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Effect of Ethanolic Extract of *Garcinia kola* on Sex Hormones of Adult Male Wistar Rats

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Abstract:

➢ ***Background:***

Sexual dysfunction is one of the major factors associated with marital failure and poor quality of life. One of the most commonly used herbs in traditional medicine is *Garcinia kola*, also known as bitter kola, which is said to have aphrodisiac properties. *Garcinia kola*, also known as bitter kola, is one of the herbs widely used in traditional medicine with controversial effects. The objective of the present study was to examine the effects of ethanolic extract of seeds of *Garcinia kola* on sexual behavior, hypothalamic – pituitary – gonadal axis and reproductive morphology in adult Wistar rats.

➢ ***Methods:***

Prospective experimental study was done with 70 rats of Wistar strain (35 females and 35 males) were divided into treatment and control groups. Seed extract doses ranged from 200 to 600 mg/kg and sildenafil and estrogen/progesterone were used as standard controls. Phytochemical Screening, LD_{50} determination, hormonal assays (LH, FSH, testosterone), sexual behavior tests (copulatory arenas and pacing chambers), and histological examination of the gonads and hypothalamus were performed. Descriptive and inferential statistics were used to analyse data.

➢ ***Result:***

The phytochemical findings showed that the contents of carbohydrates (+++), crude fiber (++), flavonoids (++), and saponins (++), were high. The LD50 was found to be above 4800 mg/kg, which is safe. Male rats showed dose-dependent increases in testosterone (0.3 ± 0.1 ng/ml at baseline vs. 6.2 ± 0.8 ng/ml at 600 mg/kg, p = 0.01), LH (3.2 ± 1.0 vs. 6.4 ± 1.9 mIU/ml), and FSH (4.7 ± 0.8 vs. 11.0 ± 0.7 mIU/ml). Female rats exhibited significant increases in LH (2.3 ± 0.7 vs. 15.9 ± 1.0 mIU/ml, p = 0.05) and FSH (4.9 ± 1.3 vs. 26.0 ± 0.7 mIU/ml, p = 0.01), but no rise in testosterone. Sexual behavior improved markedly: male mount frequency rose from 5.5 ± 3.5 (control) to 23.1 ± 2.1 (500 mg/kg, p = 0.03), while intromission frequency increased from 12.5 ± 3.5 to 36.5 ± 6.3. Female solicitation rose from 3.0 ± 0.1 to 25.8 ± 0.7 (600 mg/kg, p = 0.01),

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with lordosis quotient reaching 86.5 ± 15.1 compared to 0.0 in controls. Histology confirmed that the treated groups had larger seminiferous tubules and improved gonadal morphology.

➢ ***Conclusion:***

Ethanolic seed extract of *Garcinia kola* exhibited excellent aphrodisiac activity in male and female Wistar rats, which was primarily attributed to its action of enhancing hormones and stimulating the central nervous system. The results confirm its ethnopharmacological applications in sexual dysfunction and warrant further research to isolate active constituents and evaluate the long-term effects.

Keywords: *Garcinia kola, Aphrodisiac, Sexual Dysfunction, Wistar Rats, Hypothalamic-Pituitary-Gonadal Axis, Reproductive Behavior.*

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I. INTRODUCTION

Human marriages are about sexual fulfillment and procreation, and sexual dysfunction is a leading cause of marital breakdown (Onyinye & Godson Emeka, 2019; Shakerian et al., 2014). Sexual dysfunction is any problem associated with sexual activity at any point in the process, from desire to arousal, to orgasm or physical pleasure. While it typically doesn't pose a direct threat to physical well-being, it can contribute to depression, anxiety, and a sense of inadequacy, which in turn can decrease quality of life (Ågmo, 1997; Damam et al., 2024).

