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Original Article

Aphrodisiac Effects of an Ethanolic Root Extract of *Ocimum fimbriatum* Briq. var. *fimbriatum* (Kafupa) on Male Wistar Rats

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ABSTRACT

Background**: **Ocimum fimbriatum** **Briq. var. fimbriatum is used traditionally *in Zambia for its aphrodisiac effect, but there is no scientific evidence to support this use.* Therefore, this study aimed to validate the aphrodisiac effects of the **plant's root extract in rats.**

Study Design: This was an experimental study in which sixty Wister rats (30 males and 30 females) were separated into 5 groups of 12 rats and treated with different doses of the extract.

Methods: The root extracts *were administered* at single oral doses of 100, 200, and 400 mg/kg to 30 male rats. Distilled water and sildenafil served as controls. Female rats were treated with 0.1 mg/kg diethylstilbesterol and paired to the male rats. Mounting frequency, intromission, ejaculation, and latency periods were monitored using recording cameras. Recordings were analyzed visually and expressed as mean ± standard error.

Results: The root extract produced significant dose-dependent increases in mounting, intromission, and ejaculation frequencies and latency periods, compared to negative controls. The 400 mg/kg dose produced highest number of mounts (31.33 ± 0.49, *p= 0.001*), intromissions (24.17 ± 0.60*, p= 0.001*) and ejaculations (7.67 ± 0.33, *p= 0.001*) compared to negative controls. At this dose, latency periods for mounting (3.00 ± 0.37min, *p= 0.001*) and intromission (2.1 ± 0.32 min, *p= 0.001*) were shortest, while the latency periods for ejaculation (6.33 ± 0.56 min, *p=0.001*) were longest.

Conclusion: Root extract of *Ocimum fimbriatum exerted significant* aphrodisiac effects in rats and justifies traditional use of the plant. Further studies are required to elucidate the active principles and mechanisms involved in this effect.

INTRODUCTION

Male sexual dysfunction includes a few clinical entities that are associated with suboptimal sexual performance. Some of these entities such as erectile dysfunction (ED), and ejaculatory disorders are well characterized and measured, while others such as orgasmic dysfunction and disorders of sexual desire may be difficult measure.[1,2] Prevalence estimates vary and are mostly old.[3-7] Among reported risk factors include aging, cardiovascular disorders, diabetes, other metabolic disorders, and socio-psychiatric problems.[8-12]

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Keywords: Aphrodisiac effects; ejaculation; intromission; mounting frequency; *Ocimum fimbriatum*

Beyond pharmacotherapy, many natural products are used to enhance male sexual performance in folk medicine. Patel and others (2005) listed over 456 Indian plants and fungi with claimed aphrodisiac effects. Some of the species reported in recent literature include *Asteracanta longifolia*, *Polygonatum verticillatum, Abelmoschus manihot,* *Anacyclus pyrethrum* and *Argyreia nervosa.*[13-16] In Zambia, ***Ocimum fimbriatum*** **Briq. var.** ***fimbriatum*** **(OFBF)** *locally known as Kafupa, is one of the* species commonly used to enhance male sexual performance. However, the authors are not aware of any studies or reports on the aphrodisiac properties of the plant. The aim of this study, therefore was to verify the claimed aphrodisiac effects of the root extract of the plant in male Wister rats. The study measured the effect of single oral doses of the extract on mounting ***frequency (MF), intromission frequency (IF), ejaculation frequency (EF), mount latency (ML), intromission latency (IL) and ejaculation latency (EL) and compared these to values in control rats treated with distilled water.***

MATERIALS AND METHODS

To achieve the above aims, an experimental study was carried out with 60 Wister rats. Fresh plant roots were collected from a natural habitat in Kapiri Mposhi area of the Central District of Zambia. The plant was identified and authenticated by qualified botanists at the University of Zambia Department of Biological Sciences.

