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GARCINIA KOLA SEEDS: IS THE AQUEOUS EXTRACT A TRUE APHRODISIAC IN MALE WISTAR RATS?

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Abstract

The age long acclaimed aphrodisiac potentials of *Garcinia kola* seeds in some parts of Western Nigeria has not been substantiated with scientific evidence. In this study, we have decided to evaluate the effect of aqueous seed extract of *G. kola* at the doses of 25, 50 and 100 mg/kg body weight on sexual behaviour of male rats. Male rats weighing 215.00 ± 18.58 g were randomized completely into four groups (A-D) of six animals each. Animals in group A received, orally, 0.5 ml of distilled water only while those in groups B, C and D received same volume containing 25, 50 and 100 mg/kg body weight of the seed extract respectively. Frequencies of mount (MF), intromission (IF), genital toilet (GTF) and ejaculation (EF) as well as latencies of mount (ML), intromission (IL) and ejaculation (EL) were evaluated following the pairing of male rats (1:1) with non-oestrous female rats. The parameters were monitored for the first (15-30 min), second (75-90 min) and third (180-195 min) observatory periods. The levels of testosterone, luteinizing (LH) and follicle stimulating hormones (FSH) were also determined. Phytochemical screening of the extract revealed the presence of saponins (2.78%), cardiac glycosides (0.26%), cardenolides and dienolides (0.24%), flavonoids (1.28%) and steroids (1.14%). The 25 and 100 mg/kg body weight increased ($P<0.05$) the MF whereas the ML was decreased by all the doses of the extract. MF and ML were not altered during the second observatory period whereas the 50 mg/kg body weight increased these parameters during the third observatory period. Other sexual behaviour parameters as well as serum testosterone, FSH and LH were not significantly altered throughout the observatory periods. Overall, the results revealed that *G. kola* seeds did not have sex enhancing potential as claimed. Therefore, the acclaimed pro sexual effect of *Garcinia kola* seeds is scientifically untrue. This study has refuted the claim that one of the rationales for consuming the seeds by the aged population of Nigeria is to enhance sexual invigoration in males.

Keywords: *Garcinia kola* seeds, *Guttifera*, aphrodisiac, sexual behaviour

Introduction

Traditional herbs have been reported to have contributed to revolutionary breakthrough in the management of sexual inadequacies and have become known worldwide as an “instant” treatment (Adimoelja, 2000). These botanicals include *Terminalia cattapa seeds (*almond fruit), leaves, roots and fruits of *Musa parasidiaca L* (plantain) as well as *Fadogia agrestis* (Ratnasooriya and Dharmasiri, 2000; Yakubu et al., 2005). In Nigeria, one plant that has been acclaimed to have sex enhancing potential is *Garcinia kola* seeds. This claim was further buttressed that it may be the rationale behind its consumption by the elderly since sexual inadequacies is most common in the aged population.

Garcinia kola Heckel (family-*Guttifera*) also known as bitter kola, false kola and male kola (English), orogbo (Yoruba-Western Nigeria), cida goro (Hausa-Northern Nigeria), Aku ilu or Ugugolu (Igbo-Eastern Nigeria), Efiari (Efik), and Igoligo (Idoma-Middle Belt) is an evergreen, dicotyledonous plant found in moist forest, riverine and swampy areas. It grows to a medium sized tree of about 12-15 m high and a girth of 1.80 m. It occurs naturally in Sierra Leone, Nigeria and Angola.

The seeds have a bitter taste; hence, it is called bitter kola in Nigeria. As a result of this bitter taste, the seeds have been consumed as a stimulant (Atawodi et al., 1995). The seeds have also been used in the treatment of liver disorders and diarrhoea (Blaide, 1991), diabetes, bronchitis and throat infections (Orie and Ekon, 1993; Tita et al., 2001), and as a natural antimicrobial (Ositelu et al., 2004). *G. kola* has also been reported to posses some hepatoprotective and aphrodisiac properties (Akintonwa and Essein, 1990; Ajibola and Satake, 1992). It has also been reported to be effective in the treatment of dermatological disorders associated with melanin pigmentation (Okunji et al., 2007).

Despite the acclaimed use of *G. kola* seeds as sex invigorator in some parts of Nigeria, there seems not to be any information in the open scientific literature on this claim. Therefore, this study was aimed at evaluating this claim with a view to substantiating or refuting it. Several authors have assessed the aphrodisiac potentials of many plants using physical and biochemical parameters such as mount frequency (MF), intromission frequency (IF), genital toileting frequency (GTF), ejaculation frequency (EF) mount latency (ML), intromission latency (IL) and ejaculation latency (EL), testosterone, follicle stimulating hormone (FSH) and luteinizng hormone (LH) (Ratnasooriya and Dharmasiri, 2000; Tajuddin et al., 2004; Zamble et al., 2008; Yakubu and Afolayan, 2009), hence, the use of these parameters in this study.

