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Evaluation of the Histopathological Changes Accompanied for the Toxic Effects of Diazinon on the Spleen of Mice

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ABSTRACT

This study was carried out to determine the histopathological changes caused by diazinon in the spleen of Swiss albino mice. Experimental groups containing low dose (30 mg/kg), medium dose (60 mg/kg) and high dose (120 mg/kg) were exposed to diazinon through oral administration for 30 consecutive days. Separation and hemorrhage in the capsule, congestion, enlarged white pulp, amyloid formation, and karyolysis in some megakaryocytes were determined in the splenic parenchyma of the low dose group. An increase in the number of enlarged white pulps, hemorrhage within splenic parenchyma, accumulation of cells into dilated sinusoids and amyloid formation were examined in the medium dose group. Some cells passing from the splenic parenchyma into dilated sinusoids were also observed. Intensive congestion, necrotic areas within spleen tissue, an increase in the number of karyolytic megakaryocytes, fibrosis and some cells passing from the splenic parenchyma into enlarged sinusoids were prominent histological lesions in the high dose group. These results showed that diazinon caused severe dose-related histopathological damages and had the capacity to disrupt functions of the spleen.

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ÖZET

Bu çalışma, diazinonun İsviçre albino farelerinin dalağında oluşturduğu histopatolojik değişiklikleri belirlemek amacıyla yapıldı. Düşük doz (30 mg/kg), orta doz (60 mg/kg) ve yüksek doz (120 mg/kg) içeren deney grupları, ardışık 30 gün boyunca oral yoldan diazinona maruz bırakıldı. Düşük doz grubunun dalak parankiminde kapsülde ayrılma ve kanama, tıkanıklık, beyaz pulpa büyümesi, amiloid oluşumu ve bazı megakaryositlerde karyoliz tespit edildi. Orta doz grubunda genişlemiş beyaz pulpa sayısında artış, dalak parankiminde kanama, hücrelerin genişlemiş sinüzoidlerde birikmesi ve amiloid oluşumu incelendi. Dalak parankiminden dilate sinüzoidlere geçen bazı hücreler de gözlendi. Yoğun konjesyon, dalak dokusunda nekrotik alanlar, karyolitik megakaryosit sayısında artış, fibrozis ve dalak parankiminden büyümüş sinüzoidlere geçen bazı hücreler yüksek doz grubundaki belirgin histolojik lezyonlardı. Bu sonuçlar diazinonun doza bağlı ciddi histopatolojik hasarlara neden olduğunu ve dalağın fonksiyonlarını bozma kapasitesine sahip olduğunu gösterdi.

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INTRODUCTION

Environmental pollution caused by pesticide residues as a result of their intensive use in agriculture and industry is a major concern. Overuse of pesticides has led to an increased risk of environmental pollution, detrimental effects on food security, biodiversity, and water sources (Carvalho, 2006; Queyrel et al., 2016). Organophosphorus pesticides have been extensively used all over the world. These pesticides have adverse effects on non-target organisms including humans (Pundir & Malik, 2019). They were reported to cause harmful impacts on mammalian organs such as liver and kidney (Çakıcı & Akat, 2013) and heart (Öğütçü, 2006). Moreover, deaths of over 100.000 birds worldwide were attributed to organophosphate insecticide monocrotophos (Hooper et al., 2002). Organophosphorus pesticides cause toxic effects by irreversible inhibition of acetylcholinesterase at the cholinergic synapses in the nervous system (Timbrell, 2001), leading to accumulation of acetylcholine at the nerve terminals and neuromuscular junctions which resulted in respiratory failure and death (Krieger, 2010).

Diazinon is one of the most widely used organophosphorus insecticides to control insects in crops, lawns, fruit, and vegetables (Marete et al., 2020). It was determined in groundwater, drinking water wells, soils, fruits, crops, and even human sera (Aggarwal, 2013; Jafari et al., 2018). Diazinon causes the inhibition of acetylcholinesterase in the nervous system of humans (Zhang et al., 2010). In addition to its neurotoxic effect, diazinon induces vascular toxicity (Razavi, 2013), oxidative stress (Hernández-Moreno et al., 2018) and cardiotoxicity (Danaei et al., 2019). It also disrupts cytochrome P450 enzymes in the human liver and can bind to human serum albumin through the mainly hydrophobic interactions, and hydrogen bond (Sams et al., 2004; Jafari et al., 2018). A report from the U.S. Environmental Protection Agency noted the existence of diazinon metabolites in the urine of children (Egeghy et al., 2011). Furthermore, diazinon induces DNA or chromosomal damage in rodent and human cells in vitro (Guyton et al., 2015). Although diazinon has low persistence in the environment, it is a nonspecific insecticide and highly toxic to animals and humans. Its major degradation products are hydroxy diazinon, diazoxon, hydroxy diazoxon and 2-isopropyl-6-methyl-4- pyrimidinol, which may cause acute toxic effects to workers employed in the manufacture and application of this pesticide. Burgess et al. (2008) reported that different physiological symptoms such as headache, abdominal cramps, difficulty in breathing, and even death can result from acute diazinon exposure in human (Kouloumbos et al., 2003; Čolović et al., 2010).

