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The Protective Role of Date Palm Pollen Suspension on Carbon Tetrachloride (CCl4)-induced Reproductive Toxicity in Male Rats

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Abstract:

Date palm pollen (DPP) is used in traditional medicine and as a dietary supplement. The present study was carried out to investigate the effect of date palm pollen suspension against the toxic effects of carbon tetrachloride (CCL4) on some aspects of reproductive performance in adult male rats. Twenty adult male rats were randomly divided into four equal groups (5 rats/group) and were treated for 63 days as follows: the first group was considered a control group, the second group received date palm pollen suspension orally (0.15 gm/kg B.W) once a day, the third group were treated intraperitoneal (IP) with 0.5 gm/kg B.W. of CCL4 mixing with an equal volume of olive oil twice a week, the fourth group was received date palm pollen suspension (0.15 /kg B.W) once a day orally and treated IP with 0.5 gm/kg B.W. of CCL4 mixing with an equal volume of olive oil twice a week. The result showed that daily oral administration of DPP caused a significant $p<0.05$ increase in serum testosterone concentration improved semen analysis and an elevated number of sertoli cells, leydig cells and diameter of seminiferous tubules. While the group treated with CCL4 showed a decrease significantly $p<0.05$ in testosterone, semen analysis and histological measurements. While, the result showed that treatment animals treated with oral administration of DPP in group T3 restore change in testosterone hormones concentration and reproductive parameters which induce by CCL4 to towards normalization. It is therefore concluded that oral administration of DPP promotes reproductive parameters and prevents CCL4 induced reproductive toxicity.

Keywords: Date palm pollen; Reproductive parameters; CCL4; Male rat.

الدور الوقائي لغبار طلع النخيل ضد السمية التكاثرية الناجمة عن رابع كلوريد الكربون (CCl4) في ذكور الجرذان

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الخلاصة

يستخدم لقاح نخيل التمر (DPP) في الطب التقليدي وكمكمل غذائي. أجريت هذه الدراسة لمعرفة تأثير معلق طلع نخيل التمر ضد التأثيرات السامة لرابع كلوريد الكربون (CCL4) على بعض جوانب الكفاءة التناسلي في ذكور الجرذان البالغة. تم تقسيم عشرين جرذاً ذكراً بالغاً عشوائياً إلى أربع مجموعات متساوية (5 فئران/مجموعة) وعولجت لمدة 63 يوماً على النحو التالي: اعتبرت المجموعة الأولى مجموعة سيطرة، وتلقت المجموعة الثانية معلق حبوب لقاح نخيل التمر عن طريق الفم (0.15 جم/كجم من وزن الجسم).) مرة واحدة يومياً، بينما المجموعة الثالثة عولجت عن طريق الحقن في الصفاق (IP) بجرعة 0.5 غم/كغم من وزن الجسم. عن طريق خلط رباعي كلوريد الكاربون مع كمية متساوية من زيت الزيتون مرتين في الأسبوع، فيما تلقت المجموعة الرابعة معلق طلع نخيل التمر (0.15 / كغم من وزن الجسم) مرة واحدة يومياً عن طريق الفم وحقن رباعي كلوريد الكاربون عن طريق الصفاق بـ 0.5 جم / كجم من وزن الجسم مع خلط كمية متساوية من زيت الزيتون مرتين في الأسبوع. أظهرت النتيجة أن تناول غبار طلع النخيل يوميًا عن طريق الفم زيادة ملحوظة <0.05 في تركيز هرمون التستوستيرون في الدم مما أدى إلى تحسين معايير السائل المنوي وزيادة عدد خلايا سيرتولي وخلايا ليديغ وقطر الأنابيب المنوية. بينما أظهرت نتائج المجموعة المعاملة بـ CCL4 انخفاضاً معنوياً $p<0.05$ في هرمون التستوستيرون وتحليل السائل المنوي والقياسات النسيجية. في حين أظهرت نتائج المجموعة الثالثة المعالجة بغبار طلع النخيل DPP استعادت التغير في تركيز هرمونات التستوستيرون معايير الكفاءة التناسلية التي تسبب بها رباعي كلوريد الكاربون نحو القيم الطبيعية . لذلك نستنتج أن تناول DPP عن طريق الفم يعزز معايير الكفاءة التناسلية ويمنع السمية التكاثرية الناجمة عن رباعي كلوريد الكاربون

1. Introduction

Phoenix dactylifera L. family Palmae is known in Arabic as Nakl; in English as Date palm, in French as Dattier [1]. The palm family is a symbol of prosperity and love to Muslims and its legend dates back to Judeo- Christian mythology [2] Date palms *(Phoenix dactylifera* L., *Arecaceae)* are one of the oldest cultivated plants which are used in folk medicine for the treatment of various diseases [3]. Iraq is characterized by the presence of a great number of palms abundantly distributed all over the country. Palms in general possess many economic uses [4].

