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Effect of hydro-methanolic extract of *xylopia aethiopica* on sexual behaviour in male wister rats

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ABSTRACT

This study aims to investigate the effect of *xylopia aethiopica* on the sexual behavior of adult male rats. Fort eight adult male wistar rats were used for the study. They were randomly divided into four groups of 6 animals each: group 1 was control, while groups 2, 3 and 4 were test groups treated daily with 0.5, 2 and 10 mg/kg body weight respectively of hydro-methanolic fruit extract of *xylopia aethiopice* for 30 consecutive days followed by 30 days recovery. Mating test was performed on six animals from each group on days 30 and 60 of the study. Also, 6 animals were sacrificed from each group and blood collected for serum testosterone assay on 31st and 61st days of the study. Results show significant ($P<0.05$) dose dependent increase in ejaculation latency (EL), and post ejaculatory interval (PEI) with significant decrease ($P<0.05$) in ejaculation frequency (EF) and in serum testosterone levels in rats in all the test groups. We conclude that *xylopia aethiopice* enhances copulatory performance without altering sexual excitement, arousal and motivation; therefore could be useful in the management of male sexual dysfunction.

Key words: xylopia aethiopice, sexual behavior, aphrodisiac, male sexual disorder.

INTRODUCTION

Natural compounds have attracted considerable attention because of their preventive, therapeutic, toxic, fertility and behavioural altering potentials. According to the World Health Organization (WHO), 80% of the population in Africa and in some Asian countries still use plant preparations to treat their illnesses. Xylopia acthiopica (Dunal) A. Rich (Annonaceae) is a valuable medicinal plant widely distributed in the West African rainforest from Senegal to Sudan in Eastern Africa, and down to Angola in Southern Africa (Burkhill, 1985). It is a West African ‘’pepper tree’’. Ethnobotanical survey of *xylopia aethiopica* shows that almost every morphological part of the plant is used in traditional medicine for managing various ailments and for a wide variety of applications. The dried fruits are used as spices in the preparation of two special local soups named “Obe ata” and isi-ewu taken widely in the Southern parts of Nigeria.

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(Tairu et al, 1999). Traditionally, the plant has been in use as a galactagogue which stimulates the secretion of breast milk. It is used as anti-helminthic and also as analgesic for chest pain (Esuoso and Odetokun, 2005). Extracts of the fruits have been reported to have various effects such as antibacterial, (Fleischer et al 2008; Konning et al 2004; Kuete 2010), haematopoetic effect (Adienbo et al, 2010; Abaidoo et al 2011), and anti-plasmodial (Boyom et al, 2003) activities. Until now there is no information about the effects of this plant on male sexual desire and potency. The current study aims to investigate the effect of *xylopia aethiopica* on the sexual behavior of adult male rats. It tests the hypothesis that daily ingestion of *xylopia aethiopica* fruits over a period of thirty days reduces the sexual behavior in adult male rats. Also, the serum testosterone is assessed in those animals in order to elucidate the mechanism of action of this plant in influencing the sexual behavior.

MATERIALS AND METHODS

Animals: The experiments have been conducted in accordance with the Declaration of Helsinki, and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutesof Health (USA). Forty eight adult male and 20 adult female wistar rats weighing between 160-225 g each bred in the Animal House unit of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria were used for the study. They were housed in standard cages and maintained under standard conditions of temperature (24–30°C), relative humidity (60–70%), 12 h light-dark cycle with free access to solid pellet diet (Livestock Feeds limited, Sapele Nigeria) and water *ad libitum* throughout the study except during the experiment.

Extraction of plant material: Authenticated dried fruits of *xylopia aethiopica* were procured from the market (Port Harcourt, Nigeria) The fruits were washed, oven dried at 40^{o}c to a constant weight. 2kg of the dried fruit was then ground to coarse powder form. Each 500g of dried and ground fruits was then refluxed in 2 liters of hydro-methanol solvent (1:4) at 60–70^{0}C for 6 hrs in a continuous extraction (soxhlet) apparatus. The extract was filtered and concentrated under reduced pressure at 60^{0}C using a rotary evaporator. The net yield was 174.6g. The yield of extract was 8.73% w/w in terms of dried starting material. The extract was preserved in a refrigerator at -4^{o}c.

