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Original Research Article

Physiological and Histopathological Effects of Two Seed Extracts (*Phoenix dactylifera* and *Raphanus sativus*) and L-Carnitine Drug on Semen Quality and Testicular Sexual Hormonal Changes in Adult Male Rabbits

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ABSTRACT

Date palm (*Phoenix dactylifera*) pollen (DPP) is widely used in traditional medicine to treat male infertility. Radish (*Raphanus sativus*) is an edible root vegetable that was domesticated in Europe in pre-Roman times. This study was aimed at comparing the effects of DPP and Radish seed extracts, as well as L-Carnitine drug on the quality of sperm and sex hormone parameters in adult male rabbits. A total of 30 male rabbits were divided into five groups. Each group was administered with: normal saline (Control 1; Group I), DPP (Group II), L-Carnitine (Group III), Petroleum ether (Control 2; Group IV), or Radish seed oil extract (Group V) every day for eight weeks. Semen quality, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were measured at the end of the experiment. Also, histopathological examinations on testicles were performed. The results showed that there was significant increase in activity, total and normal sperm counts in Group II compared to Groups I and III. Meanwhile, a significant increase in activity, total and normal sperm counts was observed in Groups V compared to IV. The hormonal levels of FSH, LH, and testosterone were significantly higher in Groups II and III compared to Group I, but significantly lower in Groups V compared to IV. The findings of this study revealed that date palm pollen suspension seems to improve sperm quality, enhance fertility in male adult rabbits compared to the Radish seed oil extract and L-Carnitine drug, therefore, it may be useful in solving infertility problems.

***Keywords*:** Date palm pollen, Fertility, L-Carnitine, Motility, Radish seed, Sperm.

Introduction

Extract of date palm (*Phoenix dactylifera*) pollen (DPP) is a herbal mixture that is widely used as a folk remedy for curing male infertility in traditional medicine. It was also used in the treatment of many other diseases by the ancient Egyptians and Chinese. DPP has been reported to have antibacterial,[2] antifungal[3], antioxidant,[3,4] anti-inflammatory,[6,7] and hepatoprotective activities.[8] Also, it contains gonadotropin stimulating substances and steroid precursors,[9,10] both of which may increase testosterone production. As a result, DPP was added to the animal mash to boost growth and an increase in plasma testosterone levels was observed.[11] *Raphanus sativus* extract protects against zearalenone-induced testicular toxicity which might be due to its ability to inhibit the oxidative process. This is achieved by counteracting reactive oxygen species as well as its interaction with estrogen receptors that are occupied by the mycotoxin zearalenone.[12] Rabbit fertility and immunity have been observed to be improved by *R. sativus*.[13,14] discovered that Radish seed increases sperm motility, decreases abnormal sperm concentration, and increases live sperm concentration when compared to rocket and black cumin's fertility-enhancing activity. Furthermore, the Radish seeds contain a high percentage of oil.[14] L-Carnitine is an important amino acid and also an essential co-factor for fatty acid metabolism. It plays an important role in the generation of metabolic energy by facilitating the transport of fatty acids into the mitochondria. L-Carnitine is found at high concentrations in mammalian epididymides and sperm. The epididymal epithelium and sperm generate energy from L-Carnitine present in the epididymal fluid.[15] The aim of this study was to compare the effects of date palm pollen and Radish seed extracts, as well as L-Carnitine drug on semen quality and testicular sexual hormonal changes in adult male rabbits.

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Materials and Methods

Preparation of herbal cocktails

The DPP and Radish seeds were collected from Salah El dine, Iraq in August, 2018. authenticated, and deposited in the Plant Protection Research Institute in the Zagazig university with voucher numbers A312 DPP and RS111A for DPP and Radish seeds, respectively.

The plant seeds were documented by the botanist, Prof. Dr. Samir Salem Talab Department of Botany, Faculty of Science, Zagazig University, Sharkia, Egypt.