Pollen grains carry the male genetic material, by a variety of means, for gametogenesis in the plant kingdom. Pollen applications in the rites and its uses in traditional and herbal medicine have been recorded throughout history. The early Egyptians and ancient Chinese used date palm pollen grains as a rejuvenating medicinal agent, it was called "Fountain of Youth". Pollen preparations are distributed wide world for dietary purposes and used as dietary supplements by increasing the total dietary intake [5]. In local medicinal practices, date palms are considered a tonic. Some consider it to be an aphrodisiac. The palm flowers are used as a purgative [6].

Date palm pollen (DPP) has been used in the treatment of sexual incapacity and weakness in some Arabic country[7]. The date palm pollen grains showed the presence of estradiol, esteriol, estrone flavonoids, alpha-amirin, triterpenoids, saponins and a crude gonadotrophic substance [8, 9]. The consumption of DPP suspensions improved the sperm count, motility, morphology, and DNA quality with a concomitant increase in weights of testis and epididymis [10].

Carbon tetrachloride (CCl4) is a haloalkane used in a variety of industrial and chemical applications. It has been widely used for its solvent properties, as an intermediate in the synthesis of chlorofluorocarbons. It is an organic compound whose chemical formula is CCl_4. CCl_4 is strong toxic agent [11]. It has long been that CCL4 is known to be an effective drug against adult liver flukes and its efficiency against immature fluke's increases as the dose rate increased. It is well known that carbon tetrachloride induces fatty liver and cell necrosis in a variety of Mammalian species [12].

CCL4 leads to generation of free radicals caused cell injury and apoptosis to cells**.** Carbon tetrachloride is an important model agent to study the pathogenesis of liver injury[13] . It is known to be hepatotoxic as well as nephrotoxic and haematotoxicity to experimental animals [14]. It also causes spermatozoa damage, oxidative stress and testicular apoptosis in experimental animals [15]. So the present study investigated the protective role of DPP suspension to prevention or suppression the toxic effects of CCL4 on some aspects of reproductive performance in adult male rats.

2. Material and Methods

2.1. Preparation of date palm pollen suspension:

Date palm pollen was purchased from the local market in Baghdad city. The water suspension was prepared as 2ml of suspension contains 150 mg of date palm pollen (75 mg/ml) and each animal of treated group received 150 mg/Kg from weight of animal.

2.2. Experimental animals

Forty male albino rats aged 12-14 weeks, weighed 260-300 gm was used. Rats were housed in an animal house of the departments of animal resources in college of Agriculture / university of Sumer. Animals were fed on the pellet of freshly prepared ration and animals were allowed for free access to water along the experimental period.

2.3. Experimental design

The animals (male rats) were divided randomly into (4 groups) 5 animals per group & handled as follows: Control group: 5 Rats treated orally with distal water daily via Gavage needle and olive oil 0.5ml/kg B.W. intraperitonally twice a week for nine weeks. Second group DPP: 5 Rats treated orally with 0.15 gm/kg B.W. of DPP suspension daily via Gavage needle and olive oil 0.5ml/kg B.W. intraperitonally twice a week for nine weeks [10]. Third group CCL4: 5 Rats treated intraperitonally with 0.5 gm/kg B.W. of CCL4 and mixed with an equal volume of olive oil 0.5 ml/kg B.W. twice a week for nine weeks [16]. Fourth group DPP&CCL4: 5 Rats treated orally with 0.15 gm/kg B.W. from DPP suspension via Gavage needle and intraperitonally with 0.5 gm/kg B.W. of CCL4 mixed with an equal volume of olive oil 0.5 ml/kg B.W. twice a week for nine weeks.

2.4. Collection of Samples

After nine weeks of treatment, the animals were sacrificed under anesthetized by intramuscular injection of ketamine 90 mg/Kg B.W & Xylazine 10 mg/Kg B.W. Blood samples were collected by cardiac puncture, 5 ml of blood samples were collected from the heart, allowed to clot for 30 min and centrifuged (3,000×g, 15 min), was stored at − 80 °C for hormonal assays. The left tail of the epididymis was excised and used to determination of sperm concentration, viability, motility and morphology. Testis was fixed in formalin solution for histological examination.

2.5. Serum testosterone Determination

Testosterone hormone concentration was measured by Radioimmunoassay (RIA) kit, after treating the samples with I^{125} (Labeled testosterone tracer), then by Gamma Counter the reaction between I^{125} with testosterone hormone was measured in ng/ml unit.

2.6. Sperm evaluation

The tail of the left epididymus was taken and embedded in one ml of normal saline at 37 ^{0}C in a watch glass ,and then tail was cut into at least 200 sections by microsurgical scissor, to perform the following microscopic examination of sperm parameters. Sperm motility was assessed according to Bearden and Faquay [17]. Morphology (abnormality) was evaluated on sperm from caudal epididymis. The total morphological abnormalities were observed as described by Siegmund [18]. Assessment of live and dead sperms was carried out by the method of Baril *et al* [19]. Sperm count was done according to Mohan *et al* [20]. Sperm counts were done by using a haemocytometer.