Drug preparation: Since the hydromethanolic extract of *xylopia aethiopica* was more soluble in olive oil, therefore, the extract was suspended in olive oil to prepare fresh stock solutions of 0.1mg/ml and 0.5mg/ml respectively for oral administration. The volume of solution corresponding to the dose administered to each animal in the various groups was obtained using the formula: Volume (ml) = (Dosage ($mg.kg^{-1}$) x Body weight (kg))/ (Concentration($mg.ml^{-1}$)). Ethinyl oestradiol was suspended in distilled water, for oral use. Progesterone was also dissolved in olive oil for subcutaneous injection. All the drug solutions were prepared just before administration.

Experimental Design: Following 10 days of acclimatization, the adult male rats were randomly selected into 4 groups of 12 animals each. Group 1 (control group) was administered with the vehicle (olive oil) only, while groups 2, 3 and 4 were treated with the test substance (hydro-methanolic extract of *X. aethiopica)* at 0.5mg/kg body wt, 2mg/kg body wt, and 10mg/kg body wt respectively, each in 1ml volume of the vehicle and as single daily doses using animal-feeding intubation needles (Popper and Sons, New Hyde Park, NY). Six animals from each group were sacrificed, by cervical dislocation after chloroform anaesthesia, on day 31 of the study, twenty four hours after the last dose. The remaining 6 animals from each group were allowed another 30 days of recovery during which extract administration was discontinued; they were then sacrificed on day 61 of the study.

Mating behaviour test: The test was carried out by the methods of Dewsbury and Davis (1970) and Szechtman et al (1981), modified by Amin et al (1996). The test was carried out between 19:00 and 22:00 h under a dim light. Female rats were only allowed to mate when they were in oestrus. Females were brought into oestrus chemically by giving a single dose of 500 µg/animal oestradiol benzoate 48 h prior to pairing and then injecting progesterone subcutaneously at a dose of 5 mg/kg, 4 hours before the experiment. Six male rats from each of the groups were each monitored for sexual behaviour for 30 minutes observatory period on day 30, during the treatment period, as well as on day 60, during the recovery period. Sexual behavioral experiments began 3h after extract administration with the introduction of a male rat in an observation cage (56×35×31 cm).The stimulus female was introduced to the male after a 10 minute adaptation period by the male in the wooden cage. The receptive female and male rats were observed from the cage side for proceptive and precopulatory behaviours, respectively. The following parameters of sexual behavior were measured as previously described by Agmo,(1997), Gauthaman et al. (2002). and Zanoli et al,(2003):

- Mount latency (ML): time from the introduction of the female until the first mount
- Intromission latency (IL): time from introduction of the female to the first intromission (vaginal penetration)
- Ejaculation latency (EL): time from the first intromission to ejaculation
- Post-ejaculatory interval (PEI): time from ejaculation to the first intromission of the second copulatory series
- Mount frequency (MF): number of mounts preceding ejaculation
- Intromission frequency (IF): number of intromissions preceding ejaculation.
- Ejaculation frequency (EF): number of ejaculations in a copulatory series.

Serum Testosterone: 4ml of blood was obtained by cardiac puncture from each of the 6 rats in each study group after sacrification. Each blood sample was spun at 2500 rpm for 10 minutes in a desktop centrifuge. Serum samples were assayed for testosterone using the enzyme linked immunoassay (EIA) technique.

Statistical analysis: Statistical tests were performed using the general linear models procedure in the Statistical Package for Social Sciences Software (SPSS; version 17.0, USA). Data were analyzed using one-way ANOVA (analysis of variance of means). Post hoc comparisons between the groups after ANOVA were made using post hoc Tukey HSD test. Differences at the probability level $P \leq 0.05$ were considered statistically significant. The results, expressed as mean and standard error of mean (M±S.E.M) were presented in tables and figures.