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Materials and methods

Plant material and authentication

G. kola seeds purchased from herbsellers at Agor Market, Ilorin, Nigeria, was authenticated by Mr. Michael Onadeji of the Forestry Research Institute of Nigeria, Ibadan, Nigeria. A voucher number (F.H.I. 10847) was deposited at the herbarium of the Institute.

Assay kits

Testosterone assay kit was a product of Diagnostic Automation Inc., Calabasas, USA, while those of LH and FSH were products of Syntron Bioresearch, Inc., Carlsbad, USA.

Other reagents

All other reagents used were of analytical grades and were prepared in volumetric flasks using glass-distilled water.

Experimental Animals

Healthy, sexually matured, male albino rats (*Rattus norvegicus*) weighing 215.00 ± 18.58 g and female albino rats weighing 195. 90 ± 10.97 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in aluminium cages placed in a well-ventilated house with optimum conditions (temperature: 22 ± 3^0C; photoperiod: 12 h natural light and 12 h dark; humidity: 40-45%) with free access to rat pellets (Bendel Feeds and Flour Mills Ltd, Ewu, Nigeria) and tap water. The animals were handled humanely according to the guidelines of National Institute for Health, USA (NIH, 1985).

Preparation of extract

G. kola seeds were peeled, sliced into thin pieces and oven-dried at 40^0C for 72 h to a constant weight using Uniscope Laboratory Oven, (model- SM9053, Surgifriend Medicals, England). The materials were then pulverized with an electric blender (Crown Star Blender-model CS- 242B, Trident (H.K) Ltd, China). A portion of the powder (300 g) was extracted in 1000 ml of distilled water for 48 h at room temperature with constant shaking (SO1 orbital shaker, Stuart Scientific, Stone, UK). The extract was then filtered with Whatman No. 1 filter paper. The filtrate was concentrated on steam bath to give a yield of 16.42 g of the residue. This was later reconstituted in distilled water to give the required doses of 25, 50 and 100 mg/kg body weight used in this study.

Phytochemical screening

A portion of the extract (1% w/v) was subjected to standard chemical tests as described for alkaloid (Harborne, 1984), steroids, anthraquinones, cardenolides and dienolides (Trease and Evans, 1989), phenolics and flavonoids (Awe and Sodipo, 2001), cardiac glycoside and saponins (Sofowora, 1993), tannins, phlobatannins and triterpenes (Odebiyi and Sofowora, 1978). The detected phytochemicals were quantitatively determined for saponins (Obadoni and Ochuko, 2001), flavonoids (Boham and Kocipai-Abyazan, 1974), steroids, cardiac glycosides, cardenolides and dienolides (El-Olemy et al., 1994).

Animal grouping, extract administration and monitoring of sexual behaviour indices

The animals were randomly grouped into four (A-D) of six rats each. Animals in groups A, which served as the control, received 0.5 ml of distilled water while those in groups B, C and D were treated like the control except they received same volume containing 25, 50 and 100 mg/kg body weight of the extract. All administrations were done orally using metal oropharyngeal cannula. Immediately after their respective administration (T = 0), the animals were allowed an acclimatization period of 15 min. A non-oestrous female was introduced into the plastic cage (T = 15 min), in ratio 1:1 and sexual behavioural parameters were then monitored for observatory periods of first (15-30 min), second (75-90 min), and third (180-195 min) after administering the extract (Ramachandran et al., 2004; Zamble et al., 2008). Adopting the standard procedures described by Amin et al. (1996) and Agmo (1997), the male sexual behaviour indices monitored during the three observatory periods included: MF (the number of mounts from the time of introduction of the female until ejaculation), IF, (the number of intromissions from the time of introduction of the female until ejaculation), GTF (number of times the male rat cleaned up the tip of the copulatory organ), EF (the number of times semen was ejected from the male copulatory organ), ML (the time interval between the introduction of the female and the first mount by the male), IL (the time interval between the introduction of the female and the first intromission by the male) and EL (the time interval between the first intromission and ejaculation).

Preparation of serum

Immediately after the termination of the third observatory period, male rats were anaesthetized in a jar containing

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cotton wool soaked in ether fume. The neck areas were quickly cleared of fur and skin to expose the jugular veins. The jugular veins were slightly displaced from the neck region (to prevent contamination of the blood with interstitial fluid) and then cut with a sharp sterile blade. The rats were held head downwards and allowed to bleed into a clean, dry centrifuge tube, which were left at room temperature for 10 min to clot. The tubes were later centrifuged at 503 *g* x 10 min using Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, England). The sera were thereafter aspirated using a Pasteur pipette into clean, dry, sample bottles and were used within 12 h of preparation for the hormonal assay.

