Original Article

Protective Effect of Flaxseed Oil on Diazinon-Induced Cardiotoxicity in Rats in Sub-Chronic Exposure

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Abstract

Background: *Diazinon (DZN), an organophosphate insecticide, exerts various adverse effects on human organs. In this study, we investigated the potential cardiotoxic effects of flaxseed oil (FS oil) on oxidative stress caused by DZN in the heart tissue of rats.*

Methods: *Thirty male rats were divided into five groups: a control group receiving normal saline, an FS oil group administered 200 mg/kg/d of FS oil, a DZN group treated with 70 mg/kg/d of DZN, and two co-treatment groups receiving DZN alongside FS oil at 100 and 200 mg/kg/d. We assessed oxidative stress biomarkers in heart tissue, including malondialdehyde, total oxidant status, catalase, superoxide dismutase, and total thiol content. Heart tissue morphology was analyzed using hematoxylin and eosin staining.*

Results: *DZN significantly increased the levels of malondialdehyde (12.04±1.16) and total oxidant status (0.43±0.01) and also decreased the content of catalase (23.09±1.99), superoxide dismutase (5.52±0.61), and total thiol content (6.31±0.77) in heart tissue. FS-oil + DZN decreased malondialdehyde (10.88±0.31) and total oxidant status (0.41±0.01) and also increased the content of catalase (29.34±1.77), superoxide dismutase (6.64±0.21), and total thiol content (8.20±0.15) in heart tissue. FS oil supplement consumption reversed oxidative stress status in a dose-dependent manner. Furthermore, FS oil ameliorated heart histopathological alterations induced by DZN.*

Conclusion: *Our findings confirmed that DZN induced heart toxicity and that FS oil had protective effects. Additionally, FS oil supplementation conferred protective effects on the heart against toxicity induced by DZN.*

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Keywords: *Diazinon; Flaxseed oil; Cardiotoxicity; Oxidative stress; Rats, sprague-dawley; Malondialdehyde; Superoxide dismutase; Hematoxylin and eosin staining; Heart tissue pathology; Antioxidants*

Introduction

Diazinon (DZN) is well known as a high-consumption organophosphate pesticide across the world to control insects in vegetables, fruits, and crops, domestic animals, and agriculture. Long-term exposure to pesticide residues

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can lead to environmental pollution and acute and chronic toxicity in different tissues of humans and animals.[1] The main mechanism of DZN toxicity in different tissues can occur through the inhibition of the acetylcholinesterase enzyme. Other mechanisms reported in various studies include toxicity in the reproductive, urinary, hepatic, and nervous systems, and also hematological and biochemical changes.[2] In addition to kidney and liver tissues, exposure to DZN in other vital tissues such as the heart, lungs, and brain can induce health risks by oxidative stress production.[3]

Various studies have shown that DZN can increase the generation of reactive oxygen species (ROS), disturb the pro-oxidant and antioxidant balance, and result in oxidative stress toxicity in various tissues (Rahbari A, Nazem H, Fazilati M, Mehri F. Protective effect of resveratrol against sub-acute diazinon-induced oxidative stress in rat kidney. Koomesh 2021;23:794-800). Oxidative stress exerts destructive and harmful effects on the function of the heart, lungs, and brain, including neurotoxicity, paralysis, filtration system disruption, and blood pressure changes, by inducing apoptosis and necrosis and altering the expressions of various genes.[4, 5] The administration of antioxidants was confirmed to be useful in protecting the heart, lungs, and brain from oxidative damage induced by organophosphate.[6]

Flaxseed (FS), also known as linseed, is a significant source of plant lignans from the Linaceae family and contains natural antioxidants in the form of flavonoids. It has various applications in the production of essential oils. Flaxseed (FS) is a rich source of oil, fiber, protein, moisture, ash, and essential fatty acids, particularly α-linolenic acid and omega-3 fatty acids. It also contains various lignans, including secoisolariciresinol diglucoside, matairesinol, isolariciresinol, and pinoresinol. Additionally, flaxseed exhibits protective effects against oxidative damage through its antibacterial, antifungal, antiviral, and antioxidant activities. Consequently, it has become a focal point in diet and disease research due to its numerous health benefits.

