

**The Effect of Aqueous Extract of *Phoenix Dactylifera* Pollen
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Abstract

The *Phoenix dactylifera* date palm pollen (DPP) is used for curing male infertility and impotency in traditional medicine. However, the validity has not been scientifically tested. Therefore, the present study was undertaken to evaluate the effect of aqueous extract of *Phoenix dactylifera* pollen on the sexual behavior of male rats. Sixty male rats were randomized into 6 groups (A-F). Group A received 0.2 ml of Normal Saline mixed with Dimethyl Sulphate (DMSO), while groups B-F were injected same volume containing 35, 70, 105, 140 and 350 mg/kg of DPP extract, respectively. Sexual behavioral parameters including mounting, intromission and ejaculation frequencies and latencies were recorded in male rats one hour after injection of extract by mating with a receptive female (1:1). The male serum testosterone and estradiol concentrations were also determined. All doses stimulated male sexual behavior. Extract significantly increased mount, ejaculation, intromission frequencies and ejaculation latency in comparison to controlled ones ($p < 0.001$). Mount and intromission latencies significantly reduced ($p < 0.001$). Maximum effect was observed at a dose of 140 mg/kg. This extract was found to enhance Testosterone, Estradiol and the orientation of males toward female ones by increasing mounting and ano-genital investigatory behavior. Our results revealed that the aqueous extract of *Phoenix dactylifera* pollen can be used as a sex enhancer and seems to cure male infertility. Also, our findings support the traditional use of this plant as acclaimed aphrodisiac and for the treatment of pre-ejaculation and impotency.

Keywords: Aphrodisiac activity, aqueous extract, male rat, *Phoenix Dactylifera*, pollen grain, pre-ejaculation, sexual behavior

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Introduction

Male sexual behavior comprises a complex pattern of genital and somatomotor responses, elicited, directed, and maintained by external and internal signals. It includes copulation as well as precopulatory behaviors that allow the male to detect and locate a mate, assess her potential mating appropriateness, and stimulate a receptive response (Jung *et al.*, 2008; Anil Kumar *et al.*, 2009). For a normal sexual intercourse in males, the sexual organs and factors relating to erection of the copulatory organ must function as normally (Miesel and Sachs, 1994).

Male reproductive capacity was found to be deficient in no less than 50% of infertile couples (WHO, 2000). The incidence of sexual inadequacy in human males has led to the development of a number of available treatment options. These options are expensive with serious side effects. This problem, has necessitated the need for less side effect drugs Cicero *et al.*, 2001; Adimoelja, 2000). Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine (Krentz and Bailey, 2005).

Traditional medicines are gaining importance and nowadays are being studied to find the scientific basis of their therapeutic actions (Gupta and Briyal, 2004; Islam *et al.*, 1991). Plant-derived chemicals are used to relieve sexual dysfunction and they have sex enhancing potentials. These phytochemicals increase libido, sexual potency and sexual pleasure (Cicero *et al.*, 2001; Adimoelja, 2000). The use of herbal medicine has become increasingly popular worldwide especially in the Asian and African countries (Bahmanpour *et al.*, 2006).

The various parts of *Phoenix dactylifera* widely are used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness and nervous disorders (Biglari *et al.*, 2008; Al-Qarawi *et al.*, 2004). Suspension of *Phoenix dactylifera* date palm pollen (DPP) is an herbal mixture that is widely used as a folk remedy for curing male infertility in traditional medicine. Date palm fruit suspensions improve the sperm count, motility, morphology, and DNA quality with

a concomitant increase in the weights of testis and epididymis (Bahmanpour *et al.*, 2006). However, there is no scientific research about the effect of DPP on sexual behavior in literature. Therefore, the present study was undertaken to determine the aphrodisiac activity of aqueous extract of *Phoenix Dactylifera pollen grain* on the sexual behavior in male rats.

Materials and Methods

Plant material

Samples of the plant were collected from botanical garden at Bushehr city (South of Iran), IRAN.