The spleen is the biggest and most important secondary lymphoid organ closely associated with the circulatory system. It is responsible for initiating immune response, filtering the foreign substances from blood, removing old or damaged erythrocytes and reutilization of iron of hemoglobin (Mebius & Kraal, 2005; Mescher, 2016). The spleen has a prominent function in hematopoiesis particularly throughout fetal life in humans. However, it actively contributes to blood cell production during the lifespan of mice (Sieff & Williams, 1995). While the marrow compartment alone is the major lifelong blood-forming organ in humans, both the spleen and bone marrow are the primary site of blood cell production throughout the postnatal life in mice (Palis & Yoder, 2001). Neishabouri et al. (2007) reported toxic effects of diazinon on some internal organs, including spleen of mice. Zeinali et al. (2022) investigated the potential protective impact of chyrsin against immunotoxicity induced by diazinon. In general, these studies mentioned limited histopathologic effects of diazinon. To that end, we aimed to study detailed histopathological impacts of diazinon on the spleen of Swiss albino mice. Therefore, the current study will provide a better understanding of the possible side effects of diazinon on other nontarget organisms including humans.

MATERIAL and METHOD

Experimental Animals and Design

The mean weight range of the mice was 25-30 g. Body weight was measured weekly. After 15 days of acclimatization, the mice were randomly classified into a control group or diazinon-treated group, each containing 10 mice which were maintained with same sex in cages. Experimental groups contained low dose (30 mg/kg), medium dose (60 mg/kg), and high dose (120 mg/kg). Diazinon (purity 99%) was supplied by AgroBest Grup (Izmir, Turkey). All experimental groups were kept under standard laboratory circumstances at 12-h dark/light cycles, 45±5% relative humidity, and 22±3°C temperature. The control group received normal laboratory chow. Experimental mice were fed daily with laboratory chow mixed with diazinon for 30 consecutive days. Animals were given standard laboratory chow and tap water ad libitum. There was no death in any diazinon-treated group during these experiments. All animal care and experimental procedures were approved by Ege University Animal Experiments Ethics Committee (permit no: 2011-047). The animals were treated humanely and with regard for alleviation of suffering.

Histopathological Analyses

After 30 days following exposure to diazinon, the mice

were euthanized under ether anesthesia. Spleens of the experimental mice were quickly removed and fixed in Bouin's solution for 24 hours. They were then transferred in ethanol (70%, 96%, 100%) for dehydration and put into xylol for transparency. Thereafter, the samples were embedded in paraffin following standard histological methods. Processing was manual and 5µm-thickness deparaffinized sections were stained with Harris hematoxylin-eosin (H&E, Sigma Aldrich, St Louis, Mo, USA) to demonstrate the general morphology of the tissue. After the stain with Periodic acid Schiff (PAS, Sigma Aldrich, St Louis, Mo, USA), counterstaining was carried out with H&E. PAS staining showed severe fibrosis in the splenic parenchyma. They were examined and photographed with a Zeiss Axioscope light microscope connected to an AxioCam Erc5S digital camera (Carl Zeiss, Oberkochen, Germany).

RESULTS

The present study was designed to determine harmful effects of diazinon on the splenic parenchyma of mice. Based on our results, there was no difference between male and female mice. Light microscopic observations showed that diazinon caused dose-related destructive changes in the spleen such as congestion, enlarged white pulp, amyloid formation, karyolysis in megakaryocytes, sinusoidal dilation, accumulation of cells in enlarged sinusoids, necrotic areas, and fibrosis.

Control Group

The spleen is mainly composed of two functional parts called the white and red pulps. The organ was mainly composed of the red pulp which was clearly distinguishable from the white pulp. In the splenic parenchyma, the splenic artery was divided into small arterioles that constituted the central arterioles. The central arteriole was enveloped by lymphoid tissue forming the splenic white pulp. Lymphocytes were predominant in the white pulp surrounded by the red pulp. Numerous sinusoids were observed in the splenic tissue (Fig. 1a). Erythrocytes, macrophages, and megakaryocytes were also present in the red pulp (Fig. 1b).