Extraction of date palm pollens

Using the maceration method, 200 g of the powdered plant material (DPP) were extracted with (800 mL) water for 24 hours. The macerates were then filtered through filter paper (Whatman) in a Buchner funnel. The filtered solution was evaporated in a rotary

evaporator under vacuum at 45°C. The dry extract and stock solutions were stored at +4°C until further analysis.[16]

Extraction of Radish seed oil
For the Soxhlet extraction, 10 g sample of radish seeds were weighed and placed in an extraction thimble, using 300 mL petroleum ether. Oil was extracted continuously for 8 h at 60-80°C.[17] After extraction, the solvent was evaporated and the extract was dried at 103°C to remove residual solvent, cooled for 30 min in a desiccator and weighed. All determinations were done in triplicate. Remaining pomade was again subjected to Soxhlet for further extraction process. For oil extraction the dried radish seeds grounded & converted into fine powder using an electric grinder, which proper fixed cutter/chopper for solvent extraction, 150 g of ground seeds placed into a cellulose paper cone and extracted using light petroleum ether in a 5 L Soxhlet extractor for 8 h.[18] Radish oil was then recovered by evaporating off the solvent using rotary evaporator and residual solvent removed by drying in an oven at 60°C for 1 h. Residual moisture in the oil was removed by gentle heating.[17,18] The oil obtained from both extractions was stored at 2°C until it was analyzed.

Source of experimental animal
Adult male rabbits of New Zealand White (NZW) species were obtained from the Faculty of Agriculture, Zagazig University. They were housed in standard conditions of temperature (22 ± 2), relative humidity (55 ± 5%), and photoperiod of 12h light-dark cycles. The animals were fed with a standard pellet diet and water *ad libitum*.

Ethical approval
The study protocol was approved by the Institutional Animal Ethics Committee according to the regulation of the Committee for Control and Supervision of Experiments on Animals (Protocol No.: 902, dated: 26-09-2009). The experiment was conducted following an internationally accepted standard guideline for the use of animals.

Animal grouping and treatment
A total number of 30 male New Zealand rabbits, weighing 2.5 – 3.0 kg were divided into five groups (6 rabbits in each group):
Group I: Control 1 (Normal Saline Control), was administered 10 mL normal saline.
Group II: (DPP group), was administered 120 mg/kg (10 mL of DPP).[19]
Group III: (L-Carnitine group), was administered with 350 mg/kg of L-Carnitine (dissolved in 5 mL distilled water).[20]
Group IV: Control 2 (Petroleum ether Control), were given Petroleum ether (397.5 µL/kg).
Group V: (Extract of Radish seed oil group), were given 397.5 µL/kg of extract.[21] In all the groups, treatments were administered orally for 60 days on daily basis.

Semen evaluation
Adult male rabbit semen samples were collected for 8 weeks on an artificial vagina at a modified inner temperature of 42-45°C, Total sperm counts, sperm activity, normal sperm counts were measured.[22]

Blood collection and biochemical analyses
Blood samples were collected into clean sterilized plain tubes from the ear vein on the last day of the experiment. After blood clotting, serum was separated from the blood by centrifugation at 4000 rpm for 15-20 minutes and stored at -20 C° until the tests were carried out.

Measurement of hormones
On the final day of the experiment (after two months of continuous doses of adult male rabbits), testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured. The DRG International, Inc., USA Kit was used to determine testosterone level, and the ELISA (Enzyme-Linked Immunosorbent Assay) technique was used to determine FSH and LH levels according to Finley and Tietz.[23] The kit was obtained from Fortress Diagnostic Limited in the United Kingdom and Northern Ireland.

Histopathological examination
The formalin-preserved testis specimens were prepared in an automated tissue processor. The processing consisted of an initial 2 step fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70, 90, and 100%). The tissue was initially exposed to 70 % alcohol for 120 minutes followed by 90 % alcohol for another 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hours. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 µm) were stained with hematoxylin and eosin.[24] Stained sections were examined for circulatory disturbances, inflammation, degenerations, apoptosis, necrosis, and any other pathological changes in the examined tissues.

