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Effect Evaluation of Iraqi Date Palm Pollen on Sex Hormones Level of Male Local Rabbits

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Abstract

This study investigates the biochemical and histological effects of Iraqi date palm pollen (IDPP) (Phoenix dactylifera L. variety El-Ghanmni Ahmar) on the levels of sex hormones in the sera of adult male rabbits. Twenty eight adult male rabbits were used, divided into 4 groups (C, G1, G2 and G3). C is a control group, while G1, G2 and G3 were orally treated with 30, 60 and 90 mg/kg/day of oily IDPP for 4 weeks, respectively. The results indicated that the level of Luteinizing hormone (LH) was significantly (p≤0.05) increased in G1 and G2, while there was no significant change in G3 as compared with C. The level of Follicular Stimulating hormone (FSH) was significantly (p≤0.05) increased in G2, as compared with C and not significant in G1 and G3. In addition, no significant change in prolactin levels, while the level of testosterone significantly (p≤0.05) improved in all groups. The effect of IDPP on testicular of treated male rabbits was studied histologically. The histological study indicated that IDPP increased the active spermatogenesis with significant rise in the number of mature sperms in G1, G2 and G3 as compared with C. It could be concluded that IDPP improved fertility by boost production of testosterone and increase the spermatogenesis at seminal tubules by increasing the primary and secondary spermatocytes.
1. Introduction

Date palm (*Phoenix dactylifera*, L.) is the most important and economical species grown in Iraq, West Asia and North Africa, extensively planted in the Arab countries and also grown to some extent in southern Europe [1, 2]. Pollens are the male reproductive cells of flowers, the pollen viability differs greatly from male palms to another [3]. Suspensions of date palm pollen-DPP (an herbal mixture) are widely used as a folk remedy for curing male infertility in traditional medicine. Pollen grains of the date palm are also used to promote fertility in women in ancient Egypt [4, 5]. Investigations have revealed that pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male and female infertility [6-8].

The effect of *Phoenix dactylifera*, pollen, on sperm parameters and reproductive system of adult male rats is studied and the results indicate that the consumption of Iraqi date palm pollen (IDPP) suspensions improves the sperm count, motility, morphology, and DNA quality with a concomitant increase in the weights of testis and epididymis [4, 9]. On the other hand, the palm pollen grain extracts have shown a significant increase in spermatogenesis by increasing the primary and secondary spermatocytes in compared with control animals [5].

Therefore, the aim of this study is to investigate the effect of IDPP [Iraqi cultivar] on the levels of sex hormones.

2. Materials and Methods

2.1 Plant materials

DPP (*Phoenix dactylifera* L.) variety El-Ghanimmi Ahmar was collected from Samarra city, Salah Al-Din, Iraq and separated from the kernels by fine gauze sieve and left in an incubator at 35 °C for 3 h.

2.2 Animals

Twenty eight of local male rabbits purchased from General Company for Drug Industries/Samarra. Male rabbits of weight ranged from 1.2 to 1.55 kg were used at 3.0-3.5 months average age. Groups of rabbits were housed at room temperature with a lighting schedule of 12 h light and 12 h dark. Animals had free access to a standard pellet diet and tap water as drinking solution. The rabbits have been divided randomly into four groups (7 animals in each group). The groups G1, G2 and G3 were orally administrated daily dose 30, 60 and 90 mg/kg/day of oily IDPP, respectively, for 4 weeks. In addition, a control group (C) without IDPP treatment was used as a reference.

After 4 weeks, animals were fasted for 12 h before taking the blood samples. The blood serum was collected by centrifugation the blood at 2500 rpm for 15 min, then it was divided into 2 parts in an eppendorf tube and stored at -20 °C until analyzed. Determination of serum levels of LH, FSH, testosterone and prolactin were performed using micro plate enzyme immunoassay ELISA. All testicular tissues were fixed in 10% formalin and then treated as a procedure in [10, 11] in which, the cervical of the rabbits was removed and placed in a Petri dish containing physiological saline. Then embedded in paraffin, cut at 4 μm thickness, stained with Harris hematoxylin and eosin, and then examined under light microscope for histopathological evaluation.