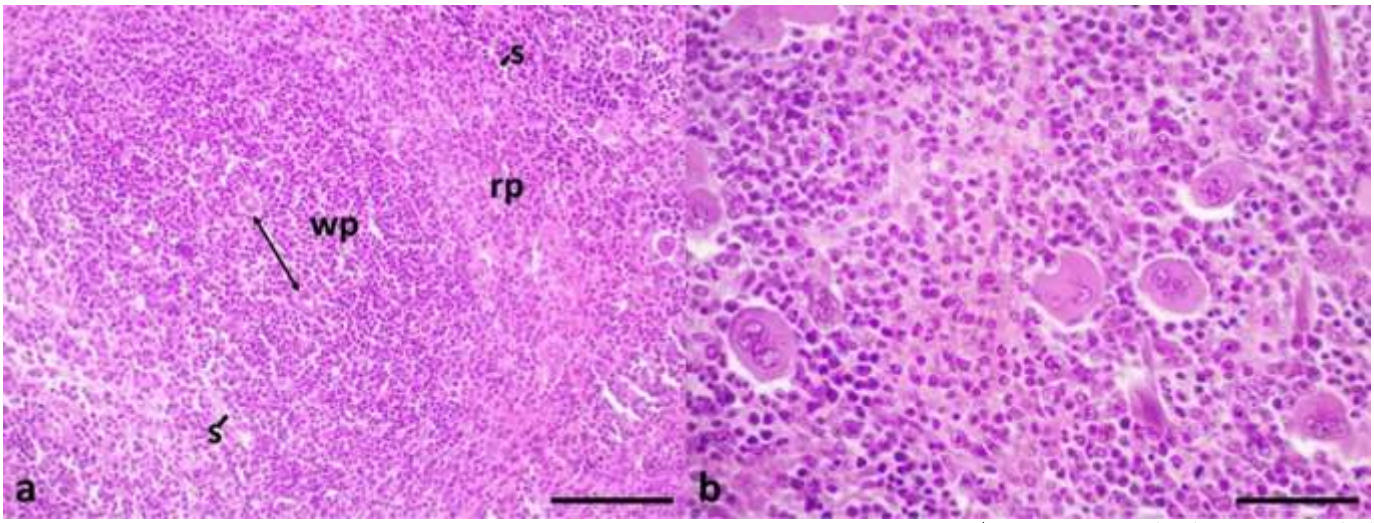

Figure 1. Light microscopic view of spleen in the control group after 30 days, **a)** White pulp (wp), red pulp (rp), central arterioles (arrows) in white pulp, sinusoids (s), scale bar: 100 µm, **b)** Detailed appearance of megakaryocytes, scale bar: 50 µm, H&E: Hematoxylin and Eosin

Şekil 1. *30 gün sonra kontrol grubundaki dalağın ışık mikroskobu görünümü,* ***a)*** *Beyaz pulpa (wp), kırmızı pulpa (rp), beyaz pulpada merkezi arteriyoller (oklar), sinüzoidler (s), ölçek: 100 µm,* ***b)*** *Megakaryositlerin ayrıntılı görünümü, ölçek: 50 µm, H&E: Hematoxylin ve Eosin*

Low Dose: 30 mg/kg diazinon exposed group

Separation and hemorrhage in the splenic capsule were determined in the low dose group. Congestion within the splenic parenchyma was also detected when compared with the control group (Fig. 2a). Enlarged white pulps (Fig. 2b) and amyloid formation in dilated sinusoids were important findings (Fig. 2c). Additionally, karyolysis in some megakaryocytes was examined (Fig. 2d).

Medium dose: 60 mg/kg diazinon exposed group

In the medium dose group, an increase in the number of enlarged white pulps was observed (Fig. 3a). Hemorrhage within the splenic parenchyma and accumulation cells in dilated sinusoids were also detected (Fig. 3b). Amyloid formation and some cells passing from the splenic parenchyma into dilated sinusoids were also determined (Fig. 3c).

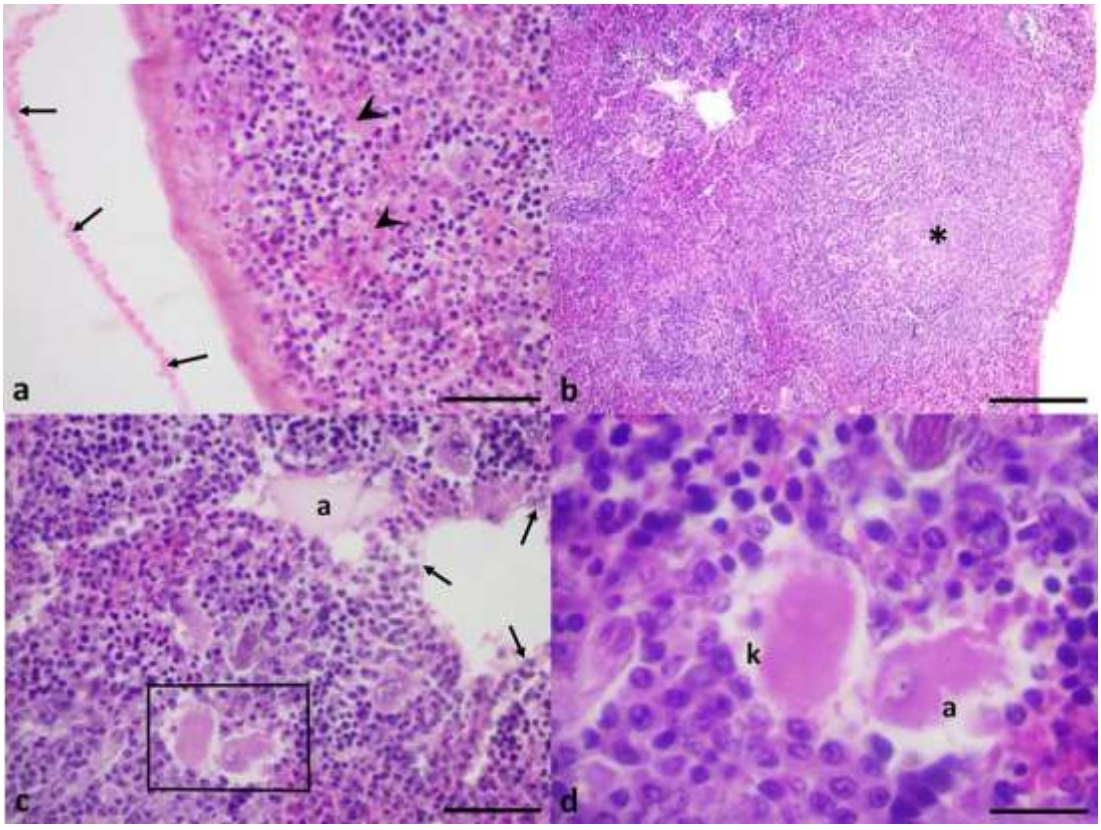

Figure 2. Light microscopic view of spleen in the low dose group after 30 days of diazinon exposure, **a)** Hemorrhage (arrow) in separated capsule. Congestion (arrowheads) within the splenic parenchyma, scale bar: 50 µm, **b)** Enlarged white pulp (asterisk), scale bar: 200 µm, **c)** Amyloid formation in dilated sinusoid (a) and enlarged sinusoid (arrow), scale bar: 50 µm **d)** Karyolysis (k) and amorphous (a) in megakaryocytes, scale bar: 20 µm, H&E: Hematoxylin and Eosin