Preparation of aqueous extract of Phoenix dactylifera pollen grain

Phoenix Dactylifera pollen grain is a small and oval shape gametocyte with a fine bark. After removing the bark, pollen grain washed with distilled water and then dried. Dried pollen grain was pulverized with a small electric blender. 100 gram of powder was extracted in 0.5 liter of warm distilled water (30° C) with constant shaking (magnetic shaker model). The solution was passed through a filter paper and lyophilized. After vaporizing of water, the resultant yield was reconstituted in normal saline to give the required doses of 35, 70, 105, 144 and 350 mg/kg used in our study. Solutions were stored in refrigerator (2-8 ° C). The doses were mixed with Dimethyl Sulphate (DMSO) and IP injection was done 1 hour before experimentations with volume of 0.2 ml / rat.

Animals

The study adheres to the principles of laboratory care established by Ethic Committee of Tehran University of Medical Sciences. A total of 120 animals made up of equal number of male and female rats were used for this study in a complete randomized design. Healthy sexually experienced male rats (wistar albino), 4 months old, weighting between 280-320 grams and females, 3 months old weighting between 220-250 grams were selected from our department (physiology department of Tehran university of medical sciences). All animals were kept in clean glass cages (4 in each cage) placed in well-ventilated animal room. Room

temperature was 24 °C with humidity of 60 %. Animals were housed in a reversed light-dark cycle (light on: 7 p.m to 7 a.m). Animals were acclimated for about 4 weeks before the experimentation. They were allowed free access to food pellets and water ad libitum. Experiments were done in dark period in a dim red florescent lamp

Estrous females

Sixty female rats were used as mating stimulus. The females were bilaterally ovariectomized via lumbar incisions under ketamine (100 µl/0.1 kg of rat) and xylazine (10 µl /0.1 kg of rat) anesthesia at least two weeks before the experiments began. The females were rendered sexually receptive by single subcutaneous injections of estradiol benzoate (10 µg) 52 h and progesterone (1 mg) 4 h, prior to pairing. Estrous females displayed a high degree of lordosis responding and proceptivity.

Animal grouping and Extract administration

Sixty male rats were randomly grouped into 6(A–F) consisting of 10 animals each. Animals in group A, which served as the control, received 0.2 ml of saline mixed with DMSO (IP injection). Animals in groups B, C, D, E and F were treated with doses of 5,10,15,20 and 50 percent of the extract, respectively, with volume of 0.2 ml/rat. All administrations were done daily at the time of during 8:00 am and 9:00 am. The experimental rats allowed free access to rat pellets and water.

Male rat sexual behaviors

A Plexiglas observation cage (0.3 m×0.5 m×0.3 m) with a mesh plate in the middle was put on a metal box that has an oblique mirror within. One hour after the treatment, the male rat was placed in this cage. After 10 minutes, a sexually receptive female rat was introduced on the other side of the cage (Anticipatory phase) and after 10 minutes, the mesh plate was removed for 30 minutes (Consummatory phase). Sexual behaviors of a male with female were observed from the cage side for proceptive and precopulatory behaviors. Sexual behavior parameters were monitored for 30 min observatory period by camera (Panasonic model with O-Lux) and direct observations.

If male rat couldn't have an intromission within the first 15 minutes, that rat was removed and replaced with a new one

According to the standard and basic procedures, the following male sexual parameters were recorded or calculated for the observatory period. 1-Mount Latency (ML), time from introduction of the female until the first mount.2-Intromission Latency (IL), time from introduction of the female until the first intromission.3-Ejaculation Latency (EL), time from the first intromission until ejaculation.4- Mount Frequency (MF), the number of mounts in a series.5-Intromission Frequency (IF), the number of intromissions in a series. 6- Ejaculation frequency (EF), the number of times there was expulsion of semen by males after vaginal penetration-characterized by rhythmic contraction of the posterior abdomen.7- Post-Ejaculatory Interval (PEI), the time from the occurrence of ejaculation until the initiation of a new series, as indicated by the next intromission. Other computed male sexual behavioural parameters 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 intromissions/number of mounts) × 100; intercopulatory efficiency = average time between intromissions (Agmo, 1997; Meisel *et al.*, 1984; Ageel *et al.*, 1994;Schiavi and Sergraves ,1995).

Assay Kits

The testosterone and estradiol enzyme immunoassay test kits were used according to their manufacture' s instruction (ABO Switzerland Co.), while estradiol benzoate and progesterone were products of Sigma Chemical (Germany).