Substances believed to increase sexual interest or activity are known as aphrodisiacs and have been used in many cultures including the use of Panax ginseng in China and chocolate (and oysters) in contemporary diet (Pratap Singh, 2024; Singh et al., 2011). Animal study has shown the aphrodisiac activity of plants like Terminalia catappa, Syzygium aromaticum and Fadogia agrestis in Nigeria (Yanuarty et al., 2024). Of these, *Garcinia kola* (bitter kola) has a long history of use as a stimulant, and is traditionally reputed to have aphrodisiac properties (Adaramoye & Arisekola, 2013; Tauchen et al., 2023a).

But there is mixed data out there. Enhanced libido and improved testicular function has been reported in rats while others have concluded that there is no aphrodisiac activity of *Garcinia kola* or it can even reduce sexual behavior and hormonal activity (Akpantah et al., 2005; Yakubu et al., 2005). This is a discrepancy that requires further investigation of its effects on sexual behaviour and the hypothalamic-gonadal axis.

Sexual dysfunction is rising around the world, and the side effects of mainstream treatments may not be beneficial, or they can become resistant. The scientific documentation on the aphrodisiac effects of *Garcinia kola* is limited and contradictory, especially when it comes to both males and females. The aim of this study is to bring clarity to this by investigating the ethanolic seed extract of *Garcinia kola* in adult Wistar rats.

The aim is to assess the effect of the ethanolic extract of the seed of *Garcinia kola* on sexual behavior, hypothalamus, pituitary glands and gonads of adult male Wistar rats. Test the impact on sexual behaviour (in copulatory arenas for males). Study changes in both the morphology of the hypothalamus and the gonad. Measure levels of hormones (LH, FSH, testosterone). Identify dose dependent actions on hypothalamus and gonads.

➢ *Null Hypotheses*

- H_o1: There is no significant difference in libido and sexual behavior of rats given *Garcinia kola* extract.
- H_o2: The extract will not significantly affect LH, FSH, and testosterone levels.
- H_o3: There is no significant difference in hypothalamus and gonad histo-architecture between treated and untreated rats.

II. MATERIALS AND METHODS

➢ *Study Design and Setting*

This was a prospective experimental study to evaluate the effect of ethanolic extract of seeds *Garcinia kola* on sexual behaviour and hypothalamic–pituitary–gonadal axis in adult Wistar rats. The study was carried out at Ebonyi State University, Abakaliki, Nigeria. The geographical position of Abakaliki is 6.32N latitude, 8.12E longitude and 117m above sea level. It is an agricultural trade centre where yam, cassava, rice, palm oil and kernel are produced, which served as a natural laboratory for ethnopharmacological studies.

➢ *Study Population and Animal Selection*

The number of animals used was 70 adult rats (70 males (200- 250 g)). The animals used were bought from the animal house of Faculty of Medical Sciences Ebonyi State University. They were acclimatized for two weeks under the normal laboratory conditions before the experiment was started. The rats were kept in well-ventilated wire-bottom steel cages at a temperature of $28\pm2\circ C$, relative humidity of 45-50% and a 12-hour light/dark cycle. The pelletized growers feed was fed to them and they were provided water ad libitum. Every day fecal droppings were cleaned up to ensure cleanliness.

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➢ *Sample Size Determination*

A total of 70 male rats were recruited. As some dropped out, mortality were replace so there would be sufficient number of cases to perform the statistical analyses.

- *Phytochemical Analysis*

The phytochemical screening was done at the Project Development Institute. The powdered *Garcinia kola* was quantitatively and qualitatively analysed with 500 g. Qualitative tests were performed and secondary metabolites including saponins (frothing test), tannins (bromine water test), flavonoids (lead acetate test), alkaloids (Wagner's reagent test) and glycosides (sodium picrate test) were detected. These metabolites were quantitatively determined by quantitative tests.

- *Determination of LD50*

The oral acute toxicity of *Garcinia kola* was carried out using the modified Lorke method of Atsukwei et al. (2015). In the first phase, 9 mice were divided into three groups and given 10, 100 and 1000 mgs/kg of the extract orally. They were monitored for 24 hours for toxicity. In the second phase, fresh groups were administered 1600, 2900 and 4800 mg/kg, with mortality and behavioural changes (restlessness, sedation and gnawing) noted. The LD50 was determined as the geometric mean of the lowest lethal dose and highest non-lethal dose. The highest dose used in the experiment was less than 30% of LD50 so it is safe for ethnopharmacological testing.