Şekil 2. *30 günlük diazinon maruziyetinden sonra düşük doz grubunda dalağın ışık mikroskobu görünümü,* ***a)*** *Ayrılmış kapsülde kanama (ok). Dalak parankiminde konjesyon (ok başları), ölçek: 50 µm,* ***b)*** *Genişlemiş beyaz pulpa (yıldız), ölçek: 200 µm,* ***c)*** *Genişlemiş sinüzoidde amiloid (a) oluşumu ve genişlemiş sinüzoid (ok), ölçek: 50 µm,* ***d)*** *Karyoliz (k) ve megakaryositlerde şekilsizlik (a), ölçek: 20 µm, H&E: Hematoxylin ve Eosin*

High dose: 120 mg/kg diazinon exposed group

In the high dose group, intensive congestion (Fig. 4a) and necrotic areas within the splenic parenchyma (Fig. 4b) were detected. An increase in the number of karyolytic megakaryocytes was clearly examined (Fig. 4c). Moreover, fibrosis and some cells passing from spleen tissue into enlarged sinusoids were observed (Fig. 4d).

All histopathological changes were presented in Table 1. Evaluation of histopathological defects were made in a blinded manner by two persons.

Table 1. Histological alterations on the spleen of mice after exposure to diazinon (30, 60 and 120 mg/kg)
Çizelge 1. *Diazinona maruziyet sonrasında farelerin dalağındaki histolojik değişiklikler (30, 60 and 120 mg/kg)*

Tissue	Histopathological changes	Control	30 mg/kg	60 mg/kg	120 mg/kg
Spleen	Congestion	0	++	++	+++
	Separation of capsule	0	+++	0	0
	Dilated sinusoids	0	++	+++	++
	Enlarged white pulp	0	++	+++	0
	Karyolysis in megakaryocytes	0	+	+	+++
	Amyloid	0	++	++	0
	Cells in dilated sinusoids	0	0	+++	+
	Fibrosis	0	0	0	+++
	Necrotic areas	0	0	0	++

Note: Histopathological defects were presented based on their severity (None=0; Mild=+; Moderate=++; Severe=+++)
Not: *Histopatolojik hasarlar ciddiyetlerine göre belirtildi (Hiç=0; Zayıf=+; Orta=++; Şiddetli=+++)*

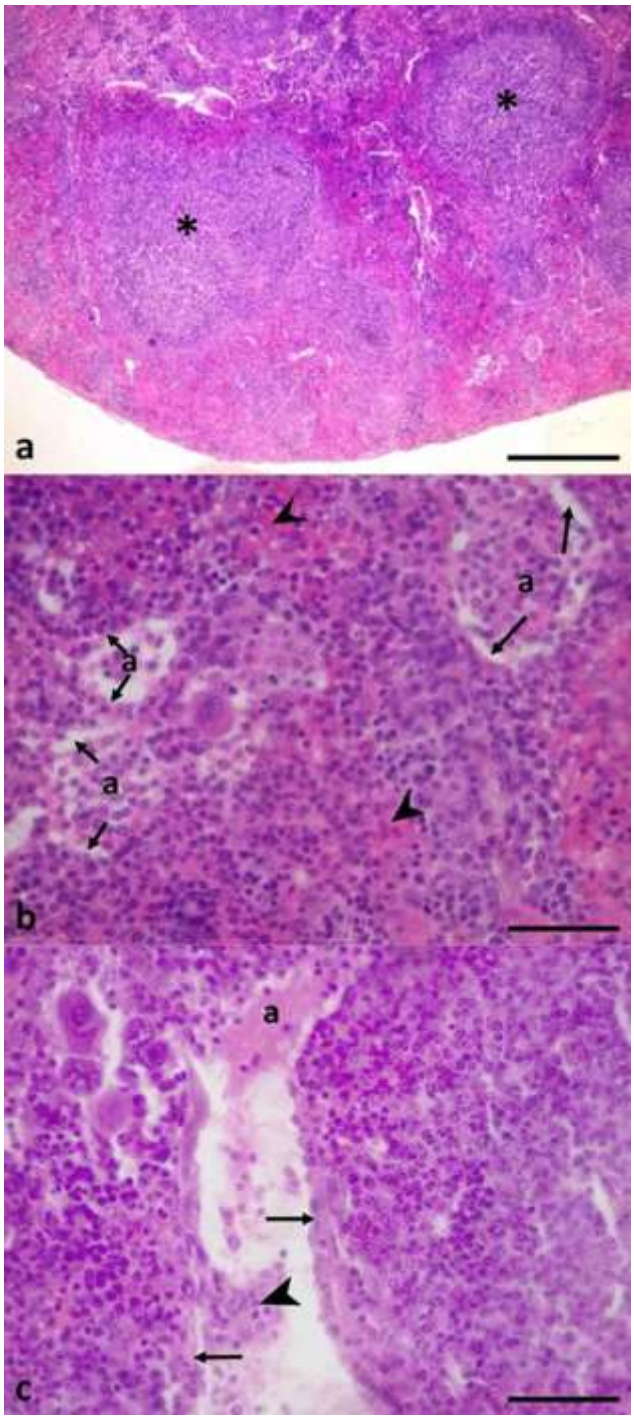

Figure 3. Light microscopic view of spleen in the medium dose group after 30 days of diazinon exposure, **a)** Enlarged white pulp (asterisk), scale bar: 400 µm, **b)** Congestion within splenic parenchyma (arrowheads) and accumulation cells (a) in dilated sinusoids (arrows), scale bar: 50 µm, **c)** Amyloid formation (a) and some cells (arrowhead) passing from spleen parenchyma into dilated sinusoids (arrows); scale bar: 50 µm, H&E: Hematoxylin and Eosin