Determination of Serum Testosterone and Estradiol Concentration

The animals were deeply anaesthetized With Ketamine and Xylazine. Blood samples were collected from the aorta, centrifuged and the serum separated, Samples stored at -20°C for the measurement of testosterone and estradiol.

Adverse effects

All treated rats were observed any signs of toxicity (salivation, lachrymation, ptosis, squinted eyes, writhing, convulsions, tremors) stress (erection of fur), diarrhea and changes in behaviour (such as spontaneous movements in the cage, climbing, cleaning of face, nongenital self grooming). In addition, food and water intake were noted.

Statistical Analysis

After recording the sexual behavioral parameters, Data was expressed as mean ±SD and analyzed. Statistical analysis was done by Kruskal Wallis test (ANOVA). The comparison of means between control and each experimental group was done by the Wilcoxon-test. P<0.05 was regarded as significant. SPSS software version 11 and Excel 2007 were used for analyzing.

Results

The effects of various doses of DPP on sexual behavior are summarized in table 1 and 2. Intraperitoneally (IP) injection of various concentration of DPP modified all parameters. Maximum effect was observed in rats treated with dose 140 mg/kg of DPP. The aqueous extract of DPP produced a significant increase in MF, IF, EIJ, EIL, PEI and decrease in ML, IL (p<0.05). Treated male rats displayed vigorous ano-genital sniffing and mounting on females and restricted them to one side of cage (table 1). Computed index in treated rats showed that all index increased especially in doses 105 mg/kg, 140 mg/kg and 350 mg/kg (table 2).

Table 1: Effect of various doses of aqueous extract of DPP on Sexual behavior in male rats

behavior	Vehicle	35 mg/kg	70 mg/kg	105 mg/kg	144mg/kg §	350mg/kg
ML (Sec.)	229±24.69	148±13.16 *	126±9.66 §	123±9.48 §	99±14.49 ¥	120±9.42 §
IL (Sec.)	339±24.68	250±6.66 *	227±13.37 §	224±12.64 §	182±11.35 ¥	224±10.74 §
EL (Sec.)	231±21.83	290±14.14 *	326±14.12 §	324±28.75 §	387±21.10 ¥	328±21.49 §
MF	10.2±1.47	16.5±3.02 *	19.2±2.85 §	23.3±2.63 §	28.3±2.35 ¥	24.2±2.25 §
IF	6.9±1.19	8.6±0.84 *	11.5±1.35 §	14.5±2.27 §	18.6±2.31 ¥	14.9±2.07 §
EF	3±0.81	5±1.05 *	6.4±1.07 §	8±1.05 §	13.2±2.25 ¥	9±0.81 §
PEI(Sec.)	3±0.81	5±1.05 *	6.4±1.07 §	8±1.05 §	13.2±2.25 ¥	9±0.81 §

Values are presented as mean± SD. All latencies and interval are expressed in seconds. Significant from control: * p<0.05, § p<0.01, ¥ p<0.001; N/group=10 rats; §= Max. Effect.

Table 2: Effect of various doses of aqueous extract of DPP on computed male rat sexual behavior parameters

parameters	Vehicle	35 mg/kg	70 mg/kg	105 mg/kg	144mg/kg	350mg/kg
Index of libido(%)	70	90	100	100	100	100
% mounted	70	90	100	100	100	100
% intromitted	60	80	90	100	100	100
% Ejaculated	40	60	80	100	100	100
Copulatory efficiency (%)	85.71	88.88	90.00	100	100	100
Intercopulatory interval(Sec.)	249±8.75	210±11.54 *	149.5±10.24 §	125±20.138 ¥	100±13.33 ¥	121±7.37 ¥

Values are presented as mean± SD. Significant from control. * p<0.05, § p<0.01, ¥ p<0.001; N/group=10.

The extract produced a significant increase in the penile erection index as compared to control

value. There were homosexual mountings in the treated animals after experimentations.