- *Inclusion and Exclusion Criteria*

Males were adult rats weighing ≥ 200 g.

Male rats < 200 g and any rat that died during the study were excluded and replaced.

➢ *Data Collection Instruments*

The methods used for the collection of data were light microscopy, glass pipettes, slides and histological stains. To assess the functioning of the hypothalamic–pituitary–gonadal axis, biochemical reagents were used in hormonal assays.

➢ *Data Analysis*

Descriptive and inferential statistics were used to code and analyse the data. Groups were compared with regard to mortality rate, behavioural change, and biochemical parameters.

➢ *Ethical Considerations*

The Postgraduate Committee of the Faculty of Medicine, Ebonyi State University, Abakaliki has given ethical clearance. All procedures followed the guidelines for the humane treatment of laboratory animals.

III. RESULTS

➢ *Phytochemical Analysis of Garcinia kola*

The qualitative phytochemical analysis of *Garcinia kola* extract revealed the presence of different amounts of phytochemical compounds as shown in the table 1.

➢ *LD50 of Garcinia kola*

The oral LD50 of *Garcinia kola* seed extract was estimated to be >4800 mg/kg, which indicated a large margin of safety of *Garcinia kola* (See Table 2).

➢ *Effect of Ethanolic Extract of Garcinia on the Body Weight of the Male Rats*

Descriptive statistics revealed a positive trend in weight gain and weight increase in the male rats in comparison with both normal and standard control groups when fed with an increasing dosage of *Garcinia kola* extract (See Table 3).

➢ *Effect of Ethanolic Extract of Garcinia kola on Histo-Architecture of Male Rats*

The stained micrographed slides were then studied after the tissue processing. For the male rats, the hypothalamus, testes and epididymis were examined and the following results were observed (See Table 4).

Table 1 Result of the Qualitative Analysis of *Garcinian kola* Extract

Phytochemicals	**Degree of Concentration**	
Carbohydrate	+++	
Crude fibre	++	
Ether extract	+	
Tanins	+	
Phenols	+	
Saponins	++	
Phytic acid	+	
Flavonoids	++	
Alkaloids	++	
Caffeine	+	
Hydrogen cyanide	-	
Oxalate	+	
Sterol	+	
Nitric oxide	++	

Keys: - = Not detected, + = Low concentration, ++ = Moderate concentration, +++ = High concentration

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Table 2 Oral LD50 for Ethanolic Extract of *Garcinia kola* Seeds in Wistar Rats

Group	N	Dose(mg/kg)	%Mortality
Phase 1			
1	3	10	0
2	3	100	0
3	3	1000	0
Phase 2			
1	3	1600	0
2	3	2900	0
3	3	4800	0

Table 3 Result of the Effect of Ethanolic Extract of Garcinia on the Body Weight of the Male Rats

Parameter	A1 Normal saline	A2 (200mg)	A3 (300mg)	A4 (400mg)	A5 (500mg)	A6 (600mg)	A7 (standard drug sildenafil)
Week 1	233.75±2.1	233.89±1.1	204.14±1.6	224.14±1.6	203.33±1.4	203.33±1.4	213.80±3.3
Week 2	239.50±1.9	242.67±3.3	212.17±3.1	230.17±3.5	212.50±5.0	212.50±5.0	222.75±3.5
Week 3	248.00±3.6	249.83±4.0	227.00±2.2	243.67±3.9	225.67±5.8	225.67±5.8	232.50±3.8
Weight change	14.25	15.94	19.34	22.34	22.35	22.80	18.7
Increase in weight	6.1	6.8	8.6	1I.0	11.2	11.8	8.7
P. Value	0.002	0.000	0.000	0.000	0.000	0.000	0.005

