

Protective Effects of Pistachio Extract Against Diazinon: Mitigating Uterine Damage and Oxidative Stress

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Information	Abstract
<p>Article Type: Original Article</p>	<p>Background and Objectives: The widespread use of the organophosphate pesticide diazinon has raised concerns about its possible harmful effects on reproductive health. This study aimed to examine the protective effects of pistachio pericarp essential oil against uterine damage and oxidative stress caused by diazinon in mice.</p> <p>Materials and Methods: Six groups were formed: control, corn oil vehicle, diazinon, diazinon with 5% pistachio extract, diazinon with 10% pistachio extract, and 10% pistachio extract alone. After 28 days of daily gavage treatments, uterine tissues were collected for histomorphometric analysis and assessment of oxidative stress markers like MDA and SOD.</p> <p>Results: Diazinon significantly raised malondialdehyde (MDA) levels and lowered superoxide dismutase (SOD) activity compared to the control, indicating heightened oxidative stress. It also decreased endometrial thickness and uterine diameter, suggesting structural damage. Importantly, treatments with pistachio extract, especially at 10%, effectively alleviated these negative effects, reducing MDA levels, increasing SOD activity, and improving uterine tissue morphology.</p> <p>Conclusion: The results indicate that diazinon may impair fertility by causing oxidative stress and uterine tissue damage. Conversely, pistachio pericarp extract offers protective benefits, likely due to its antioxidant properties. This study underscores the potential of natural extracts to counteract the reproductive toxicity of pesticides.</p>
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1. Introduction

Pesticides have dramatically increased food production, addressing the needs of a growing world, but their widespread use has created major environmental problems. While designed to enhance crop yields and protect against pests, the heavy reliance on these chemicals has resulted in their excessive use, turning them into a widespread environmental threat [1]. This overdependence has contaminated soil, water, and air, harmed ecosystems, and endangered non-target organisms [2]. Diazinon, a commonly used organophosphate insecticide, exemplifies this issue. Its long-lasting presence in the environment and ability to be absorbed through the skin and transformed into toxic metabolites in the liver pose a considerable threat to human and animal well-being. Diazinon's effects go beyond basic toxicity, as it can disrupt normal cell processes, induce genetic changes, stop crucial cell division, cause fetal abnormalities, inhibit DNA synthesis, and even initiate programmed cell death in various tissues [3].

The pervasive impact of pesticides, particularly those like diazinon, extends beyond general environmental contamination, targeting specific organs and systems within living organisms [4]. The uterus, a critical component of the female reproductive system essential for implantation, gestation, and successful reproduction, is among the vulnerable targets increasingly recognized as susceptible to the adverse effects of pesticide exposure [4]. Several studies have documented the detrimental influence of diazinon and similar toxins on reproductive systems, revealing a disturbing pattern of increased abnormalities. These abnormalities manifest in various forms, ranging from disruptions in hormonal balance [5] and alterations in uterine tissue structure to

compromised implantation and fetal development [6, 7]. Furthermore, research indicates that these toxins can interfere with the delicate interplay of cellular processes within the uterus, potentially leading to impaired fertility and adverse pregnancy outcomes [8]. The observed increase in reproductive abnormalities underscores the urgent need to understand the precise mechanisms through which these pesticides exert their toxic effects on the uterus and to develop strategies for mitigating their impact on reproductive health.

For centuries, across diverse cultures, medicinal plants have played a vital role in therapeutic practices, offering natural remedies for a multitude of ailments. Pistachios, a nut revered not only for its culinary appeal but also for its medicinal properties, stand as a testament to this historical reliance on botanicals [9, 10]. These nuts are a treasure trove of bioactive compounds, notably potent antioxidants like tocopherols [11], flavonoids [12], and carotenoids [11], as well as anti-inflammatory agents such as unsaturated fatty acids and polyphenols [10, 13]. These compounds work synergistically to combat oxidative stress and quell inflammatory responses, which are implicated in the pathogenesis of numerous diseases [14, 15].

Pistachios, supported by modern research, show potential for treating various health issues, particularly those involving hormone regulation (steroidogenesis) [16]. The often-discarded pistachio green peel is a rich source of beneficial compounds, exceeding the kernel's content, highlighting the value of exploring agricultural byproducts for therapeutic use [10]. The uterus is susceptible to oxidative damage due to its cyclical changes and hormonal fluctuations.

Pistachio compounds, like limonene, with their antioxidant properties [17], may protect the uterus by neutralizing free radicals, suggesting potential therapeutic applications for supporting uterine health.

The objective of this research is to evaluate the impact of pistachio pericarp essential oil on the structural integrity, oxidative stress markers, and apoptotic activity within the uterine tissue of adult female NMRI mice exposed to diazinon. If the pistachio pericarp essential oil positively influences tissue changes, reduces oxidative stress levels, and decreases the number of apoptotic cells in these mice, it may be recognized as a potential substance containing antioxidant compounds to protect the female reproductive system from the adverse effects of pesticides, pending further investigation.