2.7. Histological measurement

2.7.1.Leydig cell counting

Counting of leydig cells was done by reading between of every three seminiferous tubules and 20 readings per group or 5 cross-sections per rat was recorded. Readings were done under 40X magnifications [21].

2.7.2. Sertoli cell counting

The quantitative analysis of the seminiferous epithelium was performed based on the relative number of non-germ cell nuclei/section of seminiferous tubule for counting the non-germ cell number, 4 or 5 round cross sections of tubule per rat were used a total of 20 cross-sections in each group [22].

2.7.3. The diameter of seminiferous

The diameter of seminiferous tubules was measured by calculating horizontal and vertical diameters of the 5 tubules which were selected randomly for each sample by using an Ocular micrometer when matched with the stage micrometer by different magnifications then calculated the means of these diameters.

2.8. Statistical analysis

Data were expressed as means ± SE. The results were computed statistically GraphPad Prism (GraphPad Sofware, version 8) using one-way analysis of variance (ANOVA). Values of $p<0.05$ were considered statistically significant.

3. Result

The effect of date palm pollen ,carbon tetrachloride and date palm pollen plus carbon tetrachloride on serum testosterone hormone concentration is shown in figure -1, the result showed that during the pretreatment period, there were non-significant differences ($P>0.05$) among the four groups in testosterone level. The testosterone concentration was (4.170 ± 0.57), (4.014 ± 0.16 ng/ml), (4.046 ± 0.47 ng/ml) and (3.980± 0.62 ng/ml) in control, DPP, CCL4 and DPP&CCL4 groups respectively. During the treatment period, the results showed a significant ($P<0.05$) increment in the testosterone level in animals of DPP treated group as compared with the control group, also the results showed a significant ($P<0.05$) decrement in the testosterone level in animals of group CCL4 treated group as compared with the control and DPP group during all periods of experiment. There were significant differences ($P<0.05$) in testosterone levels between T1 and T2 at the 21, 42 and 63 days after treatment when compared to each other's.

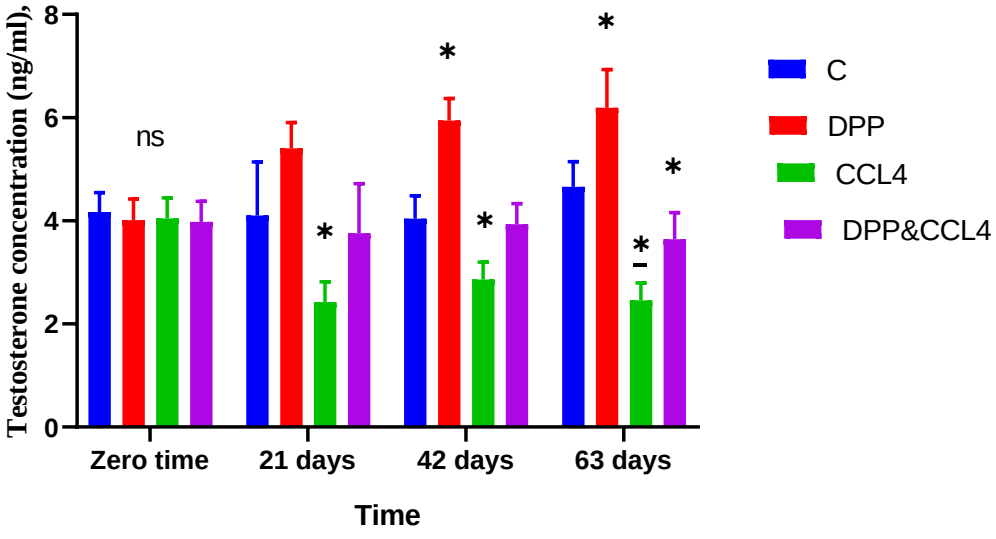

Figure -1 Effect of date palm pollen, carbon tetrachloride and date palm pollen plus carbon tetrachloride on serum testosterone hormone concentration (ng/ml), in adult male rats.

