



Effects of date palm pollen on fertility and development of reproductive system in female Balb/C mice

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ABSTRACT

Introduction: The *Phoenix dactylifera* or date palm pollen (DPP) is widely used for male infertility in traditional medicine. The aim of this study was to evaluate the effect of DPP on fertility and development of female reproductive system in Balb/C mice.

Methods: Ten groups were assigned to 2 control and 8 experimental groups. On the control groups 1 and 2 no treatment was conducted, but in the control group 2, after 10 days the mice were mated. In experimental groups 1 and 2, the animals received DPP (100 and 200 mg/kg, respectively) by oral administration for 10 days. In experimental groups 3 and 4 percentage of mating was evaluated after 10 days. Experimental groups 5 and 6 received DPP during gestation. Embryos were removed to evaluate ovaries histology. For experimental groups 7 and 8, DPP was administered until 21th day after birth. The offspring ovaries were removed to evaluate histological parameters. The levels of sexual hormones were also measured.

Results: Several parameters of ovaries in offspring, including mass index, diameter of ovaries, number of primary and secondary graph follicles and corpora luteal, percentage of mating, body mass index and Crown rump (CR) of embryos, diameter of ovary, basic sexual cell number in embryos, and mass index increased in experimental groups in comparison to the controls. However, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the experimental groups were not significantly different from those in control groups ($P>0.05$), while estrogen and progesterone considerably increased in experimental groups ($P<0.05$).

Conclusion: Results of our investigation propose that DPP can create an appropriate situation for oogenesis and maintain efficient fertility in female mice which it may be considered as useful nutraceuticals for potentiation of fertility in future human studies.

Implication for health policy/practice/research/medical education:

Date palm is effective in elevation of fertility rate in male subjects and might be used for potentiating fertility rate in females.

Isolation of date palm bioactive compounds is recommended.

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Introduction

Infertility is referred to the state in which a couple wanting children cannot conceive after 12 months of unprotected intercourse (1). Infertility is a complicated disorder that could lead to significant medical, psychosocial and economic consequences (2). By World Health Organization (WHO) estimates, 8%–12% of couples worldwide experience difficulty conceiving a child (3), making 50-80 million individuals face challenges in

becoming an integrated family (4). In most developing countries, approximately 10% of all women's visits to doctors are due to childlessness-related problems (5). Why a couple is not able to conceive, or may not be able to conceive without medical intervention could be due to a variety of reasons (6). Reproductive alterations, such as infertility, could be attributed to environmental toxic agents (7). Daily use of antioxidants and some vitamins can protect sperm DNA against free radicals and also

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increase blood-testis barrier stability (8).

Historically, medicinal plants were considered as the only form of health care to which majority of populations had immediate access (9). By WHO reports, 70%-80% of the people worldwide trust in traditional medicine as primary health care (2). Over 50% of all modern clinical drugs are natural product-derived and therefore natural products contribute greatly to developing drugs (10).

Herbs should be taken with adequate knowledge about the toxicity, purity, dosage, appropriate solvent for extraction, and adverse effects (11). Plant-derived chemicals may be used to treat sexual dysfunction and they can improve sex potentials (12).

Date palm (*Phoenix dactylifera* L.), one of the oldest cultivated plants, could be used as a shade for a person to sit or for other small plants to grow in. *P. dactylifera* is thought to have origin in crops cultivated in desert and semi-desert regions (13).

Suspension of *P. dactylifera* date palm pollen (DPP) is a herbal mixture widely used as a folk remedy for treatment of male infertility in traditional medicine. The male flowers of date palm are eaten as a fresh vegetable to improve fertility. Egyptian scientists have already demonstrated the gonad stimulating potency of DPP. Pollen grains of date palm were used to strengthen women's fertility in ancient Egypt (14). *P. dactylifera* is a tree species belonging to the family Arecaceae. All species of this genus are dioecious, with male and female flowers growing on separate trees (15).

In North Africa and Middle East *P. dactylifera* is a staple food that can be produced easily in unfavorable natural conditions (16). For centuries, *P. dactylifera* has been used in the Middle East as a tonic main food. *P. dactylifera* is also an alleged aphrodisiac and symbol of fertility. The Arab believe that date pollen juice increases the possibility of pregnancy. Many studies have shown that pollen contains estrogen-like hormones. Phytochemical studies have demonstrated the presence of sterols, oestrone-like compounds and steroidal saponin glycoside in DPP grains (17). Date is an old fruit crop which has been cultivated in North Africa and the Middle East for about 5000 years. Dates grow in extremely hot and dry climates, and to some extent tolerate salty and alkaline soils (18). Dates contain vitamin C, and vitamins B1 and B2, nicotinic acid and vitamin A. In addition 23 different amino acids have been observed in date's proteins, many of which are absent in most fruits (19).