Diverse studies have indicated that the omega-3 fatty acids composition of FS oil has immunomodulatory, antidiabetic, and cardioprotective effects.[7, 8] FS oil has been reported to possess probiotic properties, suggesting its potential application in treating heart disease, neurological and hormonal disorders, and cancer.[9, 10] Experimental studies indicate that FS oil may protect against toxicity induced by organophosphate pesticides, demonstrating its protective effects across various tissues.

This study aimed to assess the toxicity effects of DZN induced by oxidative stress on heart tissues and evaluate the protective effects of FS oil in a rat model.

Methods

DZN (Merck Co, 99% purity) was purchased from the Agricultural Research, Education, and Development Organization (AREDO) (Tehran, Iran). FS oil capsules were prepared by Barij Essence Pharmaceutical Company, Iran. All reagents and chemicals used in this study were of analytical grade and sourced from Sigma-Aldrich (USA). Thirty Wistar rats, weighing between 150 to 200 g, were obtained from the Animal Care Center at Hamadan University of Medical Sciences. The rats were housed in plastic cages at a controlled temperature of 21 to 23 ˚C, provided with standard food pellets and water ad libitum, and were quarantined for 4 weeks. All ethical guidelines were adhered to, as approved by specialists at Hamadan University of Medical Sciences (code No. 140104282930). The optimum dose of DZN used in the study was 70 mg/kg, which corresponds to one-fifth of its estimated LD50 of 350 mg/kg, as indicated in previous research.[11, 12] The animals were monitored daily for weight changes, food and water intake, signs of abnormalities, and mortality across different groups. DZN was dissolved in corn oil, while FS oil was dissolved in water. Both substances were administered orally by gavage for 4 weeks, with the control group receiving an equivalent volume of the vehicle.

The rats were randomly allocated into 5 groups (n=6 each): 1) the control group, which received normal saline; 2) the DZN group, which received DZN at a dose of 70 mg/kg, administered orally; 3) the FS oil group, which received FS oil at a dose of 200 mg/kg, administered orally; 4) the DZN + FS oil group, which received a combination of 100 mg/kg FS oil and 70 mg/kg DZN, administered orally; and 5) the DZN + FS oil 200 mg/kg group, which received a combination of 200 mg/kg FS oil and 70 mg/kg DZN, administered orally.

Twenty-four hours after the final dose, the rats were anesthetized using a ketamine/xylazine mixture (8:1, intraperitoneally).[13] The heart tissues from each rat were carefully excised and rinsed in a cold phosphate-buffered saline solution (pH=7.4). Fragments of the tissues were stored at −80 °C for subsequent evaluation of oxidative stress markers, while others were fixed in formalin for histological assessment. Protein content in the various samples was measured using the Bradford method with the Coomassie blue reagent.[14] Lipid peroxidation levels were assessed by measuring malondialdehyde (MDA), the end product of lipid peroxidation. This analysis was conducted according to protocols established in our previous studies, where a red-colored complex is formed through the reaction of MDA with thiobarbituric acid, as set up in the laboratory.[15] The absorbance was measured at 532 nm, and the results were expressed as nmol of MDA per mg of protein. A colorimetric technique using DTNB (2,2-dithionitrobenzoic acid) was employed to assess the total thiol content in heart tissue. This reagent reacts with sulfhydryl groups to form a yellow-colored complex, which exhibits maximum absorption at a wavelength of 412 nm. The procedure was conducted following the methodologies established in our group's previous studies.[16] The findings were expressed as

nmol/mg of protein. Levels of superoxide dismutase (SOD), total oxidant status (TOS), and catalase (CAT) in heart tissues were determined using an assay kit, following the manufacturer's instructions (Carmona Pars Gene, Kerman, Iran). Immediately after the heart tissue was separated, all samples were immersed in a 10% neutral buffered solution for proper fixation. The tissues were then embedded in paraffin and sectioned into 5-µm thick slices, which were stained with hematoxylin and eosin. After staining, the sections were photographed using a digital camera (Nikon E800, Japan) connected to a microscope. Histological changes in each group were assessed by analyzing 5 serial coronal sections at 400x magnification.[17] The SPSS-20 software was utilized for statistical analysis of the data. Findings were reported as mean ± standard error. The Shapiro–Wilk test was conducted to assess the normality of the data distribution in each group, followed by the Tukey post hoc test to determine mean differences between experimental groups. A P value of less than 0.05 was considered statistically significant.