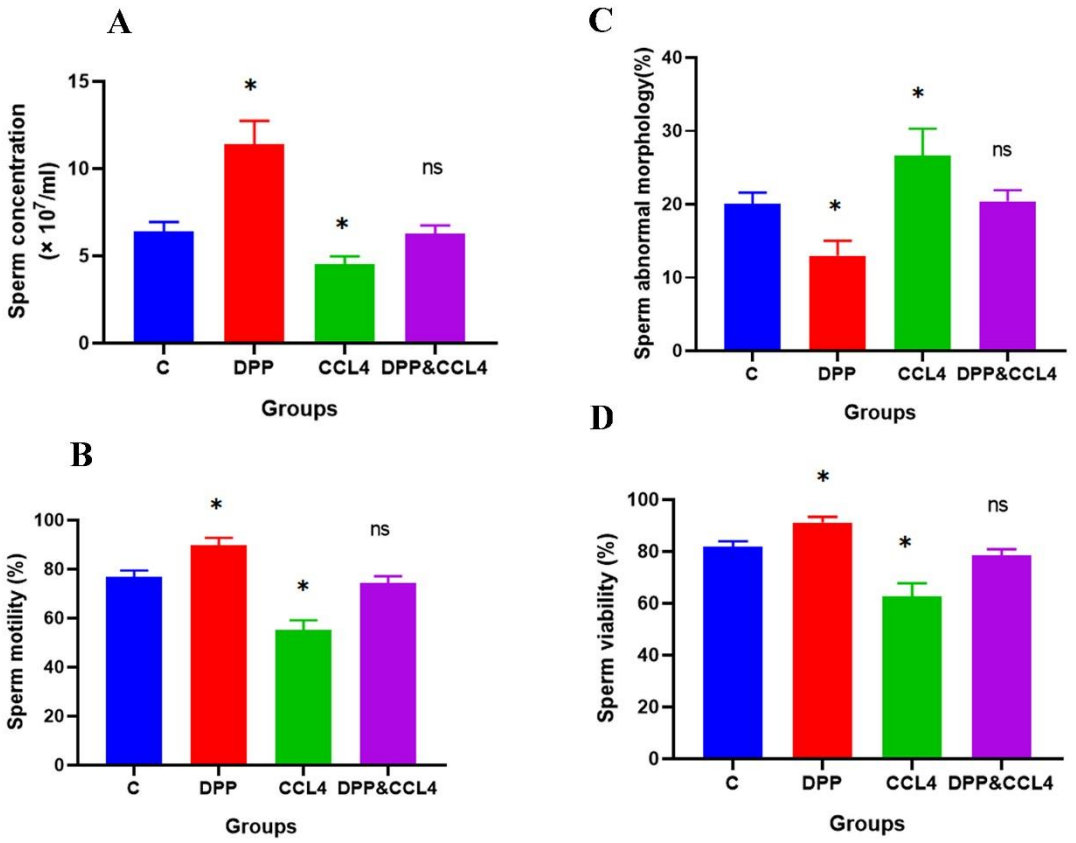

Figure -2 Effect of date palm pollen, carbon tetrachloride and date palm pollen plus carbon tetrachloride on sperm parameters in adult male rats after 63 days of treatment.

The results of figure-3 demonstrated that number of sertoli, leydig cells and diameter of seminiferous tubules were increased significantly ($P<0.05$) in DPP treated rats as compared with the control groups, CCL4 group and DPP&CCL4 group, while CCL4 causes a significant decrease ($P<0.05$) in several sertoli, leydig cells and diameter of seminiferous tubules in CCL4 group as compared with the control group after 63 days of the experiment.

The microscopic examination of testicular tissue in the group treated with date palm pollen (0.15gm/kg) showed a full differentiation and proliferation of spermatogensis in the centre of seminiferous tubules were compact with sperm and a large number of leydig cells (fig.4, DPP). While, the group treated with carbon tetrachloride showed a poor proliferation and differentiation of spermatogenesis and dilatation of the space between seminiferous tubules and few numbers of Leydig cells (Fig. 4, CCL4). The histological section of testicular tissue in the group treated with date palm pollen and Carbon tetrachloride showed a moderate proliferation and differentiation of spermatogenesis and mild few leydig cells as well as small size of seminiferous tubules and together mild dilate space between seminiferous tubules (fig. 4, DPP&CCL4).