2. Materials and Methods

2.1. Preparation of Pistachio pericarp essential oil

The research team obtained the pistachio pericarp essential oil from Mr. Seyed Mohammad Bagher Marashi, who holds patent number 86343 for its extraction ('Process for extracting essential oil or aromatic oil from pistachio pericarp').

2.2. Ethical Approval

This research project received approval from the Ethics Committee of Rafsanjan University of Medical Sciences, under ethics code IR.RUMS.REC.1402.022 and animal care throughout the study followed the established standards for laboratory animal welfare, as detailed in the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

2.3. Animals and Treatments

Adult female NMRI mice (n=48) with weights of approximately 25 ± 5 grams were procured from the animal house of Rafsanjan University of Medical Sciences. These mice were randomly allocated to six groups, each containing eight individuals, and monitored for 28 days [18]. They were housed in clean cages under controlled conditions, specifically a temperature of 22-24 °C and a 12-hour light/dark cycle, and provided with ad libitum access to standard rodent chow and water.

The mice were divided into six experimental groups, each containing eight individuals, with the following treatments:

Group 1 (Control): Healthy mice receiving no treatment.

Group 2 (Oil): Mice were administered 10 mg/kg of corn oil every 24 hours via gavage.

Group 3 (Diz): Mice were administered 30 mg/kg of diazinon every 24 hours via gavage.

Group 4 (5% Pis+Diz): Mice were administered 30 mg/kg of diazinon every 24 hours and 100 µL/kg of 5% pistachio pericarp essential oil every 24 hours via gavage.

Group 5 (10% Pis+Diz): Mice were administered 30 mg/kg of diazinon every 24 hours and 100 µL/kg of 10% pistachio pericarp essential oil every 24 hours via gavage.

Group 6 (10% Pis): Healthy mice were administered 100 µL/kg of 10% pistachio pericarp essential oil every 24 hours via gavage.

At the study's outset, estrous cycles and weights were evaluated to ensure consistent physiological baselines. Vaginal lavage samples were examined microscopically to confirm estrous cycle synchronization, identifying

proestrus, estrus, metestrus, and diestrus stages within the expected four- to five-day cycle. Vaginal smear analysis validated estrous cycle synchronization, with uniform presence of epithelial, leukocyte, and cornified cells across all groups, confirming successful synchronization [19].

To evaluate the chronic impact of diazinon and the experimental treatment, the study was conducted over 28 days, equivalent to seven mouse estrous cycles, as this duration falls within the reported range of one week to three months for laboratory animal studies [18]. After this period, the uteri of the euthanized mice were collected and subjected to histological processing. This involved initial fixation in Bouin's solution, followed by dehydration, embedding, thin sectioning, and specific staining. The resulting tissue sections were mounted on slides and examined under a light microscope, with images subsequently recorded.

2.4. Histological evaluation

Uterine tissue morphology was assessed using hematoxylin-eosin (H&E) staining, including measurements of uterine diameter and endometrial thickness. The process included: sequential dehydration in graded alcohols, paraffin embedding with labeled group numbers and precise sample orientation, microtome sectioning followed by wrinkle removal and slide mounting, H&E staining with dewaxing, and light microscopy examination. Morphometric data were then compared across experimental groups [20].

2.5. Examination of Apoptosis Rate

To assess the level of apoptosis, the Cell Death Detection kit from Roche was used. This kit labels the free 3' OH DNA ends with fluorescence duTP using terminal

deoxynucleotidyl transferase (TdT). Then, an anti-fluorescein POD antibody conjugated with peroxidase (Horseradish Peroxidase = HRP) was used, and diaminobenzidine was used as a substrate to visualize POD. The slides were incubated with the TUNEL reaction mixture for 60 minutes in a humid environment at 73 degrees Celsius and then washed twice in PBS. After several washes, diaminobenzidine (DAB) was added for 10 minutes at room temperature. Then, they were washed in PBS and mounted on slides for light microscope study. The number of TUNEL-positive cells in the tissue samples was counted in the different groups [20].

2.6. Measurement of Oxidative Stress

2.6.1. Malondialdehyde (MDA) Test

To assess lipid peroxidation in uterine tissue, MDA levels were measured using the thiobarbituric acid (TBA) method. A homogenate of uterine tissue (1 mL) was mixed with 10% KCl and then with 2 mL of TBA reagent. The resulting solution was heated to 100°C for 15 minutes and subsequently centrifuged at 10,000 rpm for 10 minutes. After cooling, the absorbance of the supernatant was read at 550 nm using an ELISA reader (Biottech-ELX-808iu). Protein content was determined via the Bradford assay, and MDA levels were expressed as nanomoles per milligram of protein [21].

2.6.2. Superoxide Dismutase (SOD) Test

The activity of SOD was measured using a kit from Zell Bio GmbH (Germany, Cat. No. Z185-S), adhering to the manufacturer's instructions. The assay utilizes the superoxide radical scavenging activity of SOD. Superoxide radicals, generated through the xanthine/xanthine oxidase system, react with nitro blue tetrazolium (NBT) to produce a purple

formazan product (NBTH2). SOD in the sample competes with NBT for superoxide radicals, thereby inhibiting NBTH2 formation. The extent of this inhibition, quantified by measuring the absorbance change at 560 nm, reflects SOD activity. The results are presented as the percentage inhibition of NBTH2 production, expressed in millimoles per minute [22].