Statistical analysis
All statistical analyses were done by the Statistical Package for the Social Sciences (v14.0 for Microsoft Windows, SPSS Inc.). Numerical data were expressed as mean ± SD. Values were considered statistically significant at a two-sided $P < 0.05$. The levels of markers were analyzed by ANOVA, while the Mann-Whitney U-test was used for comparisons between independent groups.[19]

Results and Discussion

There was a significant ($p < 0.001$) increase in the total sperm count (TSC) in the DPP, control 1 and L-Carnitine groups with values of 225.0 ± 4.5 x 10^6, 225.3 ± 7.4 x 10^6, and 255.5 ± 5.9 x 10^6, respectively as shown in Table 1. On the other hand, a significant ($P<0.001$) decrease in the volume of Ejaculation (mL) was observed in Group IV in the eighth week compared to Group V (201.6 ± 6.1 x 10^6). Since ancient times, date palm has been used as a herbal remedy with no scientific basis. This animal model was created to test the efficacy of palm pollen suspension on male infertility. The results indicated that the effects of palm pollen grain extract were significantly increased throughout the experimental period (Table 1). Also, it was observed that the total sperm cell counts were higher in DPP group in week 8 than in Control 1, showing the presence of estrogen-like hormones in the pollen grains.[26] Proteins, sugars, ions, and small organic molecules work together to control sperm motility. It is one of the primary factors that facilitate the sperm-to-egg journey and the subsequent process of fertilization.[27] The antioxidant properties of date extract, which can prevent the unnecessary generation of free radicals in the sperm cells, could be attributed to the improvement in sperm characteristics, including motility and abnormality. These observations are in support of the previous findings of improved sperm motility in connection with a significant reduction in sperm abnormality without affecting sperm livability.[28] Palm pollen is an effective material for improving sperm quality and male fertility because it contains vitamins A, E, and C, as well as minerals such as zinc, selenium, iron, copper, and cobalt.[29]
Table 2 shows the results of the % activity of semen fluid in all the study groups. A significant ($P<0.001$) increase in Group 2, DPP (91.0 ± 2.6) was observed compared to Control 1 (90.3±2.6) and L-Carnitine (89.1±2.6) groups. However, the effect of petroleum ether was observed as there was a significant ($P<0.001$) decrease in % activity of semen fluid (25±4.472) from the first to the eighth week compared to Group V (the Radish seed oil group) which showed a significant ($P<0.001$) increase in % activity of semen fluid (97.1±1.471) in the eighth week. There was a significant ($P<0.001$) increase in the normal sperm counts in the DPP, Control 1, and L-Carnitine groups with values of 95.0 ± 0.9, 96.0 ± 0.9, and 94.5 ± 1.2, respectively as highlighted in Table 3. Meanwhile, a significant ($P<0.001$) increase in normal sperm count was noticed in the Radish seed oil group (93.6 ± 1.6) compared to the Control 2 group (28.3 ± 2.6).
Studies have shown that L-Carnitine increases sperm parameters via androgenic activity, as evidenced by increased stimulation of spermatogenic activity. Jacyno also reported increased sperm counts in the ejaculate of rabbits administered with L-Carnitine.[30]

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Table 1: Total sperm counts ($x10^6$/mL) in semen fluid of experimental adult male rabbits