Results

The MDA level in heart tissue samples significantly increased in the DZN group compared with the control group (P<0.012). Treatment with DZN combined with FS oil at a dose of 200 mg/kg resulted in a significant decrease in heart MDA levels compared with the DZN group (P<0.053). Additionally, administration of FS oil at 100 mg/kg reduced MDA levels, although these changes were not statistically significant. These findings are illustrated in Figure 1 and summarized in Table 1.

As shown in Figure 2 and Table 1, a more significant increase was shown in the TOS level in the heart tissues of the DZN group compared with the control group (P<0.051). However, TOS levels in heart tissues were lower in the DZN + FS oil (200 mg/kg) group than in the DZN group (P<0.050). While TOS levels in heart tissues were lower, these changes did not constitute statistical significance. Figure 3 and Table 1 demonstrate that total antioxidant capacity (TAC) in the DZN group was significantly lower than that in the control group (P<0.001). Nonetheless, FS oil remarkably increased the TAC level compared with the DZN group. In the DZN + FS oil (200 and 100 mg/kg) groups, the TAC level was higher than that in the DZN group, but the difference was not statistically significant. Total thiol content was measured in the heart tissues of rats treated with DZN (Figure 4 and Table 1). In the DZN group, total thiol content was lower than that in the control group (P<0.001); nevertheless, FS oil administration after DZN treatment proved to reverse the DZN-induced alterations in total thiol content. Based on the results, total thiol content rose significantly (P<0.010) with the administration of high-dose FS oil.

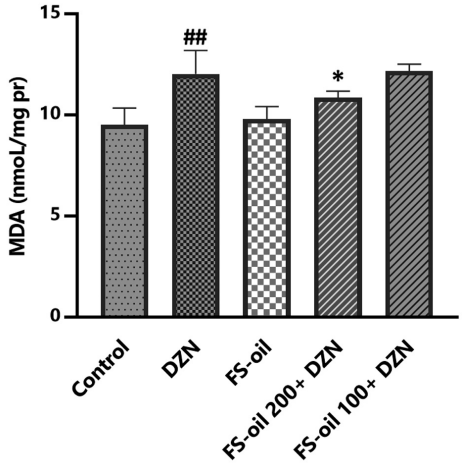

Figure 1. The image illustrates the effects of flaxseed oil (FS-oil) on MDA levels in the heart tissues of rats treated with diazinon (DZN). The data are reported as mean±SEM (n=5).
*P<0.05, ** P<0.01, and *** P<0.001 compared with the control group
# P<0.05, ## P<0.01, and ### P<0.001 compared with the DZN group

According to Figure 5 and Table 1, a significant (P<0.001) reduction in SOD activity in heart tissue was observed in DZN-exposed animals compared with the control group. In contrast, SOD activity was enhanced significantly (P<0.001) in animals that received DZN + FS oil (200 mg/kg) compared with the DZN group. Furthermore, SOD activity in the heart tissues of rats treated with FS oil (100 mg/kg) in a dose-dependent manner rose significantly (P<0.050) compared with the DZN group. As illustrated in Figure 6, histopathological analysis of heart tissue revealed that the group receiving FS oil exhibited myofiber alignment with oval, bright

Table 1. Effects of flaxseed oil (FS-oil) on stress biomarker levels in the heart tissues of rats treated with diazinon (DZN)

	Control	DZN	FS-oil	FS-oil 200+ DZN	FS-oil 100+ DZN	P
MD	9.53±0.81	12.04±1.16	9.82±0.60	10.88±0.31	12.18±0.35	<0.001
TOS	0.27±0.01	0.43±0.01	0.28±0.01	0.41±0.01	0.42±0.01	<0.001
CAT	36.42±0.93	23.09±1.99	37.44±2.41	29.34±1.77	24.57±1.52	<0.001
Thiol	11.04±0.92	6.31±0.77	11.38±0.32	8.20±0.15	7.26±0.11	<0.001
SOD	8.93±0.52	5.52±0.61	9.17±0.23	6.64±0.21	6.08±0.61	<0.001

