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EVALUATION OF APHRODISIAC POTENTIAL OF METHANOL EXTRACT OF *GARCINIA KOLA* SEED IN MALE RODENTS

Essien Grace Emmanuel[1], Effiong Grace Sylvester[2]* and Nwafor Paul Alozie[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P. M. B. 1017, Uyo, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, P. M. B. 1017, Uyo, Nigeria.

***Corresponding Author: Dr. Effiong Grace Sylvester**

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, P. M. B. 1017, Uyo, Nigeria.

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ABSTRACT

Dysfunctional erectile is a common sexual disorder among the male folk. *Garcinia kola* seed is being utilized by the folklore for treating erectile dysfunction disorder. Investigation was carried out to ascertain the acute toxicity of *Garcinia kola* seed and the behavior of male Albino Wistar rats sexually, using the methanol extract. Result shows the median lethal dose (LD_{50}) to be 1000±0.66 mg/kg. Extract administration (100 mg/kg–300 mg/kg) altered significantly the various sexual behaviours in the experiment. There were significant decreases ($p< 0.01$-$p<0.001$) in mount (100mg/kg=21.55±0.04 (s), 200 mg/kg=10.02±0.68 (s) & 300 mg/kg = 4.02±0.17 (s) and intromission latencies (100 mg/kg=22.44±0.50 (s), 200 mg/kg=17.36±0. 28 (s) & 300 mg/kg=9.51±0.36 (s) respectively in comparison with controls (mount latency; 25.45±0.25 (s), intromission latency = 45.16±0.17(s), with corresponding increases in mount (100 mg/kg=32.50±0.22(MF), 200 mg/kg=36.00±1.39(MF), 30 0mg/kg = 39.33±0.46 (MF); and intromission (100 mg/kg=30.00±0.60(EF), 200 mg/kg=34.16±0.32(EF), 300 mg/kg=34.16±0.28(EF) frequencies respectively compared with the controls (mount frequency = 23.80±1.45(MF) & intromission frequency = 20.00±1.05(EF). The 300 mg/kg extract portrays a similar significant decreasing effect on mount and intromission latency with the standard drug (mount latency=3.90±0.55(s); intromission latency=3.60±0.55(s). Similarly, the effect of the higher doses on mount and intromission frequencies were significantly increased almost as that of the standard drug (mount frequency=39.50±1 .03(MF) and intromission frequency=35.87±1.02 (EF). Contrary, there was increase in ejaculation latency (100 mg/kg=2.00±0.06(s), 200 mg/kg1.88±0.11(s)=, 300 mg/kg=1 .55±0.39(s) and decrease in ejaculation frequency (100 mg/kg=27.01±1.28(EF), 200 mg/kg=31.00±0.67(EF), 300 mg/kg=32.00±0.82(EF) respectively compared with the controls(ejaculation latency=1.21±0.04(s), ejaculation frequency=35.00±1.04(EF). These also were similar with the effect produced by the standard drug (ejaculation latency=1.21±0.04(s), ejaculation frequency=35.00±1.04 (EF). The combination of 200 mg/kg G. kola extract with testosterone (standard drug) produced more efficacies (2.81±0.31, 3.40±0.39, 1.18±0.30, 7.84±0.18, 41.72±0.45, 38.22±0.78 and 35.00±0.24 for their Mount Latency ml(s), Intromission Latency ll(s), Ejaculation Latency EL (s), Penile Erection (PE), Mount Frequency (MF), Intromission Frequency (EF) and Ejaculation Frequency (EF) respectively compared with administration of the standard drug alone. Conclusively, these findings agree with the folkloric reports that *G. kola* seeds are used as aphrodisiacs in men and may be a rationale for an erectile dysfunction drug origin.

KEYWORDS: Erectile Dysfunction, *Garcinia Kola* Seeds, Acute Toxicity, Sexual Behavior, Aphrodisiac.

INTRODUCTION

Reproductive health can be defined as a state of emotional, physical, mental and social well-being relating to reproduction and sexuality and may not be necessarily in the absence of reproductive disease or physical weakness (WHO, 2002). At all stages of life, sexual and reproductive health affects the reproductive processes, system and functions. Therefore, the implication of reproductive health is that people are capable of having a sex life that is responsible, satisfying and safer and they are able to reproduce and have the freedom to decide how often, when and if to do so (WHO, 2000).

