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Protective Efficacy of *Phoenix dactylifera* Pollen Aqueous Extract Against Male Rat Infertility Caused by Lead

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Abstract

Background: Lead produces excessive reactive oxygen species (ROS) and accelerates lipid peroxidation. A wide range of tropical plants and their extracts, including fertility-boosting compounds and antioxidants, have demonstrated significant therapeutic benefits. One of the promising therapeutic plants is *Phoenix dactylifera* pollen, which is a tropical and subtropical tree. It is rich in flavonoids, phenolics, antioxidants, ferulic, p-coumaric, and sinapic acids.

Methods: Thirty-two male rats were studied and divided into four groups: (1) control (normal saline), (2) lead acetate exposure (80 mg/kg) group, (3) date palm pollen treatment (300 mg/kg) group, and (4) lead exposure + date palm pollen treatment group. After 30 days, organ weights (liver, testes, kidneys, spleen) and sperm health (count, motility, viability) were compared.

Results: In lead-exposed rats, improvements were noted in the mass of liver, testes, kidneys, and spleen after treatment with *P. dactylifera* extracts. The results also showed a noteworthy surge in testicular weight as contrasted with the lead-treated group. Furthermore, rats treated with both lead and Phoenix dactylifera pollen extract showed a significant increase in sperm count (10^6/mm^3), motility, and live sperm percentage, along with a decrease in dead sperm percentage, compared to the group treated with lead alone.

Conclusion: It appears that date palm pollen of *P. dactylifera* serves as a potentially effective remedy for male infertility. Data showed antioxidant effects of aqueous pollen extract from *P. dactylifera* in ameliorating the toxic effect of lead on testes and weight of organs by improvements in fertility parameters as compared to the lead-treated group.

Introduction

According to reports, environmental contamination endangers animal health and reduces productivity [1]. Scientists are increasingly concerned about the harmful effects of metal pollution in the environment, and extensive research has been conducted globally [2]. There is growing worry about environmental pollutants entering the systems used to produce livestock because the effects of toxic metal pollution on animals result in significant economic losses [3]. Pollutants in the environment and industry frequently contain lead. Industrial pollutants, soil, car exhaust gases, and contaminated crops like spinach and cabbage are often cultivated near lead sources. These may contain elevated amounts of lead and are the main sources of lead exposure [4]. Lead is used to make rust-proof paint, alloys, cable sheathing, lead-acid batteries, and pigments [5]. Lead is known to increase lipid peroxidation and cause an excess of reactive oxygen species (ROS) [6]. Moreover, nuclear factor E2-related factor Nrf2, which is closely associated with antioxidant response components, is a key regulator of the cellular oxidative stress response [7]. Lead may prevent the development of spermatocytes, immature spermatids, and mature spermatids [8]. A greater variety of tropical plants and their extracts have demonstrated significant medicinal effects, including chemicals that increase fertility as well as antimicrobial, aphrodisiac, antioxidant, anti-inflammatory, and anti-cancer properties [9]. The application of herbal remedies has grown in popularity across the globe, particularly in Asian and African nations. Traditional medicine uses a variety of *P. dactylifera* plant parts extensively to treat a variety of ailments, such as neurological disorders, fever, inflammation, paralysis, coma, and memory issues [10-12]. The extent of the biologic activities of this widely used botanical medicine has to be evaluated in light of the issues over male animal infertility in our cattle business. *P. dactylifera* pollen is one of the promising medicinal plants. Phytochemicals such as phenolics, sterols, carotenoids, anthocyanins, procyanidins, and flavonoids are said to be abundant in date fruit pulp [13-15]. According to certain theories, date fruit may help prevent cellular death because of these inherent phyto-constituents [16,17]. The use of herbal remedies is growing, and two of them are currently being researched to determine the scientific basis for their therapeutic effects [18]. *P. dactylifera* is a species that originated in the Middle East many years ago [19,20]. Date palm pollen (DPP) suspension is a common folk remedy in traditional medicine for treating male infertility, together with phenolic diterpenes, anthocyanins, procyanidins, and coumaric acid. Date seeds have been shown to have high nutritional benefits in studies, especially regarding their antioxidant and fiber levels [21,22]. Date seeds contain a variety of chemical substances, including protein, fat, dietary fiber, zinc (Zn), calcium (Ca), potassium, and unsaturated fatty acids that may impede the 5-reductase enzyme, such as oleic and linoleic acids [23,24]. Moreover, seeds are rich in phenolics, antioxidants, flavonoids, procyanidins, p-coumaric, ferulic, and sinapic acids, and lipids, which can be wax, fat, or oil [25,26]. Traditional medicine practitioners frequently employ the herbal mixture suspension of DPP to treat male infertility. Date palm fruit suspensions boost testicular and epididymal weights while enhancing sperm motility, morphology, and DNA quality [27].