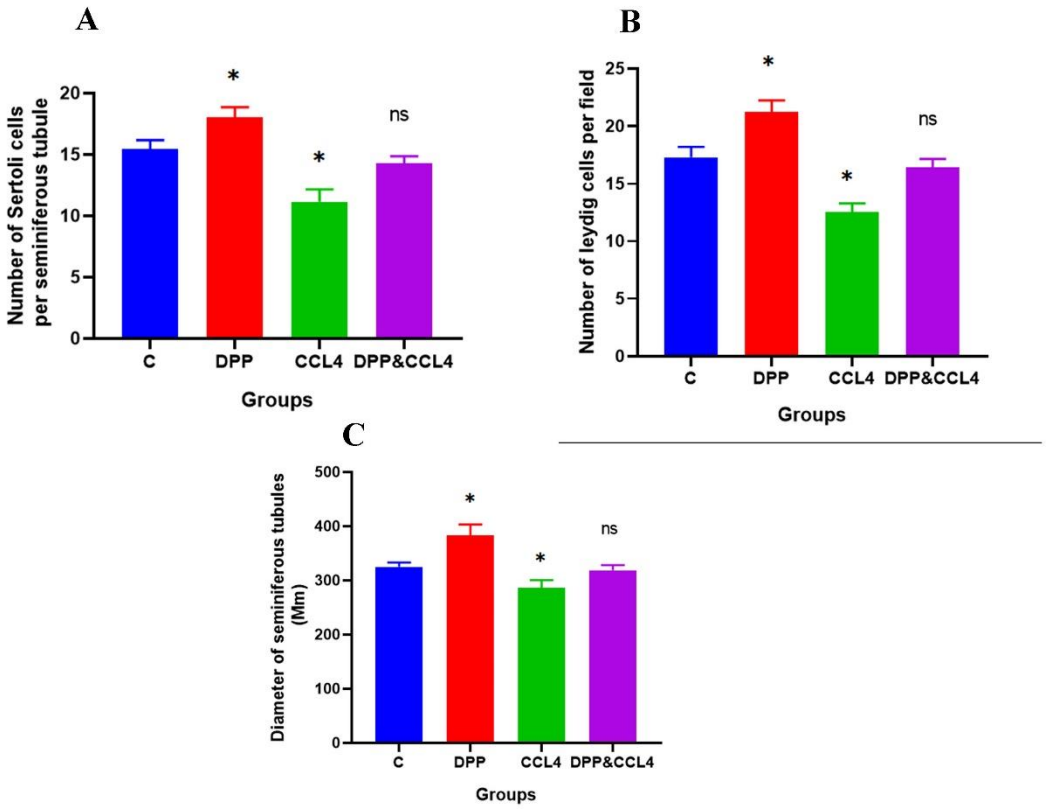

Figure -3 Effect of date palm pollen, carbon tetrachloride and date palm pollen plus carbon tetrachloride on number of sertoli, leydig cells and diameter of seminiferous tubules (Mm) in adult male rats after 63 days of treatment.

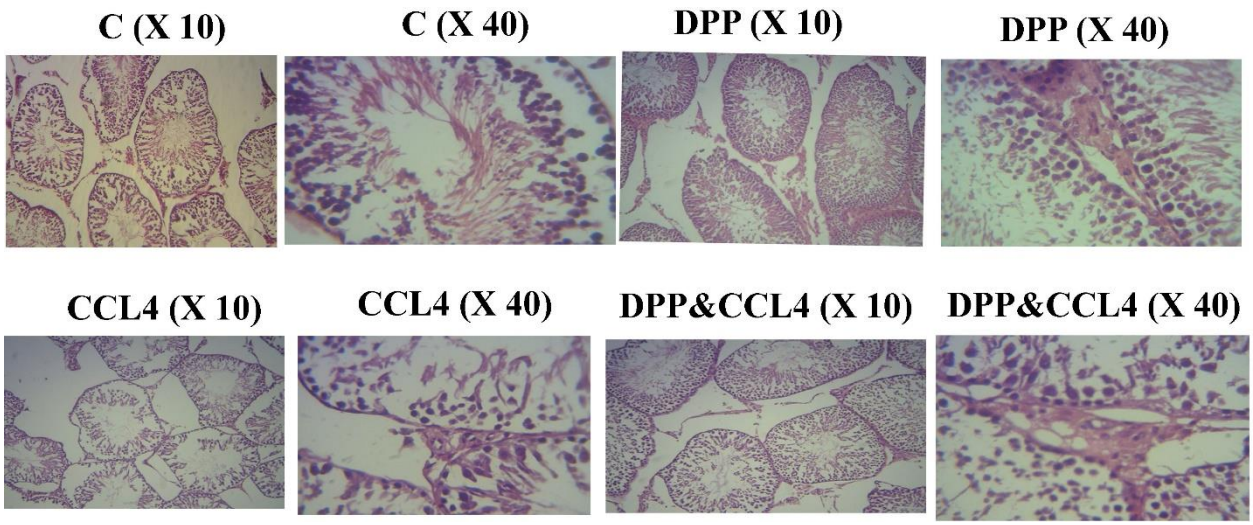

Figure -4 Effect of date palm pollen, carbon tetrachloride and date palm pollen plus carbon tetrachloride on testicular tissue adult male rats after 63 days of treatment.