Herbal medicine is a form of alternative and complementary medicine that is increasingly becoming popular in developed and developing countries, (Eisenberg et al. 1998). World Health Organization (WHO) did a survey that indicates the reliance of about 70 – 80% of the world population with the developing countries in particular on non-conventional medicines mostly of herbal sources in their primary healthcare. Today many drugs that are commonly used have herbal origin. Indeed, in the United States about 25% of the prescription drugs dispensed contain at least one active ingredient derived from plant material. The drugs are

either synthesized to mimic a natural plant compound or are made from plant extracts (WHO, 1999).
Erectile dysfunction is common in middle-aged and older men and can be said to occur when there is an inability to maintain or achieve an erection for sexual activity or penetration at least 50% of the time for the last 6 months (Laumann *et al*., 1999). It is also known as impotence and is a major sexual dysfunction commonly faced by men. The etiological factors which contribute towards ED are multifactorial including aging, diabetes mellitus, neurogenic factors and iatrogenic factors (Post *et al*., 2009). Erectile dysfunction can also be caused by a condition called Peyronie's disease (scar tissue in the penis) (Mayo clinic, 2006).

The plant, *Garcinia kola* Heckle (Guttiferae) is known in commerce as a highly valued ingredient bitter cola in the Africa traditional medicine. It is an evergreen tropical plant tree, up to 10-25m high and is a dicotyledenous plant commonly found in the rainforest (Iwu, 1993).

The seeds are obtained from the decayed fruits and are covered with a light brown testa, which may be peeled off to reveal the white seed, which is elliptically shaped about 5cm long. The whole being is extremely bitter, resinous and astringent (Iwu, 1993). *Garcinia kola* is widely known and used in African traditional medicine and every part of it has been found to be medicinally important thus it is referred to as wonder plant (Dalziel, 1937).

Sexual dysfunction may occur as erectile dysfunction, reduced or loss of desire (libido), problems with ejaculation and failure of the testicles to make the normal amount of male sex hormones and this could be a very distressing condition for men. It can erode the male essence (NIH, 1992) hence the need for a cure of these conditions. *G. kola* has been reported to posses some hepatoprotective and aphrodisiac properties (Ajibola and Satake, 1992), hence there is need to justify methanol extract of *Garcinia kola seed* effect on sexual dysfunction.

MATERIALS AND METHODS

Collection and Identification of Plant Sample

Garcnia kola fresh seeds were bought from a local market in Uyo, Akwa Ibom State, Nigeria. Dr (Mrs.) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo Nigeria authenticated the plant and assigned it a voucher specimen number UUH220; it was deposited at the herbarium of Department of Botany and Ecological Studies, University of Uyo, Nigeria.

Extraction of Plant Material

Gradient method was applied for the plant's extraction. The seeds were peeled to remove the pericarp, cut into small pieces and then crushed using a household blender (mortar and pestle). It was then dried and weighed to be 900 g. The powder was air-dried and cold macerated in a chromatographic tank using 1000 ml of 90% N-hexane, 1250 ml of 90% Chloroform, 1250 ml of Ethyl Acetate and 1250 ml of Methanol respectively in order of polarity, the pieces were extracted for 72 hours each. A water bath was used to concentrate the crude liquid extracts to dryness and then stored at -4^{o}c until used for bioassays.

