Effects of *Phoenix dactylifera* on the testes, epididymal sperm pattern and hormonal profiles of male Wistar rats

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**Abstract**

**Background:** *Phoenix dactylifera* belongs to the family *Arecaceae*. It is rich in vitamins, steroids, flavonoid, saponins and simple sugars. The aim of the study is to evaluate the effect of date palm fruit on testes, epididymal sperm pattern and hormonal profiles of male Wistar rats.

**Methods:** Twenty (20) male Wistar Rats were divided into four groups of five rats each. Three (3) experimental groups were treated with the extract at 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight for 35 days while the control receives distilled water. The rats were sacrificed on the 36th day and the testes and epididymis were dissected and weighed, the testes were processed for light microscopic study; blood samples were collected for hormonal assay [Testosterone, Follicle Stimulating Hormone and Luteinizing Hormone]. Sperm samples from the epididymis were collected and counted; a smear was made and stained with cresyl violent to determine sperm morphology. Morphometric analysis was performed to measure seminiferous tubular diameter, size of interstices and epididymal epithelial thickness.

**Results:** The result showed degeneration of spermatogenic cells, destruction of seminiferous tubular membranes and Leydig cells with significant decrease in serum testosterone levels but no significant change in FSH and LH levels. There was also significant decrease in sperm count, motility and morphology with decrease in size of interstices at \( P \leq 0.05 \).

**Conclusion:** In conclusion, the components of the extract have the potentials of causing infertility in male Wistar Rats by decreasing serum testosterone levels, sperm count, sperm motility and sperm morphology.

**Keywords:** *Phoenix dactylifera*, Leydig cells, Testes, Testosterone, Epididymis

1. **Introduction**

*Phoenix dactylifera* (Date palm), family *Arecaceae* is cultivated for its edible sweet fruit. The fruits are nutritious, high-energy food, and important part of the diets of people in the Arab countries and are consumed fresh, dried, or in various processed forms (1). The dried fruit is more than 50% sugar by weight although the glucose, fructose and sucrose contents depends on fruit types, also contains about 2% of protein, fat, and mineral matter, 20–70 calories, vitamin C, riboflavine and thiamine depending on size and variety (2, 3). The date fruit extract also contains antioxidants such as coumaric and ferulic acids (4).

The Ancient Egyptians used the fruits to make date wine, and ate them at harvest. They are an important traditional crop in Iraq, Arabia, North Africa and Morocco where they are used in the treatment of various ailment/illness (5). Infertility is defined as the inability of couples to achieve pregnancy after one year of continuous unprotected sexual intercourse or six months, if the woman is 35 years or older (6, 7). The prevalence varies widely, being less in developed countries where resources for investigation and treatment are readily available and accessible but more in developing countries where limited resources for investigation and treatment are available, infertility is considered as a public problem. It does not affect the couples’ life only, but it also affects the healthcare services and social environment (8). The aim of the present study is to evaluate the effect of aqueous extract of *P. dactylifera* on the testes, epididymal sperm pattern and hormonal profiles of Wistar rats.

2. **Materials and Methods**

2.1. Preparation of Extract

Date palm fruit was purchased from Samaru market in Zaria and the extraction was done in the

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Department of Pharmacognosy, ABU Zaria, Kaduna State. The fruit was opened and the fleshy part was oven dried and grounded to powder. It was soaked in maceration apparatus with distilled water for 24 hours, filtered and allowed to settle down, it was then decanted and oven dried at 50°C.

2.2. Experimental Design

Twenty Wistar rats were purchased from the animal house, Department of Human Anatomy, ABU Zaria and the experiment was approved by the ABU ethical committee. The Rats were randomized into four groups of five rats each and were kept under standard laboratory condition (12 hours light/12hours dark cycle), fed with standard feeds pellets (Grower’s marsh, Vital Feed, Grand Cereal, Nigeria) and water ad libitum. They were acclimatized to the animal house condition for two week prior to the start of the experiment. The rats in group I (control) were given distilled water while Group II-IV received aqueous extract of P. dactylifera at (250 mg/kg, 500 mg/kg, & 1000 mg/kg, respectively) oro-gastrically by intubation once daily for 35 days (9).