3. Results

3.1. Histological analysis

Analysis of endometrial thickness revealed that the Diz, 5% Pis+Diz, and 10% Pis+Diz groups exhibited significantly lower thickness than the control and 10% Pis (P=0.001) (Figure 1-A and Figure 2). Uterine diameter

measurements revealed that the Diz and 5% Pis+Diz groups exhibited significantly smaller diameters than the control and 10% Pis groups (P=0.001). The 10% Pis+Diz group also showed a decrease in diameter compared to the control group (Figure 1-B and Figure 2). Analysis of apoptotic cell counts in uterine tissue revealed that the diazinon group exhibited the highest number of apoptotic cells across all groups. Specifically, the Diz and 5% Pis+Diz groups demonstrated a statistically significant increase in apoptotic cells compared to both the control and 10% Pis groups (P=0.001). Conversely, the 10% Pis+Diz group shows a statistically significant difference in apoptotic cell counts when compared to the control and 10% Pis groups (Figure 1-C and Figure 3).

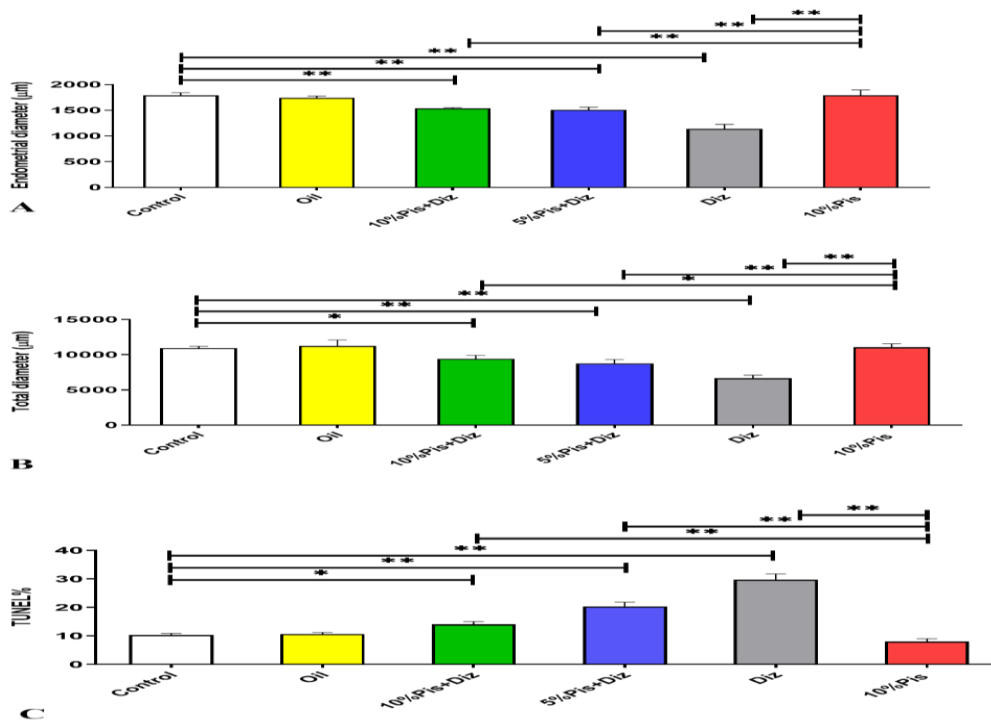


Fig 1. Endometrial and total diameters and TUNEL assay were assayed after mice treatment with 30 mg/kg/day diazinon (Diz), 30 mg/kg/day diazinon + 5% pistachio pericarp essential oil (5% Pis+Diz), 30 mg/kg/day diazinon + 10% pistachio pericarp essential oil (10% Pis+Diz) and 10% pistachio pericarp essential oil (10% Pis) for 28 days. The control group obtained only a routine diet and water and the sham group orally received 10 mg/kg/day corn oil throughout 28 days.

The data are given as the mean ±SD.

p value is *: p≤0.05, **: p≤0.001.