Table 4 Result of the Effect of Ethanolic Extract of *Garcinia kola* on Relative Organ Weight of the Male Rats (Organ Weight-Body Weight Ratio)

Parameter	A1 Normal saline	A2 (200mg)	A3 (300mg)	A4 (400mg)	A5 (500mg)	A6 (600mg)	A7 (standard drug sildenafil)
TESTIS	0.0074	0.0075	0.0077	0.0075	0.0082	0.0085	0.0084
Epididymis	0.0015	0.0016	0.0018	0.0019	0.0020	0.0023	0.0021

- *Effect of Ethanolic Extract of Garcinia kola (Low Doses) on Sexual Behavior of Male Rats*

Garcinia kola extract was seen to enhance the sexual performance parameters in male rats at low doses. The mount frequency, the intromission frequency, and the number of ejaculations per training session were all higher in the treatment groups than in the control group, and the mount and intromission latency were lower in the treatment groups than in the control group, suggesting increased sexual motivation. Ejaculation latency and PEI ranged in their changes. Overall, the extract showed dose dependent improvement in sexual behavior at low doses (See Table 5).

- *Effect of Ethanolic Extract of Garcinia kola (High Doses) on Sexual Behavior of Male Rats*

Parameters of sexual behavior were significantly improved significantly after high doses of *Garcinia kola* extract. Mounts and intromissions were found to be significantly higher, and mount and intromission latencies were significantly lower, suggesting improved sexual motivation. Higher doses resulted in longer PEL and L and higher frequency of ejaculation, indicating prolonged sexual activity. These results highlight the potential of *Garcinia kola* extract to improve sexual performance in male rats at high doses (See Table 6).

- *Effect of Ethanolic Extract of Garcinia kola (Low Doses) on Sexual Behavior of Male Rats*

In male rats, low doses of the ethanolic extract of *Garcinia kola* gave significant changes in the sexual behaviour parameters. Mount frequency and intromission frequency were significantly higher than controls and mount and intromission latency were significantly less, reflecting increased sexual motivation. There was a mild increase of frequency and variable changes in an increase of an interval following ejaculation and an interval before the next ejaculation. The overall result shows there was a progressive improvement in sexual performance at low doses (See Table 7).

- *Effect of Ethanolic Extract of Garcinia kola (High Doses) on Sexual Behavior of Male Rats*

S. extract of *Garcinia kola* had a greater effect on sexual behavior at high doses. Increased mount and intromission frequency and decreased mount latency and intromission latency, indicating improved sexual drive. The higher doses caused an increase in ejaculation frequency and a decrease in ejaculation latency and post-ejaculation interval, indicating prolonged sexual activity. From the above results, it can be concluded that the extract of *Garcinia kola* at high doses can enhance the sexual performance of male rats similar to the standard drug (sildenafil) (See Table 8).

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Table 5 Result of the Effect of Ethanolic Extract of *Garcinia kola* (Low Doses) on Sexual Hormone Levels of Male Rats.

Parameter	A1 Normal saline	A2 (200mg)	A3 (300mg)	A7 (Standard drug; sil)	P. value (btw normal and high dose of rind
Testosterone(ng/ml)	0.3 ± 0.1[b]	0.9 ± 0.4[b]	2.9 ± 0.1	5.0 ± 0.2[a]	0.15
LH miu/ml	3.2 ± 1.0	3.9 ± 0.1	4.6 ± 0.2	4.7 ± 0.1	0.00
FSH miu/ml	4.7 ± 0.8	6.8 ± 0.1	8.6 ± 1.2	9.8 ± 1.1	0.02

Value expressed as mean ± SD, N = 5, P value ≤ 0.05

- significant when compared with negative control
- Significant when compared with positive control

Table 6 Result of the Effect of Ethanolic Extract of *Garcinia kola* (High Doses) on Sexual Hormone Levels of Male Rats.

