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Aphrodisiac Effect of Ethanol Extract of *Piliostigma thonningii* Leaf on Male Albino Wistar Rats

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ABSTRACT

In an attempt to evaluate the acclaimed aphrodisiac activity of ethanol leaf extract of *Piliostigma thoningii*, twenty (20) male wistar albino rats were weighed and grouped into four study groups (A-D) of five animals each. Rats in group A (control) were administered with 1ml of distilled water orally while those in groups B, C, and D were given same volume orally, corresponding to 50, 100, and 200 mg/kg body weight of the extract respectively for 21 days. Sexual behaviour parameters were monitored in the male rats for 3 days after administration by pairing with a receptive female (1:1). The male serum testosterone concentration was also determined. Cage side observation on the animals revealed prospective behaviours by the receptive female rats and pre- copulatory behaviours by the extract-treated male rats. The extract at 50, 100, and 200 mg/kg body weight significantly ($P < 0.05$) increased the frequencies of mount and intromission. In addition, the ejaculation latency was significantly ($P < 0.05$) prolonged. The latencies of mount and intromission were reduced signifi-cantly ($P<0.05$) whereas ejaculation frequency increased. The extract also reduced the post- ejaculatory interval of the wistar albino rats. Computed percentages of index of libido, mounted, intromitted, ejaculated and copulatory efficiency were higher in the extract- treated animals than the control whereas the intercopulatory interval decreased significantly. The extract also significantly ($P<0.05$) increased the serum testosterone content of the animals. Data from this study suggest that the ethanol extract of *Piliostigma thonningii* leaf enhanced sexual behaviour in male rats.

INTRODUCTION

Plant materials are central to tradiomedical practices and have remained usefulsources of new drugs (O'Brien, 2004). Although, orthodox medical practice is generally acceptable, alternative health care is still relied on all over the world (O' Brien, 2004; Leckridge, 2004). In the developing countries of the world, traditional herbal medicine is often used side by side Western medicine with herbal medicine taking the upper hand when the cost of Western medicine is beyond reach (Busia, 2005). Management of degenerative diseases such as mental illness, sexual dysfunction and microbial infections is one area in which a lot of people in developing countries depend on herbal medicine (Adimoelja, 2000; Ajali, 2002; Fang and Schinkle, 2007). However, in an attempt to control the spread of these degenerative diseases, some strategies have been proposed. The search for new ways to treat them stimulates the investigation of cheap and effective natural compounds as an alternative treatment of the aforementioned diseases (Odukoya, 2002; Yakubu, 2011). Erectile dysfunction being one of the sexual dysfunctions can be caused by psychological disorders like anxiety, depression, stress, cerebral trauma, Alzheimer's, Parkinson disease; chronic disorders – diabetes, hypertension, vascular insufficiency, atherosclerosis; penile diseases - phimosis, peyronies; life style – chronic alcohol abuse, cigarette smoking; ageing – decrease in hormone level with age; Systemic diseases – Cardiac, hepatic, renal, pulmonary; Organ diseases like hypogonadism and hyperprolactinaemia; and cancer (Mendoza *et al*., 2008;). The incidence of sexual inadequacy in human males has led to the development of a number of available treatment options. Unfortunately, these options are too expensive, not easily accessible and with some serious side effects such as aching in the penis, urethral burning, infection, pains and bleeding (Yakubu, Akanji, and Oladiji, 2007). These problems, coupled with the increasing number of men seeking help for the treatment of male sexual dysfunction (MSD) with negligible side effect, have necessitated the need for more pharmacological research on plant-derived chemicals that have sex enhancing (aphrodisiac) potentials

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in animals (Adimoelja, 2000; Cicero, 2001). In Africa, *Piliostigma thonningii* is one of the plants with diverse ethnomedical and economic applications (Igoli *et al*., 2005). It is known locally as abefe in Yoruba, kalgo in Hausa, okpoatu in Igbo, nyihar in Tiv, omepa in Igede ,ejei-jei in Igala, obepa in Yala and Kidakpam in Obudu languages (Rabo and Sanusi, 2001; Odukoya, 2002; Igoli *et al*., 2003; Edeoga *et al*., 2005; Jimoh and Oladiji, 2005; Aderogba *et al*., 2006; Brummitt *et al*., 2007; Sofowora, 2008; Ozolua *et al*., 2009; Dasofunjo *et al*.,2012; Dasofunjo *et al*., 2013).