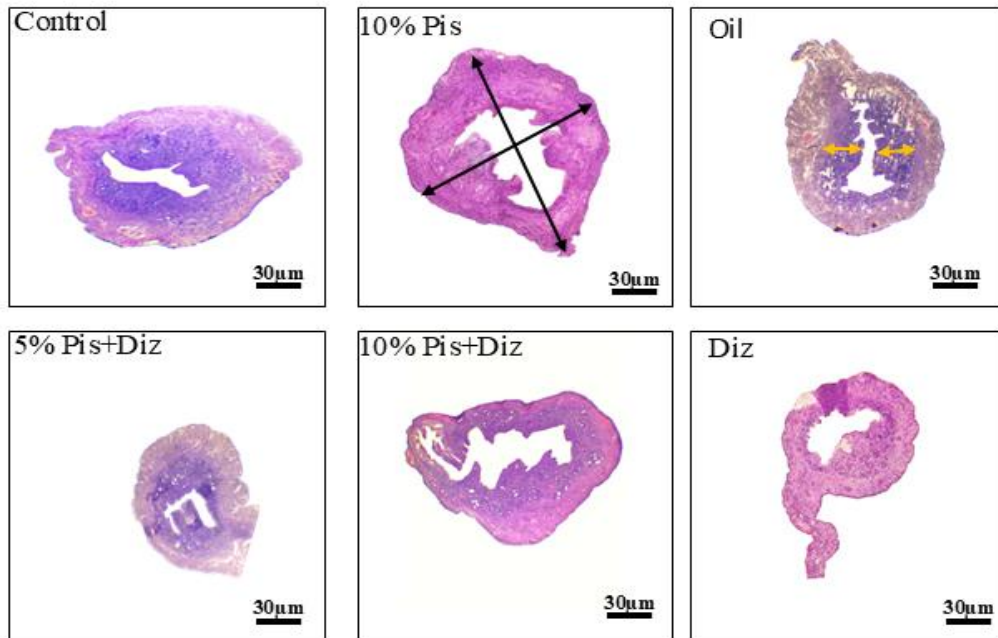


Fig 2. Histological changes in uterus tissue after hematoxylin and eosin staining in control and experimental mice. The endometrial diameter (yellow arrow) and total diameter (black arrow) were determined by measuring two perpendicular diameters in each uterine horn.

Control, Healthy mice receiving no treatment; Oil: Mice were administered 10 mg/kg of corn oil; Diz, Mice were administered 30 mg/kg of diazinon; 5% Pis+Diz, Mice were administered 30 mg/kg of diazinon and 100 µL/kg of 5% pistachio pericarp essential oil; 10% Pis+Diz, Mice were administered 30 mg/kg of diazinon and 100 µL/kg of 10% pistachio pericarp essential oil; 10% Pis, Healthy mice were administered 100 µL/kg of 10% pistachio pericarp essential oil.

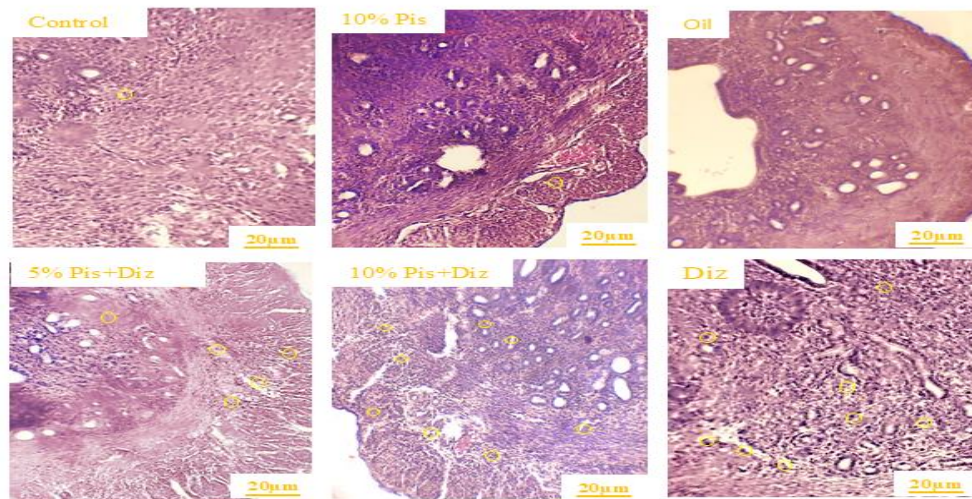


Fig 3. TUNEL assay was performed to detect apoptotic cells (yellow circles). Control, Healthy mice receiving no treatment; Oil: Mice were administered 10 mg/kg of corn oil; Diz, Mice were administered 30 mg/kg of diazinon; 5% Pis+Diz, Mice were administered 30 mg/kg of diazinon and 100 µL/kg of 5% pistachio pericarp essential oil; 10% Pis+Diz, Mice were administered 30 mg/kg of diazinon and 100 µL/kg of 10% pistachio pericarp essential oil; 10% Pis, Healthy mice were administered 100 µL/kg of 10% pistachio pericarp essential oil.

3.2. MDA Level

Analysis of MDA levels, an indicator of oxidative stress, revealed that the Diz and 5% Pis+Diz groups exhibited significantly elevated MDA levels compared to both the control

and 10% Pis groups ($P=0.001$). In contrast, the 10% Pis+Diz group did not demonstrate a statistically significant increase in MDA levels when compared to the control groups ($P>0.05$) (Figure 4-A).