Methods

Plant material

Plant samples were obtained from the Shatt Al Arab Botanical Park, Basra, Iraq. Using an affine gauze sieve, the pollen was removed from the kernels and stored in the refrigerator at 4°C. Powdered pollen was mixed with distilled water using a magnetic stirrer for ten minutes to ensure complete dispersion. The aqueous suspension was prepared fresh daily.

Study Design

In this investigation, 32 mature male albino rats weighing between 200 and 210 grams were employed. The experiment was carried out in the Veterinary Medicine Student Animal Building at the University of Basrah. Throughout the trial, rats were housed in clean, well-ventilated polypropylene cages with adequate food and water. At least two weeks prior to the commencement of the study, they were accustomed to the lab environment. Rats in this investigation were split into four test groups.

Group I:
Served as a control and administered 0.1 ml of N.S. for 30 days.

Group II:
Rats received lead acetate (80 mg/kg body weight) only through intraperitoneal injection for 30 days.

Group III:
Rats received aqueous extract of pollen, only 300 mg/kg orally for 30 days.

Group IV:
Rats received lead acetate (80 mg/kg) via intraperitoneal injection and aqueous extract of Phoenix dactylifera pollen (300 mg/kg) for 30 days. Rats received lead acetate (80 mg/kg body weight) through intraperitoneal injection, and after one hour, received aqueous extract of pollen 300 mg/kg orally for 30 days.

Rats were euthanized humanely under general anesthesia in accordance with the institutional animal ethics committee guidelines. The organs (testes, liver, kidney, and spleen) of all animals were weighed, and the number of sperm was calculated using the hemocytometers, utilizing Majumder and Biswas' methodology [24].

Statistical Analysis

Data expressed as Mean ± SEM was analyzed, and two-way ANOVA was used to evaluate the data. It was performed to compare the means of each experimental group and the control group; A p-value < 0.05 was considered significant, and SPSS software version 17.0 was used for analysis.

Results

Parameters	Testes (g)	Liver (g)	Kidney (g)	Spleen (g)
Control Group (CG)	1.426 ± 0.025^{a}	4.710 ± 0.010^{b}	1.368 ± 0.183^{c}	0.240 ± 0.018^{c}
Lead Group (LG)	0.733 ± 0.052^{c}	7.163 ± 0.036^{a}	3.141 ± 0.028^{a}	1.565 ± 0.677^{a}
P. dactylifera Group (PDG)	1.316 ± 0.052^{a}	4.798 ± 0.026^{b}	1.233 ± 0.028^{c}	0.371 ± 0.033^{c}
Lead + PDG	1.115 ± 0.013^{b}	5.345 ± 0.020^{c}	1.893 ± 0.023^{b}	1.313 ± 0.155^{b}

Table 1: The effect of *P. dactylifera* pollen on the weight of organs in lead-treated male rats.

Parameters	Sperm count (10^6/mm^3)	Motility %	Live sperm %	Dead sperm %
Control Group (CG)	19.55 ± 2.835^{a}	91.833 ± 2.136^{a}	89.000 ± 0.894^{a}	9.500 ± 0.547^{c}
Lead Group (LG)	7.66 ± 2.765^{c}	12.333 ± 2.338^{c}	6.166 ± 1.471^{c}	94.166 ± 1.834^{a}
P. dactylifera Group (PDG)	1.888 ± 2.289^{a}	84.500 ± 4.324^{b}	81.833 ± 1.834^{a}	10.833 ± 2.857^{c}
Lead + PDG	1.242 ± 2.350^{b}	74.600 ± 3.361^{c}	68.000 ± 2.549^{b}	30.400 ± 1.673^{b}

Table 2: The effect of aqueous extract of *P. dactylifera* pollen on sperm viability in lead-treated male rats.