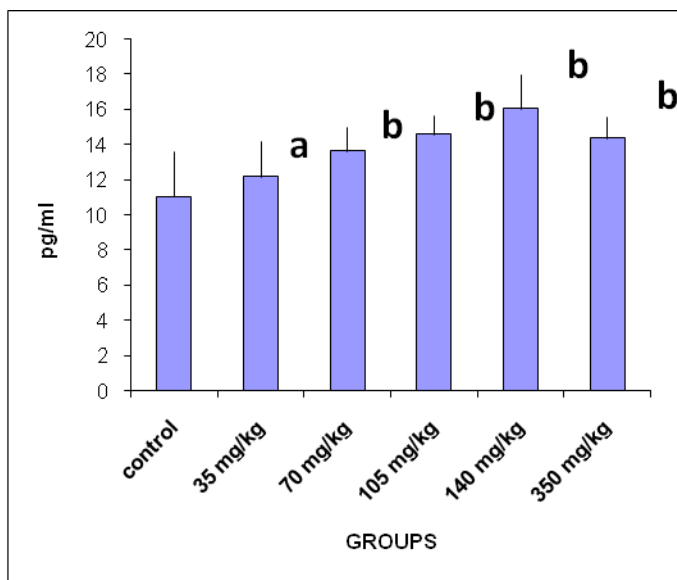


Fig. 1: Effect of DPP extract on the blood level of Estradiol.

Values are Means \pm SD. Different from control. Bar carrying letter 'a': non-significant. Bars carrying letter 'b', different from control. $P < 0.01$. $n = 10$ for each group.

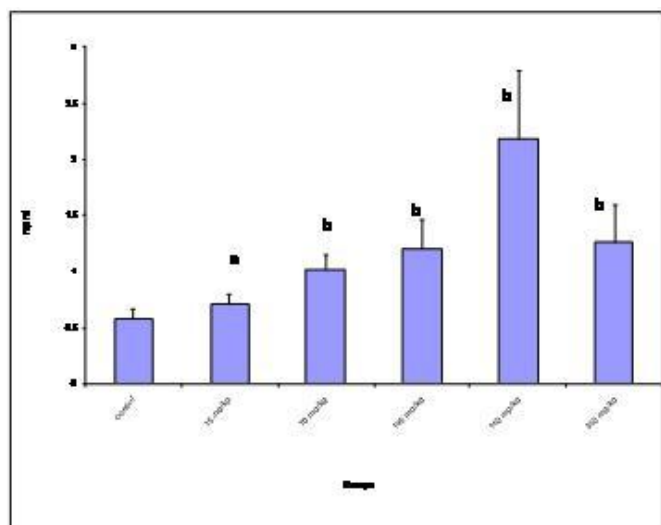


Fig. 2: Effect of DPP extract on the blood level of Testosterone.

Values are Means \pm SD. Bars carrying letters 'a' and 'b', different from control. $P < 0.01$ and $p < 0.005$, respectively. $n = 10$ for each group.

Our studies on the orientation of male towards the female rats showed that males treated with the extract displayed more frequent and vigorous anogenital sniffing and mounting on females, as compared to controlled cases. The orientation towards environment (climbing on cage wall, raring and exploration) was found to be inconsistently

reduced whereas towards self (genital grooming) was increased. The rats treated with the extract tended to show confinement to a particular area of the cage (around female) showing restriction in movement.

There was an increase in the blood level of Estradiol and Testosterone (Fig. 1 and 2). As shown in Figures, the effective dose for estradiol and testosterone was 140mg/kg.

There were no defects and clinical signs of toxicity, stress or changes in behaviour and appearance evident. Diarrhea was observed in animal treated with 350 mg/kg. The food and water intake of all treated rats was similar to those of controls.

Discussion

Many plants with medicinal properties are effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins¹⁷. Treatments that alter the concentration of circulating sex hormones may also modify sexual behaviour (Padashetty and Mishra, 2007).