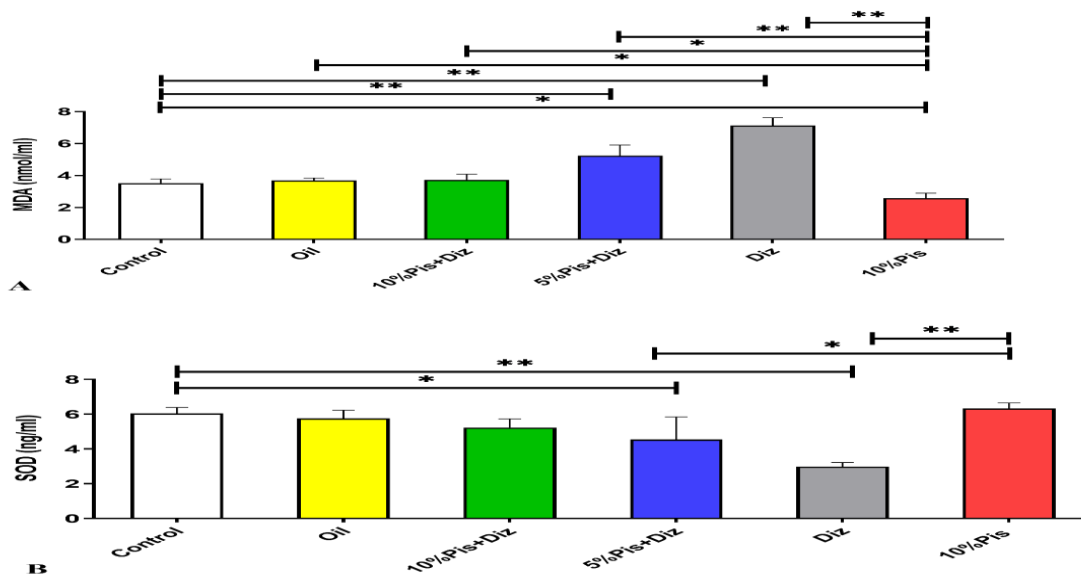


Fig 4. Malondialdehyde (MDA) Test and Superoxide Dismutase (SOD) were assayed after mice treatment with 30 mg/kg/day diazinon (Diz), 30 mg/kg/day diazinon + 5% pistachio pericarp essential oil (5% Pis+Diz), 30 mg/kg/day diazinon + 10% pistachio pericarp essential oil (10% Pis+Diz) and 10% pistachio pericarp essential oil (10% Pis) for 28 days. The control group obtained only a routine diet and water and the sham group orally received 10 mg/kg/day corn oil throughout 28 days.

The data are given as the mean \pm SD.

p value is *: $p \leq 0.05$, **: $p \leq 0.001$.

3.3. SOD Level

Statistical analysis of SOD levels revealed a significant decrease in the Diz group compared to both the control and 10% Pis groups ($P=0.001$). The 5% Pis+Diz group exhibits a statistically significant decrease in SOD levels when compared to the control and 10% Pis groups (Figure 4-B).

3.4. Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics 22 and GraphPad Prism (v. 8.4.3). To assess the significance of differences between group means, a one-way analysis of variance (ANOVA) was conducted,

supplemented by a Tukey post hoc test for multiple comparisons. Results are reported as mean \pm standard error of the mean (SEM), with statistical significance defined as $p < 0.05$.

4. Discussion

This study examined the harmful effects of diazinon on mouse uterine tissue, specifically oxidative stress markers, apoptosis, and structural alterations, while also assessing the potential protective role of pistachio pericarp essential oil. The results indicated that diazinon significantly elevated MDA levels and reduced SOD activity in uterine tissue, confirming oxidative stress. This aligns with existing

research highlighting the pro-oxidant properties of organophosphate insecticides.

Our results align with observations from Sargazi et al. [23], Erthal-Michelato et al. [24], and Zarein et al. [25], who reported increased MDA levels in ovarian and uterine tissues following exposure to diazinon, endosulfan, and malathion, respectively. Similarly, studies by Bohlool et al. (133), Abou Hasan et al. [26], Sargazi et al. [27], and Ahmadi et al. [28] corroborate our findings regarding the reduction of SOD activity upon diazinon exposure. These studies collectively highlight the ability of organophosphate insecticides to disrupt the oxidant/antioxidant balance, leading to oxidative damage across various tissues.

Diazinon exposure significantly increased apoptotic cells in the mouse uterine tissue. This novel finding, the first to evaluate diazinon-induced uterine apoptosis and the protective effects of pistachio pericarp essential oil, likely results from diazinon-induced oxidative stress. Reactive oxygen species can trigger mitochondrial dysfunction, cytochrome C release, and caspase activation, culminating in cell death [29].

Notably, our findings also revealed a significant reduction in endometrial thickness and uterine diameter in diazinon-exposed groups. This suggests a potential disruption of the hypothalamic-pituitary-ovarian axis, as evidenced by previous studies showing that organophosphates can affect LH levels and steroidogenesis [30, 31]. Given the critical role of sex hormones in endometrial growth and function [32-34]. The observed reduction in endometrial thickness likely reflects an imbalance in estrogen, progesterone, LH, and FSH levels.

These findings are consistent with the broader body of research highlighting the detrimental effects of pesticide exposure on female reproductive health. Mona et al. [35] reported a strong association between organophosphate pesticide exposure and recurrent miscarriages, attributing this to increased oxidative stress and apoptosis in the placenta. Similarly, Zhou et al. [36] found that organophosphates disrupt endometrial reconstruction by inhibiting key molecular pathways in the uterine decidua, crucial for fetal growth and maintenance. El Mazoudy et al. [37] also documented reduced uterine weight and impaired endometrial development in rats exposed to diazinon, and Sheffield et al. [38] reported uterine dysfunction in mice. However, it is important to note that Johari et al. [39] did not find significant changes in uterine tissue after diazinon exposure, highlighting potential variations in study design, dosage, or exposure duration.