From the results in Table 1, when animals were treated with lead acetate only, there was a significant increase in the weight of liver, kidney, and spleen, but a significant decrease was observed in testicular weight between the lead-treated group and the control group. On the other hand, when the animals were treated with lead and aqueous extract of *P. dactylifera* pollen, there was a notable decline in the weight of liver and kidney as compared with the lead-treated group only, and these organs' weight shifted towards the normal values recorded in the control group animals. Concerning testes weight, the group treated with both lead and the extract showed a significant improvement, with the weight shifting towards that recorded in the control group animals.

It is evident from Table 2 that the semen viability (sperm count (10^6/mm^3), live and dead sperm) of animals treated with the aqueous extract of plant pollen shows approximately normal levels, while sperm motility is significantly decreased compared with the control group. Also from Table 2, it can be observed that the reproductive parameters declined when animals were exposed to toxic material (lead treatment), while the percentage of dead sperm increased as compared to the control group.

Discussion

The findings of this study revealed that lead significantly ($p < 0.05$) reduced the weights of the testes, sperm viability, and the number of live sperm, while it increased the weights of the liver, kidneys, and spleen compared to the control group. After undergoing aqueous extract therapy of the plant pollen for 30 days, these values in the lead-exposed rats group showed significant improvement, shifting towards the control group. *P. dactylifera* pollen contains a phytochemical that has a calming effect. For instance, it is thought that the saponin present in *P. dactylifera* pollen helped to cause the reported rise in sperm motility. The highest percentage of sperm motility was seen in the group that was given 300 mg/kg body weight, the highest dosage. This rise in sperm motility was detected with high doses of saponin extract. This could have been accomplished by raising internal cAMP, which is observed to be responsive to lead toxicity and plays a role in sperm kinematics, by inhibiting the cAMP phosphodiesterase using saponins in aqueous extract. Hence, therapies that raise intracellular cAMP levels also raise kinematics and sperm motility [28]. Also, it is likely that the phytochemicals in the plant directly benefited the testes through direct therapeutic effects. According to Roberts *et al.,* [29], dates contain flavonoids and estradiol, which improve sperm health and boost male reproductive activity. *P. dactylifera* has gonadotrophin-like actions, which may be caused by its steroidal components, according to a report by Eustache *et al.,* [30]. The phytochemical genistein from dates and plant-derived compounds has the potential to enhance sex and is used to treat sexual dysfunction [31,32]. These phytochemicals stimulate sexual arousal, desire, and enjoyment during sexual activity [28]. The male sexual function is facilitated by *P. dactylifera* according to analysis. DPP has a high concentration of sterols, steroids, alkaloids, and flavonoids, all of which have the ability to enhance and control sexual behavior [33]. Given that the DPP's flavonoid components change testosterone levels, it could make sense to link these behaviors to them. DPP's alkaloid concentration seems to have ergogenic qualities by causing blood vessel vasodilation, which occurs during an erection. They can alter the production of neurotransmitters and receptor expression in brain regions involved in sexual arousal and regulation, enhance the processing of sensory stimuli, and increase responsiveness through genomically mediated actions [34-36]. Like our findings, other studies have demonstrated that when

compared to the control, segments of the Quassinoid-rich Eurycoma longifolia extract and pomegranate juice dramatically boosted the concentration and motility of epididymal sperm, the density of spermatogenic cells, and reduced the rate of aberrant sperm [37]. The tests showed increased secretions after giving the animals DPP. This observation is consistent with other studies where doses of 120 mg/kg and 240 mg/kg were administered. This boosted activity was reflected in their higher testosterone and estradiol levels, which might explain why the relative weights of their tests and epididymides increased compared to their overall body weight [38]. Findings of the current study were in line with those of a study by Abedi *et al.,* [39], which found that taking Bulbine natalensis Baker stem extract at doses of 25 and 50 mg/kg increased testicular weight. These findings support the traditional use of *P. dactylifera* as an aphrodisiac and a remedy for premature ejaculation [40]. The available information points to its use in treating sexual dysfunction and its effects on sexual desire. The presence of steroids, alkaloids, and flavonoids may cause *P. dactylifera* pollen effects through a variety of cerebral and peripheral pathways [40].

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Author Contributions

All of the authors contributed to the work that is being presented here. Each author has contributed to the study's conception and design, the gathering of data, the analysis and interpretation of the findings, and the report's composition. The writers are grateful for their assistance and encouragement.

Competing Interest

No conflicts of interest have been disclosed by the writers.

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