Hormonal assay

The hormones were assayed in the serum of the animals following the procedure outlined in the manufacturers' instruction manual as described for testosterone (Chen et al., 1991), FSH (Kapen et al., 1973) and LH (Uotila et al., 1981).

Statistical analysis

Data were mean ± SEM of six replicates. They were analyzed for statistical significance using Duncan Multiple Range Test and complemented with Student's t-test. Differences were considered statistically significant at $P<0.05$ (Mahajan, 1997).

Results

Aqueous extract of *G. kola* seeds gave positive tests to cardiac glycosides, flavonoids, steroids, saponins, cardenolides and dienolides while tannins, anthraquinones, phenolics, phlobatannins and triterpenes were not detected (Table 1). Quantitatively, the extract was found to have saponins in highest concentration followed by flavonoids and steroids while cardiac glycosides, cardenolides and dienolides were weakly present (Table 1).

Among all the physical indices of sexual behaviour monitored in male rats, the extract only produced significant effects on the MF and ML. For instance, the extract at 25 and 100 mg/kg body weight increased the MF during the first observatory period (Table 2), whereas it was all the doses that significantly reduced the ML during the same period (Table 3). In contrast, no effect was produced on the MF and ML by the extract during the second observatory period. This incidence of null effect on the MF and ML by the extract was extended to the third observatory period in all the treatment groups except those administered with the 50 mg/kg body weight of the extract where the MF and ML increased significantly (Tables 2 and 3).

Furthermore, all the doses of the extract did not produce any effect on the IF, IL, EF, EL and GTF throughout the exposure period (nil values not shown). Again, the levels of FSH, LH and testosterone in the serum of the animals treated with the extract were not significantly altered at the end of the observatory period when compared with their respective control values (Table 4).

Discussion

This study has refuted the acclaimed use of *G. kola* seeds as an aphrodisiac in some parts of Western Nigeria. Male rats in the presence of non-oestrous female did not improve their sexual performance when orally administered with varying doses of aqueous seed extract of *G. kola*. Observation at each experimental period in this study revealed that sexual function in male rats were not enhanced, more so, when some indices of sexual performance such as IF, IL, EF, EL and GTF had no value.

Table 1: Phytochemical constituents of aqueous extract of *Garcinia kola* seeds

Phytochemicals	**Concentration (%)**
Cardiac glycosides	0.26±0.02
Tannins	Not Detected
Anthraquinones	Not detected
Phenolics	Not Detected
Flavonoids	1.28±0.01
Steroids	1.14±0.05
Saponins	2.78±0.03
Frothing time	4 h 20 min
Frothing height	4.85 cm
Alkaloids	Not Detected
Cardenolides and dienolides	0.24±0.03
Phlobatannins	Not Detected
Triterpenes	Not Detected

Results are mean ± SEM of three replicates

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According to Singh and Mukherjee (1998), aphrodisiac is any substance that (a) stimulate the production of semen (b) improve and purify the quality of semen (c) help sexually and in ejaculation (d) delay the time of ejaculation and (e) arouse sexual desire. Therefore, the aqueous extract of *G. kola* seeds may not be considered as a sex enhancer and or sexual invigorator since it did not satisfy the aforementioned properties most especially, sexual arousal, prolonged ejaculation latency and many fold increase in mounting during the observatory periods. Furthermore, the significantly enhanced MF and ML, which are useful indices of sexual vigour, libido and arousability (Tajuddin et al., 2004; Mbongue et al., 2005), in the first observatory period, does not necessarily qualify the seed extract as an aphrodisiac because the indices were not sustained beyond this period. All these, coupled with the nil values of IF, GTF, EF, IL and EL obtained in this study further suggests that sexual appetitive behaviour was not enhanced by the extract (Yakubu and Afolayan, 2009).

Table 2: Effect of administration of aqueous seed extract of *G. kola* on mount frequency of male rats

	Observatory Periods (min)		
Doses	**First (15-30)**	**Second (75-90)**	**Third (180-195)**
Distilled water (2.33 ml/kg body weight	0.33±0.12[a]	0.00±0.00[a]	0.00±0.00[a]
25 mg/kg body weight of the extract	1.67±0.37[b]	0.00±0.00[a]	0.00±0.00[a]
50 mg/kg body weight of the extract	0.33±0.08[a]	0.00±0.00[a]	8.06±2.87[b]
100 mg/kg body weight of the extract	0.67±0.03[c]	0.00±0.00[a]	0.00±0.00[a]

Values are mean ± SEM of six replicates
Values carrying superscript different from the control are significantly different (P<0.05).

