INVESTIGATING THE EFFECTS OF PAUSINYSTALIA YOHIMBE AQUEOUS LEAVES EXTRACT ON PARAMETERS FOR REPRODUCTIVE PERFORMANCE IN EXPERIMENTAL RATS

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ABSTRACT

BACKGROUND: Herbal plants and herbal preparations are widely used as immunomodulatory medicines that helps in improving reproductive health in humankind, and they are globally used and have rapidly grown in economic developmental importance.

OBJECTIVE: The study was conducted to investigate the effects of the aqueous extract of P. yohimbe leaves on sexual reproductive performance in Wistar rats. The primary outcome of the study is the reproductive performance sexual rate.

METHODS: The experimental study was carried out on albino rats of the Wistar strain, weighing between 150 and 200g sexually naive. The animals were raised in polyethylene cages and divided into four groups, which received a saline solution (control group), 2, 5, and 10 mL/kg of the aqueous extract of P. yohimbe leaves for fourteen days orally. The sexual behavior test was performed according to three types of crossing.

RESULTS: The results of the treated groups showed a significant increase in mating frequency compared to the control group. Overall, the results showed that P. yohimbe significantly affects sexual behavior. The aqueous extract of P. yohimbe leaves increased sexual behaviour and orientation activity performance recorded in the treated rats.

CONCLUSION AND RECOMMENDATION: The enhanced reproductive performance appetitive detected in the study, justify the indigenous use of the herbal plant in reproductive medicine and could be a precursor in the synthesis of useful drugs. However, based on the current findings, we recommend that careful administration be considered as a better option justifying human circumstances for patient with advanced mankind age in low dosage resource settings, as it achieves a better reproductive performance rate at a lower dose dependent increase.

KEYWORDS: Aphrodisiac effect; herbal plant; reproductive performance parameters; experimental rat.

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INTRODUCTION

Medicinal plants serve as critical therapeutic agents and valuable raw materials for manufacturing numerous traditional and modern medicines. In many developing countries, traditional medicine is still the mainstay of health care, and most of the drugs and cures come from natural sources of plant origin[1]. Many herbal Plants and plant preparations produces a wide range of secondary metabolites such as phenolic compounds, alkaloids, flavonoids, and other secondary metabolites with proven aphrodisiac[2], spermatogenic[3] and antioxidant activities[4, 5, 6].

The aphrodisiac therapy, Pausinystalia yohimbe (K. Schum.) Pierre ex Beille, belongs to the family Rubiaceae. It is an evergreen species growing in West and Central Africa in lowland forests. The tree grows about 30 m tall, with a straight boles/trunk that is rarely larger than 50-60 cm in diameter. The leaves grow in groups of three, with short (about 2 cm) petioles. The blades are oval-shaped, 11-47 cm long and 5-17 cm wide. Its geographical spread is from South-Western Nigeria to Gabon and Zaire[7, 8].

The aphrodisiac activity of some plants like Tribulus terrestris and Microdesmis keayana have been implicated due to their androgen increasing properties[9, 10]. Since the Pausinystalia yohimbe leaf has been acclaimed to be used as an aphrodisiac8, there is the need to provide scientific information on its androgenic potentials which appear not to be, to the best of my knowledge, in existence of the open scientific literature. This study therefore attempts to provide scientific evidence to the androgenic potentials inherent in the aqueous extract of Pausinystalia yohimbe leaf, and focuses on evaluating the effect on sexual behavior and its sequences leading to the mating of Wistar rats.

MATERIALS AND METHODS

Animals

The Animal, Herbal Plants and Plant Preparations Ethics Committee of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria has approved the experimental protocol. We used adult rats, Wistar strain for all experiments. These animals were acclimatized to laboratory conditions (temperature 25±2 °C and humidity 70-80% and photoperiod 12:12h); with free access to rat's pellet and water. And after extract administration, they were subjected to various sexual behavioural study using the methods of Yakubu *et al*[11].