Recent studies indicate that the aqueous extracts of date have potent antioxidant activity. The antioxidant activity of date is attributed to the wide range of phenolic compounds including p-coumaric, ferulic acid, sinapic acids, flavonoids, and procyanidins (20).

Objectives

In the present study, the effect of DPP on fertility and development of reproductive system in female Balb/C

mice was investigated.

Materials and methods

Balb/C mice were obtained from animal house of Razi Institute, Mashhad and kept under 12-hour light/dark cycle and temperature of $22 \pm 2^\circ\text{C}$. Standard pellet food and distilled water were freely available to the mice. The animals were acclimatized to the laboratory conditions for one week till the start of the experiments. Two doses, 100 and 200 mg/kg, of drug were selected. The animals were divided into 10 groups of 10 each (8 experimental and 2 control groups).

Control groups

Control 1 received no drug. After 10 days the mice were deeply anesthetized with anesthetic ether and sacrificed. Histological changes in ovary were measured.

Control 2 received no drug. After 10 days the mice were mated overnight at a 3:1 ratio of female and male, respectively.

Experimental groups

Experimental groups 1 and 2: Animals treated once daily by oral administration of 100 and 200 mg/kg of the drug under study. Body weight (BW) DPP suspension was given via drinking water for 10 days. The weight of the ovary was also determined, and after tissue processing, their histology were studied by light microscope.

Experimental groups 3 and 4: Animals treated once daily by oral administration of 100 and 200 mg/kg. BW DPP suspension was given via drinking water for 10 days. Then percentage of mating was determined.

Experimental groups 5 and 6: Animals in these groups received DPP via drinking water during gestation at doses of 100 and 200 mg/kg. After 18 days, embryos were removed from uterus. The ovary of embryos was studied by light microscope.

Experimental groups 7 and 8: Animals received 100 and 200 mg/kg DPP via drinking water during gestation and lactation until day 21 after birth. The ovary of embryos was removed after sexually maturation and fixed in Bouin's solution. By preparing serial tissue sections, structural changes in ovary were studied.

Statistical analysis

Data were presented as mean \pm SD by statistical software of *GraphPad InStat* V2.01. Statistical analysis was by one-way analysis of variance (ANOVA) and the comparison between the control and experimental groups was done by Tukey-Kramer test.

Results

Regarding the obtained results, both ovary mass index and diameter significantly increased in experimental groups 1 and 2 in comparison with the control group ($P < 0.05$, $P < 0.01$, respectively). The remarkable differences have

been observed in BW (g) in animals of the groups treated with DPP (Table 1).

The obtained results demonstrated that DPP caused a considerable enhancement of the mean number of primary, secondary and graph follicles and corpora luteal when compared to the corresponding variables in the control group (Table 2, Figure 1).

In the studied groups, both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) showed no considerable difference compared to the control group ($P > 0.05$). On the other hand, for estrogen and progesterone, a significant enhancement was observed in the studied groups ($P < 0.05$; Table 3).

Also, a significant increase was observed for both mass index and CR of embryos in treated mice in contrast to control group ($P < 0.001$; Table 4).

The comparative evaluation between control and experimental groups revealed that consumption of DPP suspensions leads to significant increase in diameter of embryos ovary ($P < 0.05$; Table 4).

The basic sexual cells count in female embryos obviously increased in experimental groups when compared to the control group ($P < 0.01$; Table 4).

A significant increase was seen in the number of primary, secondary and graph follicles and corpora luteal in ovary of female offspring as compared to control group (Table 5; Figure 1).

Based on our findings, a significant increase in offspring ovary mass index and diameter were observed in animals of the groups treated with DPP as compared to the control group ($P < 0.01$; Table 6).

The comparative evaluation between control and experimental groups revealed that consumption of DPP suspensions led to a significant increase in percentage of

mating ($P < 0.05$; Table 7).

Discussion

We conducted this study to investigate the efficacy of palm pollen suspensions at different concentrations on female infertility.