Administration of the aqueous extract at all dose levels modified rat copulatory behavior as well as orientation activities, the main determinant for measuring male sexual behavior (Morales *et al.*, 1982). The results of the present study revealed a significant increase in penile erection and genital grooming. The increase of penile erection induced by Pheonix dactylifera could be due to their androgenic effects (Bahmanpour *et al.*, 2006). Also, our study showed that DPP can increase plasma level of testosterone and estradiol, it has been documented that sexual behavior and erection are dependent on androgen which may act through central and peripheral mechanisms. Androgens regulate the magnitude of penile erectile response, partly by regulating the venous outflow from cavernous spaces (Giuliano *et al.*, 1993; Mills *et al.*, 1994). Therefore, the increase in serum testosterone concentration by extract might be responsible for improved sexual behavior in males and are essential for libido (Bahmanpour *et al.*, 2006). In fact, it has been shown that copulatory behaviour is maintained in castrated rats by treatment with testosterone

(Meisel and Sachs, 1994; Mills *et al.*, 1994). 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 libido²². Increasing of testosterone of serum by the aqueous extract might be responsible for the enhanced sexual behaviour in the animals (Majewska *et al.*, 1995).

Extract prolonged the ejaculatory latency in male rats, indicating an increase of ejaculatory threshold (Yakubu *et al.*, 2008). These findings support the use of this medicinal plant for the treatment of premature ejaculation in traditional medicine.

Increased MF and IF in treated rats (indicating the sexual motivation and efficiency of erection and penile orientation) and Increasing of the libido might be the result of increase in the several hormones that are secreted from pituitary. The main components of DPP are steroids, flavonoids, saponins, and lipid. These agents can increase sexual behavior. They stimulate endogenous testosterone levels probably by raising the level of luteinizing hormones (LH) (Yakubu *et al.*, 2008; Suresh *et al.*, 2000; Yakubu *et al.*, 2005). Following adequate erection and activity of muscles, intromission is possible and increasing of IF by the extract show the activation of penile erection mechanism. Alkaloids of DPP have estrogenic properties (Mahran *et al.*, 1976; Amine *et al.*, 1996) that induce vasodilation of blood vessels of penis which results in erection Zarrindast *et al.*, 2003). Saponin of PDD acts as nitric oxid and causes smooth muscle relaxation of corpus cavernosum. DPP has gonadotrophin effects (Adimoelja, 2000). Steroidal saponins increase LH and FSH levels that in turn increase testosterone. Our data showed that using DPP extract increases the plasma levels of estradiol and testosterone. These hormones are found at high concentrations in rat testis and seminal fluids. Estrogen is synthesized in male reproductive system by at least three different cell types, Sertoli, Leydig and germ cells. Estrogen regulates the reabsorption of luminal fluid in the head of the epididymis (Adimoelja, 2000). Testosterone enhances sexual desire, index of libido, motivation and sexual performance. Another component of DPP is alkaloid that elevates

testicular cholesterol in male testis ((Putnam *et al.*, 2001; Ballard and Wood, 2007).

Steroidogenesis produces steroid hormones that they increase dehydroepiandrosterone. The last agent enhances sexual behavior and prolongs EF and reduces EL, PEI and intercopolatory intervals. All of these mechanisms are central. Peripheral acting of alkaloids can relax smooth muscles of corpus (Guathaman and Adaikan, 2008). Our study showed that extract increases dopamine from accumbance nucleus (Unpublished Data). Enhanced dopamine efflux causes facilitation of sexual behavior and has effects on sexual motivation, copulatory proficiency and genital reflexes. Dopamine influences motor activity in mesolimbic tract and activates numerous behavior and genital reflexes (Padashetty and Mishra, 2007; Guathaman and adaikan, 2008). Also, dopamine increases IF and reduces IL and ML (Adimoelja, 2000; Sandroni, 2001).

Conclusion

Finally, our study showed that DPP possess facilitatory effects that increase sexual arousal, the state of sexual excitement or desire during sexual interaction. Dose 140 mg/kg had greater activity in stimulating of erection, genital grooming and orientation activities of male rats. In addition of improving the sperm quality (Bahmanpour *et al.*, 2006; Unpublished data), DPP can enhance penile erection and other sexual behaviors. It seems, the primary site of DPP action may be the testis, however, its effect on the hypo-pituitary axis could not be ruled out (Ligha and Oyibo, 2012). Therefore, it may be useful to solve the sexual problems such as pre-ejaculation and impotency. It can influence the sexual arousal and performance. The aphrodisiac effect of the DPP extract may be due to the presence of alkaloids, saponins and flavonoids through a central and peripheral pathway.

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