Şekil 3. *30 günlük diazinon maruziyetinden sonra orta doz grubunda dalağın ışık mikroskobu görünümü,* ***a)*** *Genişlemiş beyaz pulpa (yıldız), ölçek: 400 µm,* ***b)*** *Dalak parankimasında konjesyon (ok başları) ve genişlemiş sinüzoidlerde hücrelerin birikimi (a), ölçek: 50 µm,* ***c)*** *Amiloid oluşumu (a) ve dalak parankimasından genişlemiş sinüzoidlere (oklar) geçen bazı hücreler (ok başı); ölçek: 50 µm, H&E: Hematoxylin ve Eosin*

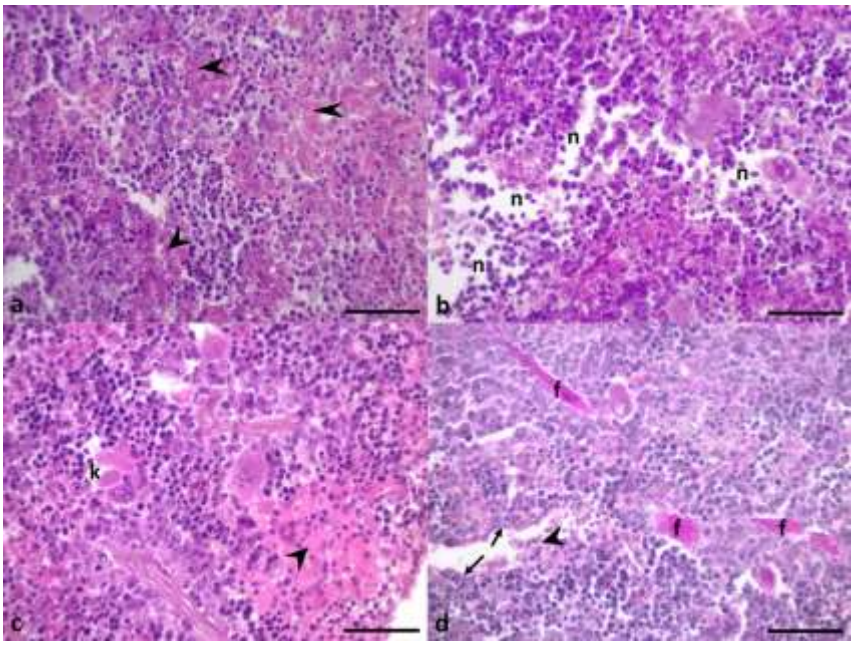

Figure 4. Light microscopic view of spleen in high dose group after 30 days of diazinon exposure, **a)** Intensive congestion within the splenic parenchyma (arrowheads), **b)** Wide necrotic areas (n), **c)** Karyolytic (k) megakaryocytes and congestion (arrowhead) in splenic parenchyma; H&E: Hematoxylin and Eosin, **d)** Fibrosis (f) and some cells (arrowhead) passing from spleen parenchyma into enlarged sinusoids (arrows); scale bars: 50 µm, PAS: Periodic Acid Schiff

Şekil 4. *30 günlük diazinon maruziyetinden sonra orta doz grubunda dalağın ışık mikroskobu görünümü,* ***a)*** *Dalak parankiminde yoğun konjesyon (ok başları),* ***b)*** *Geniş nekrotik alanlar (n),* ***c)*** *Karyolitik (k) megakaryositler ve dalak parankimasında konjesyon (ok başı); H&E: Hematoxylin ve Eosin,* ***d)*** *Fibrözis (f) ve dalak parankimasından genişlemiş sinüzoidlere (oklar) geçen bazı hücreler (ok başları); ölçekler: 50 µm, PAS: Periodic Acid Schiff*

DISCUSSION

The spleen, which is the largest lymphoid organ in the body, acts a prominent role in the defense mechanisms of organisms. Additionally, it serves as a blood cell reservoir and performs the destruction of old or damaged erythrocytes. It also acts to eliminate abnormal neutrophils and platelets, and it is a phagocytic filter to remove bacteria. Abnormality in this role, e.g. hypersplenism, results in an increase in the number of abnormal neutrophils, erythrocytes, platelets or any composition of these blood factors (Podder et al., 2010).