Parameter	A1 (Normal saline)	A4 (400mg)	A5 (500mg)	A6 (600mg)	A7 (Standard drug; sil)	P. value (btw normal and high dose of seed
Testosterone(ng/ml)	0.3 ± 0.1[b]	4.2 ± 0.1a	6.1 ± 0.7[a]	6.2 ± 0.8[a]	5.0 ± 0.2[a]	0.01
LH miu/ml	3.2 ± 1.0	3.1 ± 0.6	6.3 ± 1.8	6.4 ± 1.9	3.9 ± 0.1	0.32
FSH miu/ml	4.7 ± 0.8	9.5 ± 5.4	10. ± 0.8[ab]	11. ± 0.7[ab]	9.8 ± 1.1	0.05

Value expressed as mean ± SD, n = 5, P value ≤ 0.05

- Significant when compared with negative control
- Significant when compared with positive control

Table 7 Results of the Effect of Ethanolic Extract of *Garcinia kola (Low Doses)* on Sexual Behavior of Male Rats.

PARAMETER		Al (Normal Saline)	A2 (200mg)	A3 (300mg)	A7 (Standard drug:Sil)	P Value btw normal and low doses of G.kola
Mount Freq	WK1	5.5±3.5	7.0 ± 2.3	10.0 ± 0.0	15.0 ± 12.7	0.02
	WK2	9.0±4.2	9.5 ± 2.1	16.0 ± 7.1	19.0 ± 7.0	0.05
Intro. Freq.	WK1	12.5 ± 3.5	17.5 ± 6.3	19.0 ± .0	22.5 ± 2.1	0.05
	WK2	19.0 ±7.1	19.5 ±1.4	27.51± 3.5	22.5 ± 7.7	0.40
Eja. Freq	WK1	2.0 ± .00	2.2 ± 0.7	2.51 ± 0.7	2.5 ± 0.7	0.10
	WK2	2.0 ± .0	2.5 + 0.0	2.51 ± 0.7	2.5 ± 0.7	0.5
Mount Lat.	WK1	115.5 ± 96.9	22.5 ± 36.1[a]	21.0 ± 28.3[a]	59.5± 44.6	0.20
	WK 2	88.5 ± 18.3	17.5 ± 17.7	13 ± 5.7	41.5 ± 38.9	0.01
Intro Lat.	WK l	84.0 ± 28.3	67.0 ± 8.5	52.5 ± 13.4	68.5 ± 6.4	0.20
	WK2	55.5+ 62.9	50.5± 34.6[a]	40.0 ± 94.8	51.0 ± 7.0	0.08
Eja Latency	WK l	135.5 ± 57.70	150,5 ± 12.0	198.8 ± 80.6	216.5 ± 10.6	0.10
	WK2	145.0± 39.6	147.5 ± 28.4	207.0 ± 107.5[a]	276.0 ± 48.1	0.03
Post – Eja Interval	WK l	364.0 ± 60.8	321.5±14.0	255.0 ±.7.0	168.5 ± 9.1	0.20
	WK2	319.5± 40.7	300.5± 22.7	228.5 ± 89.8	126.0 ±77.8	0.30

Values expressed as mean ±SD, n=5, P value < 0.05, a- significant when compared with negative control, b- Significant when compared with positive control

Table 8 Result of the Effect of Ethanolic Extract of *Garcinia kola (High Doses)* on Sexual Behavior of Male Rats.

Parameter		Al (Normal Saline)	A4 (400mg)	A5 (500mg)	A6 (600mg)	A7 (Standard drug; Sil.)	P Value btw normal and high doses of G. kola
Mount Freq	WK1	5.5 ± 3.5	12.5 ± 3.5	18.0 ± 9.2	18.5 ± 9.2	15.0±12.7	0.20
	WK2	9.0 ± 4.2	7.5 ± 0.7	22.1 ± 2.1[ab]	23.1 ± 2.1[ab]	19.0 ± 7.0	0.03
Intro. Freq.	WK1	12.5 ±3.5	16.5± 10.6	26.5 ± 6.3	27.5 ± 6.3	22.5 ± 2.1	0.50
	WK2	19.5 ±7.1	23.0 ±14.1	35.5 ± 6.3[ab]	36.5 ± 6.3[ab]	22.5 ±7.7	0.02
Eja. Freq	WK1	2.0 ±.00	2.0 ± .0	3.5 ± 0.7	4.0 ± 0.7	2.5 ± 0.7	0.20
	WK2	2.0 ±.0	2.0 ±1.4	3.5 ± 0.7	4.0 ± 0.7	2.5 ± 0.7	0.21
	WK1	115.5± 96.9	36.0±29.7[a]	17.8 ±1.4	17.0 ±1.4	22.5 ± 36.1[a]	0.05