Conversely, Davis et al. [40] suggested that fruit and vegetable consumption, despite potential pesticide residues, may be associated with a lower risk of uterine fibroids due to their antioxidant properties. This aligns with our findings regarding the protective effects of pistachio pericarp essential oil, which is rich in antioxidants.

Indeed, our study demonstrated that pistachio pericarp essential oil effectively mitigated the detrimental effects of diazinon. Specifically, 10% pistachio pericarp essential oil significantly reduced MDA levels and increased SOD activity compared to the diazinon-exposed groups, demonstrating its potent antioxidant properties. This is consistent with Taghizadeh et al. [41], who reported the free radical scavenging activity of pistachio pericarp essential oil. Moreover, pistachio pericarp essential oil reduced the

number of apoptotic cells and improved endometrial thickness, suggesting its protective role against diazinon-induced uterine tissue damage. This is further supported by Poormoosavi [42], who showed that *Pistacia atlantica* pistachio peel extract improved antioxidant activities and uterine structure in diabetic mice.

Finally, Lerro et al. [43] reported an increased risk of uterine cancer in women occupationally exposed to organophosphate pesticides. This highlights the long-term implications of pesticide exposure on female reproductive health and underscores the importance of developing strategies to mitigate these risks.

While our study yielded results consistent with existing research and provided a solid foundation for further investigation, it was not without limitations. We did not assess sex hormone levels (LH and FSH), nor did we evaluate implantation and fertility rates in the mice. Consequently, future studies should incorporate these parameters to provide a more comprehensive understanding of diazinon's effects and the protective mechanisms of the pistachio pericarp essential oil. Additionally, exploring the underlying molecular pathways would greatly enhance our understanding of both diazinon's toxicity and the pistachio pericarp essential oil mechanism of action. Therefore, we recommend that future research designs include molecular pathway analysis.

References

- 1- Khan BA, Nadeem MA, Nawaz H, Amin MM, Abbasi GH, Nadeem M, Ali M, Ameen M, Javaid MM, Maqbool R, Ikram M. Pesticides: impacts on agriculture productivity, environment, and management strategies. In: Emerging contaminants

5. Conclusion

In conclusion, our study offers significant evidence regarding the harmful effects of diazinon on uterine tissue and highlights the potential protective role of pistachio pericarp essential oil. These findings contribute to the increasing interest in natural products as therapeutic agents to reduce pesticide-induced reproductive toxicity. Future research should aim to clarify the specific mechanisms through which pistachio pericarp essential oil operates, examine its impact on sex hormone levels, and assess its effectiveness in clinical settings.

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Conflicts of interest

Competing interests All authors declare that they have no conflict of interest.

and plants: interactions, adaptations and remediation technologies. Cham: Springer International Publishing; 2023. p. 109-134.