Week / Group	Control 1	D.P.P	L-Carnitine	Control 2	Oil
Week 1 / $\overline{X}$ ±SD	180±0.894[b]	194±1.673[b]	194±8.124[a]	177.6±8.310[a]	169.3±4.926[a]
% Change		8%	8%		-5 %
Week 2 / $\overline{X}$ ±SD	185.6±1.861[b]	202.2±6.337[b]	201.3±11.673[a]	174±8.809[a]	174.1±2.994[a]
% Change		9%	8%		0.05%
Week 3 / $\overline{X}$ ±SD	194.6±4.033[b]	220.5±5.958[b]	218.3±13.662[a]	153.3±8.115[a]	178.6±3.265[b]
% Change		13%	12%		17%
Week 4 / $\overline{X}$ ±SD	196.6±2.581[b]	221.6±5.163[b]	224.16±11.673[a]	136.3±8.809[a]	182.6±2.994[b]
% Change		13%	14%		34%
Week 5 / $\overline{X}$ ±SD	206.6±6.831[b]	225.3±4.273[b]	231.5±9.027[a]	121.6±6.831[a]	184.6±5.428[b]
% Change		95	12%		52%
Week 6 / $\overline{X}$ ±SD	215±4.472[a]	232±4.020[a]	249±7.359[a]	112.3±10.053[a]	189.1±3.970[b]
% Change		8%	16%		68%
Week 7 / $\overline{X}$ ±SD	220±4.472[a]	244±6.186[a]	252±5.163[a]	95±3.898[a]	194±4.119[b]
% Change		11%	15%		104%
Week 8 / $\overline{X}$ ±SD	225±4.472[b]	225.3±7.393[b]	255.5±5.890[a]	88±2.683[a]	201.6±6.055[a]
% Change		0.1%	14%		129%

Values having different letters are significantly different ($P \leq 0.001$). Letter "a" represented the highest value, followed by letter "b". *
*Control 1: Normal saline, * DPP: Date Palm Pollen, * Drug: L_Carnitine, * Control 2: Petroleum ether, * Oil: Seeds of radish oil*

Table 2: Sperm activity (%) of semen fluid of experimental adult male rabbits

Week / Group	Control 1	D.P.P	L-Carnitine	Control 2	Oil
Week 1 / $\overline{X}$ ±SD	58.3±5.163[a]	64.1±3.763[a]	63.3±5.715[a]	46.6±2.581[a]	65.8±5.845[b]
% Change		10%	9%		41 %
Week 2 / $\overline{X}$ ±SD	70±4.472[b]	73±5.099[b]	81.1±4.622[a]	45±4.472[a]	68.6±5.853[b]
% Change		4%	16%		52%
Week 3 / $\overline{X}$ ±SD	78.3±2.581[b]	81.915±2.562[b]	86.6±4.082[a]	43.7±1.366[a]	73.2±6.047[b]
% Change		5%	11%		68%
Week 4 / $\overline{X}$ ±SD	81±1.549[b]	83±4.690[b]	88.1±3.125[a]	38.3±2.581[a]	74.3±8.334[b]
% Change		2%	9%		94%
Week 5 / $\overline{X}$ ±SD	82±5.440[a]	84.1±3.430[a]	87.1±4.020[a]	35±4.472[a]	76.3±7.865[b]
% Change		3%	6%		118%
Week 6 / $\overline{X}$ ±SD	86.6±2.581[a]	86.3±3.141[a]	89.1±2.041[a]	35±4.636[b]	82.5±0[a]
% Change		0.3%	3%		136%
Week 7 / $\overline{X}$ ±SD	88.3±2.581[a]	88.8±2.562[a]	89.1±2.041[a]	28.3±2.581[a]	85.5±2.345[b]
% Change		0.5%	1%		202%
Week 8 / $\overline{X}$ ±SD	90.3±2.581[a]	91±2.562[a]	89.1±2.639[a]	25±4.472[a]	97.1±1.471[b]
% Change		1%	-1%		288.4%

Values having different letters are significantly different ($P \leq 0.001$). Letter "a" represented the highest value, followed by letter "b".
** Control 1: Normal saline, * DPP: Date Palm Pollen, * Drug: L_Carnitine, * Control 2: Petroleum ether, * Oil: Seeds of radish oil*

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Table 3: Normal sperm count (%) of semen fluid in experimental adult male rabbits