Preparation of the Root Extract

Fresh roots of OFBF were air dried in the shade at room temperature (26-28 ° C) for 14 days, and the dried roots were pulverized using an industrial blender. The resulting powder was weighed, and 100 g extracted with 400 mls of 75 % ethanol using soxhlet extraction at 60 ° C. The extract was concentrated using Rotavapor R11 (Buchi Labortechnik AG, Switzerland) at 40 ºC, and dried to a constant weight at 110 ° C. The resulting gummy residue was stored in the refrigerator at 4-8 ° C, until required for experiment.

Experimental Animals

Sixty healthy male and female Wistar albino rats weighing 160-230 g were purchased from animal holding unit, Department of Biological Sciences, School of Natural Sciences, University of Zambia. The animals were kept in well ventilated rodent cages and allowed acclimatization period of 14 days in the animal facility of Physiological Sciences Department, School of Medicine, UNZA before the commencement of the experiment. They were kept at room temperature of 24±2 ºC with relative humidity of 70 % and 12 hours natural light and dark cycle. The rats were allowed free access to standard Laboratory rat diet and tap water given ad libitum.

Animal studies were conducted according to standard guidelines for use of laboratory animals.[17]

Experimental Protocol

The 30 male rats were separated into 5 groups of 6 rats each (denoted A to E) and treated with oral doses of the root extract, sildenafil, or distilled water as follows:

Group A (test):	100 mg/kg root extract
Group B (test):	200 mg/kg root extract
Group C (test):	100 mg/kg root extract
Group D (positive control):	5 mg/kg sildenafil citrate suspension
Group E (negative control):	1 ml distilled water

The drugs were administered with 22G gastric feeding needles attached to syringes.

The 30 female rats were primed with single subcutaneous injection of 0.1 mg/kg Diethylstilbestrol 24 hours prior to paring with the male rats to induce heat.[18]

Recording Sexual Behaviour

After the administration of each dose, the animals were observed for sexual behaviour using surveillance cameras (Zosi Technology Co., Ltd, china) for 3 hours. The surveillance cameras were mounted for 3 hours in a secure place free from disturbances. Recordings were stored in an inbuilt

storage device of the surveillance cameras. Recordings were later played and viewed on the monitor with the help of qualified veterinary technician.

Data Analysis

Documented data for mounting frequency (MF), intromission frequency (IF), ejaculation frequency (EF), mount latency (ML), intromission latency (IL) and ejaculation latency (EL) were analyzed using SPSS software version 20. The descriptive statistics included the mean of six replicates and standard error of the mean for each group. For inferential statistics, the dataset was first tested for normality of distribution using Shapiro-Wilk test. Upon confirmation of normality of data distribution, one-way analysis of variance (ANOVA) and Post Hoc Tests were used to make comparison between individual groups. The value of $p < 0.05$ was considered statistically significant.

Ethical Considerations

The protocol for this study was approved by ERES converge IRB an independent research ethics committee in Lusaka, Zambia. Permission to use laboratory for root extraction was sought from Head of Chemistry Department, School of Natural Sciences at the University of Zambia. Animals were purchased from the Head of Department of Biological Sciences, School of Natural Sciences at the University of Zambia. All Animals involved in the study were handled humanely in accordance to standard guidelines for use of laboratory animals.[17] The researcher was well trained from the University of Zambia in animal handling.

RESULTS

Qualitative Phytochemical Screening

Table 1 below shows the relative concentration of the secondary metabolites in the samples, which were determined by considering the colour intensity of the positive results. An intense colour was taken as an indication of a high concentration of the metabolite. The same was done for moderate and low intensity coloration of the positive results.

Table 1: Results of Phytochemical Screening

Phytochemicals	OFBF
Tannins	+++
Flavonoids	+
Saponins	-
Sterols and triterpenoids	+++
Alkaloids	++
Cardiac glycosides	+++

Key: high (+++); moderate (++); low (+); absent (-); OFBF, *Ocimum fimbriatum Briq. var. fimbriatum*

EFFECT OF OFBF ON SEXUAL BEHAVIOUR OF MALE RATS

The effects of ethanolic root extracts of OFBF on sexual beahiour of male rats is shown in table 2 below.