RESULTS

Mating behaviour test: The results for sexual behaviour parameters are as presented in table 1. Several female proceptive behavior parameters (ear-wiggling characterized by a rapid anteroposterior vibration of the ears, a short run where the female rats suddenly stops and present her posterior to the male rats (darting) and a short jump with stiff legs followed by immobility and presentation (hopping)) were observed when the stimulus female rats were introduced to the extract-treated male rats. The male rats, in all the groups, responded with immediate advances towards the females and displayed precopulatory behaviour such as chasing, anogenital sniffing which eventually culminated into mounting. Lordosis was also displayed by the receptive female rats before, at the beginning and during the mounts. There was

genital toileting after every mount that resulted in intromission. The mount latency (ML) and intromission latency (IL) were observed to insignificantly ($p>0.05$) decrease while the Mount frequency (MF) and intromission frequency (IF) both increased insignificantly ($p< 0.05$) in a dose dependent manner in all the test groups when compared with their respective control groups. On the other hand, while the ejaculation frequency (EF) significantly decreased, the ejaculation latency (EL) and post ejaculatory interval (PEI) both demonstrated significant ($p<0.05$) dose dependent increase in all the test groups, when compared with their respective control groups. Also, a significant ($P<0.05$) dose dependent decrease in serum testosterone level (figure 1) was observed in all the treatment groups when compared with the control group. Such reductions in testosterone levels were however reversed after 30 days of extract withdrawal.

Table 1: Effect of hydro-methanolic extract of *xylopia aethiopica* on sexual behavioural parameters (Mean ± S.E) of male rats after 30 days treatment and 30 days reversal respectively.

Parameter	**Study period**	**Study group**			
		Control	**0.5 mg/kg**	**2 mg/kg**	**10 mg/kg**
Mount latency (secs)	Treatment	51.20±8.07	44.60 ± 11.4	43.40±9.03	39.80 ± 6.80
	Reversal	42.25±7.08	26.34±5.83	30.00±5.20	27.19±8.42
Mount frequency (n)	Treatment	9.6 ±1.29	10.0 ±0.45	10.40 ±0.4	11.0 ± 0.55
	Reversal	13.50±2.96	15.05±2.07	14.67±2.73	15.86±3.03
Intromission latency (secs)	Treatment	62.5.00±18.19	48.0±14.11	46.0 ±8.75	44.0 ±7.79
	Reversal	42.75±5.84	47.55±6.60	58.33±13.35	35.12±8.30
Intromission frequency (n)	Treatment	6.20 ±0.86	6.40 ±0.51	7.00 ±0.45	7.60 ±0.25
	Reversal	11.35±1.32	10.70±1.20	9.67±0.67	9.90±0.58
Ejaculation latency (secs)	Treatment	168.80±21.69	326.6±15.22 ‡	382.4 ±28.55 ‡	387.4 ±17.71 ‡
	Reversal	226.5±29.43	276.39±20.0	313.55±2.50	304.40±21.70
Ejaculation frequency (n)	Treatment	4.60 ±0.25	3.20 ±0.49*	2.60 ±0.4*	2.40 ±0.25*
	Reversal	4.25±0.48	3.50±0.34	3.00±0.00	3.01±0.64
Post Ejaculatory Interval (secs)	Treatment	187.60±16.60	228.00 ±4.90*	230.00±0.00 *	230.0 ±0.00 *
	Reversal	247.00±28.00	268.49±10.03	276.50±28.50	280.22±14.21

[a] Signifies significant difference ($p\leq0.05$). ‡Signifies highly significant difference ($p\leq0.01$)

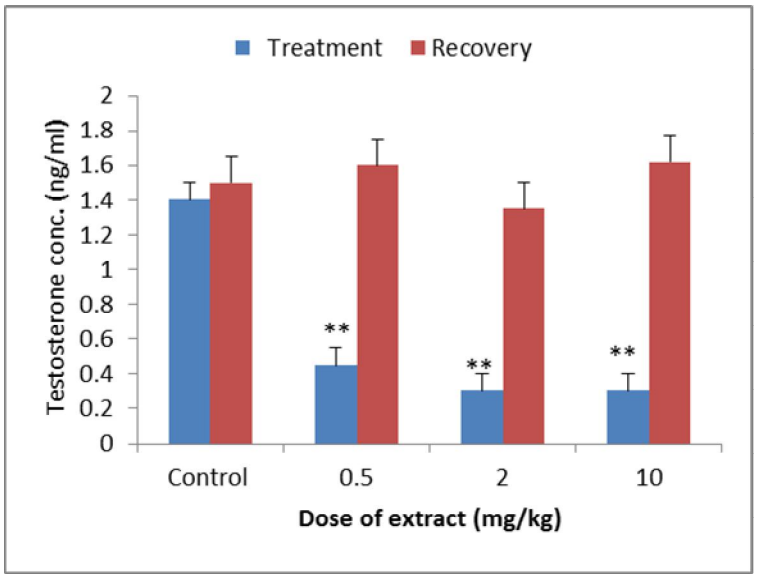

Figure 1: Effect of hydro methanolic extract of *xylopia aethiopica* on plasma testosterone concentration of male rats after 30 days treatment and 30 days reversal respectively. **significant difference ($p<0.01$).