The medicinal value of different parts of the plant has been examined, of which various preparations of its parts have been used to arrest bleeding, treat fevers and bacterial infections (Fakae *et al*., 2000; Igoli *et al*., 2005). It also acts as laxative, anthelmintic, anti-inflamatory, hypocholesterolemic, hypoglycemic, hepactoprotective and haematopoietic agent (Fakae *et al*., 2000; Igoli *et al*., 2005; Togola *et al*., 2005; Dasofunjo *et al*., 2012 Dasofunjo *et al*., 2013a, Dasofunjo *et al*.,2013b).

The need to solve the problems of male sexual dysfunction as stated above has led to the use of *Piliostigma thonningii* extract in this research work to ascertain its aphrodisiac effect as used in folk medicine by the people of Benue State, Nigeria.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Piliostigma thonningii* were obtained from Okuku, Cross River University of Technology (CRUTECH), Cross River State, Nigeria. The plant was identified and authenticated at the Federal College of Forestry, Jos, Plateau State, Nigeria with the voucher number #25.

Experimental Animals

Albino rats were obtained from the animal holding unit of Medical Biochemistry, CRUTECH. The animals were allowed to undergo acclimatization period of seven (7) days and were housed in a well ventilated wooden cage. They were kept at room temperature of 29±2ºC with relative humidity of 70% and 12hours natural light and dark cycle. The rats were allowed free access to standard feed from Vital Feeds, Ogoja Cross River State and supplied with portable water. Good hygiene was maintained by constant cleaning and removal of faeces and supplied feed from the cage on daily basis.

Assay Kits

The automated analyzer (902) for testosterone assay and other assay kits are products of Randox Laboratories Limited, United Kingdom. Diethyl-Stilboesterol is a product of Sigma Chemicals (St. Louis, USA). All reagents used were of analytical grade.

Preparation of Plant Material

Fresh leaves of *Piliostigma thonningii* were collected and air dried for 14days until constant weight was obtained. They were pulverized using a blender and sieved to obtain the powdered form.

Three hundred grams (300g) of the powdered form was dissolved in 1000ml of 75% ethanol for 72hours to achieve maximum extraction. The solution was filtered using Whatman No.1 filter paper and the filtrate concentrated in water bath at 50ºC. The slurry from ethanol extract was later weighed and reconstituted in distilled water to the required dosage for administration.

Animal Grouping And Administration of Extract

A total of 20 male rats (weighing 230-300g) were selected for study. They were randomly divided into four groups (A, B, C, D) and ear tags and colour codes were given to identify each animal. The control group (A) received 1ml of distilled water orally for 21days. The three test groups (B, C and D) were administered orally with the ethanol leaf extract of *Piliostigma thonningii* on a daily dosage of 50, 100 and 200mg/kg body weight respectively in 1ml of distilled water for 21days.

Three days (72hours) prior to final administration of the leaf extract, 25 virgin female rats were selected and each of them was given sub-cutaneous injection of diethyl-stilboesterol (0.1mg/kg body weight in 0.5ml olive oil). On the last day of the administration, the diethyl-stilboesterol injection was administered to ensure that the female rats were in oestrous, this being the time when they were most receptive to fertilization (Mills *et al*., 1996). Half hour after the last dose administration, on the last day, the male rats were individually placed in separate cages.

The animals were handled humanly in accordance with the guidelines of European Convention for the protection of vertebrate animals and other scientific purposes – ETS – 123 (European Treaty Series, 2005).

Determination of the ethanol extract of *Piliostigma thonningii leaf* on male rats sexual behaviour

The male sexual behaviour test was carried out by the method of Ratnasonviva and Dhamasiri (2000) modified by Tajudeen *et al*., (2004) and that of Agmo (1997) modified by Gauthaman *et al*., (2002) and Yakubu and Akanji (2011).

The receptivity of the females was confirmed before the test by exposing them to the male rats that were not used for the experiment. Twenty most receptive females (observed when females firmly raised their hind limb quarters and tails to accept male sexual advances) were selected for the study. The experiment was carried out 2 to 3hours for 3days after the onset of the dark in a quiet room (under red light) as this is the time when these wistar rats (Crepuscular) are most active (Mills *et al*., 1996).