Data are indicated as the mean ±standard deviation of 5 separate animals in each group.

central nuclei, similar to the control group. In contrast, the DZN group displayed significant abnormalities, including irregular myofibril arrangement, rupture of the myofibril membrane, acidification of the sarcoplasm, displacement and darkening of myofiber cores, congestion, and bleeding. The increased number of fibroblasts observed suggests the presence of fibrosis in the heart tissue.

Administration of FS oil at different doses significantly reduced the irregularity of myofibers, acidification of the sarcoplasm, and other pathological conditions in heart tissues. However, in the group receiving the higher dose of FS oil, an increase in blood presence within the heart tissue was observed.

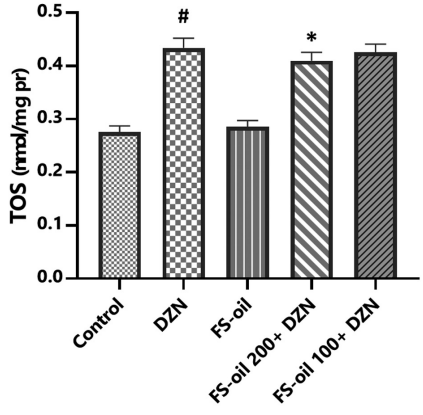

Figure 2. The image depicts the effects of flaxseed oil (FS-oil) on TOS levels in the heart tissues of rats treated with diazinon (DZN). The data are reported based on Mean±SEM (n=5).

*P<0.05, **P<0.01, and ***P<0.001 compared with the control group

#P<0.05, ##P<0.01, and ###P<0.001 compared with DZN group

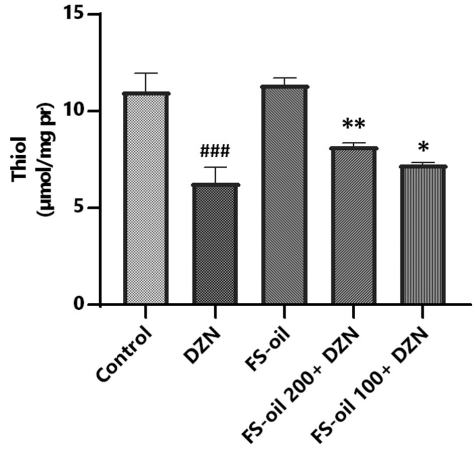

Figure 4. The image depicts the effects of flaxseed oil (FS-oil) on thiol levels in the heart tissues of rats treated with diazinon (DZN). The data are reported as mean±SEM (n=5).

*P<0.05, **P<0.01, and ***P<0.001 compared with the control group

#P<0.05, ##P<0.01, and ###P<0.001 compared with the DZN group

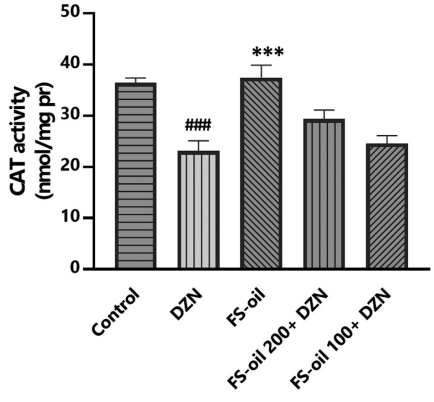

Figure 3. The image illustrates the effects of flaxseed oil (FS-oil) on CAT levels in the heart tissues of rats treated with diazinon (DZN). The data are reported as mean±SEM (n=5).