2.3 Statistical analysis

Results were analyzed statistically by Analysis of Variance Test-ANOVA using the statistical program Minitab. Averages were compared to calculations of the characteristics of the application Duncan’s Multiple Range Test by probability level P ≤ 0.05.
3. Results and Discussion

3.1 Effect of IDPP on the levels of sex hormones in sera of adult male rabbits

The level of sex hormones was measured in sera of male rabbits groups treated with IDPP and control group C. The mean ± SD values of hormones for the groups were shown in Table 1. The results indicate that the level of LH significantly increased (p≤ 0.05) in sera of G1 and G2 as compared with C, with no significant differences in G3 (high dose of IDPP). Date palm (Phoenix dactylifera) and its pollen have been used as an herbal remedy since ancient time without any scientific rationale [4]. We designed this animal model to investigate the efficacy of palm pollen suspensions with various concentrations on male infertility. The obtained results indicate that IDPP might impact the levels of LH with the best dose 30 mg/kg of body weight. The results are compatible with a study carried out by Salman et al [12] and the results of Ibrahim et al [13] in experimental male animals. Similarly, Jiheel and Arrak [14], reported that an alcoholic extract of IDPP increased the level of LH after 28 days of treatment with 50 and 100 mg/kg. The concentrations higher than 100 mg/kg shows no dose related effects.

3.1.1 Follicular stimulating hormone (FSH) levels

Results obtained from Table 1 suggest that the most effective doses of FSH were 60 mg/kg for IDPP with no significant change in G1 and G3 as compared with C. Our findings are in agreement with those of other authors who showed that the alcoholic extract of IDPP led to increase the level of FSH [14] and with those found that the effective dose of IDPP suspension was 216 mg/kg [13].

3.1.2 Prolactin levels

Results obtained from the Table 1 indicated that the date palm pollen grains did not affect the level of prolactin in all groups under investigation as compared with C. Similarly, the powdered bark of the Pygeum tree (contains high phytoesterols) and used for centuries to treat urinary disorders, and diminish circulating prolactin and decreases the prostate uptake of testosterone [15].

3.1.3 Testosterone levels

Results indicated that the levels of hormone largely affected by the treatment of IDPP, in which the level of hormone significantly increased in the sera of all groups G1, G2, G3 as compared with control one. In our study, the effective dose for IDPP was found to be 90 mg/kg, while other studies suggest that the most effective dose for IDPP was 120 mg/kg of body weight [4] and 140 mg/kg of body weight in another study [16]. The gonadotrophin effects of IDPP have already been reported [17]. This effect might be due to the presence of gonadotropins like substances or steroidal component present in the IDPP [18, 19]. Bahmanpour, et al. [4] suggested that IDPP contains estradiol and flavonoid components that have positive effects on the sperm quality, and the suspension of IDPP significantly increased the plasma levels of testosterone. The result was in agreement with those of other authors who showed that the aqueous extract of IDPP significantly elevated the level of hormone in the sera of albino male rats treated with sodium fluoride [20].

3.2 Histopathological change in rabbits testes treated with IDPP

The histological test of testicular of male rabbits was tested for the control group (non-treated) group Fig. 1. The cross sections for the testicles rabbits treated by IDPP of G1, G2 and G3 were shown in Figs. 2, 3 and 4, respectively. It indicates that normal seminiferous tubule, spermatocytes with releasing sperms in large amounts in seminiferous tubule is due to increase active spermatogenesis with significant rise of number of mature sperms in all the three groups.
(G1, G2 and G3) which increased with the increase of the dose of treatment with IDPP. The connective tissue between seminiferous tubule become more clear. The Setroli cell was notified with clear appearance in Figs. 2 and 3. Pre-building of seminiferous tubule also appeared in Fig. 4. No organic pathological lesions (ie. absent interstitial fibrosis, congestion vascular injury or inflammation with no tubular necro-degenerative changes or atrophy) was found in the section of testicles in all three groups as compared with normal one in control group which appeared normal in histological structure and production of sperms.