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Mount Lat. (secs)	WK 2	88.5 ±18.3	29.5 ±11.7	11.9 ± 2.8[ab]	11.0 ± 2.8[ab]	13 ± 5.7	0.04
Intro Lat.	WK l	84.0±28.3	50.0±7.1	15.5 ± 7.8	15.0 ± 7.8	16.5 ± 6.4	0.30
	WK2	50.5±62.9	37.5±20.5[a]	10.5 ± 0.7[ab]	10.0 ± 0.7[ab]	16.0 ± 7.0	0.02
Eja Latency	WK l	135.5±57.70	177.0± 17.0	254.5 ± 12.0[a]	264± 12.0[a]	216.5 ± 10.6	0.01
	WK2	145.0±39.6	182.0±32.5[a]	292.5 ± 37.5[ab]	298± 37.5[ab]	276.0 ± 48.1	0.05
Post – Eja Interval	WK l	364.0 ±60.8	204:5± 17.5	158.0 ± 41.0	154.0 ± 41.0	168.5 ± 9.1	0.05
	WK2	319.5±40.7	181.5±41.7	110.0± 22.6[ab]	109± 22.6[ab]	126.0±77.8	0.03

Values expressed as mean ±SD, n=5, P value 5 ≤ 0.05

- significant when compared with negative control
- Significant when compared with positive control

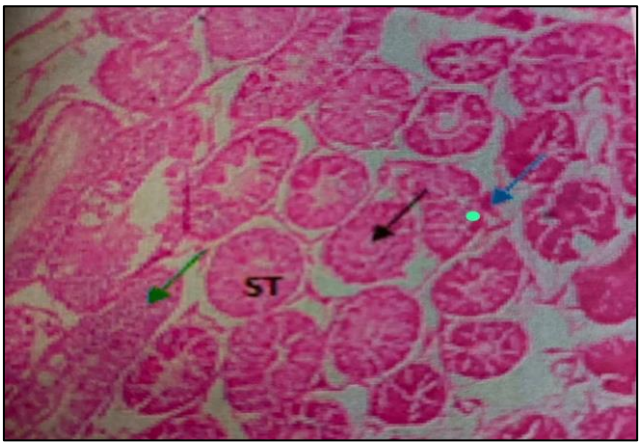

Plate 1 Photomicrograph of Testis of Rats in Group A1 (Normal Control Rats), Treated with Normal Saline.

The testis shows normal testicular histological features, consisting of seminiferous tubules (ST), which are held together by connective tissue interstitium consisting of interstitial cells and Leydig cells (blue arrow). The ST are lined by several types of cells (green arrows) including the spermatogonia cells, spermatocytes, spermatids as well as the spermatozoa that fill the lumen of the tubules (black arrows). (H & E) X 100

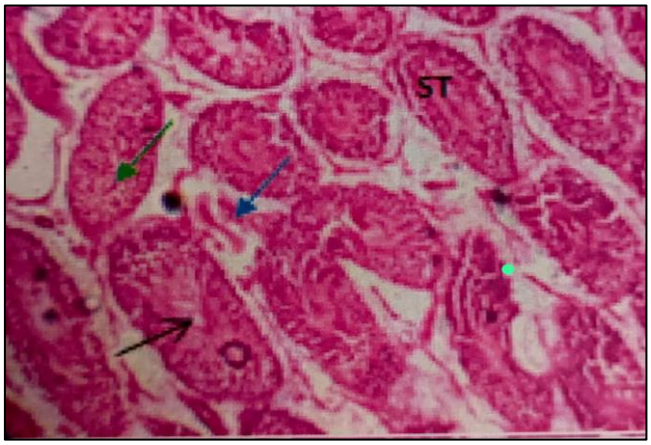

Plate 2 Photomicrograph of testis of rats treated in Group A2 (200mg/kg of *Garcinia kola* extract) showing normal testicular histo-architecture with larger sized seminiferous tubules when compared with normal control group (A1) and smaller sized seminiferous tubules when compared with (7A) (H & E) X 100