*P<0.05, **P<0.01, and ***P<0.001 compared with the control group

#P<0.05, ##P<0.01, and ###P<0.001 compared with DZN group

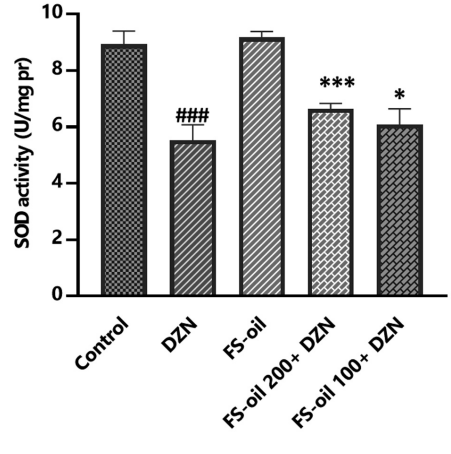

Figure 5. The image demonstrates the effects of flaxseed oil (FS-oil) on SOD levels in the heart tissues of rats treated with diazinon (DZN). The data are reported as mean±SEM (n=5).

*P<0.05, **P<0.01, and ***P<0.001 compared with the control group

#P<0.05, ##P<0.01, and ###P<0.001 compared with the DZN group

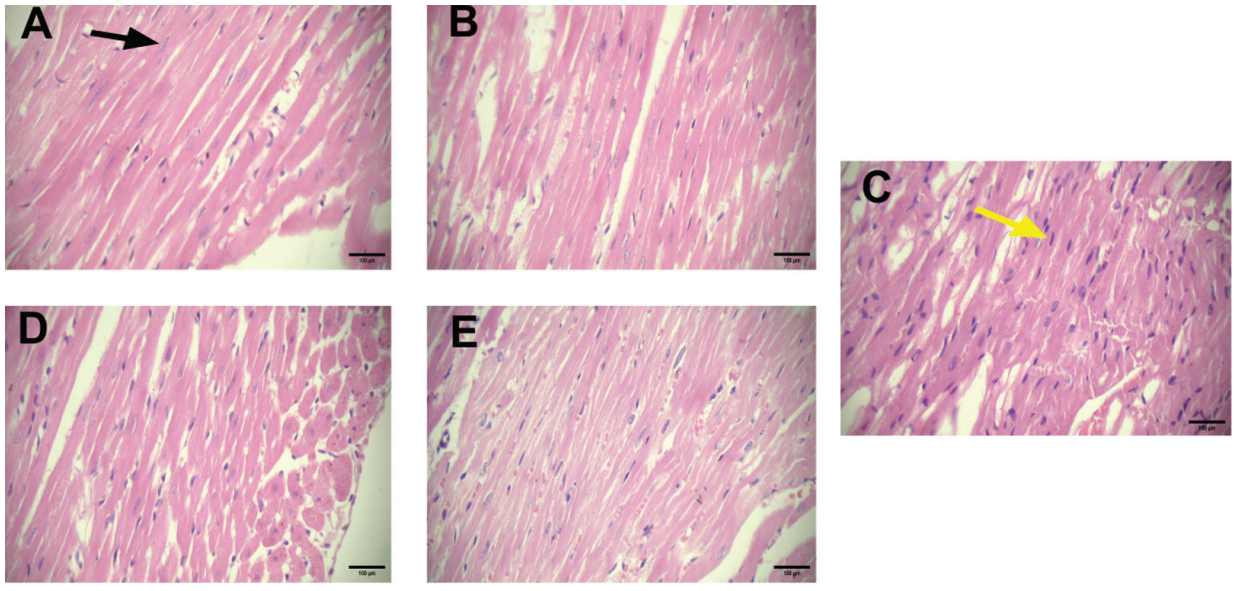

Figure 6. The images depict the effect of flaxseed oil on the histopathological changes in the heart.
Control group (A), flaxseed oil (B), DZN (C), diazinon + flaxseed oil (100 mg/kg) (D), diazinon + flaxseed oil (200 mg/kg) (E) magnification 1000 x, scale bar=20um
The black arrow shows the healthy core of myofibers, and the yellow arrow shows the compression of the core of myofibers.