Table 1: Mean ± SD of sex hormones in sera of rabbits treated with IDPP

<table>
<thead>
<tr>
<th>Groups</th>
<th>LH (mIU/mL)</th>
<th>FSH (mIU/mL)</th>
<th>Prolactin (ng/mL)</th>
<th>Testosterone (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.0523±0.0443</td>
<td>0.004±0.002</td>
<td>9.571±0.976</td>
<td>4.540±1.711</td>
</tr>
<tr>
<td>G1</td>
<td>0.2170±0.1660</td>
<td>0.006±0.002</td>
<td>10.900±1.084</td>
<td>7.321±2.119</td>
</tr>
<tr>
<td></td>
<td>p≤ 0.050</td>
<td>NS</td>
<td>NS</td>
<td>0.050</td>
</tr>
<tr>
<td>G2</td>
<td>0.1060±0.0760</td>
<td>0.007±0.004</td>
<td>11.000±1.225</td>
<td>11.055±1.607</td>
</tr>
<tr>
<td></td>
<td>p≤ 0.050</td>
<td>0.050</td>
<td>NS</td>
<td>0.050</td>
</tr>
<tr>
<td>G3</td>
<td>0.0500±0.0440</td>
<td>0.006±0.003</td>
<td>10.714±1.286</td>
<td>11.842±0.980</td>
</tr>
<tr>
<td></td>
<td>p≤ NS</td>
<td>NS</td>
<td>NS</td>
<td>0.050</td>
</tr>
</tbody>
</table>

* NS = non-significant

Figure 1: Cross section for normal rabbits testes, shows the seminiferous tubule contain spermatocytes, sperm and connective tissue and Leydig cell (C) (HE)-300X: [CT: Connective tissue, L: Leydig cell; S: Sperm, SC: Spermatocytes, ST: Seminiferous tubule].

Figure 2: Cross section of rabbits testes, shows the seminiferous tubule contain spermatocytes, sperm and connective tissue (G1) (HE)-300X: [TF: Thin Fibrous, SC: Spermatocytes, S: Sperm].
Figure 3: Cross section of rabbits testes, shows the seminiferous tubule contain spermatocytes, sperm and connective tissue (G2) (HE)-300X: [CT: Connective Tissue, S: Sperm, SC: Spermatocytes].

Figure 4: Cross section of rabbits testes, shows the seminiferous tubule contain spermatocytes, sperm with pre-building of seminiferous tubule (G3) (HE)-300X: [S: Sperm, SC: Spermatocytes, Se: Sertoli cell].

The results showed that the oral administration of IDPP has a positive effect on the testicular function of male rabbits. Furthermore, the tubules of testicular showed an increased active spermatogenesis with a significant rise of a number of mature sperms, with a notable decrease of tubular dysfunction is encountered. This may be due to the IDPP which caused a significant increase in testosterone in the blood. This explanation agreed with the current finding in which the testosterone levels were significantly increased in the sera of all male rabbits treated with IDPP. The increases in the serum levels of testosterone caused an increased active spermatogenesis with a significant rise in a number of mature sperms [7, 21]. It has also shown that oestrogen is synthesized in the male reproductive system by at least three different cell types, Sertoli, Leydig and germ cells [22]. A differentiation of Leydig cells in Figs. 3 and 4, due to the maturation of the cells and increasing its size, thus improving its function for secretion of testosterone and also the maturation of sperm, so increased the number of sperm [4].

4. Conclusions

The biochemical and histological effects of Iraqi date palm pollen (IDPP) on the levels of sex hormones in the sera of adult male rabbits were investigated. The results showed that the oral administration of IDPP has a positive effect on the testicular function of male rabbits. Moreover, the tubules of testicular showed an increased active spermatogenesis with a significant rise of a number of mature sperms, with a notable decrease of tubular dysfunction is encountered. The histological study indicated that IDPP increased the active spermatogenesis with significant rise in the number of mature sperms in all three groups G1, G2, G3 as compared with normal tissue in control one. IDPP could improve fertility by boost production of testosterone and enhance the spermatogenesis at seminal tubules by boosting the primary and secondary spermatocytes.
References