2.3. Histopathology

All the rats were sacrificed on the 36th day and the testes and epididymis were dissected and weigh using a chemical balance. The testes were fixed in bouin’s fluid, embedded in paraffin wax, sectioned at 5µm and stained with hematoxylin and eosin.

2.4. Sperm Count

The right epididymis was teased and placed in a vessel containing 5ml (9.5%) normal saline to make a suspension, a pipette was used to introduce the suspension onto an improved Neubauer haemocytometer (Hawksley Christalite) that was fitted with cover slip and placed under the microscope for counting. A smear was made on a glass slide from which cresyl violent staining was done to determine sperm cells with normal morphology by counting in different fields.

2.5. Morphometry

The seminiferous tubular diameter, size of interstices and epidydimal epithelial thickness were measured using a standardized ocular micrometer (Graticules ltd. Ton bridge Kent England) in the Department of Microbiology, ABU Zaria.

2.6. Hormonal Profile

Blood samples were collected in a plain bottle using the cardiac puncture method, it was centrifuged at 2500 revolution per minute for 5 minutes and the serum obtained was used to determine the levels of testosterone, follicle stimulating hormone and luteinizing hormone using Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer’s instruction.

2.7. Statistical Analysis

The Data were analyzed using Instant Statistic Package version 3. One way analysis of Variance (ANOVA) was used to compare the mean difference between and within the groups and a P-value (P<0.05) was considered statistically significant.

3. Results

The result showed that, there were no significant difference between the control and treated groups for the body and organ weights (Testes and epididymis) of the rats treated with aqueous extract of P. dactylifera but there was significant decrease in sperm count, motility and morphology (abnormal sperm cells consist of sperm cells with no tail or coiled tail figure 1) between the control group and groups treated with the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight at P≤0.05 (Table 1). There was a significant decrease in the levels of testosterone between the control group and the rats treated with aqueous P. dactylifera extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg at P≤0.05 with no significant difference in FSH and LH between the control and the treated groups (Table 2). There was a significant increase in seminiferous tubular diameter between the control and the groups that receive the extract at 250 mg/kg and 1000 mg/kg. There was a significant decrease in size of interstices and epididyimal epithelial thickness between the control and the groups that receive the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg at P≤0.05 (Table 2). The photomicrograph of the testes of control rats showed the typical structure of the seminiferous tubules illustrating all the stages of spermatogenesis from spermatogonia to mature sperm cells (Figure 2A). The testes of rats treated with P. dactylifera at 250 mg/kg and 500 mg/kg showed distortion of spermatogenic cells and reduction in size of interstices (27.30±1.38 µm and 26.60±1.98 µm respectively) compared to that of the control (36.50±2.18 µm) (Figure 2B and 2C) while the rats treated with the extract at 1000 mg/kg showed degeneration of Leydig cells and seminiferous tubular capsule and reduction in size of interstices (27.30±1.38 µm) compared to that of the control (Figure 2D).

Figure 1. Photomicrograph of sperm cells in a field showing normal cells (Black arrows), sperm cells without tail (light blue arrow) and sperm cell with coiled tail Orange arrow) Cresyl violent stain x400.
Table 1. Body weight, organs weight and Epididymal sperm count, Motility and Morphology of Rats treated with aqueous extract of P. dactylifera