Discussion

The current study aimed to evaluate the protective effects of FS oil against DZN-induced heart toxicity, focusing on morphological damage and oxidative stress. Results indicated that DZN significantly increased oxidative stress in heart tissue. As anticipated, co-treatment with FS oil at doses of 100 mg/kg and 200 mg/kg reduced heart toxicity in a dose-dependent manner. The mechanism underlying DZN-induced heart injury is complex. While the primary mode of toxicity is the inhibition of the cholinesterase enzyme, numerous studies suggest that oxidative stress and the production of free radicals also play critical roles in DZN toxicity.[18, 19] In stressful situations and the presence of various toxic substances, the balance between antioxidants and ROS is often disrupted. This imbalance can lead to increased ROS production, which can attack sensitive biomolecules, resulting in lipid peroxidation, protein damage, and DNA alterations. Consequently, this oxidative stress can decrease the activity of crucial antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (GPX) in the body.[20, 21]

During the Fenton reaction, the presence of superoxide anions (O_2^-) and iron can generate numerous hydroxyl radicals in the body.[22] These free radicals interact with the lipid structure of the cell membrane and activate the generation of aldehydes such as MDA, which is an important marker of lipid peroxidation.[23] We found that DZN increased MDA and TOS levels, while FS oil administration reduced the levels of these biomarkers, indicating a protective effect against heart injury. These results align with findings from other studies regarding various toxicities induced by organophosphate pesticides.[24, 25]

Razavi et al[27] reported DZN-induced cardiotoxicity as a result of elevated MDA levels in sub-chronic exposure. In our previous study, we detected DZN-induced toxicity subsequent to increased TOS and MDA levels in the kidneys of animals.[3] Regarding the protective effects of FS oil, Chavan et al[26] indicated that FS oil, given its richness in omega-3 fatty acids, had a hepatoprotective effect on DZN-induced toxicity. Hendawi et al[6] concluded that FS had antioxidant activity, as it reversed the effect of thiacloprid on MDA levels. According to our findings, DZN intoxication induced oxidative stress as evidenced by depleted antioxidant defenses (eg, TAC, TTG, and SOD activity), accumulated free radicals, and exhausted antioxidant agents in the body. These observations are in agreement with previously reported findings. For instance, Esfahani et al[3] and El-Shenawy et al[29] presented different antioxidant enzyme activities, such as CAT, TTG, and TAC, as the first line of defense against oxidative stress reduced in liver and kidney tissue treated with DZN. Likewise, Messarah et al[30] concluded that DZN could mitigate antioxidant systems, including TAC, GPX, and CAT, in multiple organs. The mechanism of action related to the SOD enzyme involves the conversion of superoxide anions (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2), which can subsequently be detoxified to water and oxygen by the CAT enzyme.[31] Furthermore, oxidative damage can result in high sensitivity of protein thiols (P–SH).[32] On the other hand, it was found that FS oil remarkably elevated the activities of SOD, total thiol content, and TAC enzymes in animals administered with DZN. It is recommended that the protective effects

of FS oil on heart tissue be mediated by phytoestrogenic lignans and omega-3 essential fatty acids, which, through free radical scavenging and singlet oxygen quenching, can prevent lipid peroxidation of the cell membrane and enzyme leakage.[33] Our results chime with the findings of Naqshbandi et al,[34] who reported FS oil had cardiac protection effects by increasing the activities of SOD, CAT, and GPX. Yang et al[35] concluded that FS oil could preserve glutathione levels in erythrocytes at high glucose levels.

These findings, supported by histopathological examinations of the heart tissue, show irregularity in the arrangement and rupture of the myofibril membrane, acidification of the sarcoplasm, darkening of the myofiber core, and an increase in the number of fibroblasts, indicating fibrosis in heart tissue due to exposure to DZN. It is worth mentioning that the toxic effects of DZN were mitigated by co-administration of FS oil, which may be attributed to the antioxidant protective effects of this combination on the cardiac cell membrane. These results were confirmed by Hana et al,[37] who showed that FS oil co-administration reduced histotoxicity in heart tissue.

Conclusion

Our study indicated the potentially hazardous effects of DZN toxicity in the heart tissue of rats. FS oil ameliorates oxidative stress induced by DZN by scavenging ROS, inhibiting the production of lipid peroxidation, and promoting the development of antioxidant enzymes.

This study had some limitations that should be considered in future research. For DZN-induced heart injury, it should be reported that other possible routes of toxicity exist. More studies could explore the molecular mechanisms involved in the anti-inflammatory and antioxidant effects of FS oil against heart injuries induced by DZN.