Two independent observers, blind to the conditions (test versus control) manually scored by monitoring the behaviour of the male rats. The male rats in separate cages were allowed 10minutes adaptation period with the receptive females (1 female to 1 male). The following parameters of sexual behaviour were monitored for 15minutes after pairing.

Mount frequency (MF)

The number of times the males assumed copulatory position but failed to achieve intromission – characterized by lifting of the male's fore body over the hindquarter of the female and clasping her flanks with his forepaw.

Intromission frequency (IF)

The number of vaginal penetration made by the male.

Ejaculation frequency (EF)

The number of times there was expulsion of semen by the males after vaginal penetration – characterized by rhythmic contraction of the posterior abdomen. The female rats were also observed for the presence of vaginal plug. In addition, other standard parameters of sexual behaviour obtained through manual data acquisition using stopwatch included

Mount latency (ML)

The time from the introduction of the female until first mount made by the male.

Intromission latency (IL)

The time from the introduction of the female until the first intromission by the male that is usually characterized by pelvic thrusting and springing dismount.

Ejaculation latency (EL)

The time from the first intromission until ejaculation – usually characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of reduced activity.

Post ejaculatory interval (PEI)

The time interval from ejaculation to intromission of the next series.

Some additional male sexual behaviour parameters computed include:

% index of libido = (number mated /number paired) × 100; % mounted = (number mounted/number paired) × 100; % intromitted = (number of rats that intromitted/number paired) × 100; % ejaculated = (number of rats that ejaculated/number paired) × 100; Copulatory efficiency = (number of intromission/number of mounts) × 100; and intercopulatory efficiency = average time between intromissions.

The next morning (08:00hours), the vaginal smear of each female rat was examined under a microscope for the presence of spermatozoa; and the number of sperm-positive females were calculated for the control and experimental groups and the mated females were watched for pregnancy and birth of offspring.

Preparation of Serum

The animals (male rats) were anaesthetized in a jar containing cotton wool soaked in ether. When the rats became unconscious, they were quickly brought out of the jar and the abdominal region was opened along the linear Alba cut with scalpel blade to expose the organs and blood was collected into a sterile sample container by a cardiac puncture. Later, the blood was transferred into a clean, dry centrifuge tube and allowed to clot for 30min before centrifuging at 300rpm ×10min using Uniscope Laboratory Centrifuge (model SM800B, a product of Surgifriend Medicals, Essex, England). The sera were thereafter aspirated into clean, dry sample bottles using Pasteur pipette and used for the determination of testosterone concentration within 12hours of preparation as described by Malomo (2000).

Determination of serum testosterone concentration

The serum testosterone concentrations of the animals were determined using the procedure outlined in the manufacturer's instruction manual (Elecsys and cobas e analysers - Products of Roche Diagnostics GmbH, Germany) which operates with the chemiluminescence immunoassay (CLIA) method.

The principle of the CLIA test follows the typical competitive binding scenario as described by Tietz (1995), Ekins (1998), Ooi (1998) and modified by Yakubu and Akanji (2011). Competition occurs between an unlabeled antigen (present in standards, control and samples) and an enzyme labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the luminescence substrate solution is added. The relative luminescence units (RLUs) are measured on a microtiter plate luminometer. The RLU values are inversely proportional to the concentration of free testosterone in the sample. A set of calibrators were used to plot a standard curve from which the amount of free testosterone in samples and controls can be directly read (Tietz, 1995; Ekins, 1998; Ooi, 1998; Winter, 1998; Yakubu and Akanji, 2011).

Statistical analysis

Data were presented as a mean ± SD of five determinations. Statistical analyses were carried out using one way analysis of variance (ANOVA). Differences were statistically significant at $P<0.05$ (Mahaja, 1997).

RESULTS

Several female proceptive and male precopulatory behaviour parameters were observed from the cage side when the extract-treated male rats were introduced to the receptive female rats. The proceptive behaviour displayed by the female rats included ear-wiggling characterized by a rapid antero posterior vibration of the ears, a short run where they suddenly stop and present their posterior to the male rats (darting) and a short jump with stiff legs followed by immobility and presentation (hopping). The male rats, upon introduction, responded with immediate advances toward the females and displayed precopulatory behaviour such as chasing, anogenital sniffing which eventually culminated into mounting. The extract produced no sedative effect on the male rats since none of the animals showed any evidence of tiredness throughout the observatory period.