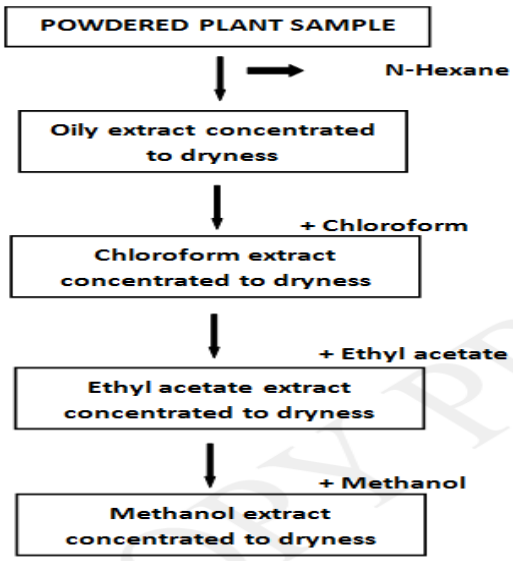

Fig. 1: Extraction procedure of *Garcinia kola.*

Acute toxicity study

Animals: A safe dose for *Garcinia kola seed* was determined using the acute toxic study. Swiss Albino mice (20-25g) of both sexes obtained from the Department of Pharmacology and Toxicology, University of Uyo, Nigeria were used for the experiment. Animals of the same sex were kept together in clean plastic cages under standard conditions. Studies were carried out in accordance with the principles of good laboratory practice and animal handling of the ethical guide for care and use of laboratory animals of the Faculty of Pharmacy, University of Uyo, Nigeria.

Methods

The method of Miller and Tainter (1944) was used to estimate the median lethal dose (LD_{50}) of the plant. Different doses of the extract at 100 – 1000 mg/kg were administered to 10 groups of three mice each by intraperitoneal (i.p) route. In 24h, the manifestations of physical signs of toxicity observed in the animals were; decreased body/limb tone, writhing, decreased motor activity, decreased respiration and death. The LD_{50} was determined by plotting the probit versus the log of the dose concentration.

Sexual Behaviour Study

Experimental Animals: Seventy two Albino rats of Wistar strain (both sex), aged twelve weeks and weighing (150- 180g) were included in the study. All animals were kept in cages at room temperature under standard conditions (temperature 28±2^{o}C, relative

humidity 50±5%) and maintained in a 12h light/dark cycle and feed with water and solid pellet food *ad libitum*. The study was approved by the Faculty of Pharmacy, University of Uyo, Nigeria's ethical committee thus the experiments were conducted in compliance with their ethical guide for the care and handling of experimental animals. The male rats were randomly divided into 6 groups comprising of 6 rats each.

Sexual Behaviour Indices Assessment: Sexual behaviour monitoring was conducted in a darkened room after 8 h of extract administration for 19.00 and 22.00 hours. It was carried out in a plexiglass chamber under a dim light (using a red bulb). After a 30 minutes adaptation period in a plastic cage, the receptive female was introduced to the male and the female and male rats were observed for proceptive and precopulatory behaviours respectively. On a daily bases after extract administration, the rats were monitored for sexual behaviour for an observational period of 30 minutes. Female responsiveness was confirmed by exposing them to males that were not part of the study. The females with most responsiveness were selected for the study. Animals exhibiting low activity were excluded. Adopting the standard procedure of Agmo, (1997) and Gauthaman *et al.,* (2002). Male and female rats were paired in the ratio 1:1, in groups with access to food and water *ad libitum.* Prior to the experimental commencement all male rats were given training for sexual experience. This was done by allowing each male rat 30 min exposure to a female (as mating stimulus) thereby determining their estrous behaviour. Males showing ejaculation latency shorter than 15 min at least the last three sessions are referred to as being sexually active considered sexually experienced and were selected into the study.

Experimental Procedure

Thirty-six sexually experienced male rats were divided randomly into six groups of six rats each. Tween 80 (5ml /kg p.o) was administered to Group I and it served as control. II, III and IV Groups received (100 - 300 mg/kg; p.o) doses of the extract respectively. Group V received 1 mg/kg of testosterone while group VI was pretreated with 200 mg/kg of extract 10 minutes before 1 mg/kg of testosterone. The animals were treated with the extract 8 h prior to the commencement of the sexual behavior experiment. The male rats were kept in a plexiglass box for 10minutes to acclimatize them before introduction of the female. The test began when the female was introduced into the cage and terminated; after 15 minutes the females were brought in the estrous state. Administration of 17-β estradiol (10µg/kg body weight) and progesterone (500µg/kg body weight) to the females induced estrous in them, 48 h and 8 h respectively prior to the commencement of the experimental study.

The following parameters were documented

- Mount Latency(ML) (the time interval between the introduction of the female and the first mount by the male)

- Intromission Latency (IL) (the time interval between the introduction of the female and the first intromission by the male).