Week / Group	Control 1	D.P.P	L-Carnitine	Control 2	Oil
Week 1 / $\overline{X}$ ±SD	75.6±3.614 [b]	86.6±2.065 [b]	76.3±8.164 [a]	62.5±2.581 [a]	77.6±4.412 [b]
% Change		15%	0.9%		24 %
Week 2 / $\overline{X}$ ±SD	79.3±4.589 [b]	90.6±3.326 [b]	85.8±7.194 [a]	26.5±8.273 [a]	41.85±12.695 [b]
% Change		14%	8%		58%
Week 3 / $\overline{X}$ ±SD	85±4.472 [a]	94.1±2.041 [b]	87.2±4.021 [a]	58.3±2.581 [a]	87±3.464 [b]
% Change		11%	3%		49%
Week 4 / $\overline{X}$ ±SD	87.6±3.141 [a]	92.2±2.714 [b]	89.2±4.401 [a]	49±3.224 [a]	87.1±2.786 [b]
% Change		5%	2%		78%
Week 5 / $\overline{X}$ ±SD	91±2.366 [a]	92.6±2.065 [a]	92±3.834 [a]	46.6±2.788 [a]	91.3±2.503 [b]
% Change		2%	1%		96%
Week 6 / $\overline{X}$ ±SD	94±1.549 [a]	92.8±2.483 [a]	94.1±2.041 [a]	43.3±2.581 [a]	92.3±2.065 [b]
% Change		-1%	0.1%		113%
Week 7 / $\overline{X}$ ±SD	95±0 [a]	94.8±0.752 [a]	93±2.449 [b]	33.3±2.581 [a]	94.8±2.136 [b]
% Change		-0.2%	-2%		176%
Week 8 / $\overline{X}$ ±SD	95±0.894 [a]	96±0.894 [a]	94.5±1.22 [b]	28.3±2.581 [a]	93.6±1.632 [b]
% Change		1%	-0.5%		231%

Values having different letters are significantly different ($P \leq 0.001$). Letter "a" represented the highest value, followed by letter "b".
** Control 1: Normal saline, * DPP: Date Palm Pollen, * Drug: L_Carnitine, * Control 2: Petroleum ether, * Oil: Seeds of radish oil*

Increased sperm count in ejaculates is most likely due to the supplement leading to increased spermatozoa survival in the epididymis, rather than L-Carnitine enhancing spermatogenesis. In the mitochondria, LC is involved in the conversion of acetyl-CoA to acetyl carnitine. This prevents acetyl groups from accumulating and inhibiting the activity of pyruvate dehydrogenase, which is responsible for mitochondrial energy metabolism.[31] The function of L-Carnitine improves sperm survival and thus increases the total number of sperm cells in the ejaculate.[32,33] Semen evaluation is the collection of a sperm sample for analysis, total sperm count, sperm activity, and normal morphology of sperm. On the other hand, indicated an important tool for determining the effects of Radish seed extract, as well as the petroleum ether (Control 2) that was used to extract the Radish seed oil on the semen quality. The results (Tables 1-3) from the present study revealed that the oil group outperformed the Control group in most of the total sperm counts. The oil of Radish is free of any substance used to extract it, as demonstrated by the effect of petroleum ether, which was used in Control 2 that resulted in a decrease in total sperm counts, activity, as well as an increase in abnormal sperm. On the other hand, the oil group exhibited a normal result in all tests after 8 weeks, with an increase in semen quality to the best limit. According to Zaman,[34] the Radish group produced the fewest free radicals, as evidenced by the lowest concentrations of malondialdehyde and reactive nitrogen species, particularly the powerful oxidant (ONOO-) molecule of peroxynitrite. Furthermore, due to the presence of white cells in sperm, the production of reactive species is normal within certain limits because of cell activity. As a result of the presence of a powerful antioxidant in Radish, it may be reasonable to reduce the production of these later products,[35] that control the production of reactive species. These findings are in agreement with Magda's investigation.[36] Antioxidants and other stimulating compounds found in the seeds may help to increase sperm qualities.