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>250 mg/Kg</th>
<th>500 mg/Kg</th>
<th>1000 mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>77.20±2.69</td>
<td>60.00±4.52</td>
<td>82.00±2.98</td>
<td>71.80±7.23</td>
</tr>
<tr>
<td>Left Testis (g)</td>
<td>0.740±0.033</td>
<td>0.748±0.012</td>
<td>0.692±0.016</td>
<td>0.638±0.024</td>
</tr>
<tr>
<td>Right Testis (g)</td>
<td>0.748±0.028</td>
<td>0.740±0.027</td>
<td>0.686±0.018</td>
<td>0.618±0.023</td>
</tr>
<tr>
<td>Left Epididymis (g)</td>
<td>0.506±0.292</td>
<td>0.196±0.014</td>
<td>0.160±0.014</td>
<td>0.180±0.014</td>
</tr>
<tr>
<td>Right Epididymis (g)</td>
<td>0.226±0.023</td>
<td>0.212±0.013</td>
<td>0.156±0.016</td>
<td>0.190±0.018</td>
</tr>
<tr>
<td>Sperm count (×10^6/ml)</td>
<td>130.14±8.18ªᵇ</td>
<td>93.90±3.08ª</td>
<td>91.15±3.33ª</td>
<td>93.55±10.59ª</td>
</tr>
<tr>
<td>Sperm Motility (10^6/ml)</td>
<td>116.60±7.43ªᵇº</td>
<td>79.50±8.51ª</td>
<td>70.75±4.03ª</td>
<td>68.60±8.91°</td>
</tr>
<tr>
<td>Normal sperm (%)</td>
<td>87.16±1.94ªᵇº</td>
<td>74.49±1.38ª</td>
<td>79.59±0.75ª</td>
<td>70.58±1.50°</td>
</tr>
</tbody>
</table>

All values expressed as Mean±SEM. Values with the same super script in the same row are significantly different at P≤0.05 using one-way ANOVA.

Table 2. Levels of testosterone, FSH and LH and morphometric parameters of Testis and Epididymis of Rats treated with aqueous extract of P. dactylifera

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>250 mg/Kg</th>
<th>500 mg/Kg</th>
<th>1000 mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>2.02±0.07ªᵇº</td>
<td>1.14±0.09ª</td>
<td>1.00±0.11ª</td>
<td>1.14±0.09ª</td>
</tr>
<tr>
<td>FSH (mlu/ml)</td>
<td>0.41±0.015</td>
<td>0.39±0.014</td>
<td>0.39±0.017</td>
<td>0.40±0.013</td>
</tr>
<tr>
<td>LH (mlu/ml)</td>
<td>0.59±0.012</td>
<td>0.58±0.012</td>
<td>0.58±0.012</td>
<td>0.58±0.008</td>
</tr>
<tr>
<td>Seminiferous Tubular Diameter (µm) x100</td>
<td>23.35±0.07ªᵇ</td>
<td>25.72±0.46ª</td>
<td>24.83±0.56</td>
<td>25.14±0.55ª</td>
</tr>
<tr>
<td>Size of Interstices (µm x400)</td>
<td>36.50±2.18ªᵇº</td>
<td>27.30±1.38ª</td>
<td>26.60±1.98ª</td>
<td>27.30±1.38ª</td>
</tr>
<tr>
<td>Epididymal Epithelial Thickness (µm x400)</td>
<td>10.06±0.09ªᵇº</td>
<td>7.20±0.22ª</td>
<td>6.64±0.25ª</td>
<td>6.12±0.12°</td>
</tr>
</tbody>
</table>

All values expressed as Mean±SEM. Values with the same super script in the same row are significantly different at P≤0.05 using one-way ANOVA.

Figure 2. A: Photomicrograph of the testis of control rats illustrating the typical structure of the seminiferous tubule showing the stages of spermatogenesis, spermatogonia (light blue arrows), sperm cells (black arrows), and the interstitial cells of Leydig (light green arrow). B: Photomicrograph of tests of rats treated with aqueous P. dactylifera extract at 250 mg/kg showing scanty sperm cells in the lumen of seminiferous tubule (black arrow), distortion of spermatogenic cells (light blue arrows) and compacted interstices (light green arrows). C: Photomicrograph of the tests of rats treated with aqueous extract of P. dactylifera at 500 mg/kg showing distortion of sperm cells (black arrows) within the lumen of seminiferous tubule and compacted interstices (Light blue arrows). D: Photomicrograph of tests of rats treated with aqueous P. dactylifera extract at 1000 mg/kg showing seminiferous tubule with scanty sperm cells (black arrow), distortion of capsule (green arrows), compacted interstices (light blue arrow), and degeneration of Leydig cells (orange arrow). H and E x250.