- 2- Poudel S, Poudel B, Acharya B, Poudel P. Pesticide use and its impacts on human health and environment. *Environ Ecosyst Sci.* **2020**;4:47-51.
- 3- Reshi MS, Mustafa RA, Javaid D, Haque S. Pesticide toxicity associated with infertility. In *Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility Volume II*. Cham: Springer International Publishing; **2022**. p. 59-69.
- 4- Jain D, Verma RK, Sharma V, Kaur A, Rai AR, Kumari P, Nagar V, Sankhla MS, Parihar K. Associations between high levels pesticide and adverse reproductive outcomes in females: A comprehensive review. *Mater Today Proc.* **2023**;95:50-60.
- 5- Siavashpour A, Ghasemi Y, Khalvati B, Jeivad F, Azarpira N, Niknahad H. Diazinon Interrupts Ovarian Steroidogenic Acute Regulatory (StAR) Gene Transcription in Gonadotropin-Stimulated Rat Model. *Iran J Pharm Res.* **2018**;17:535-42.
- 6- Saraei F, Sadraie SH, Kaka GR, Sadoughi M, Afzal Nejad N, Sarahian N. Effects of maternal diazinon exposure on frontal cerebral cortical development in mouse embryo. *Int J Neurosci.* **2023**;133:152-8.
- 7- Hashem HR. Evaluation of the postnatal effects induced by Diazinon on the growth of the mice offspring and the development of their cerebellar cortex. *Cells Tissues Organs.* **2022**;211:539-54.
- 8- Medithi S, Kasa YD, Jee B, Venkaiah K, Jonnalagadda PR. Alterations in reproductive hormone levels among farm women and their children occupationally exposed to organophosphate pesticides. *Women Health.* **2022**;62:454-64.
- 9- Bagheri F, Hassanshahi G, Kahanamani Falahatipour S, Dini A, Mohamadi M, Ahmadi Z, Noroozi M, Khanamani Falahati-pour S. Effects of Pistachios and Their Different Plant Parts on Various Disorders: Evidence about Their Therapeutic Effects on Diabetes Mellitus, Gastrointestinal and Liver Disorders, as well as Blood Pressure. *Pistachio Health J.* **2021**;4:28-47.
- 10- Mandalari G, Barreca D, Gervasi T, Rousell MA, Klein B, Feeney MJ, Carughi A. Pistachio nuts (*Pistacia vera* L.): Production, nutrients, bioactives and novel health effects. *Plants.* **2021**;11:18.
- 11- Özdikicierler O, Öztürk-Kerimoğlu B. Bioactive phytochemicals from pistachio (*Pistacia vera* L.) oil processing by-products. *Bioactive Phytochemicals from Vegetable Oil and Oilseed Processing By-products: Springer*; **2023**. p. 577-94.
- 12- Nobari A, Marvizadeh MM, Sadeghi T, Rezaei-Savadkouhi N, Nafchi AM. Flavonoid and anthocyanin pigments characterization of pistachio nut (*Pistacia vera*) as a function of cultivar. *J Nuts.* **2022**;13:313-22.
- 13- Irshad S, Iftikhar S, Riaz M, Mahmood A, Mushtaq A, Saleem Y, Shamim R, Akter QS. Chemical fingerprinting, antimicrobial, antioxidant, anti-inflammatory, and anticancer potential of greenly synthesized silver nanoparticles from pistachio (*Pistacia vera*) nuts and senna (*Cassia angustifolia* Vahl.) leaves. *Food Sci Nutr.* **2024**;12:4989-5006.
- 14- Garrido JR, Patrignani M, Puppò MC, Salinas MV. Nutritional and bioactive characterization of pistachio—a review with special focus on health. *Explor Foods Foodom.* **2024**;2:363-90.
- 15- Boutemine IM, Amri M, Dorgham K, Amir ZC, Benazzouz S, Ameer F, Layaida K, Yssel H, Touil-Boukoffa C. Beneficial role of *Pistacia lentiscus* aqueous extract in experimental colitis: anti-inflammatory and potential therapeutic effects. *Inflammopharmacology.* **2021**;29:1225-39.
- 16- Dogani M, Askari N, Hesari AK. A diet enriched with *Pistacia atlantica* fruits improves the female rats' reproductive system. *J Tradit Complement Med.* **2024**;14:335-42.
- 17- Shahdadi F, Khorasani S, Salehi-Sardoei A, Fallahnajmabadi F, Fazeli-Nasab B, Sayyed RZ. GC-MS profiling of *Pistacia vera* L., and effect of antioxidant and antimicrobial compounds of its essential oil compared to chemical counterparts. *Sci Rep.* **2023**;13:21694.
- 18- Santacruz-Márquez R, Solorio-Rodríguez A, González-Posos S, García-Zepeda SP, Santoyo-Salazar J, De Vizcaya-Ruiz A, Hernández-Ochoa I. Comparative effects of TiO₂ and ZnO nanoparticles on growth and ultrastructure of ovarian antral follicles. *Reprod Toxicol.* **2020**;96:399-412.
- 19- Hosseini E, Jahandideh M. Effects of the alcoholic extract of ginger on sex hormone serum levels and