In the spleen there are two main functional parts, the red and white pulps. The localization of white pulp is around central arterioles, and it consists of the periarteriolar lymphoid sheath (PALS), primary or secondary follicles and marginal zone. PALS is known as the T-cell region. The localization of splenic B cells are mainly primary or secondary follicles adjacent to the PALS. The adjacent follicles and PALS are surrounded by a marginal zone that contains a mixture of cell types, and the junction between the marginal zone and the red pulp is not always apparent (Elmore, 2006). The spleen acts a vital role in hematopoiesis throughout the life of mice (Sieff & Williams, 1995). Therefore, many megakaryocytes were observed in the histological section of the spleen in both control and treatment groups in this study. Megakaryocytes are giant polyploid cells predominantly scattered in the bone marrow typically near sinusoidal capillaries. Their polyploid nuclei are large and irregularly lobulated with coarse chromatin. Myeloid stem cells give rise to megakaryocytes. Thrombopoietin, a humoral factor formed by the liver, is known to arrange megakaryocytes and the production of platelets (Mescher, 2016). Hematopoiesis is the dynamic process whereby all formed elements of the blood arise from the multipotent precursor cells produced by hematopoietic stem cells. While hematopoiesis in the adult human occurs primarily within the red bone marrow, several other tissues including yolk sac, liver, and spleen serve as primary sites of hematopoiesis during mammalian embryogenesis (Tavassoli, 1991; Zon, 1995). Both the spleen and bone marrow serve as the primary sites of blood cell production throughout the life of the postnatal mouse. On the other hand, bone marrow is the major lifelong blood-forming organ in humans (Sieff & Williams, 1995).

Due to fact that reports related to the negative impacts of pesticides on mammalian splenic tissue

are limited and the spleen is the site of direct and indirect toxicity, the current work was performed to elucidate the harmful impacts of diazinon on the splenic parenchyma of Swiss albino mice. Therefore, the results of this study can only be discussed with a few other searches. Separation and hemorrhage in the capsule and congestion within the splenic parenchyma were determined in the low dose group. After exposure to the aromatic amine 3,4-dichloroaniline (herbicide), a dose-related enlargement of splenic red pulp was described with prominent vascular congestion and increased red pulp cellularity in a fish species, *Pomatoschistus microps* (Monteiro et al., 2006). In our study, enlarged white pulp and amyloid formation in dilated sinusoids were important findings in the low dose group. Lovat et al. (2015) reported that mouse models of Non-Hodgkin Lymphomas showed splenomegaly with enlargement of the white pulp secondary to expansion/accumulation of B lymphocytes. Neishabouri et al. (2007) investigated some toxic effects of diazinon on mice internal organs. Authors found that the ratio of spleen red pulp to white pulp demonstrated an important increase. In addition, diazinon caused white pulp atrophy with capsular and trabecular damages in the spleen.

In the medium dose group, congestion within the splenic parenchyma of Swiss albino mice was observed. Similar observations were noticed by Mohany et al. (2011) who reported that imidacloprid insecticide caused congestion in the red pulp of the spleen of male albino rats. We also examined accumulation of cells in dilated sinusoids, amyloid formation, and some passing cells from splenic cords into dilated sinusoids, an increase in the number of enlarged white pulps in the medium dose group. Korani et al. (2011) noted hypertrophic white pulps after exposure to $AgNO_3$. The white pulp of mice also revealed hypertrophy after being exposed to zinc oxide, cyclodextrin and cefepime (Oprea et al., 2016). Dkhil (2009) reported that there was an apparent enlargement of the white pulp because of cellular proliferation. Handy et al. (2002) mentioned the diazinon effects on some organs of mice. However, they did not present detailed findings on these organs including spleen. They reported the hyperplasia of white and red pulps and hemorrhage after exposure to diazinon. In this study, intensive congestion and some cells passing from spleen tissue into enlarged sinusoids were observed in the high dose group. An increase in the number of karyolytic megakaryocytes were also examined. Studies of diazinon's mutagenicity showed that it can damage DNA in human blood cells and cells from laboratory animals (Grover et al., 2003). Moreover, disintegration of the splenic tissue and fibrosis were observed. Similar lesions were determined in the spleen of rats exposed to Bisphenol A (Ahmed et al., 2015) and Levantine frog, *Pelophylax bedriagae* exposed to Carbaryl (Çakıcı, 2018). All these histopathological changes may be attributed to functional disruption of the spleen. Due to the primary role of megakaryocytes in blood cell production, an increase in the number of karyolytic megakaryocytes showed that diazinon has the capacity to hinder blood cell formation. According to Handy et al. (2002), chronic diazinon exposure resulted in necrotic degeneration of the trabeculae in both spleen and thymus of mice. El-Bendary et al. (2014) showed that profenofos and chlorpyrifos caused hepatic and splenic lesions including congestion and hemorrhage in male mice. Due to the splenic necrose, it exhibited a reduction in the number of lymphocytes in the white pulp of the high dose group in this study. On the other hand, Dkhil (2009) reported that it was difficult to differentiate the limit between white and red pulps in the spleen of malaria-infected mice. Similar finding was observed in the high dose group of our study.

CONCLUSION

Diazinon induced considerable histological defects in the splenic tissue of Swiss albino mice. Since there is little research related to the impacts of pesticides on the spleen of mammals, the current research will be useful for other mammalian toxicologic investigations.

Researchers' Contribution Rate Statement

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

Authors declare no conflict of interest.

REFERENCES

Aggarwal, V., Deng, X., Tuli, A., & Goh, K.S. (2013). Diazinon-chemistry and environmental fate: a California perspective. *Reviews of Environmental Contamination and Toxicology 223*, 107-140. https://doi.org/10.1007/978-1-4614-5577-6_5

Ahmed, W.M., Moselhy, W.A., & Nabil, T.M. (2015). Bisphenol A toxicity in adult male rats: hematological, biochemical and histopathological approach. *Global Veterinaria 14*(2), 228-238.

