

Effect of *Phoenix Dactylifera* Pollen on Sperm Parameters and Reproductive system of Adult Male Rats

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Abstract

Background: There is a rapidly growing trend in the consumption of herbal remedies in the developing countries. The *Phoenix dactylifera* date palm pollen (DPP) is used in the traditional medicine for male infertility. The aim of this study was to determine the effects of orally administered DPP on the reproductive system of adult male rats.

Methods: Fifty Sprague-Dawley rats were maintained according to standard laboratory conditions. They were divided into five groups (n=10) and received daily gavages of aqueous suspensions DPP containing 30, 60, 120 and 240 mg/kg, for 35 consecutive days. At the end the sperm was collected from ductus deferens under anesthesia and their numbers, motility, and morphology were determined under light microscopy. The DNA integrity or denaturation was also evaluated by acridine orange staining. The weight of the testis and reproductive appendages was also determined, and after tissue processing, their histology were studied by light microscope.

Results: The comparative evaluation between control and experimental groups revealed that consumption of DPP suspensions improved the sperm count, motility, morphology, and DNA quality with a concomitant increase in the weights of testis and epididymis. It did not significantly affect the weight of the prostate and the seminal vesicle or the histology of the reproductive tissues.

Conclusion: Date palm pollen seems to cure male infertility by improving the quality of sperm parameters. However, further studies are needed to see its beneficial effects in man.

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Keywords • Date palm pollen • male reproductive system • sperm • infertility

Introduction

The use of herbal medicine has become increasingly popular worldwide especially in the Asian countries. There are many ancient records of herbal medical plants. 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.^{1,2} The male flowers of date palm are also eaten directly by people as a

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fresh vegetable to enhance fertility.³ Egyptian scientists have reported the gonad stimulating potency of DPP.³ Pollen grains of date palm were also used to promote fertility in women in ancient Egypt.⁴

Infertility affects 13-18% of married couples, and growing evidence exist from clinical and epidemiological studies suggesting an increasing incidence of male reproductive problems.^{5,6} The pathogenesis of male infertility can be reflected by defective spermatogenesis due to failure in germ cell proliferation and differentiation.⁵ Over the past decades growing evidences are also indicating a steady decline in human sperm counts and motility.⁷

In recent years, it has been suggested that estrogen, may be involved in the regulating the renewal of spermatogonial stem cells,⁸ and male reproductive tissues with estrogen receptors.⁹ Investigations have revealed that palm kernels and date pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility.¹ Reports have also pointed that isolation of micro elements from DPP has estrone, sterols, and other agents that may influence male fertility.^{4,10,11} With regard to these components, snack foods have been supplemented with date pollen to improve male infertility.¹² Pollen mixed with honey is eaten as an aphrodisiac from ancient time.¹³ DPP mainly contains cholesterol, rutin, carotenoids, as estrone which is known to exhibit gonadotrophin activity in the rat.¹⁴

The fertility effects of DPP in traditional medicine are not supported by scientifically and the literature does show little reports on its effects on sperm parameters or male genital system. Therefore, the present investigation was designed to determine the effects of DPP on the histology of the testis, reproductive tracts, and sperm parameters of adult male rats.

Materials and methods

Preparation of the herbal cocktails

The DPP was collected from Busher Province Botanical garden, South of Iran, authenticated and deposited in the Herbarium of Department of Biology of Science School of Shiraz University with the voucher specimen number of 40010.

Animal treatment

Fifty sexually mature 6-8 weeks-old male Sprague-Dawley rats were obtained from Animal House of Shiraz University of Medical Sciences. The study adheres to the principles of laboratory care established by Ethic Committee of Shiraz University of Medical Sciences. The animals were acclimatized to the laboratory conditions for two weeks prior to the start

of the experiments. The rats were housed individually in metal cages in a room with controlled temperature of 22-24°C with 12 hours dark/ light cycle. They had access to food and water *ad libitum*.

The doses chosen here were selected as those that used in the traditional medicine. However, unlike the traditional medicine which DPP dose was 60 mg/kg, in these study four doses with 30, 60, 120 and 240 mg/kg were selected. The animals were divided into five groups (n=10) of equal number, four experimental and control groups. The animal of the experimental groups received one ml of suspension of DPP in distilled water orally containing 30, 60, 120, 240 mg/kg for 35 consecutive days by gavage method, whereas, the control group only received an equal volume of distilled water.