IV. DISCUSSIONS

Garcinia kola phytochemicals found were phenols, steroids, alkaloids, flavonoids, tannins, saponins, crude fibers, carbohydrates, oxalate and caffeine. The higher levels of carbohydrates, crude fiber, flavonoids and saponins were recorded as reported earlier (Mazi et al., 2013). The moderate levels of flavonoids and saponins might account for their aphrodisiac activity since they are capable of binding to hormone receptors or enzymes that participate in the synthesis of hormones (Safe et al., 2021). The same findings were observed in the case of Tribulus terrestris studies (Singh et al., 2011).

Oral LD50 of *Garcinia kola* is estimated to be >4800 mg/kg, which means it is relatively safe even with high usage. The amount of the highest dose used was below 30% of LD50, which is considered safe for ethnopharmacological assays (Chinedu et al., 2013; Erhirhie et al., 2018).

Findings showed significant increase in body weight and reproductive organ weights (testes, epididymis) particularly at 600 mg/kg, which is a biological indicator of increased steroidogenesis (Sardar et al., 2023).

Motivation & Performance: Decreased mount latency (ML) and intromission latency (IL); increased mount frequency (MF), intromission frequency (IF) and ejaculation frequency. Activity was significantly higher (600 mg/kg) than sildenafil.

Potency & Recovery: Post-ejaculatory interval (PEI) has improved, as recovery from ejaculation has become quicker. Ejaculatory latency (EL) was found to be increased which indicates a longer period of coitus with greater rigidity. The effects are related to the presence of saponins and flavonoids that increase the production of androgens and nitric oxide (Damam et al., 2024; Tauchen et al., 2023b).

V. CONCLUSION

Garcinia kola seed extract has a number of phytochemicals such as saponins, flavonoids, phenol and steroids. This could have led to increased aphrodisiac effect in the seed extract. The study has demonstrated the aphrodisiac property of ethanolic seed extract of *Garcinia kola*, based on the histological, sexual behavioral and hormonal assay results and these results have given evidence to support the usage of these extracts as aphrodisiac in traditional medicine. *Garcinia kola* ethanolic seed extract have aphrodisiac properties, therefore, it may be used for the

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treatment of men and women with sexual dysfunction. The aphrodisiac property of the seed extract observed in this study was found to be stronger when compared to that seen in the normal control group. This effect can be mediated partly through a central stimulatory effect and/or by a sexual hormonal enhancing effect. This was also found to be dose dependent.

RECOMMENDATION

The present study aimed at studying the effect of ethanolic seed extract of *Garcinia kola* on the Hypothalamic-Pituitary-Gonadal axis and sexual behavior of adult wistar rats of both sexes. Based on the results of this research study, these plant materials should be used in the daily diet of couples facing any sexual difficulty. Further research need to be carried out to isolate the exact active compound having the aphrodisiac property and to determine if all the effects observed in this study will be sustained, reversible or irreversible after a longer period of time. Pharmaceutical institutes should also explore the use of the extract from the seeds of *Garcinia kola* as an effective aphrodisiac in modern day.

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- *Competing Interests*

The authors declare that there are no competing interests involved with this work.

- *Authors Contribution*

All authors were involved in Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Original Draft, as well as Review & Editing.

- *Conflict of Interest*

The authors declare no conflict of interest.

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- *Data Availability*

Data are available upon request and will be deposited in the public domain in due course.

- *Declarations*

The authors declare no conflicts of interest.

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