DISCUSSION

In the present study, *xylopia ethiopica* fruit extract was tested in animal experimentation for its effect on sexual behaviour. The study showed that the extract possesses varied sexual function activity as observed in sexual behaviour tests. Mating behaviour test revealed that the extract does not have effect on the Mounting Frequency (MF), Intromission Frequency (IF), Mounting Latency (ML) and Intromission Latency (IL) in all the test groups. Mounting Frequency (MF) and Intromission Frequency (IF) are considered as indices of both libido and potency. Therefore, it does appear that the extract does not affect libido and potency at the doses used. Mounting and intromission latencies are considered to be inversely proportional to sexual motivation or desire (Beach, 1956). The non affectation of these parameters in this study suggests that at the doses used, the extract does not alter sexual desire and motivation. However, the extract was observed to significantly increase the ejaculation latency (EL) and reduce the ejaculation frequency (EF). The mating potential of a male rat is determined by the number of ejaculations during a time-limited behavioral test or by the number of ejaculations prior to his attaining sexual satiety (Sachs. and Meisel,1988).The significant prolongation in ejaculation latency as well as reduction in ejaculation frequency in all the test doses of the extract after 30 days of administration imply that *xylopia ethiopica* causes a prolongation in the duration of coitus, which indicates a significant and sustained increase in sexual activity and therefore an enhancement of copulatory performance. Post Ejaculatory Interval (PEI) which is considered as an index of potency and libido, and also a parameter of the rate of recovery from exhaustion after first series of mating (Tajuddin et al 2004) was in this study, found to significantly increase dose dependently in all the test groups, suggesting that the extract could cause a delay in recovery after an exhausting mating series, possibly due to a reduction of mitochondrial activity (Breitbart et al 1984; Randel et al 1992), from impairment of ATPase system, leading to energy depletion, hence the exhaustion. Male sexual behavior, consisting of both appetitive and consumatory components, is regulated by gonadal steroid hormones (Testosterone) secreted from the testes (Hull et al,

2006). In adult males, lack of testosterone (T) after castration abolishes both sexual drive and copulatory behavior, and the declined sexual ability can be restored by testosterone replacement (Hull et al, 2002). In this study, plasma Testosterone was reduced, but not abolished. There is a threshold for testosterone below which sexual function could be impaired (Carani et al,1992). The non affectation of ML and IL, which are androgen dependent, shows that this threshold was not exceeded and hence the sustenance of the appetitive component of the sexual response since there is a general and shared opinion that an affectation of these parameters (ML and IL) points out an affectation of the appetitive component of the sexual response (Paola et al,2008). Also, mount latency (ML), intromission latency (IL), and the post-ejaculatory interval (PEI) are commonly used for evaluating male's sexual motivation (Everitt 1990). Since ML and IL are not affected, the prolongation in PEI may be through a testosterone-independent mechanism. Reports show that lesions in the ventral tegmental area (VTA) or nucleus acumbens (NAc) increase PEIs, but did not affect copulation (Hull et al., 2006). This is similar to the results obtained in our study where mount and intromission latencies and frequencies were not affected, but the post ejaculatory interval was prolonged in the test groups, suggesting that the extract may have similar mode of action (likely direct inhibitory), on the ventral tegmental area (VTA) or nucleus acumbens (NAc). In conclusion, this study has shown that in male rats, *xylopia aethiopica* does not alter the appetitive and precopulatory components of sexual behavior such as sexual excitement, arousal and libido; but only affects the consumatory phaseby prolonging the duration of coitus, which indicates a sustained increase in sexual activity and therefore an enhancement of copulatory performance which may be through testosterone-independent mechanisms, possibly by altering the activities of some hypothalamic regulatory centres . It however, causes a delay in recovery after an exhausting mating series. These results show that the extract may have similar effects in male humans by maintaining sexual desire (libido) and arousal, while sustaining a prolongation in the duration of sexual intercourse, which may be useful in the management of male sexual dysfunction (MSD) such as premature ejaculation.

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