Table 2: Effects of Ethanolic Root Extract of OFBF on Sexual Behaviour

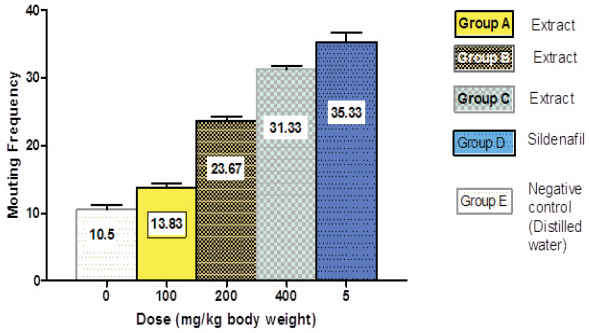
Groups	MF	IF	EF	ML (MIN)	IL (MIN)	EL (MIN)
Control	10.5±0.76	5.50±0.76	3.33±0.49	9.17±0.60	7.00±0.37	1.17±0.31
Sildenafil (5mg/kg)	35.33±1.3**	32.83±1.01**	6.67±0.49**	3.67±0.42**	2.10±0.32**	8.33±0.49**
Extract 100mg/kg	13.83±0.60	8.17±0.60	3.33±.49	6.22±0.33**	5.85±0.33	3.00±0.37*
Extract 200mg/kg	23.67±0.67**	14.17±0.60**	5.67±0.56*	5.17±0.48**	4.46±0.44**	5.00±0.37**
Extract 400mg/kg	23.6±0.49**	24.17±0.60**	7.67±0.33**	3.00±0.37**	2.10±0.32**	6.33±0.56*

Key: all values are expressed as mean ± SE; N=6; *p<0.05 considered significant, ** p<0.01 considered extremely significant compared to control; MIN, minutes; MF, Mounting Frequency; IF, Intromission Frequency; EF, Ejaculation Frequency; ML, Mounting latency; IL, Intromission latency; EL, Ejaculation latency

Mounting Frequency

The results in table 2 above and figure 1 below showed that increase in MF between groups corresponded with an increase in extract doses. MF was statistically significantly different between the groups as determined by one-way ANOVA (F (4, 25) = 172.770, p= 0.001). A Dunnet T3 post-hoc test revealed that the increase in MF was statistically significant at extract doses of 200 mg/kg (23.67 ± 0.67, p=0.001) and 400 mg/kg (31.33 ± 0.49,

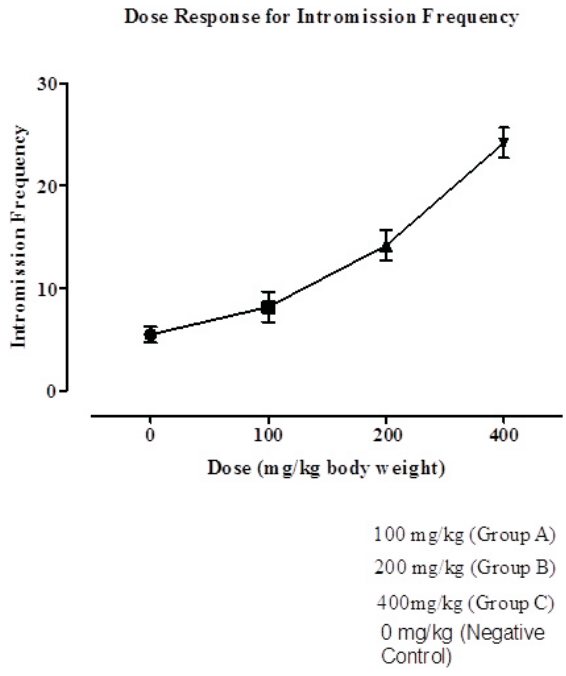
p=0.001) compared to 100 mg/kg (13.83 ± 0.60). Furthermore, the increase in MF was also significant at extract doses of 200 mg/kg (*p*=0.001) and 400 mg/kg (*p*=0.001) as compared to distilled water (0 mg/kg) treated control group (10.50 ± 0.76). However, there was no statistically significant increase in MF between extract dose of 100 mg/kg (*p*=0.056) and distilled water treated control group. Data was normally distributed with *p*>0.05 (Shapiro-Wilk Test).