Results obtained in this study showed that the DPP leads to significant increase in the serum values of estrogen and progesterone whereas the serum values of LH and FSH in the experimental groups have shown no significant difference compared to the controls. Moshtsghi et al showed that the serum density of LH and FSH in the experimental group was not significantly different compared to the control group. The groups in which values of 200 and 400 mg/kg DPP were administered showed remarkable increase in estrogen and progesterone. Also in this study, it was suggested that DPP contains flavonoid and alkaloid compounds that have estrogenic activity (21). Based on obtained results in the present study it can be concluded that the DPP significantly increases the number of ovarian follicles (primary, secondary and graph) and corpora luteal. Previous studies have shown that date palm contains estradiol and flavonoid (22). An experimental study on black seed (*Nigella sativa*) showed that hydro-alcoholic extract of black seed led to a significant increase in ovaries weight and follicles number. According to the findings in the study on *N. sativa*, it was suggested that the presence of linoleic acid in *N. sativa* stimulates several molecular pathways and consequently increases oogenesis (23). Our obtained results confirmed that consumption of DPP suspensions during gestation led to considerable impacts on development of reproductive system in mice. In conclusion, present study showed that both basic sexual cells count and ovary diameter in female

Table 1. Effect of date palm pollen (DPP) on ovary mass index (mi), ovary diameter (mm) and Body mass (g)^a

	Ovary mass index (mi)	Ovary diameter (mm)	Body mass (g)
C 1	0.024 ± 0.002	2.98 ± 3.06	29.6 ± 2.10
E 1	0.812 ± 2.57	5.04 ± 3.06	33.06 ± 2.07
E 2	1.05 ± 0.018	5.06 ± 7.32	35.8 ± 2.03
Value	$P < 0.05$	$P < 0.01$	$P < 0.05$

Abbreviations: C 1, Control 1; E 1, Experimental group 1; E 2, Experimental group 2.

^aValues have been shown as mean ± SD.

Table 2. Effect of date palm pollen on ovarian follicles (primary, secondary and graph) and corpora luteal

	Primary follicle	Secondary follicle	Graafian follicle	Corpora lutea
C 1	7.25 ± 3	5.51 ± 1.7	1.38 ± 0.52	4.2 ± 1.3
E 1	16 ± 3.53	8 ± 1.58	2.8 ± 0.83	8.4 ± 2.7
E 2	19.2 ± 7.32	1.2 ± 3.7	3.4 ± 1.14	10.8 ± 2.58
Value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Abbreviations: C 1, Control 1; E 1, Experimental group 1; E 2, Experimental group 2.

Table 3. Effect of date palm pollen (DPP) on the serum values of LH, FSH, estrogen and progesterone

	Serum density of LH (mlu/ml)	Serum density of FSH (mlu/ml)	Serum density of estrogens hormones (pg/ml)	Serum density of progesterone hormones (g/ml)
C 1	0.132 ± 0.026	0.584 ± 0.054	4.1 ± 0.91	49.5 ± 14.59
E 1	0.132 ± 0.022	0.594 ± 0.042	10.6 ± 1.15	66.3 ± 5.66
E 2	0.131 ± 0.011	0.594 ± 0.78	11.6 ± 1.60	68.5 ± 7.63
Value	$P > 0.05$	$P > 0.05$	$P < 0.001$	$P < 0.05$

Abbreviations: C 1, Control 1; E 1, Experimental group 1; E 2, Experimental group 2.

Table 4. Effect of date palm pollen (DPP) on embryos weight (g)

	Weight embryos (g)	CR (mm)	Diameter of embryos ovary (μm)	Basic sexual cells of female embryos
C 2	0.66 ± 0.053	16.11 ± 1.1	173.8 ± 9.35	17.6 ± 6.58
E 5	1.25 ± 1.31	21.3 ± 1.73	179.4 ± 6.01	48 ± 10.79
E 6	1.11 ± 0.17	24.55 ± 1.76	178.82 ± 7.88	39 ± 4.94
Value	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.05	<i>P</i> < 0.01

Abbreviations: CR (mm), diameter of embryos ovaries (μm) and basic sexual cells count of male embryo; C 2, Control 2; E 5, Experimental group 5; E 6, Experimental group 6.

Table 5. Effect of date palm pollen (DPP) on number of offspring primary, secondary and graph follicle and corpora luteal

	No. of offspring primary follicle	No. of offspring secondary follicle	No. of offspring Graf follicle	No. of offspring corpora lutea
C 2	5.68 ± 1.14	6.65 ± 1.8	1.84 ± 0.54	5.1 ± 2.3
E 7	16.8 ± 1.58	7.4 ± 0.82	2.4 ± 1.14	8.4 ± 2.3
E 8	17 ± 6.76	10.6 ± 3.43	2.8 ± 1	10.2 ± 4.43
Value	<i>P</i> < 0.001	<i>P</i> < 0.05	<i>P</i> < 0.01	<i>P</i> < 0.001

Abbreviations: C 2, Control 2; E 7, Experimental group 7; E 8, Experimental group 8.