There was a significant increase ($P<0.001$) in FSH levels in the L-Carnitine, DPP, and Control 1 groups (11.6 1.1, 10.3 1.3, and 5.5 0.5 ng/dL, respectively) compared to the Control 2 group. The Radish seed oil group increased significantly ($P<0.001$) (7.7 0.4 ,3.6 0.6 ng/dL) during the experimental period. On the other hand, a significant ($P<0.001$) increase in LH level was observed in the DPP, L-Carnitine, and Control 1 groups with values of 6.50.529, 5.60.374, and 3.50.322 mIU/mL, respectively. There was an increase in LH level in the Radish seed oil group compared to Control 2 group (5.4 0.7 and 2.7 0.2 mIU/mL, respectively) during the experimental period. This study revealed a significant ($P<0.001$) increase in testosterone level of the adult male rabbits in the DPP (16.3 1.5 ng/mL), L-Carnitine (12.5 1.8 ng/mL), and Control 1 (9.6 0.4 ng/mL) groups. The current study showed no significant increase ($P<0.001$) in testosterone level in the Radish seed oil group (8.41.229 ng/mL) during the experimental period compared to the Control 2 group (4.11.2 ng/mL). According to the observation in this study, date palm extract affected sexual behavior and libido by decreasing reaction time due to its androgenic effects (increase in plasma level of testosterone, FSH, and LH). This result is consistent with the findings of Bahmanpour and Mil,[19,37] who confirmed that erection and sexual behavior are primarily influenced by androgen, which regulates the ability of penile erectile reaction. As a result, aqueous date extract may improve male libido by increasing serum testosterone concentrations. The use of date palm extract increased testosterone, FSH, and LH levels in the blood compared to the control group. This finding is similar to that of Kostyu,[38] who found that taking date palm increased plasma testosterone levels. Date extract increased follicle-stimulating hormone, luteinizing hormone, and testosterone levels, according to the findings of Zarga.[39] Table 4 revealed that there was a 30% increase in the level of L-Carnitine compared to the Control 1 group. L-Carnitine plays an important role in increasing testosterone and other steroidal hormone levels such as FSH and LH in the bloodstream. This is due to fatty acid transport to the mitochondria, where they undergo ß- oxidation, resulting in the generation of metabolic energy in the form of adenosine triphosphate, which the cells require to function. The cytoplasm of Leydig cells contains a lot of smooth endoplasmic reticulum, Golgi complexes, and mitochondria.[40] In a study conducted by Austin and Short, it was revealed that the smooth endoplasmic reticulum and mitochondria of interstitial cells contain the majority of the enzymes involved in testosterone synthesis.[41] The L-Carnitine system performs two major functions: firstly, it facilitates the transport of long-chain fatty acids into mitochondria for use in energy-generating processes; and secondly, it facilitates the removal of short-chain and medium-chain fatty acids that accumulate as a result of normal and abnormal metabolism.[42]

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Table 4: Hormonal changes in experimental adult male rabbits

Test/ Group	Control 1	D.P.P	L-Carnitine	Control 2	Oil
FSH (ng/dL)					
$\overline{X}$ ±SD	5.5 ± 0.536 [c]	11.6 ± 1.108 [b]	10.3 ± 1.265 [a]	3.6 ± 0.558 [a]	7.7 ± 0.404 [a]
% Change		111%	87%		114%
LH (mIU/mL)					
$\overline{X}$ ±SD	3.5 ± 0.322 [c]	6.5 ± 0.529 [a]	5.6 ± 0.374 [b]	2.766 ± 0.225 [a]	5.366 ± 0.677 [b]
% Change		86%	60%		94%
Testosterone (ng/mL)					
$\overline{X}$ ± SD	9.6 ± 0.403 [c]	16.3 ± 1.499 [a]	12.5 ± 1.796 [b]	4.1 ± 1.173 [a]	8.4 ± 1.229 [b]
% Change		70%	30%		105%

Values having different letters are significantly different (P ≤ 0.001). Letter "a" represented the highest value, followed by letter "b" and "c".
** Control 1: Normal saline, * DPP: Date Palm Pollen, * Drug: L_Carnitine, * Control 2: Petroleum ether, * Oil: Seeds of radish oil*

Babu *et al* made a similar observation in their investigation, reporting a rise in mean FSH and LH levels in all fertile males studied, with significant differences (p<0.05). However, the difference in mean testosterone levels between the two arms was insignificant.[43] The Radish seed oil extract affected sexual behavior with significant differences (P <0.001) in plasma levels of testosterone, FSH, and LH compared to the Control 2 group as revealed by Table 4.

