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Research Article

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Spleen Toxicity Assessment of Propanil Exposure in Swiss Albino Mice

İsviçre Albino Farelerinde Propanil Maruziyetinin Dalak Toksisitesi Değerlendirmesi

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ABSTRACT

Pesticides or their metabolites may have the capacity to disturb and hinder the functions of some essential organs including spleen. This study examined the side effects of propanil on the spleen of mice. The experimental groups were exposed to propanil through oral administration for 30 consecutive days. The following histopathological changes were observed in the low-dose group: separation of capsule from the splenic parenchyma, thickening of the capsule, congestion in the splenic parenchyma, dilated sinusoids, arteriolar enlargement, karyolysis in megakaryocytes and amyloid formation. In the medium-dose group, the following changes were detected: hemorrhage and separation in the capsule, amorphous megakaryocytes, karyolysis in megakaryocytes, congestion in the splenic tissue, sinusoidal enlargement, fibrosis, lobule formation, enlargement of the white pulp, deformation of arterioles. The high-dose group showed the following changes: hemorrhage in separated capsule, karyolysis in megakaryocytes, congestion in the splenic parenchyma, enlargement of the white pulp, fibrosis, necrosis in the white pulp, congestion in the enlarged sinusoid and cellular swelling in the splenic red pulp. These results clearly demonstrated that propanil induced important dose-related histopathological damages. Based on our results, we can confidently state that it may have the capacity to cause failure of this organ.

Key Words

Mice, spleen, propanil, herbicide, histopathology.

ÖZ

Pestisitler veya metabolitleri, dalak dahil olmak üzere bazı temel organların fonksiyonlarını bozma ve engelleme kapasitesine sahip olabilir. Bu çalışmada, propanilin farelerin dalakları üzerindeki yan etkileri incelenmiştir. Deney grupları, ard arda 30 gün boyunca oral uygulama yoluyla propanile maruz bırakıldı. Düşük doz grubunda kapsülün dalak parankiminden ayrılması, kapsülde kalınlaşma, dalak parankiminde konjesyon, genişlemiş sinüzoidler, arteriyolar genişleme, megakaryositlerde karyoliz ve amiloid oluşumu şeklinde histopatolojik değişiklikler gözlendi. Orta doz grubunda hemoraji ve kapsülde ayrılma, amorf megakaryositler, megakaryositlerde karyoliz, dalak dokusunda konjesyon, sinüzoidal genişleme, fibrozis, lobül oluşumu, beyaz pulpada genişleme, arteriollerde deformasyonu tespit edildi. Yüksek doz grubunda ayrılmış kapsülde kanama, megakaryositlerde karyoliz, dalak parankiminde konjesyon, beyaz pulpada genişleme, fibrozis, beyaz pulpada nekroz, genişlemiş sinüzoidde konjesyon ve dalak kırmızı pulpasında hücresel şişkinlik gibi histopatolojik değişiklikler gözlendi. Bu sonuçlar açıkça propanilin doza bağlı önemli histopatolojik hasarlara neden olduğunu gösterdi. Sonuçlarımıza dayanarak propanilin dalak yetmezliğine yol açabilecek kapasiteye sahip olabileceğini net bir şekilde söyleyebiliriz.

Anahtar Kelimeler

Fare, dalak, propanil, herbisit, histopatoloji.

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Propanil (3′,4′-Dichloropropionanilide) is one of the most excessively used herbicides to control weeds [1] and belongs to the phenylamide class of herbicides. It functions as an inhibitor in photosynthesis by blocking photosynthetic electron transfer in broad-leaf weeds [2]. Herbicide applications in the agricultural industry significantly contribute to an increase in food production and save labor resources [3]. However, in nature, propanil can be converted into 3,4 dichloroaniline (DCA), which undergoes biodegradation at very slow speed [4]. Both propanil and DCA have a wide range of adverse effects. Many studies have indicated that propanil has caused acute toxicity in a variety of aquatic species [5-7]. Some research has also showed that DCA has toxic effects on mammals, fish, and the immune system of humans [4, 8]. This secondary pollutant, DCA, can be transformed into 3,3′,4,4′-tetrachloroazoxybenzene (TCAB) via microbial peroxidases. TCAB is an extremely toxic metabolite, known as a carcinogen and a genotoxin [4, 9].