Table 6. Effect of date palm pollen (DPP) on offspring ovary weight (g) and diameter (mm)

	Offspring ovary weight (g)	Offspring ovary diameter (mm)
C 2	0.022±0.001	2.89±0.52
E 7	0.027±0.003	4.46±0.61
E 8	0.024±0.008	4.18±0.78
Value	<i>P</i> < 0.01	<i>P</i> < 0.01

Abbreviations: C 2, Control 2; E 7, Experimental group 7; E 8, Experimental group 8.

embryos increased in experimental groups as compared to the control group. Also according to our findings, it can be concluded that use of DPP suspension during gestation and lactation increases oogenesis in offspring significantly. DPP contains cytochrome p450 enzyme and like-estrogen compounds. Cytochrome p450 enzyme is

Table 7. Effect of date palm pollen (DPP) on percentage of mating

	Percentage of mating
C 2	40±0.63
E 3	75±3.4
E 4	75±3.6
Value	<i>P</i> < 0.05

Abbreviations: C 2, Control 2; E 3, Experimental group 3; E 4, Experimental group 4.

able to transfer cholesterol to progesterone and increase progesterone hormone. Like-estrogen compounds also increase estrogen hormone (21). These compounds that exist in DPP transfer to embryos and offspring via lactate and placenta and affect reproductive system in adult mice. Finally, our study showed that DPP possesses facilitative

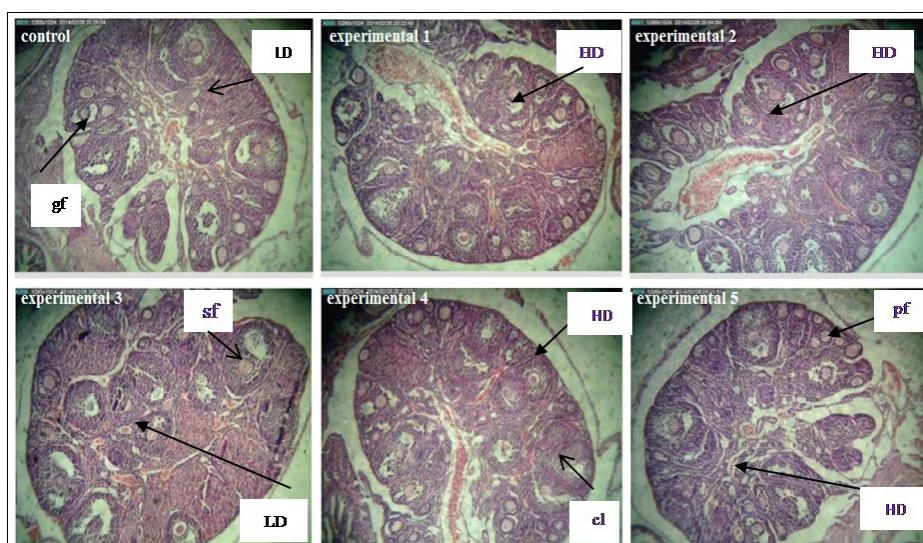


Figure 1. Effect of date palm pollen on density of follicles in control and various experimental groups (×100, H&E); ovary in treated groups shows increased number of follicles; LD: low density; HD: high density; Pf: primary follicle; sf: secondary follicle; gf: graph follicle; cl: corpora luteal.

effects that increase sexual arousal and percentage of mating. In another study, Abedi et al examined the aphrodisiac activity of aqueous extract of *P. dactylifera* pollen grain on the sexual behavior in male rats. They demonstrated that the aqueous extract of *P. dactylifera* pollen grain improved sexual behavior in male rats. The improved sexual appetitive behavior in male rats may be attributed to the alkaloids, saponins, and or flavonoids because these phytochemicals have engorgement, androgen enhancing properties (12).

Conclusion

According to the findings of this study, it can be concluded that the DPP suspension (at 100 and 200 mg/kg BW) for 10 days improves sperm quality, enhances fertility in female adult mice and also causes a positive effect on reproductive system in female embryos. These effects may be explained by the chemical composition of the plant. Therefore, it may be useful to improve infertility problems.

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Authors' contributions

Design and Supervision: JB and FM; Data Collection and/or Processing: FM and LJ; Literature Search: FN and SZB; Writing: FM and EA; Critical Reviews: JB, FN, AND SZB.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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