Burgess, P., Harper, C., Todd, G.D., & Wohlers, D. (2008). Toxicological profile for diazinon. https://www.atsdr.cdc.gov/toxprofles/tp86.pdf. (Alınma Tarihi: 20.02.2021)

Carvalho, F.P. (2006). Agriculture, pesticides, food security and food safety. *Environmental Science & Policy 9*(7-8), 685-692. https://doi.org/10.1016/j.envsci.2006.08.002

Čolović, M., Krstić, D., Petrović, S., Leskovac, A., Joksic, G., Savic, J., Franko, M., Trebse, P., &

Vasic, V. (2010). Toxic effects of diazinon and its photodegradation products. *Toxicology Letters 193*(1), 9-18. https://doi.org/10.1016/j.toxlet. 2009.11.022

Çakıcı, Ö. & Akat, E. (2013). Effects of oral exposure to diazinon on mice liver and kidney tissues: biometric analyses of histopathologic changes. *Analytical and Quantitative Cytology and Histology 35*(1), 7-16.

Çakıcı, Ö. (2018). Histopathological analysis of carbaryl-induced toxicity in the spleen of Levantine frog, *Pelophylax bedriagae* (Anura: Ranidae). Environmental Science and Pollution Research *25*(25), 24917-24922. https://doi.org/ 10.1007/s11356-018-2538-5

Danaei, G.H., Memar, B., Ataee, R., & Karami, M. (2019). Protective effect of thymoquinone, the main component of *Nigella sativa*, against diazinon cardio-toxicity in rats. *Drug and Chemical Toxicology 42*(6), 585-591. https://doi.org/10.1080/ 01480545.2018.1454459

Dkhil, M.A. (2009). Apoptotic changes induced in mice splenic tissue due to malaria infection. *Journal of Microbiology, Immunology and Infection 42*, 13-18.

Egeghy, P.P., Cohen Hubal, E.A., Tulve, N.S., Melnyk, L.J., Morgan, M.K., Fortmann, R.C., & Sheldon, L.S. (2011). Review of pesticide urinary biomarker measurements from selected US EPA children's observational exposure studies. *International Journal of Environmental Research and Public Health 8*, 1727-1754. https://doi.org/10.3390/ijerph8051727

El-Bendary, H.M., Shaker, M.H., Saleh, A.A., Negm, S., Khadey, M.E., & Eldeen, F.A. (2014). Histopathological changes associated with exposure of male mice to profenofos and chlorpyrifos. *Annual Research & Review in Biology 4*(5), 766-777. https://doi.org/10.9734/ARRB/ 2014/ 4924

Elmore, S.A. (2006). Enhanced histopathology of the spleen. *Toxicologic Pathology 34*(5), 648-655. https://doi.org/10.1080/01926230600865523

Guyton, K.Z., Loomis, D., Grosse, Y., El-Ghiassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., & Straif, K. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *The Lancet Oncology 114*(2), 70134-70138. https://doi.org/10.1016/S1470-2045(15)70134-8

Grover, P., Danadevi, K., Mahboob, M., Rozati, R., Banu, B.S., & Rahman, M.F. (2003). Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet assay. *Mutagenesis 18*, 201-205. https://doi.org/10.1093/mutage/ 18.2.201

Handy, R.D., Abd-El Samei, H.A., & Bayomy, M.F.F. (2002). Chronic diazinon exposure: pathologies of spleen, thymus, blood cells, and lymph nodesare modulated by dietary protein or lipid in the mouse. *Toxicology 172*, 13-34. https://doi.org/10.1016/ S0300-483X(01)00575-3

Hernández-Moreno, D., Míguez, M.P., Soler, F., & Perez-Lopez, M. (2018). Influence of sex on biomarkers of oxidative stress in the kidney, lungs, and liver of rabbits after exposure to diazinon. *Environmental Science and Pollution Research 25*(32), 32458-32465. https://doi.org/10.1007/ s11356-018-3258-6

Hooper, M.J., Mineau, P., Zaccagnini, M. E., & Woodbridge, B. (2002). Pesticides and international migratory bird conservation. In Handbook of ecotoxicology. CRC Press (pp. 761-778).

Jafari, F., Samadi, S., Nowroozi, A., Sadrjavadi, K., Moradi, S., Ashrafi-Khooshk, M.R., & Shahlaei, M. (2018). Experimental and computational studies on the binding of diazinon to human serum albumin. *Journal of Biomolecular Structure and Dynamics 36*(6), 1490-1510. https://doi.org/ 10.1080/07391102.2017.1329096

Kouloumbos, V.N., Tsipi, D.F., Hiskia, A.E., Nikolic, D., & van Breemen, R.B. (2003). Identification of photocatalytic degradation products of diazinon in TiO2 aqueous suspensions using GC/MS/MS and LC/MS with quadrupole time-of-flight mass spectrometry. *Journal of the American Society for Mass Spectrometry 14*(8), 803-817. https://doi.org/ 10.1016/S1044-0305(03)00333-7

Krieger, R. (2010). Hayes' handbook of pesticide toxicology. Academic press 1435-1478.