Table 3: Effect of administration of aqueous seed extract of *G. kola* on mount latency of male rats

	Observatory Periods (min)		
Doses	**First (15-30)**	**Second (75-90)**	**Third (180-195)**
Distilled water (2.33 ml/kg body weight	4.20±1.29[a]	0.00±0.00[a]	0.00±0.00[a]
25 mg/kg body weight of the extract	1.47±0.26[b]	0.00±0.00[a]	0.00±0.00[a]
50 mg/kg body weight of the extract	1.43±0.26[b]	0.00±0.00[a]	2.24±0.36[b]
100 mg/kg body weight of the extract	0.34±0.07[c]	0.00±0.00[a]	0.00±0.00[a]

Values are mean ± SEM of six replicates
Values carrying superscript different from the control are significantly different (P<0.05).

Table 4: Effect of administration of aqueous seed extract of *G. kola* on some reproductive hormones of male rats

	Observatory Periods (min)		
Doses	**Follicle stimulating hormone (mIU/L)**	**Luteinizing hormone (mIU/L)**	**Testosterone (ng/ml)**
Distilled water (2.33 ml/kg body weight	2.35±0.15[a]	1.00±0.11[a]	5.15±0.25[a]
25 mg/kg body weight of the extract	2.29±0.22[a]	1.03±0.14[a]	5.15±0.15[a]
50 mg/kg body weight of the extract	2.30±0.12[a]	1.05±0.10[a]	5.05±0.26[a]
100 mg/kg body weight of the extract	2.30±0.19[a]	1.01±0.12[a]	5.16±0.13[a]

Values are mean ± SEM of six replicates
Values carrying the same superscript as the control are not significantly different (P>0.05).

Elevated level of testosterone has been associated with a moderate but significant increase in sexual desire and penile function (Gauthaman et al., 2002). Clinical data on testosterone also suggest that a slightly increased level of testosterone in adult males results in an enhanced sexual desire and arousability (Thakur and Dixit, 2007). The level of

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testosterone has been reported to be related to LH and FSH such that increase in the levels of the gonadotropins results in corresponding increase in testosterone (Andersen and Tufik , 2006). Therefore, it is not surprising that these hormones which have specific roles to play in sexual activity of animals were not significantly altered. Such a lack of significant change in the levels of the reproductive hormones in this study is an indication that GnRH-LH signalling was not affected. This further supports the non-enhancement of the sexual appetitive behaviour of the animals and also explain the lack of effect on IF, GTF, EF, IL and EL.

Several phytochemicals have been implicated in enhancing sexual function in male rats. For example, saponins in *Fadogia agrestis* (Schweinf. Ex Hiern) and *Tribulus terrestris* (Linn.) as well as alkaloids in *Pausinystalia yohimbe* (K. Schum) and *Microdesmis keayana* (J. Leonard) have been shown to be responsible for aphrodisiac activity (Ernst and Pittler, 1998; Gauthaman et al., 2002; Yakubu et al., 2005; Zamble et al., 2008). Similarly, prosexual stimulatory property of *Mondia whitei* Hook (Skeels) has also been attributed to its steroid and triterpene contents (Drewes et al., 2003). These bioactive agents exhibit aphrodisiac activity either by increasing the biosynthesis and secretion of androgens or act directly on the central nervous system to modulate the action of neurotransmitters and gonadal tissues in animals. Specifically, saponins enhance androgen production (Gauthaman et al., 2002) whereas alkaloids may increase the dilation of blood vessels in the sexual organs (Perbot, 1982), or increase nitric oxide that plays a key role in central erection and central sexual stimulation (Zamble et al., 2008). Interestingly, some of these phytochemicals such as saponins and steroids were also detected in the seed extract in this study; it is possible that the extract lacked the specific type. For instance, steroidal saponins have been implicated to play a role in enhancing sexual behaviour by either binding to hormone receptors, which may result in conformational change that will enhance the physiological function of the hormones or bind to enzymes that are involved in the synthesis of such hormones and thus enhance its production (Gauthaman and Adaikan, 2008), whereas none of such has ever been reported for the second class of saponins, the triterpenoid saponins.

This study has thus revealed that aqueous seed extract of *G. kola* did not possess aphrodisiac activity as claimed and may not explain the consumption of the seeds mainly by the aged. Furthermore, the results taken together discourage the continued claim or assumption of *Garcinia kola* seeds as an aphrodisiac.

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