Histopathological examination of testes

The observations made for the histopathological examinations of testes are presented in Figures 1-5. In the Control 1 group, the examined sections of the testes showed normal testicular tissue with preserved seminiferous tubules, sertoli cells, ledying cells, interstitial, and vascular structures (Figure 1). It was observed in Figure 2 that in date palm pollen water extract, most of the testicular tissues showed degenerative and apoptotic changes with the partial or complete arrest of spermatogenesis. A few tubules were normal and functional. In the L-Carnitine group, most of the testicular structures were normal with preserved histomorphology of all cellular contents. A few tubules revealed mild interstitial edema and degenerative changes as illustrated in Figure 3. However, sections from testes in the Control 2 group revealed interstitial edema, partial atrophy, and degenerative changes in the seminiferous tubules with the arrest of spermatogenesis in some tubules (Figure 4). Furthermore, sections from the testes of the oil group indicated (Figure 5) normal testicular tissue in most parts, however, some parts showed interstitial edema and focal degeneration of the cellular contents of the seminiferous tubules.

The testicular tubules demonstrated increased active spermatogenesis, with a significant increase in matured sperms and a significant decrease in tubular dysfunction. This could be due to a large increase in testosterone levels in the blood caused by the DPP.[38] This explanation agrees with the current finding, which showed that testosterone levels in the sera of all the male rabbits treated with DPP were significantly higher. Increases in testosterone serum levels resulted in increased active spermatogenesis, with a significant increase in some matured sperms.[44,45] The current study found that the response to the LC treatment was detectable 8 weeks after the commencement of the experiment. LC administration was continued from the beginning to the end, which is thought to be sufficient for investigating the role of LC in the rooster's spermatogenesis process. It was found that LC has a positive effect on spermatozoa quality by increasing seminal enzymatic activity, which effectively protects the structures and functions of spermatozoa from oxidative stress. After a period of treatment, the addition of LC improved sperm quality parameters (motility, viability, and concentration), acrosomal abnormality, plasma membrane integrity, and male fertility.[46-48]

Histological examination of testes cross-sections from rabbits exposed to Radish seed, flaxseed, and Control 2 revealed an increase in diameter of seminiferous tubules as well as improved spermatogenesis compared to the oil group. Mehraban,[44] found that Radish seed oil and flaxseed increased the diameter of seminiferous tubules and promote spermatogenesis, as observed in this study.

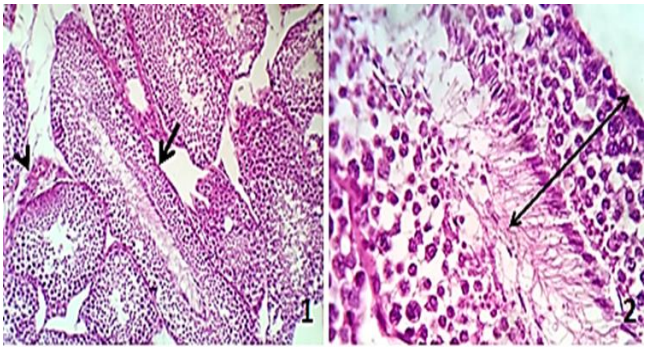

Figure 1: Photomicrograph of testes showing normal testicular tissue with preserved seminiferous tubules (open arrow), Sertoli cells, ledying cells (arrowhead), and normal spermatogenesis (double-headed arrow). H&E X 100(1), 400 (2)

