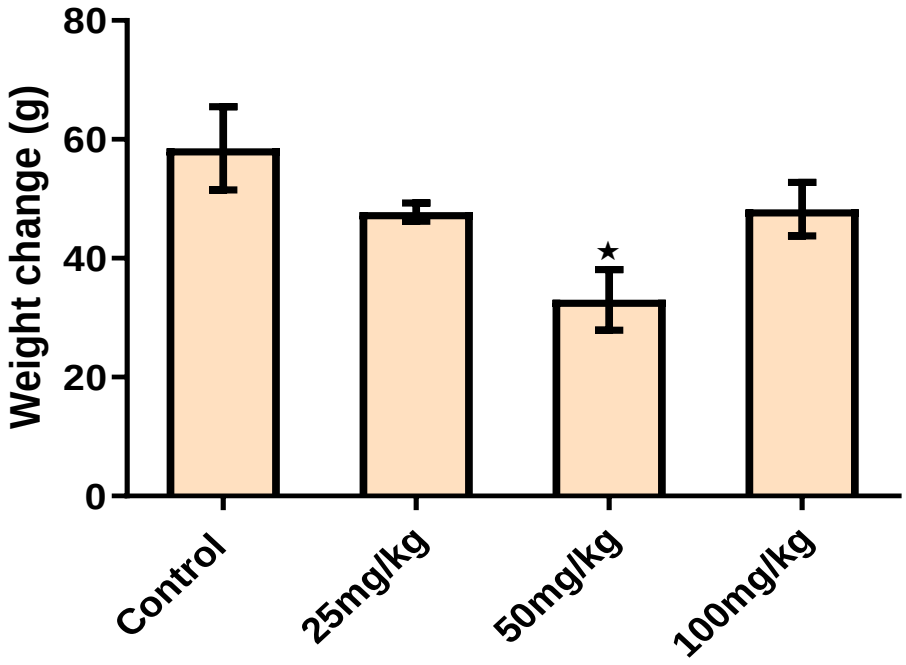

Figure 9: Effect of *G. kola* extract on body weight change.

There was significant decrease at 50mg/kg compared with control, though; there were no significant changes in 25mg/kg and 100mg/kg compared with control.

*** = Significant**

DISCUSSION

In all three hormones - Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone (T), there were no significant difference when both the 9- 18-hours starvation were compared to Control in rats fed with standard diets. This shows that both moderate and prolonged starvation have no effect on the Male Reproductive Hormones when compared to the regularly fed rats.

Body Weights: The significant decrease in the 9 hours starvation for the body weight change of wistar rats on standard diets when compared to the control indicates the suppressive effect of the moderate and intermittent fasting on the body weight of wistar rats. This was not so with the body weights change of the 18 hours intense and prolonged starvation. This is probably due to inhibition of the appetite centres of the hypothalamus and hence reduced feeding urge. That is, inhibition of the lateral hypothalamic nuclei and a corresponding stimulation of the ventromedial hypothalamic nuclei (satiety centre) causes undesirous feeding resulting in increased weight loss (Burbach *et al*, 2001). Also, stimulation of the Para ventricular nuclei and lesion of the dorsomedial nuclei reduced eating with the similar growth inhibitory effects (Lohmeier, 2003). However, beyond the moderate (9 hours) starvation, body weight began to increase towards the control. The significant decrease observed in mean Body Weight Change of rats administered with 50mg/kg of *G. kola* extracts may be due to the hypoglycaemic effect of moderate dose of the extract administered. It has been reported that biflavonoids drastically lowers the blood sugar level and induced hypoglycaemia and anorexia similar to those of tolbutamide. The crude extracts of flavonoids found in *G. kola* seed contain hydroxyl citrate which inhibits fatty acid synthesis and lipid accumulation of the rat liver (Lowenstein., 1971, Sullivan *et al*., 1974, Ibekwe *et al*., 2006)

LH and FSH are known as gonadotropins having attained stimulatory affinity with the Gonads, the male testes. They are produced by the anterior pituitary gland cells categorized as gonadotrophs. LH binds to Leydig cells receptors in the testes to stimulate the synthesis and secretion of testosterone. LH increases cAMP after binding to cells which elevates the side chain cleavage of cholesterol and protein secretion together with other similar steps to increase the steroidogenesis and testosterone synthesis of all other androgens (Adienbo *et al*., 2015; Alhrbi & AL-sowayan, 2020; Sekiita et al., 2023). Testosterone acts on the Sertoli and peritubular cells of the seminiferous tubules and directly enhances spermatogenesis stimulation (Singh *et al*., 1995; Bhasin *et al*., 1987; O'Donnell *et al*., 1994 Emily et al., 2023).

Ethanolic extract of *G. kola* at Different Doses

Significant decreases observed in mean Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) of rats administered with 25mg/kg, 50mg/kg and 100mg/kg of *G. Cola* extracts signifies that direct action of *G. kola* on the testes may have caused inhibition of gonadotropic action on the testes. This was shown by Price *et al*., (1987) who observed an irreversible combination of saponins with membranes in animal cells, thus rendering the membrane non semipermeable. Other possibilities include preventing the release of pituitary gonadotropins via secretion of inhibin by the Sertoli cells and/or elevation of blood levels of testosterone (by inhibition of hepatic metabolism) thereby inducing negative feedback effect on gonadotropin release. This may be the mechanism in which *G. kola* decreases the serum levels of LH and FSH in rats. The most plausible explanation of the observations in male rats in this study could be that *G. kola* inhibits gonadotropic action on the testes.

The significant decreases observed in mean Testosterone (T) of rats administered with 50mg/kg and 100mg/kg gives the indication that ethanolic extract of *G. Cola* possesses crude flavonoid and crude alkaloid content that either depresses the ability of the Leydig cells to secrete testosterone, interferes with the secretion of LH as observed previously or both. This finding agrees with the work of Braide *et al*., 2003 who equally reported decreased serum testosterone of rats treated with crude alkaloid extract of *G. kola* seed. This findings is contrary to Emily et al., 2024 using combination of tigernut, soybean and datefruit with similar phytochemical constituents.

CONCLUSION

The results of this study indicated a generalised deleterious effects with a slight positive change in the starvation-induced stressed rats fed with SD while prolonged administration of ethanolic extracts of *G. kola* induces remarkable weight reduction and a remarkable decrease in all three male reproductive hormones (FSH, LH and Testosterone) respectively.

CONFLICT OF INTEREST: Non declared.

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