Ethical approval was obtained before the start of the research study from the Institutional Ethical Committee on Use of Experimental Animal and Procurement of Herbal Plants of Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria.

Procurement, Authentication and Preparation of the Plant Sample

The plant material was collected at Arakhuan village in the boundary of Okomu National Park, Udo, Edo State. It was authenticated in Botanical Systematic Unit of Eureka Herbal Clinic, IgboEgunrin, Ondo State. Aqueous maceration of P. yohimbe powdered leaves sample was done by macerating 400g of plant powder for 24 hours in two liters of distilled water at (99.8%) at room temperature and in the shade for 72 days. After filtration, the solution obtained was evaporated in the shade. With the help of a magnetic stirrer to drive out the solvent using a hot plate at 50 °C until a paste was obtained, kept at 4 °C until its use.

Treatments, Dosage of Test Drug and Route of Administration

Forty rats were separated into four groups, a control group and three treatment groups (5 males and 5 females each), which were subjected to treatment by gavage, of 2, 5 and 10 mL/kg of P. yohimbe for fourteen successive days.

Reproductive Performance

In mammals, male sexual behavior includes a precopulatory phase (motivational or appetitive) and a copulatory (or consummatory) phase leading ultimately to ejaculation12. During the precopulatory phase, male rats sniff the genitals of females, emit ultrasonic vocalizations, mark their territory with urine, and show a clear preference

for oestrus (or receptive) females[12]. During the consumptive phase, the male performs climbs followed by intromissions, during which he performs back-and-forth movements corresponding to rhythmic pelvic thrusts, culminating in ejaculation[12]. Thus, in rats, copulatory parameters take place chronologically according to Yakubu *et al*[11]: (1) In the *mounting*, the male rat climbs on the receptive female in heat from the rear flank and makes pushing movements with his pelvis. The latency is generally 3 to 6 seconds; (2) The intromission occurs similarly to the mating but is immediately followed by a deep pelvic thrust when the penis enters the vagina; this penetration phase lasts about 200 to 400 ms in well-trained rats.

Reproductive Performance Measurements

After the last administration, the animals were placed in a mating cage (one couple per day) in a quiet room at room temperature (24±2 °C) with a light/dark cycle of 12:12h and a humidity of 60 to 65%. Before each test, the male rat was placed in the observation cage (42 cm x 10 cm x 21 cm), he was allowed to get used to the test chamber for 5 min. Afterward, a female was introduced into the cage, and the mating behavior observation started immediately after the introduction of the female, and the parameters were recorded as the test progressed. The copulatory activity (rise latency, intromission latency, rise frequency, and intromission frequency) of each male was evaluated in the presence of a female in a quiet room as described by Watcho et al13.

Reproductive Orientation Activities

Male rat orientation activities towards females (riding, licking, and sniffing), and towards oneself (genital grooming, non-genital grooming) were observed during the period of the copulatory behavior test, according to the method described and evaluated according to Zade et al[14]. No male was exposed to the same female more than once during the experiment. These tests are carried out according to three types of crosses: control male × control female, treated male × treated female, and treated male × control female. Examinations usually are completed immediately after the first post-ejaculatory intromission. In this test, female rats were introduced into the cages of the male animal with a ratio of one female to one male[11].

Reproductive Performance Parameters

The following parameters of sexual behavior were measured as described by Yakubu et al[11]. Mating latency time (ML) is the time between introducing a female into the cage and the first breeding; The latency time of the intromission (IL) is the time that separates the introduction of the female and the first intromission; The frequency of mounts (MF) is the mounts numbers, with or without intromissions preceding ejaculation; The frequency of intromissions (IF) corresponds to the number of intromissions preceding ejaculation; Penile licking (PE) is the number of times the rat bent over to lick the penis.