- ovarian follicles during pregnancy and lactation in the adult female offspring of rats. *J Babol Univ Med Sci.* **2015**;17:74-80.
- 20- Danafar A, Khoradmehr A, Bondarabadi MH, Mazaheri F, Tamadon A, Pourmasoumi S, Gholizadeh L, Moshrefi M, Halvaei I, Hosseini A, Golzadeh J. Impairment of sperm efficiency in mice following short-term nano-titanium dioxide exposure: An experimental study. *Int J Reprod BioMed.* **2022**;19:1045.
- 21- Han J, Yang D, Liu Z, Tian L, Yan J, Li K, Fang Z, Chen Q, Lin B, Zhang W, Xi Z. The damage effect of heat stress and psychological stress combined exposure on uterus in female rats. *Life Sci.* **2021**;286:120053.
- 22- Albishtue AA, Yimer N, Zakaria MZ, Haron AW, Babji AS, Abubakar AA, Baiee FH, Almhanna HK, Almhanawi BH. The role of edible bird's nest and mechanism of averting lead acetate toxicity effect on rat uterus. *Vet World.* **2019**;12:1013.
- 23- Sargazi Z, Nikravesh MR, Jalali M, Sadeghnia HR, Rahimi Anbarkeh F. Apoptotic effect of organophosphorus insecticide diazinon on rat ovary and protective effect of vitamin E. *Iran J Toxicol.* **2016**;10:37-44.
- 24- Erthal-Michelato RP, Frigoli GF, de Aquino AM, Punhagui-Umbelino AP, Scarano WR, Fernandes GS. Low doses of malathion impair ovarian, uterine, and follicular integrity by altering oxidative profile and gene expression of rats exposed during the peripubertal period. *Environ Sci Pollut Res.* **2024**;31:21721-36.
- 25- Zarein M, Zarban A, Shoorei H, Gharekhani M, Hassanzadeh-Taheri M. The amelioration of ovarian dysfunction by hesperidin in malathion-treated mice through the overexpression of PCNA and FSHR proteins. *Heliyon.* **2023**;9:e22484.
- 26- Abou Hasan F, Mutlu HS, Özdemir İ, Kotil T. Effects of diazinon on the ovarian tissue of rats: a histochemical and ultrastructural study. *J Mol Histol.* **2024**;55:1211-23.
- 27- Sargazi Z, Nikravesh MR, Jalali M, Sadeghnia H, Anbarkeh FR, Mohammadzadeh L. Gender-related differences in sensitivity to diazinon in gonads of adult rats and the protective effect of vitamin E. *Int J Women Health Reprod Sci.* **2015**;3:40-7.
- 28- Ahmadi S, Jafari M, Asgari A, Salehi M. Acute effect of diazinon on lipid peroxidation level and activities of antioxidant enzymes in rat spleen. *J Kermanshah Univ Med Sci.* **2012**;16:1-9.
- 29- Kalpage HA, Wan J, Morse PT, Zurek MP, Turner AA, Khobeir A, Yazdi N, Hakim L, Liu J, Vaishnav A, Sanderson TH. Cytochrome c phosphorylation: Control of mitochondrial electron transport chain flux and apoptosis. *Int J Biochem Cell Biol.* **2020**;121:105704.
- 30- Iheanacho JU, Onyeka PI, Udujih HI, Udujih OG, Okoroigbo FI, Amaechi AA, Okafor AI. Sub-lethal study of Organophosphorus (Chlorpyrifos) toxicity on reproductive biomarkers in Female Wistar Rats. *ASJ: Int J Health, Saf Environ.* **2020**;6:499-505.
- 31- Yan J, Fang L, Zhao Z, Su X, Xi M, Huang Y, Li J, Chang R, Zhang W, Qian Q, Wang Z. Adolescent exposure to tris (1, 3-dichloro-2-propyl) phosphate (TCPP) induces reproductive toxicity in zebrafish through hypothalamic-pituitary-gonadal axis disruption. *Sci Total Environ.* **2024**;953:176096.
- 32- Liu FT, Wu Z, Yan J, Norman RJ, Li R. The potential role of growth hormone on the endometrium in assisted reproductive technology. *Front Endocrinol.* **2020**;11:49.
- 33- 33. Yu K, Huang ZY, Xu XL, Li J, Fu XW, Deng SL. Estrogen receptor function: impact on the human endometrium. *Front Endocrinol.* **2022**;13:827724.
- 34- Mao R, Hou X, Feng X, Wang R, Fei X, Zhao J, Chen H, Cheng J. Recombinant human luteinizing hormone increases endometrial thickness in women undergoing assisted fertility treatments: a systematic review and meta-analysis. *Front Pharmacol.* **2024**;15:1434625.
- 35- El-Baz MA, Amin AF, Mohany KM. RETRACTED ARTICLE: Exposure to pesticide components causes recurrent pregnancy loss by increasing placental oxidative stress and apoptosis: a case-control study. *Sci Rep.* **2023**;13:9147.
- 36- Zhou YJ, Qiao QF, Wang LQ, Sheng TY, Cui MX, Chen QD, Wang CY, Zhang YX. Toxicity mechanism of peri-implantation pesticide beta-cypermethrin

- exposure on endometrial remodeling in early pregnant mice. *Toxicology*. **2023**;489:153497.
- 37- ElMazoudy RH, Attia AA, AbdElGawad HS. Evaluation of developmental toxicity induced by anticholinesterase insecticide, diazinon in female rats. *Birth Defects Res B Dev Reprod Toxicol*. **2011**;92:534-42.
- 38- Sheffield SR, Lochmiller RL. Effects of field exposure to diazinon on small mammals inhabiting a semienclosed prairie grassland ecosystem. I. Ecological and reproductive effects. *Environ Toxicol Chem*. **2001**;20:284-96.
- 39- Johari H, Shariati M, Abbasi S, Sharifi E, Askari HR. The effects of diazinon on pituitary-gonad axis and ovarian histological changes in rats. *Iran J Reprod Med*. **2010**;8:125-133.
- 40- Davis CP, Garzia NA, Cushing-Haugen K, Terry KL, Chiu YH, Sandoval-Insausti H, Chavarro JE, Missmer SA, Harris HR. Fruit and vegetable consumption, pesticide residue intake from consumption of fruits and vegetables, and risk of uterine fibroids. *F S Sci*. **2023**;4:90-9.
- 41- Taghizadeh SF, Davarynejad G, Asili J, Riahi-Zanjani B, Nemati SH, Karimi G. Chemical composition, antibacterial, antioxidant and cytotoxic evaluation of the essential oil from pistachio (*Pistacia khinjuk*) hull. *Microb Pathog*. **2018**;124:76-81.
- 42- Behmanesh MA, Poormoosavi SM, Mahmoodikouhi A, Najafzadehvarzi H. Pistacia atlantica's effect on ovary damage and oxidative stress in streptozotocin-induced diabetic rats. *JBRA Assist Reprod*. **2021**;25:28.
- 43- Lerro CC, Koutros S, Andreotti G, Friesen MC, Alavanja MC, Blair A, Hoppin JA, Sandler DP, Lubin JH, Ma X, Zhang Y. Organophosphate insecticide use and cancer incidence among spouses of pesticide applicators in the Agricultural Health Study. *Occup Environ Med*. **2015**;72:736-44.