4. Discussion

The result of this study is clear that oral administration of DPP causes significant increase in testosterone may be due to the effects of DPP which contain cholesterol which is a precursor for testosterone synthesis by its action on the Leydig cells [23, 24]. Also identified total

steroids, cholesterol, stigmasterol, campesterol and α-sitosterol in pollen grain which stimulate Leydig cells to produce testosterone [25, 26], this result agrees with Bahmanpour [10] who showed that DPP suspension increased testosterone and estrogen hormone of blood male rats. El-Ridi *et al* [27] isolated a crude gonadotrophically active substance from date palm pollen, this substance may increase LH which stimulating leydig cells to increase testosterone. DPP contains phytoestrogenic substances, it may cause an increase in testosterone, [28]. Carbon tetrachloride can cause lipid peroxidation ,damage and increase the activity of the enzyme in the testes, also the activities of all the lysosomal enzymes increased in the testis of rats, it caused oxidative stress and a decrease in anti-oxidative enzyme, and steroid biosynthesis inhibition by reactive oxygen species (ROS)which suggested a decrease in testosterone [29]. Carbon tetrachloride toxicity affected on pituitary gland causing a reduction in the level of FSH and LH leading to the inhibition of steroid biosynthesis by leydig cells causing a decrease in testosterone [30]. The results showed a significant elevation ($P<0.05$) in serum testosterone in the DPP&CCL4 group as compared with the CCL4 group may refer to the effect of DPP to increment of testosterone and reduce the negative of CCL4 toxicity, from the result, the DPP suspension contains antioxidant compounds (flavonoid, steroid phenol, alkaloid saponin and tannins) might decrease the toxicity of carbon tetrachloride. Phytoestrogen has antioxidant properties through hydrogen/electron donation via hydroxyl groups, therefore it acts as free radical scavengers and may inhibit toxicity of carbon tetrachloride [31], so that testosterone level increment demonstrated in the DPP&CCL4 group.

DPP suspension increased sperm concentration, motility, and viability in (DPP group) which may be due to an increase in testosterone level and activation of spermatogenesis and finally increase in these parameters [32]. DPP stimulates the pituitary for an increase of LH release which leads to stimulate testosterone production and thereby increases sperm evaluation, also DPP contains gonadotropin substance,flavonoid , alkaloid phenolic compounds and steroids which increases the count of sperm [10]. In CCL4 group, sperm parameters decreased when compared with the control, CCL4 may cause a decrease in sperm concentration due to oxidative stress which affects sperm quality and male fertility, also sperm cells are highly vulnerable to free radicals, therefore, oxidative stress caused spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells lead to azoospermia [33]. In the DPP&CCL4 group, the sperm parameters returned to normal values and became close to the control group, this may be attributed to the effect of DPP which is responsible for its protective effect against carbon tetrachloride toxicity (contains antioxidant compound carotenoids, and flavonoids and phytosterols), spermatozoa are protected by various antioxidants, which removed free radical and returned the sperm count to normal [34]. Also, testosterone may have a protective role against oxidative stress produced by CCL4 which effects on sperm parameters.

An increase of testosterone levels in rats that received DPP suspension, may increase testicular weight and volume, so the diameter of seminiferous tubules increase [35] On the other hand, testosterone supports spermatogenesis and increases spermatocyte maturation; facilitating round to elongated spermatid progression and release of spermatids from Sertoli cells may lead to an increase in lumen and diameter of seminiferous tubules [36]. Toxicity of CCL4 similar another Toxic compound another Toxic compound compound which causes a reduction in testicular size and weight and cause severe histopathological testicular abnormalities, including loss of the germinal line, sertoli cells and diminutions in cellular

proliferation and spermatogenesis and decreased number of leydig cells. These are good markers of the integrity of the blood-testis barrier which lead to reduced tubular diameters of seminiferous tubules may decrease testosterone concentration a good marker decrease in the number of leydig cells [37].

On the other hand, steroid compounds in DPP may moderate decrease in the diameter of seminiferous tubules by CCL4 toxicity(group T3) due to maintaining the concentration of testosterone which is important for the proliferation of spermatogonia and maturation of seminiferous tubules [38]. Furthermore, an antioxidant compound of DPP increases plasma vitamin C, which plays an important role in decreasing the toxicity of CCL4 and returning the diameter of seminiferous near to normal [39]. Based on the results of the present study, we concluded that oral administration of DPP is a beneficial effect on sperm evaluation and protects sperm and testicular tissue from CCl4- intoxication in rats. Further studies for elucidating DPP against reproductive toxicity in large animals and for humans who suffer from infertility.

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Conflict of Interest
The authors declare that they have no conflicts of interest