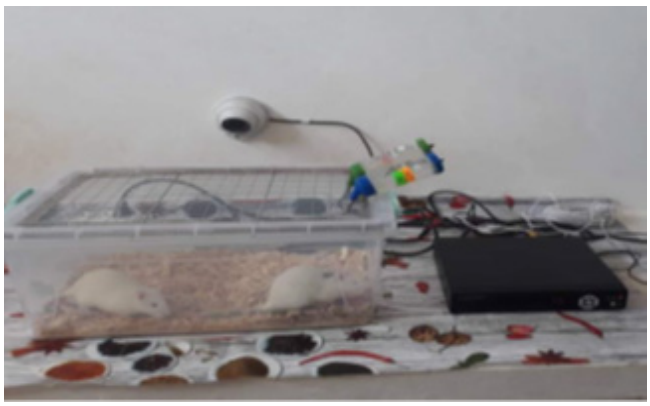

Plate 1: A pictorial relay of reproductive performance test

Data Analysis

All parameters measured in this study were statistically analyzed by SPSS computer software (version 20) and Microsoft Excel (2013) software, using descriptive metric methods giving the mean, and the standard deviation of the mean. The results were, also, analyzed by a variances comparison (ANOVA, whose significance level is $p < 0.05$).

RESULTS

From our findings, we noted that the first contact time is 25.60±5.30 seconds in the control rats, while

the treated rats took less time to establish the first contact with their partner than the control rats; these times are significantly different (Table 1). For the intromission latency time, the aqueous extract of P. yohimbe acts on this time, and we recorded less time in the treated rats compared to the control (Table 1). Aqueous extract significantly increased the intromission frequency in treated rats compared to control rats, which had lesser frequency (Table 1). Similarly, we recorded a significantly decreased mounting latency in the treated rats compared to control rats (Table 1).

Table 1 Effect of the aqueous leaves extract of P. yohimbe on 1st contact, the latency time of the intromission, the frequency of intromission, and mating latency time of Wistar rats.

Group	1st Contact time (s)	Contacts number	Intromission latency time (s)	Intromission frequency	Mounting latency time (s)
Control	25.60± 5.30	111.20± 36.51	12316.20±11444.51	3.80±1.39	14.60±6.12
2 mL/kg	19.20±8.03	120.40± 25.33	12107.60±9877.75	15.60±8.80	5.00±1.41
5 mL/kg	9.20±0.80	168.40±15.68	6693.60±3374.76	19,40±8,90	4.20±1.20
10 mL/kg	21.40±8.17	140.40± 15.37	7673.40±1334.36	17.20±6.40	6.00±3.40
P-value	0.05*	0.05	0.05	0.05**	0.05**

The mounting frequency is increased in the treated rats compared to the control (Table 2). We found highly significant differences between the mount's frequencies ($P < 0.05$; Table 2). For the rise latency time, P. yohimbe affects significantly, the rise latency time ($P < 0.05$; Table 2). The P. yohimbe aqueous extract has a highly significant influence on the mating time ($P < 0.05$; Table 2). In the end, we show that the males lick their penises; we noted that the P. yohimbe aqueous extract does not act on this sequence, and we recorded no significant effect between the licking times (Table 2).

Table 2 Effect of the aqueous leaves extract of P. yohimbe on the frequency of mounts, mating time, time of licking, and number of lickings of Wistar rats.

Group	Mounts frequency	Mating time (s)	Licking time (s)	Lickings number	Ejaculation
Control	3.80±1.39	7.60±2.78	165.20±104.30 12.	40±6.54	Absent
2 mL/kg	11.20±6.40	30.10±15.20	163.00± 75.27	14.00±3.16	Present
5 mL/kg	19.40±8.90	38,80±17,81	214.40±144.91	23.00±7.42	Present
10 mL/kg	15.60±8.80	31.20±17.60	167.00± 79.87	18.00±5.16	Present
P-value	0.05**	0.05**			

Also, administration of aqueous extract of P. yohimbe leaves affects specific performance parameters and sexual motivation in treated rats, thereby, causing a stimulatory effect, increasing sexual performance and orientation activity towards the female when compared with the control rats (Table 3).