Lovat, F., Fassan, M., Gasparini, P, Rizzotto, L., Cascione, L., Pizzi, M., Vicentini, C., Balatti, V., Palmieri, D., Costinean, S., Croce, C.M. (2015). miR-15b/16-2 deletion promotes B-cell malignancies. *Proceedings of the National Academy of Sciences 112*(37), 11636-11641. https://doi.org/10.1073/pnas.1514954112

Marete, G.M., Shikuku, V.O., Lalah, J.O., Mputhia, J., & Wekesa, V.W. (2020). Occurrence of pesticides residues in French beans, tomatoes, and kale in Kenya, and their human health risk indicators. *Environmental Monitoring and Assessment 192*(11), 1-13. https://doi.org/10.1007/ s10661-020-08662-y

Mebius, R.E. & Kraal, G. (2005). Structure and function of the spleen. *Nature Reviews Immunology 5*(8), 606-616. https://doi.org/10.1038/ nri1669

Mescher, A.L. (2016). Junqueira's basic histology: text and atlas. United States: Mcgrawhill 560 p.

Mohany, M., Badr, G., Refaat, I., & El-Feki, M. (2011). Immunological and histological effects of exposure to imidacloprid insecticide in male albino rats. *African Journal of Pharmacy and Pharmacology 5*(18), 2106-2114. https://doi.org/ 10.5897/AJPP11.625

Monteiro, M., Quintaneiro, C., Pastorinho, M., Pereira, M.L., Morgado, F., Guilhermino, L., & Soares, A.M.V.M. (2006). Acute effects of 3, 4-dichloroaniline on biomarkers and spleen histology of the common goby Pomatoschistus microps. *Chemosphere 62*(8), 1333-1339. https://doi.org/ 10.1016/j.chemosphere.2005.07.038

Neishabouri, Z.E., Hassan, Z.M., Azizi, E., & Ostad, S.N. (2007). Evaluation of immunotoxicity induced by diazinon in C57bl/6 mice. *Toxicology* 196, 173-179. https://doi.org/10.1016/j.tox.2003.08.012

Öğütçü, A., Uzunhisarcikli, M., Kalender, S., & Durak, D. (2006). The effects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial cells in rat heart tissue and protective role of vitamin E. *Pesticide Biochemistry and Physiology 86*, 93-98. https://doi.org/10.1016/ j.pestbp.2006.01.010

Queyrel, W., Habets, F., Blanchoud, H., Ripoche, D., & Launay, M. (2016). Pesticide fate modeling in soils with the crop model STICS: Feasibility for assessment of agricultural practices. *Science of The Total Environment 542*, 787-802. https://doi.org/10.1016/j.scitotenv.2015.10.066

Palis, J. & Yoder, M.C. (2001). Yolk-sac hematopoiesis: the first blood cells of mouse and man. *Experimental Hematology 29*(8), 927-936. https://doi.org/10.1016/S0301-472X(01)00669-5

Podder, S., Chattopadhyay, A., Bhattacharya, S., & Ranjan, M. (2010). Histopathology and cell cycle alteration in the spleen of mice from low and high doses of sodium fluoride. *Fluoride 43*(4), 237-245.

Pundir, C.S. & Malik, A. (2019). Bio-sensing of organophosphorus pesticides: A review. Biosensors and Bioelectronics *140*, 111348. https://doi.org/ 10.1016/j.bios.2019.111348

Razavi, B.M., Hosseinzadeh, H., Movassaghi, A.R., Imenshadidi, M., & Abnous, K. (2013). Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure. *Chemico-Biological Interactions 203*(3), 547-555. https://doi.org/10.1016/j.cbi.2013.03.010

Sams, C., Cocker, J., & Lennard, M.S. (2004). Biotransformation of chlorpyrifos and diazinon by human liver microsomes and recombinant human cytochrome P450s (CYP). *Xenobiotica*, *34*(10), 861-873. https://doi.org/10.1080/00498250400017273

Sieff, C.A. & Williams, D.A. (1995). Hematopoiesis. In: Handin RI, Stossel TP, Lux SE, editors. Blood:principles and practice of haematology. Philadelphia: JB Lippincott Company, 171-224.

Tavassoli, M. (1991). Embryonic and fetal hemopoiesis: an overview. *Blood Cells 17*(2), 269-281.

Timbrell, J. (2001). Introduction to toxicology. CRC Press, 115 p.

Oprea, A.E., Pandel, L.M., Dumitrescu, A.M., Andronescu, E., Grumezescu, V., Chifiriuc, M.C., Mogoantă, L., Bălşeanu, T.A., Mogoşanu, G.D., Socol, G., Grumezescu, A.M., Iordache, F., Maniu, H., Chirea, M., & Holban, A.M. (2016). Bioactive ZnO coatings deposited by maple—An appropriate strategy to produce efficient anti-biofilm surfaces. *Molecules 21*(2), 220. https://doi.org/10.3390/ molecules21020220

Zhang,Y., Zhang, W., Liao, X., Zhang, J., Hou, Y., Xiaou, Z., Chen, F., & Hu, X. (2010). Degradation of diazinon in apple juice by ultrasonic treatment. *Ultrasonics Sonochemistry 17*(4), 662-668. https://doi.org/10.1016/j.ultsonch.2009.11.007

Zeinali, M., Shafaei, A., Rafatpanah, H., Mosavat, A., Tayebi-Meybodi, N., Hossainzadeh, H., & Rezaee, S.A. (2022). Potential protective efects of chrysin against immunotoxicity induced by diazinon. *Scientific Reports 12*, 15578. https://doi.org/10. 1038/ s41598-022-20010-3

Zon, L.I. (1995). Developmental biology of hematopoiesis. *Blood 86*(8), 2876-2891. https://doi.org/10.1182/blood.V86.8.2876.2876