The spleen is a highly vascularized lymphoid organ covered by a dense connective tissue capsule. It is organized into two regions, the white pulp, an immune component composed of nodules, periarteriolar lymphoid sheath, and marginal zone, and the red pulp, which filters antigens, particulate materials, damaged, and aged erythrocytes, while also serving as a reservoir for erythrocytes and platelets [10, 11]. Mature blood cells have a relatively short lifespan and must be continually replaced by new cells through a process known as hematopoiesis [11]. While the spleen plays a role in hematopoiesis during fetal life in humans, it actively participates in blood cell production throughout the lifespan of mice [12, 13]. In adult humans, pluripotent hematopoietic stem cells and other hematopoietic stem cells are located in the red bone marrow, making it the main blood-forming organ throughout human life [14]. However, during the postnatal life of mice, the spleen and bone marrow cooperate in blood cell production [15].

We aimed to investigate histopathological effects of propanil on the spleen of Swiss albino mice. Since studies related to the adverse effects of pesticides on mammalian spleen are very limited, results of the present study will contribute to understanding potential side effects of propanil on other nontarget organisms as well as humans.

MATERIALS and METHODS

The animal Ethics Committee of Ege University (2011-073) confirmed the current study. The study was carried out on male and female Swiss albino mice (6-8 weeks old, 25-30 g) supplied from the Breeding Center of Experimental Animals in the Ministry of Agriculture and Rural Affairs, Turkey. After acclimation for fifteen days, each mouse was randomly moved into cages including the same sex. Each group (control or propanil-treated) containing 10 mice (5 females and 5 males) was placed in 20x35x15-cm cages. Experimental groups contained low-dose (75 mg/kg), medium-dose (150 mg/kg) and high-dose (300 mg/kg) groups. Propanil (purity 99%) was obtained from AgroBest Grup (Izmir, Turkey). All experimental animals were kept under standard laboratory conditions in a 12-hours dark/light cycle, 22±3°C temperature and 45±5% relative humidity. While the mice of the control group were fed daily with standard laboratory chow, the mice of treated groups were fed with laboratory chow containing propanil for 30 days. All animals had ad libitum access to tap water and laboratory chow. Death did not occur in both control and experimental groups. At the end of the experiment, the spleens of mice were removed after cervical dislocation under ether anesthesia, and they were fixed in Bouin's solution for 24 hours. Spleen samples were processed according to the standard histological protocols. Spleen sections of 5-µm-thickness were stained with Harris hematoxylin-eosin (H&E), Mallory's Trichrome (MT) and Periodic acid Schiff (PAS). They were examined and photographed using a Zeiss Axioscope light microscope connected to an AxioCam Erc5S digital camera.

RESULTS

The present study was designed to detect negative impacts of propanil on the spleen of mice. There were no differences between males and females in terms of histopathological alterations.

Histological examinations showed that propanil caused dose-related damages in the spleen such as the separation of capsule from the splenic parenchyma, amyloid formation, enlargement of the white pulp, congestion, karyolysis in megakaryocytes, dilation of sinusoids, lobule formation, deformation of arterioles, necrotic areas, fibrosis, and cellular swelling in the splenic parenchyma.

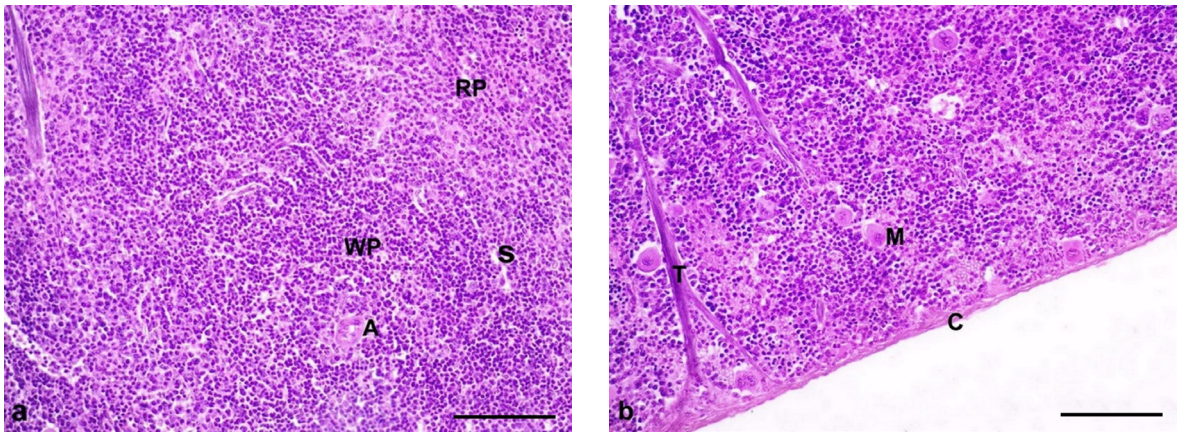

Figure 1. Histological section of spleen in control group, Scale bars = 100 µm a) white pulp (WP), red pulp (RP) and central arteriole (A) in white pulp, sinusoids (S), b) Capsule (C), trabecula (T) and megakaryocytes (M); Stain: H&E.