Similarly, dot receptivity was not displayed by any of the female rats used in the study. The parameters (MF, IF, EF, ML, IL, EL and PEI) were exhibited by both groups of the male rats (the control (A) group and the extract-treated groups-B, C, D) but were of different latency and frequency. Within the extract-treated male rats, these parameters are of different values (Tables 1, 2 and 3).

Table. 1: Effect of the ethanol extract of *Piliostigma thonningii* leaf on male rats sexual behaviours monitored on Day 1.

Parameters	A (Control)	(mg/kg body weight)		
		B (50)	C (100)	D (200)
MF	7.5±0.23	4.5±0.32	3.5±0.22	2.0±0.22*
IF	5.0±0.03	8.0±0.22*	10.0±0.21*	12.0±0.21*
EF	3.0±0.31	4.0±0.21	5.0±0.43*	6.0±0.31*
ML	30±0.42	120.0±0.22*	60.0±0.04*	40.0±0.22
IL	120.0±0.22	60.0±0.01*	50.0±0.21*	40.0±0.03*
EL	6.0±0.32	4.0±0.31*	6.0±0.11	3.0±0.21*
PEI	60.0±0.03	30.0±0.03*	10.0±0.24*	5.0±0.31*

Results were expressed as mean ± SD (n=5) * significant at P<0.05 compared with the control. Where MF=Mount frequency; IF=Intromission frequency; EF=Ejaculation frequency; ML=Mount latency; IL=Intromission latency; EL=Ejaculation latency; and PEI=Post ejaculation interval.

Table. 2: Effect of the ethanol extract of *Piliostigma thonningii* leaf on male rats sexual behaviours monitored on Day 2.

	A	Extracts (mg/kg body weight)		
Parameters	(Control)	50	100	200
MF	1.0±0.22	4.0±0.33*	6.0±0.42*	8.0±0.43*
IF	1.0±0.31	3.5±0.21*	4.0±0.31*	6.0±0.45*
EF	0.5±0.22	1.5±0.33*	2.0±0.33*	2.5±0.20*
ML (sec)	120.0±0.34	300.0±0.21*	70.0±0.22	160.0±0.24*
IL (sec)	180.0±0.40	120.0±0.31*	130.0±0.40	100.0±0.33*
EL (sec)	1.0±0.30	2.0±0.36	3.0±0.42*	5.0±0.34*
PEI (sec)	100.0±0.31	60.0±0.44*	50.0±0.44*	20.0±0.22*

Results were expressed as mean ± SD (n=5) * significant at P<0.05 compared with the control. Where: MF=Mount frequency; IF=Intromission frequency; EF=Ejaculation frequency; ML=Mount latency; IL=Intromission latency; EL=Ejaculation latency; and PEI=Post ejaculation interval.

Table. 3: Effect of the ethanol extract of *Piliostigma thonningii* leaf on male rats sexual behaviours monitored on Day 3.

	A	Extracts (mg/kg body weight)		
Parameters	Control	50	100	200
MF	1.0±0.22	3.0±0.33*	5.0±0.42*	7.0±0.43*
IF	0.5±0.31	1.5±0.21	2.0±0.31	4.0±0.45*
EF	0.5±0.22	0.8±0.33	1.0±0.33	2.0±0.20
ML (sec)	180.0±0.34	160.0±0.21	130.0±0.22*	120.0±0.24*
IL (sec)	200.0±0.40	170.0±0.31	140.0±0.40*	110.0±0.33*
EL (sec)	2.0±0.30	5.0±0.36*	8.0±0.42*	10.0±0.34*
PEI (sec)	160.0±0.31	80.0±0.44*	70.0±0.44*	40.0±0.22*

Results were expressed as mean ± SD (n=5) * significant at P<0.05 compared with the control. Where: MF=Mount frequency; IF=Intromission frequency; EF=Ejaculation frequency; ML=Mount latency; IL=Intromission latency; EL=Ejaculation latency; and PEI=Post ejaculation interval.

At day 1, the control group showed a decrease in IF, ML and increase in MF and PEI but no significant impact was observed in the other days (day2 and day3) compared to the extract-treated group (Table 1-3 respectively). The extract-treated group at 50, 100 and 200mg/kg body weight showed significant effect on the parameters in which IF and EF increased while ML and PEI decreased (p< 0.05) significantly at the days of observation (Table 1 -3 respectively) compared to the controlled group. The computed male rats sexual behaviour parameters which include percentage (%) index of libido, mounted, intromitted, ejaculated and copulatory efficiency were significantly (p<0.05) higher in the extract-treated animals when compared to the control group (Table 4). The ethanol extract of *Piliostigma thonningii* leaf at 50, 100 and 200mg/kg body weight produced a significant increase in serum testosterone content of the albino male rats compared to that of the untreated control albino male rats (Table 5). Testosterone level was highest at 200mg/kg body weight compared to that of the 100 and 50mg/kg body weight following administration (Table 5).