of OFBF on mounting frequency of male rats. Values are means of six replicates ± SE

Intromission Frequency

Intromission frequency (IF) increased significantly at extract doses of 200 mg/kg (14.17 ± 0.60, *p*=0.001) and 400 mg/kg (24.17 ± 0.60, *p*=0.001) in comparison with 100 mg/kg (8.17 ± 0.60). Furthermore, IF increased statistically significant at extract doses of 200 mg/kg (*p*=0.001) and 400 mg/kg (*p*=0.001) as compared to control (distilled water). However the increase in IF was insignificant at 100 mg/kg body weight (*p*=0.107) compared to control (5.50 ± 0.76). IF differs significantly between the groups as determined by one-way ANOVA ($F(4, 25) = 241.402$, $p = 0.001$). In addition, the Levene's Test of Homogeneity of variance gave a



p-value equal to 0.136, hence, Tukey's test was used for multiple comparisons.

Figure 2: Dose dependent increase in IF at doses of 100 mg/kg (8.17 ± 0.60), 200 mg/kg (14.17 ± 0.60) and 400 mg /kg (24.17 ± 0.60) for the treatment groups.

Ejaculation Frequency

The extract doses of 200 mg/kg (5.67±0.56, *p*=0.016) and 400 mg/kg body weight (7.67±0.33, *p*=0.001) significantly increased EF compared to 100 mg/kg (3.33±0.49). EF was also significantly increased at 200 mg/kg (*p*=0.016) and 400 mg/kg (*p*=0.001) as compared to distilled water control group (3.33±0.49). There was no change in EF between an extract dose of 100 mg/kg (*p*=1.00) and control (distilled water). In addition one-way ANOVA (F (4, 25) = 16.587, p= 0.001) revealed significant statistical difference between the groups. The Levene's Test of Homogeneity of variance gave a P-value equal to 0.635, hence, Tukey's test was used for multiple comparisons.

Mounting Latency

There was a statistically significant difference in ML between the groups as determined by one-way ANOVA (F (4, 25) =29.28, p= 0.001). A Tukey post-hoc test revealed a significant dose dependent reduction in ML between the extract dose of 200 mg/kg (5.17 ± 0.48 min, p=0.017) and 400 mg/kg (3.00 ± 0.37 min) and between 400 mg/kg (p=0.001) and 100 mg/kg (6.2 ± 0.33 min). However, the extract dose of 200 mg/kg (p=0.480) did not significantly reduce ML as compared to 100 mg/kg. The results further showed that all the mentioned extract doses significantly reduced ML in comparison with negative control (p<0.05).

Intromission Latency

The extract dose of 400 mg/kg body weight (2.10 ± 0.32 min) significantly reduced IL in comparison with extract dose of 100 mg/kg (5.85 ± 0.34 min, p=0.00) and 200 mg/kg (4.46 ± 0.44 min, p=0.001). There was no significant reduction in IL between extract doses of 100 mg/kg and 200 mg/kg body weight (p=0.077). Tukey post-hoc test further revealed significant reduction in IL at 200 mg/kg (p=0.001) and 400 mg/kg (p=0.001) as compared to distilled water control group (7.00 ± 0.37 min). However, reduction of IL between extract dose of 100 mg/kg and distilled water control group was insignificant (p=0.189).

Ejaculation Latency

The results in table 2 above showed statistically significant difference in EL between the groups as determined by one-way ANOVA (F (4, 25) =42.70, p= 0.001). In addition, Tukey post-hoc test revealed that EL was significantly prolonged at extract at doses of 200 mg/kg (5.00 ± 0.37 min, p=0.022) and 400 mg/kg body weight (6.33 ± 0.56 min, p=0.001) in comparison with extract dose of 100 mg/kg (3.00 ± 0.37 min). All the extract doses showed significant prolongation of EL as compared to distilled water treated group (p<0.05).