References

[1] N. M. Ammar, S. Y. Al-Okbi, D. A. Mohamed, and L. T. A. El-Kassem, "Antioxidant and estrogen like activity of the seed Phoenix dactylifera L. palm growing in Egyptian oases," Rep Opin,. **1**: p. 1-8, 2009.
[2] N. R.Vyawahare, R. Pujari, A. Khsirsagar, D. Ingawale, M. Patil, and V. Kagathara, "Phoenix dactylifera: An update of its indegenous uses, phytochemistry and pharmacology,". The Internet Journal of Pharmacology, 7(1), 1-9, 2009. https://ispub.com/IJPHARM/7/1/8733.
[3] H. A. Al-Shwyeh, "Date Palm (Phoenix dactylifera L.) Fruit as Potential Antioxidant and Antimicrobial Agent," Journal of pharmacy & bioallied sciences, **11**(1): p. 1-11,2019. doi: 10.4103/jpbs.JPBS_168_18.
[4] H. S. Khierallah, S. M. Bader, K. M.Ibrahim, and I. J. Al-Jboory, "Date palm status and perspective in Iraq, in Date palm genetic resources and utilization,", Springer, p. 97-152, 2015. https://link.springer.com/chapter/10.1007/978-94-017-9707-8_4.
[5] H.M. Hassan, "Chemical composition and nutritional value of palm pollen grains," Global J Biotechnol Biochem, **6**(1): p. 1-7, 2011.
[6] A. A. Al-Qarawi, H. M. Mousa, B. H. Ali, H. Abdel-Rahman, and S. A. El-Mougy, "Protective effect of extracts from dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats. Int J Appl Res Vet Med, 2004. **2**(3): p. 176-180.
[7] M. Shehzad, H. Rasheed, S. A. Naqvi, J. M. Al-Khayri, J. M. Lorenzo, , M. A. Alaghbari, and R. M.Aadil, "Therapeutic Potential of Date Palm against Human Infertility: A Review," Metabolites, **11**(6): p. 408, 2021. https://doi.org/10.3390/metabo11060408
[8] F. A. Abbas,. and A. M. Ateya, "Estradiol, esteriol, estrone and novel flavonoids from date palm pollen," Aust J Basic Appl Sci, **5**(8): p. 606-614,2011.
[9] G. Mahran, S. Abdel–Wahab, and A. Attia, "A phytochemical study of date palm pollen," Planta Medica, **29**(02): p. 171-175,1976.

[10] S. BAHMANPOUR, M.R. PANJEH SHAHIN, T. TALAEI, Z. VOJDANI, A. POUST PASAND, S. ZAREEI, and M. GHAEMIAN, “EFFECT OF PHOENIX DACTYLIFERA POLLEN ON SPERM PARAMETERS AND REPRODUCTIVE SYSTEM OF ADULT MALE RATS,” IRANIAN JOURNAL OF MEDICAL SCIENCES (IJMS), vol. 31, no. 4, pp. 208–212, 2006, [Online]. Available: https://sid.ir/paper/276058/en
[11] V. Unsal, M. Cicek, and İ. Sabancilar, "Toxicity of carbon tetrachloride, free radicals and role of antioxidants," Reviews on Environmental Health,. **36**(2): p. 279-295, 2021.
[12] E. Smuckler, "Alterations produced in the endoplasmic reticulum by carbon tetrachloride," Panminerva medica, **18**(9-10): p. 292-309, 1976.
[13] C. E. Ugwu, and S.M. Suru, "Medicinal plants with hepatoprotective potentials against carbon tetrachloride-induced toxicity: a review," Egyptian Liver Journal,. **11**(1): p. 1-26, 2021. https://doi.org/10.1186/s43066-021-00161-0
[14] Q. A. Obaid, "The protective effect of Date Palm Pollen (Phoenix dactylifera) suspension on hematological parameters in male rats which exposed to CCL4." The 2nd International Scientific Conference of Medical and Health Specialties. p. 326-332, 2015.
[15] M. Sönmez, G. Türk, S. Çeribaşı, M. Çiftçi, A.Yüce, M. Güvenç, and M. Aksakal, "Quercetin attenuates carbon tetrachloride-induced testicular damage in rats," Andrologia, **46**(8): p. 848-858, 2014.
[16] Y. K. Yim, H. Lee, K. E. Hong, Y. I. Kim, B. R. Lee, T. H. Kim, and J. Y. Yi, "Hepatoprotective effect of manual acupuncture at acupoint GB34 against CCl4-induced chronic liver damage in rats," World journal of gastroenterology, **12**(14): p. 2245-2249, 2006. doi: 10.3748/wjg.v12.i14.2245
[17] H. Bearden, and J. Faquay, "Applied animal reproduction" 3rd ed. Asimen and Schuster Company, Englewood and Cliffs, 1992.
[18] O. Siegmund, and C. Fraser, "Reproductive and urinary system," The merck veterinary manual. Siegmand, OH and Fraser, CM (ed.). Published by merck and Co. Inc. Rahway, NJ USA. p. 792-892, 1979.
[19] P. Baril, Y. Cagnie, Y. Guerin, P. Orgeur, and J. C. Vallet, "Training manual on artificial insemination in sheep and goats," Etude FAO: Production et Sante Animales (FAO), 1993.
[20] G. Mohan, N. Mazumder, and K. Goswami, "Note on semen characteristics in Indian Pashmina goats," Indian journal of animal sciences, 1980.
[21] A. Didolkar, and D. Roychowdhury, "Effect of prostaglandins A-1, E-2 and F-2α on spermatogenesis in rats," Reproduction, **58**(1): p. 275-278, 1980.
[22] F. U Wazir, I. U. Wazir, M. Javeed, M. Fida, M. Saeed, and K. Jahanzeb, "Morphological changes induced by cottonseed flour in rat testes," Biomedica, **22**: p. 105-109, 2006.
[23] W.F. Ganong, "Review of medical physiology. Dynamics of blood and lymph flow," **30**: p. 525-541, 1995.
[24] S. Azhar, and E. Reaven, "Regulation of Leydig cell cholesterol metabolism, in The Leydig cell in health and disease,", Springer. p. 135-148, 2007.
[25] S. M. Mohamed, H. A. Bosila, S. S. I. Ibrahim, and Refay, K. "A Phytochemical screening of some in vivo and in vitro date palm tissue," in The Second International Conference on Date Palms, Al-Ain, UAE, March. 2001.
[26] M. Al-Farsi, C. Alasalvar, A. Morris, M. Baron, and F. Shahidi, "Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylifera L.) varieties grown in Oman," Journal of agricultural and food chemistry," **53**(19): p. 7592-7599, 2005.
[27] M. El Ridi, L. Strait, and M.A. Wafa, "Isolation of rutin from the pollen grain of the date palm (Dactylifera Palma L.)," Archives of Biochemistry and Biophysics,. **39**(2): p. 317-321,1952.
[28] A. A. Abo-El-Soaud, A. Sabor, N. R. El-Sherbeny, and E. I. Baker, "Effect of date palm (Phoenix dactylifera L.) flavonoids on hyperglycemia," in The Second International Conference on Date Palm. 2004.
[29] P. Abraham, and G. Wilfred, "Lysosomal enzymes in the pathogenesis of carbon tetrachloride induced injury to the kidney and testis in the rat," Indian Journal of Pharmacology 32(3):p 250-251, 2000.