- Ejaculatory Latency (EL) which is the time between the first ejaculation and the first intromission;

- Mount Frequency (MF) which is the total number of mounts during the observation period;

- Intromission Frequency (IF) (the number of intromissions from the time of introduction of the female until ejaculation).

- Penile Erection (PE).

- Ejaculatory Frequency (EF) (the number of times semen was ejected from the male copulatory organ).

- frequency interferences (FI) or the total number of interferences during the hour of study.

Some additional male sexual behavior parameters computed include % mounted = (number mounted/number paired) × 100; % intromitted = (number of rats that intromitted/number paired) × 100; % index of libido = (number mated/number paired) × 100; % ejaculation = (number of rats that ejaculated/number paired) × 100; copulatory efficiency = (IF divided by MF + IF) × 100; inter-copulatory efficiency = average time between intromissions (calculated as ejaculated latency divided by intromission frequency) (Amin et al. (1996) and Agmo (1997).

After the sexual behavior test, the females were caged individually overnight with their sexually active male partners, a positive evidence of mating was the presence of spermatozoa on the vagina the following morning. Those rats which were pregnant were monitored till parturition. To determine the effect of extract on fecundity in male rats the number of pups littered, were counted (Vikas, *et al.*, 2009).

ANALYSIS OF STATISTICS

Values were expressed as Mean ± SEM. One-way ANOVA test was used to analyze the comparison of each of test groups for the statistical significance and the control with the use of SPSS 15, this was followed by Tukey Kramer multiple comparison post test. A significant level was considered with a probability of less than 5%.

RESULTS

ACUTE TOXICITY

The median lethal dose (LD_{50}) was 1000±0.66 mg/kg. The toxicity physical signs observed were; excitation, paw licking, decreased motor activity, increased respiratory rate, gasping and coma which were followed by death.

SEXUAL BEHAVIOUR'S EFFECT OF EXTRACT IN MALE RATS: Extract administration to male rats caused dose-dependent increase in mount, intromission and ejaculatory frequencies, as shown in Table 1. In the same vein, there were decreases in both mount and intromission latencies. The effects were significant statistically ($p< 0.05$) in relative to the control (Table 1).

FECUNDITY EFFECT OF EXTRACT IN MALE RATS: The fecundity effects of extract in male rats were observed as shown in Table 2. There were reductions in the number of female rats impregnated (66.67%) in all doses and in litter size (100mg/kg=5.33+1.43, 200mg/kg=5.50+1.11, 300mg/kg=3.50+95). These reductions in litter size were statistically significant ($p<0.001$) in relative to control (10.33+0.49). However, no pups were littered in the group that received testosterone (Table 2).

Table1: G. kola methanol extracts effect on male rats' sexual behaviour.

Weight of Animal (g)	Dose (mg/kg)	Mount Latency ml(s)	Intromission Latency ll(s)	Ejaculation Latency EL (s)	Penile Erection (PE)	Mount Frequency (MF)	Intromission Frequency (EF)	Ejaculation Frequency (EF)
215	Control Tween 80 (5ml/kg)	25.45±0.25	45.16±0.17	4.32±0.15	12.0±0.86	23.80±1.45	20.00±1.05	17.25±1.04
203	100	21.55±0.04^c	22.44±0.50^c	2.00±0.06^c	10.52±0.02	32.50±0.22^c	30.00±0.60	27.01±1.28^c
223	200	10.02±0.68^c	17.36±0.28^c	1.88±0.11^c	21.55±0.04^c	36.00±1.39^c	34.16±0.32	31.00±0.67^c
202	300	4.02±0.17^c	9.51±0.36^c	1 .55±0.39^c	8.22±0.04^c	39.33±0.46^c	34.16±0.28	32.00±0.82^c
223	Testosterone	3.90±0.55^c	3.60±0.55^c	1.21±0.04^c	7.45±0.35^c	39.50±1 .03^c	35.87±1,02	35.00±1.04^c
218	Testosterone +200	2.81±0.31^c	3.40±0.39^c	1.18±0.30^c	7.84±0.18^c	41.72±0.45^c	38.22±0.78	35.00±0.24^c