At the end of the experiment the animals were deeply anesthetized with anesthetic ether and sacrificed. Blood samples were collected from the aorta, centrifuged, the serum separated, and stored at -80°C for the measurement of testosterone and estradiol, using immunoassay technique and Spectra Testosterone and estradiol kits were used according to their manufacturer's instruction (Orion Diagnostica; Finland and DRG Instruments GmbH; Germany).

Sperm collection

Animals were sacrificed and their reproductive organs were exposed. The ductus deferens, due to its role in the conduction of the mature sperms, were selected for analyzing of the sperm.¹⁴ Ten millimeter tissue (segment) of the distal part of ductus deferens was immediately removed and placed in 5 ml of Hanks Balance Salt Solution (HBSS).¹⁴ The incubation continued for five minutes or until an appropriate amount of sperm was diffused into the Hanks medium and then the ductal tissue was removed.

Two smear preparations were done separately for obtaining the morphology and DNA integrity. DNA integrity was evaluated using acridine orange staining method. The samples were assayed for obtaining the percentage of sperms with normal morphology.

The experimental equipments such as Petri dishes, slides and all other supplies were maintained at 37°C to ensure the accuracy of the experiments. The sperm samples present in the Hanks medium were loaded on the hemocytometer for counting the sperm numbers. The motility of the sperm cells was calculated by randomly selecting ten fields and counting the number of motile, semi-motile, sluggish and immotile sperms in each field.

Finally the testis, epididymis, seminal vesicle and prostate were dissected and weighed, fixed, processed, sectioned, and stained with

the H & E technique and examined under light microscopy.

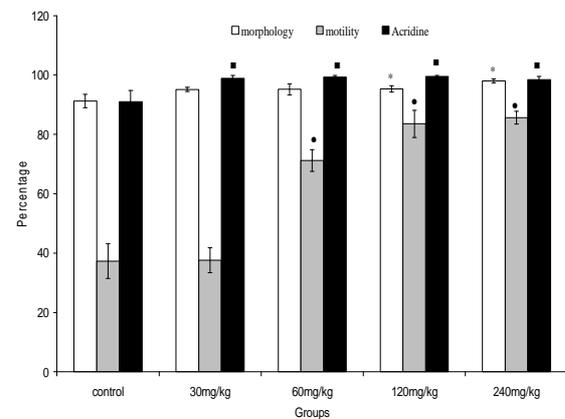
Statistical Analyses

Quantitative data are presented as Mean±SD. Sperm counts, morphology, and motility, of control and experimental groups are compared using one-way analysis of variance (ANOVA), and LSD test used to find the statistical differences among their means. A value of $p < 0.05$ was considered to be statistically significant.

Results

Oral administration of various concentrations of DPP improved the sperm parameters such as the sperm count, motility and morphology. But the significant differences were observed only in animal groups that treated with DPP concentrations of 120mg/kg and 240 mg/kg (Fig.1). Sperm motility significantly improved in all experimental groups of DPP with the exception of those treated with 30 mg/kg. Administration of DPP reduced DNA denaturation significantly, being more pronounced at higher concentrations and in this regard the most effective dose was 120 mg/kg (Fig.1).

*Different from Control $P < 0.05$



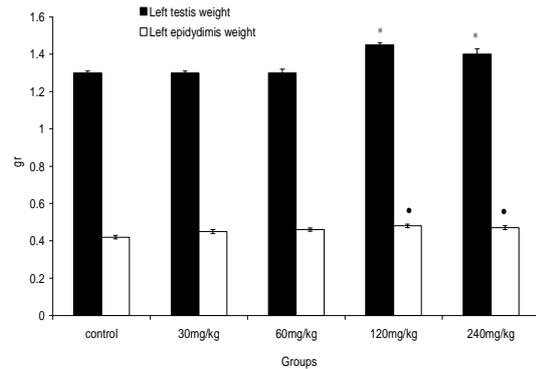
● Different from Control $P < 0.001$
 ■ Different from Control $P < 0.001$

Fig 1: The effect of date palm pollen on morphology, motility and DNA denaturation (Acridine) of sperm.

The increase in sperm count was obvious in all experimental groups as compared to the control group, but the most effective dose was 120 mg/kg. After treating the rats with DPP suspension, testes and epididymis weights increased significantly (Fig 2).