Indubitably, further research can provide new insights into the clinical management of DZN-induced heart injuries.

Acknowledgments

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References

1. Rezaee R, Hassanzadeh-Khayyat M, Mehri F, Khashyarmanesh Z, Moallemzadeh H, Karimi G. Determination of parathion, aldicarb, and thiobencarb in tap water and bottled mineral water in Mashhad, Iran. Drug Chem Toxicol 2012;35:192-198.
2. Mehri F, Ranjbar A, Shirafkan N, Asl SS, Esfahani M. The protective effect of resveratrol on diazinon-induced oxidative stress and glucose hemostasis disorder in rats' liver. J Biochem Mol Toxicol 2022;36:e23063.
3. Esfahani M, Rahbar AH, Soleimani Asl S, Mehri F. Resveratrol: a panacea compound for diazinon-induced renal toxicity. Toxin Reviews 2021;42:40-50.
4. Shahper L, Farzana F, Muneer MQ, Hassan S, Hashim R, Ilyas M. Effects of whole-ground flax seeds versus flax oil for pesticide residues-induced nephrotoxicity in male Wistar rats. Khyber Medical University Journal 2020;12:86-94.
5. El-Sharaky A, Newairy A, Eweda S, Kamel M. Bromobenzene-induced hepatotoxicity in male rats: the protective effect of flaxseed. Toxicological & Environmental Chemistry 2012;94:1000-1013.
6. Hendawi MY, Alam RT, Abdellatief SA. Ameliorative effect of flaxseed oil against thiacloprid-induced toxicity in rats: hematological, biochemical, and histopathological study. Environ Sci Pollut Res Int 2016;23:11855-11863.
7. Lee P, Prasad K. Effects of flaxseed oil on serum lipids and atherosclerosis in hypercholesterolemic rabbits. J Cardiovasc Pharmacol Ther 2003;8:227-235.
8. Newairy AS, Abdou HM. Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food Chem Toxicol 2009;47:813-818.
9. Wang Y, Li D, Wang LJ, Chiu YL, Chen XD, Mao ZH, et al. Optimization of extrusion of flaxseeds for in vitro protein digestibility analysis using response surface methodology. Journal of Food Engineering [Internet] 2008;85:59-64.
10. Zhang Z-S, Wang L-J, Li D, Jiao S-S, Chen XD, Mao Z-H. Ultrasound-assisted extraction of oil from flaxseed. Separation and Purification Technology 2008;62:192-198.
11. Mehri F, Ranjbar A, Shirafkan N, Asl SS, Esfahani M. The protective effect of resveratrol on diazinon-induced oxidative stress and glucose hemostasis disorder in rats' liver. J Biochem Mol Toxicol 2022;36:e23063.
12. Kamali M, Johari H, Hami J. Protective Effect of Flax Seed on Brain Teratogenicity Induced by Lamotrigine in Rat Fetuses. Folia Med (Plovdiv) 2020;62:372-377.
13. Makhdoomi S, Mahboobian MM, Haddadi R, Komaki A, Mohammadi M. Silibinin-loaded nanostructured lipid carriers (NLCs) ameliorated cognitive deficits and oxidative damages in aluminum chloride-induced neurotoxicity in male mice. Toxicology 2022;477:153260.
14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-254.
15. Ranjbar A, Mohsenzadeh F, Chehregani A, Khajavi F, Zijoud SM, Ghasemi H. Ameliorative effect of Matricaria chamomilla .L on paraquat: Induced oxidative damage in lung rats. Pharmacognosy Res 2014;6:199-203.
16. Hosseini A, Rasaie D, Soleymani Asl S, Nili Ahmadabadi A, Ranjbar A. Evaluation of the protective effects of curcumin and nanocurcumin against lung injury induced by sub-acute exposure to paraquat in rats. Toxin Reviews 2019;40:1233-1241.
17. Chen JL, Dai L, Zhang P, Chen W, Cai GS, Qi XW, Hu MZ, Du B, Pang QF. Methylene blue attenuates acute liver injury induced by paraquat in rats. Int Immunopharmacol 2015;28:808-812.
18. Shah MD, Iqbal M. Diazinon-induced oxidative stress and renal dysfunction in rats. Food Chem Toxicol 2010;48:3345-3353.
19. Hariri AT, Moallem SA, Mahmoudi M, Memar B, Hosseinzadeh H. Sub-acute effects of diazinon on biochemical indices and specific biomarkers in rats: protective effects of crocin and safranal. Food Chem Toxicol 2010;48:2803-2808.
20. Sams C, Cocker J, Lennard MS. Biotransformation of chlorpyrifos and diazinon by human liver microsomes and recombinant human cytochrome P450s (CYP). Xenobiotica 2004;34:861–873.
21. Buyukokuroglu ME, Cemek M, Yurumez Y, Yavuz Y, Aslan A. Antioxidative role of melatonin in organophosphate toxicity in rats. Cell Biol Toxicol 2008;24:151-158.
22. Blanco-Ayala T, Andérica-Romero AC, Pedraza-Chaverri J. New