[30] H. Ismail, and H. El-Nahari, "Therapeutic and protective role of Panax ginseng on pituitary and testicular axis in male rats treated with carbon tetrachloride," Alexandria journal of agricultural research, 54(1), 2009.
[31] J. H. Mitchell, P. T. Gardner, D. B. McPhail, P. C.Morrice, A. R. Collins, and G. G. Duthie, "Antioxidant efficacy of phytoestrogens in chemical and biological model systems," Archives of biochemistry and biophysics," **360**(1): p. 142-148, 1998.
[32] L. Johnson, D. D. Varner, M. E. Roberts, T. L. Smith, G. E.Keillor, and W. L. Scrutchfield, "Efficiency of spermatogenesis: a comparative approach," Animal Reproduction Science, **60**: p. 471-480, 2000.
[33] F. Helfenstein, S. Losdat, A. P.Møller, J. D. Blount, , and H. Richner, "Sperm of colourful males are better protected against oxidative stress," Ecology letters, **13**(2): p. 213-222, 2010.
[34] R. A. Al-Alawi, J. H. Al-Mashiqri, J. S. Al-Nadabi, B. I. Al-Shihi, , and Y. Baqi, "Date Palm Tree (Phoenix dactylifera L.): Natural Products and Therapeutic Options," Frontiers in plant science,. **8**: p. 845-845, 2017.
[35] M. Arslan, G. F. Weinbauer, S. Schlatt, M. Shahab, and E. Nieschlag, "FSH and testosterone, alone or in combination, initiate testicular growth and increase the number of spermatogonia and Sertoli cells in a juvenile non-human primate (Macaca mulatta)," Journal of Endocrinology,. **136**(2): p. 235-NP, 1993.
[36] M. Haywood, J. Spaliviero, M. Jimemez, N. J. King, D. J. Handelsman, and C. M. Allan, "Sertoli and germ cell development in hypogonadal (hpg) mice expressing transgenic follicle-stimulating hormone alone or in combination with testosterone," Endocrinology,. **144**(2): p. 509-517, 2003.
[37] S. Joursaraei, A. R. Firouzjaei, P. Y. YOUSEFNIA, M. I. TAHMASBPOUR, and E. Sarabi, "Histopathological effects of single dose treatment of diazinon on testes structure in rat," Yakhteh Medical Journal, Vol 12, No 1, 2010.
[38] R. Walczak-Jedrzejowska, K. Kula, E. Oszukowska, K. Marchlewska, W. Kula, , and J. Slowikowska-Hilczer, "Testosterone and oestradiol in concert protect seminiferous tubule maturation against inhibition by GnRH-antagonist," International journal of andrology,. **34**(5pt2): p. e378-e385, 2011.
[39] A. K. Chandra, A. Chatterjee, R. Ghosh, and M. Sarkar, "Vitamin E-supplementation protect chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats," Food and chemical toxicology, **48**(3): p. 972-979, 2010.