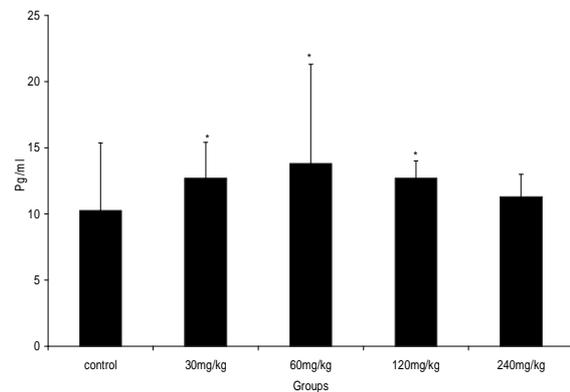
There was an increase in the blood level of estradiol and testosterone (Figs 3 and 4). As shown in Fig. 3, the effective doses for estradiol were 30, 60 and 120 mg/kg and for testosterone was 120 mg/kg.

No histological changes were observed in the seminal vesicle, epididymis and prostate; however, some minor changes were found in testes specimens. The number of interstitial cells (Leydig's cells) increased by 30% of the groups receiving 120mg/kg DPP suspension.



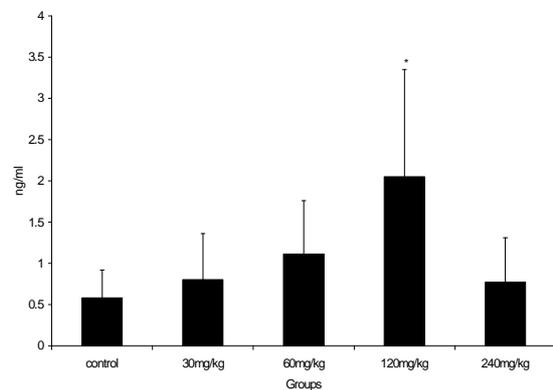
*Different from control $P < 0.001$
 ● Different from control $P < 0.05$

Fig 2: The effect of date palm pollen on on testis and epididymis weight.



*Different from the control $P < 0.05$

Fig 3: Effect of the date palm on the estradiol blood level



*Different from control $P = 0.004$

Fig 4: Effects of date palm pollen on testosterone

Discussion

Date palm (*Phoenix Dactylifera*) has been used as an herbal remedy since ancient time without any scientific rationale. We designed this animal model to investigate the efficacy of palm pollen suspensions with various concentrations on male infertility.

Reports are indicating that date palm contain estradiol and flavonoid components,^{10,11} that have positive effects on the sperm quality.^{15,16} The scavenging properties of DPP is said to be the main important effects on the sperm parameters. Our results confirmed that DPP had beneficial effects on male reproductive activity. These results showed that the DPP concentrations up to 120 mg/kg showed the best effects on sperm parameters.

Gonadotrophin effects of DPP have already been reported.¹¹ Our data showed an increase in the weight of testis and seminal vesicle in the rats that consumed DPP suspension. This effect might be due to the presence of gonadotropin like substances or steroidal component present in the DPP.^{5,8}

Our data showed that using DPP suspension increases the plasma levels of estradiol and testosterone. These hormones are found at high concentrations in rat testis and seminal fluids.¹⁵ It has also been shown that estrogen is synthesized in male reproductive system by at least three different cell types, Sertoli, Leydig and germ cells.¹⁵ Estrogen regulates the reabsorption of luminal fluid in the head of the epididymis. Disruption of this essential function causes the sperm to enter the epididymis in diluted form and reduces the sperm cell count.¹⁷ Despite increasing the weight of epididymis, no obvious changes were observed in its histological sections under light microscopy. The weight gains observed in epididymis, testes and seminal vesicles, in this study might have been due to fluid resorption effects of estradiol, which improved fertility. This might be due to the presence of phytoestrogen, as a steroidal component of DPP, which may have influenced sperm parameters.¹⁸

The sperm chromatin condensation and stability can be a valuable index of sperm quality by reflecting the possibility spermatogenesis disorders.¹⁹ In this study after staining with acridine orange, the denaturated sperm nuclei were red, whereas, that of normal DNA was green. The red and yellow nuclei is indicative of denaturation and partial denaturation of sperm DNA. DPP suspension reduced the sperm DNA denaturation and therefore, seems to improve its DNA quality compared to control group.

Conclusion

Date palm pollen suspension seems to improve sperm quality, enhance fertility in the male adult rat. Therefore, it may be useful to solve infertility problems.

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