insights into antioxidant strategies against paraquat toxicity. Free Radic Res 2014;48:623-640.
23. Yang W, Liu W, Yu W, Fei D, Meng X, Yang S, Meng S, Zhao M. Angptl2 deficiency attenuates paraquat (PQ)-induced lung injury in mice by altering inflammation, oxidative stress and fibrosis through NF-κB pathway. Biochem Biophys Res Commun 2018;503:94-101.
24. Youness ER, Agha FE, El-Toukhy SE, El-Naggar SM, Selim AA, Ibrahim AM. The protective effect of orange juice on glyphosate toxicity in adult male mice. J Chem Pharm Res 2016;8:13-28.
25. Cavuşoğlu K, Yapar K, Oruç E, Yalçın E. Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice. J Med Food 2011;14:1263-1272.
26. Razavi BM, Hosseinzadeh H, Movassaghi AR, Imenshahidi M, Abnous K. Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure. Chem Biol Interact 2013;203:547-555.
27. Chavan T, Khadke S, Harke S, Ghadge A, Karandikar M, Pandit V, Ranjekar P, Kulkarni O, Kuvalekar A. Hepatoprotective effect of polyunsaturated fatty acids against repeated subacute acetaminophen dosing in rats. Int J Pharm Bio Sci 2013;4:286-295.
28. El-Shenawy NS, El-Salmy F, Al-Eisa RA, El-Ahmary B. Ameliorative effect of vitamin E on organophosphorus insecticide diazinon-induced oxidative stress in mice liver. Pesticide Biochemistry and Physiology 2010;96:101-107.
29. Messarah M, Amamra W, Boumendjel A, Barkat L, Bouasla I, Abdennour C, Boulakoud MS, Feki AE. Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes. Toxicol Ind Health 2013;29:77-88.
30. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov 2021;20:689-709.
31. Ghiasian M, Khamisabadi F, Kheiripour N, Karami M, Haddadi R, Ghaleiha A, Taghvaei B, Oliaie SS, Salehi M, Samadi P, Ranjbar A. Effects of crocin in reducing DNA damage, inflammation, and oxidative stress in multiple sclerosis patients: A double-blind, randomized, and placebo-controlled trial. J Biochem Mol Toxicol 2019;33:e22410.
32. Bhatia AL, Sharma A, Patni S, Sharma AL. Prophylactic effect of flaxseed oil against radiation-induced hepatotoxicity in mice. Phytother Res 2007;21:852-859.
33. Naqshbandi A, Khan W, Rizwan S, Khan F. Studies on the protective effect of flaxseed oil on cisplatin-induced hepatotoxicity. Hum Exp Toxicol 2012;31:364-375.
34. Yang W, Fu J, Yu M, Huang Q, Wang D, Xu J, Deng Q, Yao P, Huang F, Liu L. Effects of flaxseed oil on anti-oxidative system and membrane deformation of human peripheral blood erythrocytes in high glucose level. Lipids Health Dis 2012;11:88.
35. Hana RS, Saeed N. Alteration in oxidants, antioxidants and cytokines levels in blood of malathion exposed human and animal groups and the effect of flaxseed oil in alleviating malathion toxic effects. European Journal of Biotechnology and Bioscience 2013;1:08-19.