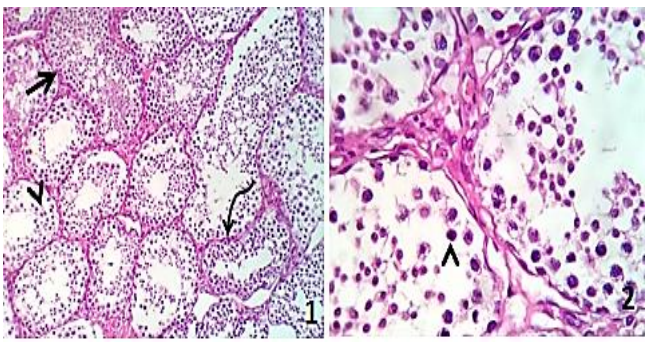

Figure 2: Photomicrograph of testes showing degenerative and apoptotic changes (arrow heads) with the partial or complete arrest of spermatogenesis (curved arrow) with normal and functional rest of tubules (open arrow). H&E X 100 (1), 400 (2).

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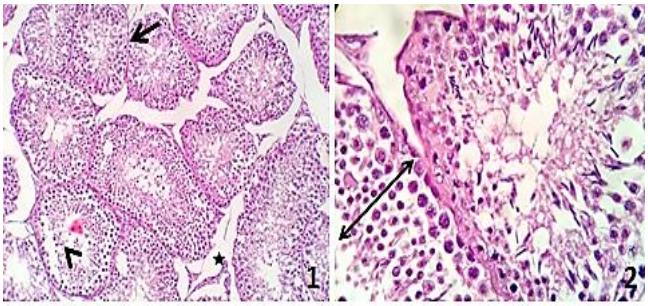

Figure 3: Photomicrograph of testes showing normal spermatogenesis (open arrow) with preserved histomorphology of all cellular contents (double arrowhead), a few tubules showing mild interstitial edema (star) and degenerative changes (arrow head). H&E X 100 (1), 400 (2).

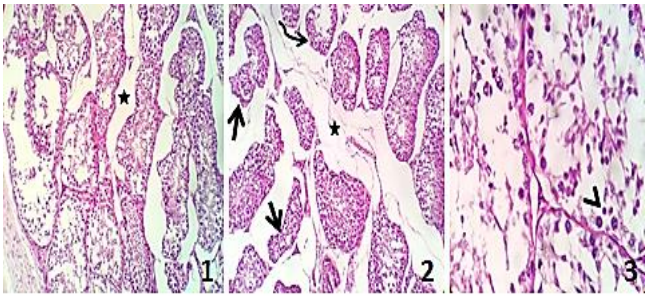

Figure 4: Photomicrograph of testes showing interstitial edema (stars), partial atrophy (open arrows), degenerative changes (arrow head) in the seminiferous tubules with the arrest of spermatogenesis (curved arrow) in other tubules. H&E X 100 (1,2), 400 (3).

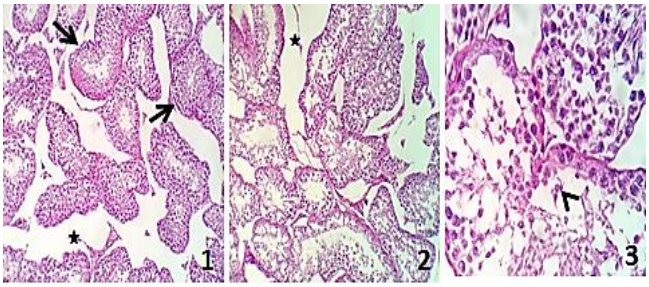

Figure 5: Photomicrograph of testes showing normal testicular tissue (open arrows) in most parts with interstitial edema (stars) and focal degeneration of the cellular contents (arrow head) in other seminiferous tubules. H&E X 100 (1,2), 400 (3).

Conclusion

Date Palm pollen suspension seems to improve sperm quality, enhance fertility in male adult rabbits than L- Carnitine and seeds of radish oil. Therefore, it may be useful to solve infertility problems.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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