DISCUSSION

The mating behavior study revealed that single oral dose administration of OFBF root extract was able to significantly increase frequency of mounting, intromission and ejaculation in comparison to untreated distilled water control animals, at the same time, decreased latency period of mount and intromission, when compared to negative control. It also significantly prolonged ejaculation latency. Ultimately, it resulted in an increased sexual performance. Comparatively, sildenafil exerted more potent action than OFBF in this study. This is quite understandable as OFBF is still used in its crude and unpurified form. However, marked increased response was observed at 400 mg/kg body weight of OFBF extract, as nearly equal to standard drug (Sildenafil) at 5 mg/kg. In addition, the pre-coital sexual behaviors, such as chasing, nosing, and anogenital sniffing, were prominently observed in this group dosed with 400 mg/kg. The increase in responses was in dose-dependent manner.

Administration of ethanolic root extract of OFBF at 100?mg/kg body weight (b.w) did not have any significant increase on the MF (13.83 ± 0.60, p=0.056), IF (8.17 ± 0.60, p=0.107), EF (3.33 ± 0.49, p=1.00) and IL (5.85±0.34 min, p=0.189) in comparison to distilled water control group. The single doses of 200 mg/kg and 400 mg/kg recorded significant (p<0.05) increase in MF, IF, EF and EL and reduction in ML and IL as compared to the control. Oral administration of 400 mg/kg body weight when compared to untreated distilled water (placebo) control animals significantly recorded the highest number of mounts (31.33 ± 0.49, p=0.001), intromissions (24.17 ± 0.60, p=0.001) and ejaculations (7.67 ± 0.33, p=0.001). Furthermore, it showed the shortest mounting latency (3.00 ± 0.37min, p=0.001), intromission latency (2.1 ± 0.32 min, p=0.001) and longest ejaculation latency (6.33 ± 0.56 min, p=0.001). However, animals treated with 200 mg/kg recorded lower sexual behavior activities when compared to 400 mg/kg treated rats, but significantly higher than untreated distilled water control animals. Reports in other studies have shown a similar dose-related increase in MF, IF and EF and reduction in mounting and intromission latency and prolonged ejaculation latency as compared to the control group (p<0.05).[5,13]

Mounting and intromission frequencies are considered to be indices of libido and potency, while ML and IL are indicators of sexual arousal.[19,20] The significant increases in MF and IF and the decreases in ML and IL in the treatment group, indicate that libido and potency were enhanced by extract of OFBF. Therefore, the decrease in the mount and

intromission latencies observed at the doses of 200 and 400 mg/kg body weight in this study might imply stimulation of sexual motivation and arousal. It may also be an indication of enhanced sexual appetitive behavior in the male rats, which further supports the sexual improvement effect of the extract. Furthermore, the prolongation of EL is an indicator of prolonged duration of coitus and increased IF and EF in treated male rats indicated the involvement of NO in the intervention.[13,21-23]

Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, glycosides, sterols and triterpenes in OFBF *ethanolic extract*. The presence of these phytochemicals in the herbal extracts which show medicinal activity was an indication of the herbal extracts' ability to exhibit physiological activities.[24] The mechanism of these agents includes steroids by raising androgen production; flavonoids by enhancing testosterone synthesis or by preventing its metabolic degradation.[19,25,26] Others are alkaloids by dilating the blood vessels in the sexual organs; and tannins by activating gonadal tissues and CNS via NO-dependent mechanism.[19,27] Thus, the improvements in sexual function demonstrated in the current study might be due to the presence of such compounds in prepared OFBF.

CONCLUSION

The study concluded that the single oral dose administration of ethanolic root extract of OFBF enhanced overall sexual function and performance in male wistar rats due to the presence of phytochemical compounds that possess aphrodisiac properties. The significant increases in MF and IF and the decreases in ML and IL in the treatment group, indicate that libido and potency were enhanced by extract of OFBF. These findings support the traditional use of OFBF as an aphrodisiac.

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