Values represented as Mean ± SEM. $^c p< 0.001$ = Significance relative to control. n=6

Table 2: Extract effect of on fecundity in male rats

Dose (mg/kg)	Female rats impregnated	Litter size
Control Tween 80 (5ml/kg)	6	10.33+0.49
100	4 (66.67%)	5.33+1.43^C
200	4 (66.67%)	5.50+1.11^c
300	4 (66.67%)	3.50+95^c
Testosterone	NIL (0%)	0.00+0.00^c
Testosterone + 200 Extract	2 (33.33%)	4.33+0.33^c

Values represented as Mean ± SEM. $^c p< 0.001$ = Significance relative to control. n=6

DISCUSSION

For centuries, traditional herbs have been used to improve sexual performance or treat erectile dysfunction. Substances that enhance both sexual drive/sexual pleasure and can also arouse sexual desire or libido are called aphrodisiacs. Impaired sexual functions can be modified by these substances (Gauthaman, *et al.*, 2002). In this research work, a remarkable change in sexual behaviour was caused in male rats by the extract as there were significant decrease ($p< 0.001$) in mount latency, with a corresponding increase in mount frequency, indicating enhanced arousal and sexual vigor. This may indicate that Garcinia kola seed extract stimulate blood flow to the penis, increase libido and decrease the periods between ejaculations. This was similar to the report of Yakubu *et al.*, 2008 on other plants. There was also decrease in intromission latency and increase in intromission frequency indicative of increased copulation rate. The extract might have activated the Cyclic Adenosine monphosphate (CAMP) pathway and Nitric Oxide/cyclic Guanosine monophosphate (NO/CGMP) thereby mediating this phenomenon which results in increased blood flow through the penile arteries. This was in accordance to the work by Murphy and Tee, (2002). These indices of libido, when taken together, pointed to the fact that, the extract may possessed aphrodisiac properties. Such increases in the frequencies of mount and intromission, suggest that libido, sexual vigor and sexual performance were enhanced in this study as it is also reported by Ratnasooriya and Dharmasiri, (2000) and Ralebona et al., (2012). Garcinia seed extract could have activated two mechanisms used to manage penile erection; the psychogenic erection which is achieved by erotic or emotional stimuli and the reflex erection, which is achieved by directly touching the penile shaft. The former uses the limbic system of the brain while the latter uses the peripheral nerve and the lower parts of the spinal cord. Thus Garcinia kola seed extract could have stimulated the parasympathetic activity, resulting in relaxation of the penile smooth

muscle increase by the penile stimulation or central sensory psychogenic stimuli or both which was similar to Saenz de Tejada *et al.,* (1996) work.

Another pathway that could have been used by the extract for inducing relaxation and erection is vasoactive intestinal peptide (VIP) mediated which was in line with the work of Ehmke *et al,* (1995). Here specific protein couples the VIP receptors in the Cavernous body forming $_{C}$AMP when stimulated by the catalytic activity of adenylate cyclase. The regulation of penile smooth muscle contractility is participated by endogenous prostanoids. Relaxation of prostaglandin E is mediated by the receptor designated "EP reception". Relaxation of the arterial and trabecular smooth muscles is caused by the stimulation of beta adrenergic receptors by catecholamines (McKenna, 1998), resulting in the fillings of the sinusoids. The plexuses of subtunical venules compress between the trabeculae and tunical albuginea occludes venous outflow and causes erection (Mayo clinic, 2006). Increased adrenergic activity enhances the termination of erection and it comprises of two components: The smooth muscle with a direct constrictor effect mediated by the alpha I and alpha II receptors and the vasodilatory nerves with an indirect effect whereby it is inhibited by a prejunctional, alpha 2 – adrenergic-mediated mechanism (De Great and Booth, 1993).

CONCLUSION

The results obtained from this research suggest that the methanol extract of *Garcinia kola* seeds might have caused improved sexual behaviour and enhance libido and potency in male rats after treatment. That the extract being able to enhance indices that favour sexual drive and vigor by increasing mount and intromission frequencies, while decreasing mount and intromission latencies, could be an indicative of its aphrodisiac potentials.

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