Table 3 Effect of the aqueous leaves extract of P. yohimbe on the exploration, raring, climbing, genital grooming, and non-genital grooming of Wistar rats.

Group	Exploration	Raring	Climbing	Genital grooming	Non-genital grooming
Control	14.33±1.86b	4.00±2.00c	0.00±0.00	0.67±0.67d	1.00±0.58b
2 mL/kg	12.33±0.33a	1.33±0.67b	0.00±0.00	3.33±0.67b	1.33±0.88b
5 mL/kg	10.33±0.33a	0.67±0.57a	0.00±0.00	4.00±2.00a	2.33±0.33a
10 mL/kg	11.67±0.33a	2.00±0.00b	0.00±0.00	2.67±0.67c	1.67±0.67b

(n=4), $p < 0.05$ – Significant, $p > 0.05$ – Not Significant; Different letters in superscript across the columns are significant from others

DISCUSSION

Several studies reported that herbal plants are good sources of aphrodisiac alternatives for improving sexual functions in mammals, human beings inclusive[15], probably due to their bioactive agents, resulting in efficacy and potency[10]. This sexual behavior may also be due to androgenic and gonadotropic activities of P. yohimbe aqueous extract; these results were also observed for M. acuminata stem in male rats[16].

The frequencies of mounts and intromission remain sufficient valuable indices of vigour, libido, and power[17]. As observed in this study, treated animals with the aqueous extract of P. yohimbe show that the treated males go directly towards their treated females in the first 30 seconds of the test, contrary to the control rats, which take a little more time to join their females. This rapid orientation step towards the conspecific indicates the extract's stimulating effect, which probably includes aphrodisiac compounds. Our results are consistent with those of Yakubu and Akanji[16], which suggest that male rats, upon introduction to the test cage, responded with immediate advances toward females and showed precopulatory behaviors such as chasing anogenital sniffing that eventually resulted in mounting.

ML and IL are considered indicators of sexual motivation16. The significative reduction in these parameters observed in rats treated with aqueous extract of P. yohimbe could imply an improvement in sexual motivation and sexual appetite. Besides, increased MF is an indicator of vigor, libido, and potency. Increased MF indicates sexual motivation[18]. The pro-sexual effect of P. yohimbe was also established by increasing the MF after treatment.

When tested with a female, the copulatory behavior of the male rat consists of a repeated series of mounts and intromissions culminating in ejaculation[19]. The administration of an aqueous extract of P. yohimbe caused a stimulatory effect, increasing sexual performance and orientation activity towards the female. These results are similar to those of Watcho et al[13], and Watcho et al[20], where long-term administration of the aqueous and methanol extracts of Ficus asperifolia and Raphia vinifera leaves significantly increased the sexual behavior of the animals during the observation period.

The precopulatory behavior of the extract-treated male rats indicated that the animals were generally aroused. Several precopulatory behavior parameters of males were observed on the cage side when extract-treated male rats were presented to receptive female rats. Also, there was genital grooming after each mounting by the male which resulted in intromission. The effect of the aqueous extract of P. yohimbe leaves in rats was similar to that of the aqueous extract of the plant Massularia acuminata[16]. The present study results demonstrate the potency of P. yohimbe leaves stimulating the

copulatory activity of sexually naïve rats.

Our findings highlight the sexually stimulating effects of the aqueous extract of P. yohimbe leaves in rats and thus give credence to its aphrodisiac reputation. It appears from this work that this extract has a sexually stimulating activity which would confirm the interest of its traditional use as a reproductive performance stimulant. Based on these findings, we recommend that further studies with larger doses be conducted to confirm the results. However, based on the current findings, we recommend that careful administration be considered as a better option justifying human circumstances for patients with advanced mankind age in low dosage resource settings, as it achieves a better reproductive performance rate at a lower dose-dependent increase.

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