Table. 4: Effect of the ethanol extract of *Piliostigma thonningii* leaves on computed parameters of male rats' sexual behaviour.

Computed Parameters	A Control	B (50mg/kg body weight)	C 100mg/kg body weight)	D (200mg/kg body weight)
% Index of Libido	75	100	100	100
% Mounted	75	100	100	100
% Intromitted	50	100	100	100
% Ejaculated	50	75	90	100
% Copulatory efficiency	58.82	83.33	92.31	96.30
Intercopulatory Interval (Efficiency) (sec)	300	200	180	140

Table. 5: Effect of ethanol extract of *P.thonningii* leaf on serum testosterone concentrations.

		Extract (mg/kg body weight)		
	Control	50	100	200
Serum Testosterone Concentration (ng/ml)	0.87±0.23	2.39±0.42*	5.50±0.21*	8.72±0.03*

Results were expressed as mean ± SD (n=5) * significant at P<0.05 compared with the control.

DISCUSSION

The need for plants with sex enhancing potential or aphrodisiacs with little or no side effect is of great concern. Various substances of animal and plant origin have been used in folk medicine of different cultures as aphrodisiac, some of which have been identified pharmacologically to exert their effects on the hypothalamic – pituitary – testicular axis (Mills *et al*., 1996; Yakubu, 2011). Plant preparations including *Piliostigma thonningii* as sex enhancer has become prominent in folk medicine. To understand the scientific reasons behind these folk claims instigated the need for investigation of the effects of ethanol extract of *P.thonningii* leaf in this study. The parameters of the sexual behaviour of the male albino rats such as MF, IF, EF, ML, EL, PEI and testosterone levels were used in this study.

Mount frequency and intromission frequency are useful indices of vigour, libido and potency. While the number of mount (MF) reflects sexual motivation, increase in the number of intromission (IF) shows the efficiency of the erection, penile orientation and the ease by which ejaculatory reflexes are activated (Agmo, 1997). Therefore, the significant increase in MF and IF following administration on day 2 and day 3 of the observation days suggests enhanced libido (Tajuddin *et al*., 2004). Such enhancement of libido might have arisen from increase in the concentration of several anterior pituitary hormones and serum

testosterone which in turn stimulated dopamine receptor synthesis and sexual behaviour (Taha *et al*., 1995; Yakubu *et al*., 2008). It may therefore be logical to suggest that the sex-enhancing behaviour of the albino rats maybe due to flavonoid/saponin constituents of the plant since they have been reported to alter androgen levels (Dasofunjo *et al*., 2012; Dasofunjo *et al*., 2013).

The disparity in the values of MF and IF in this study suggests that it was not every mount by the male rats that resulted in intromission. Similarly, the increase in EF by the leaf extract of *P.thonningii* at 200mg/kg body weight on day 2 and day 3 is an indication of enhanced aphrodisiac potential of the plant. The presence of plug in the vagina of the female rats indicated that ejaculation occurred.

Furthermore, since intromission is not possible without adequate erection and coordinated activity of penile muscle (Agmo, 1997; Yakubu, 2011), the increase in IF by the extract in this study suggests that the mechanism of the penile erection was activated. Therefore, ethanol extract of *Piliostigma thonningii* leaf may increase potency by allowing or sustaining erection.

The observed changes in testosterone levels of the experimental male rats was dose dependent which also suggest that the plant extract exhibited aphrodisiac effect as supported by Mills *et al*.(1996). Clinical data on testosterone also suggest that a slight increase in the levels of the hormone in adult males results in a moderate but significant increase in sexual desire and libido (Thakur and Dixit, 2007).

CONCLUSION

These results are clear indications that ethanol extract of *Piliostigma thonningii* leaf at graded doses of 50, 100 and 200 mg/kg body weight could be used to positively enhance sexual behaviour in male rats. Thus, this study supports the acclaimed aphrodisiac potential of the plant in folk medicine.

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