4. Discussion

The rats treated with P. dactylifera extract did not show any significant change in body/organ weight compared to that of the control, this shows that the extract may not affect metabolism. This is in agreement with earlier studies by (10, 11).

The significant decrease in epididymal sperm count, motility and percentage of sperm cells with normal morphology in rats treated with the extract compared to that of the control suggested that the extract could affect spermatogenesis or had the potentials of destroying sperm cells. The extract contained high content of steroids, flavonoids and
saponins (12), steroids are reported to affect male fertility by reduction in sperm quality and serum testosterone levels (13, 14). Saponins are reported to have antifertility, abortifacient and anti-implantation properties in rodents (15, 16). Flavonoids and saponins have tumor suppressive activities and are used as anticancer agents (17, 18) and any substance/compound with tumor suppressive activity inhibits proliferation of stem cells (19). Therefore, the steroids, flavonoids and saponin content of *P. dactylifera* extract might be the cause of decrease sperm count, motility and morphology. There was no significant change in the serum levels of FSH and LH in Rats treated with the extract compared to that of the control, this showed that the extract may have no effect on the hypothalamus and pituitary gland which are the glands responsible for production of FSH and LH (20). The significant decrease in serum testosterone level of rats treated with the extract compared to that of the control is an indication that the extract could affect spermatogenesis as testosterone is necessary for normal development of the cells of the spermatogenic lineage (21). The steroid and Flavonoid contents of the extract might be the cause of decrease in serum testosterone levels as steroids are reported to decrease testosterone levels (22). Flavonoids are also reported to decrease plasma testosterone levels in rodents (23, 24). There is reported malformation of male reproductive system in infants of rats exposed to flavonoids during gestation (25). The decrease in testosterone level might be the cause of decrease sperm count, motility and morphology because testosterone is the principal hormone responsible for development of the spermatogonial stem cells to mature sperm cells (20, 26). The significant increase in seminiferous tubular diameter of rats treated with the extract at 250 mg/kg and 1000 mg/kg, compared to that of the control might be as a result of inflammation of the spermatogenic cells. The destruction of the basement membrane might be the cause of increased size, as membranes control the passage of substances/fluid in and out of the cell (20). The decrease in epididymal epithelial thickness of rats treated with *P. dactylifera* extract compared to that of the control showed that the extract might have potentials of destroying epididymal epithelium. The decrease in size of interstices might be the cause of decrease serum testosterone as interstitial cells of Leydig’s primary function is testosterone Production (20). The compaction of interstices and destruction of Leydig cells might be the cause of decrease serum testosterone as impaired/damaged Leydig cells could affect steroidogenesis (testosterone production) leading to scanty and abnormal sperm cells in the lumen of seminiferous tubules and distortion of spermatogenic cells (26-28) which were noticed in the rats treated with the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg as compared to that of the control rats. The destruction of basement membrane showed that the extract may affect membrane of some cells, saponin content of the extract might be the cause of basement membrane destruction as saponins are reported to affect cell membranes by forming pores, and they also exert a lytic action on erythrocytes membranes (29, 30). Administration of aqueous extract of *P. dactylifera* at 250 mg/kg, 500 mg/kg and 1000 mg/kg to male Wistar rats might affect fertility by degeneration of Leydig cells and distortion of spermatogenic cells and decrease in sperm count, sperm motility, sperm morphology and serum testosterone levels. This might be the cause of declining fertility in Middle East and North Africa as reported by (31, 32) where date palm is a key food source (33).

**Conclusion**

From this study, it can be concluded that administration of aqueous extract of *P. dactylifera* at 250 mg/kg, 500 mg/kg and 1000 mg/kg to male Wistar rats for 35 days might affect fertility by decreasing serum testosterone levels, sperm count, sperm motility and sperm morphology, distortion of spermatogenic cells and degeneration of Leydig cells. Therefore, caution should be taken in the consumption of *P. dactylifera* fruit even though it has numerous therapeutic effects.

**Acknowledgement**

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