The spleen primarily consists of two main parts, the white and red pulps. The large part of this organ is the red pulp which is clearly discernible from the white pulp. The central arteriole is covered by lymphoid tissue constituting the white pulp. Lymphocytes are predominantly found in the white pulp that is encircled by the red pulp. There are many sinusoids in the splenic parenchyma (Figure 1a). A wide variety of blood cells including macrophages, erythrocytes, megakaryocytes, and lymphocytes are present in the red pulp (Figure 1b).

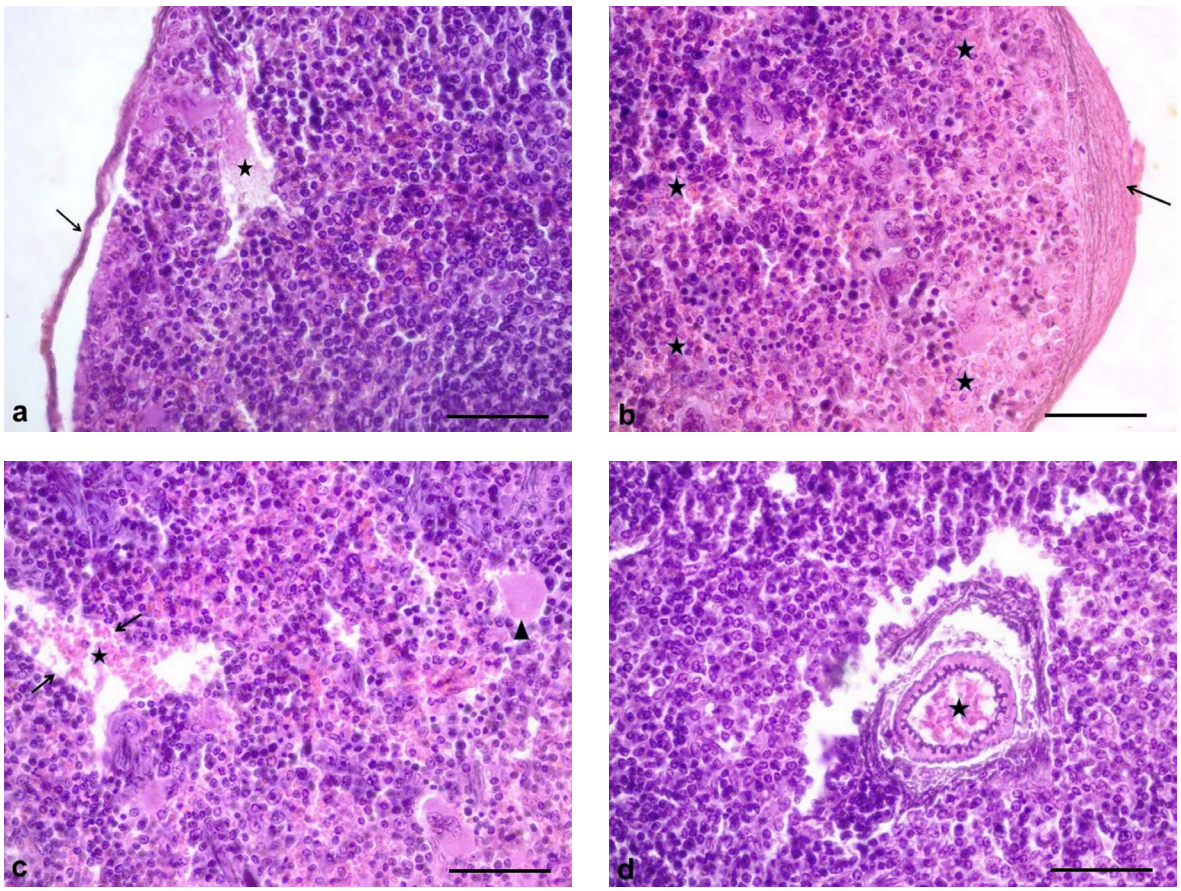

Figure 2. Histological section of low dose propanil-treated group. Scale bars = 50 µm a) The separation of capsule from the splenic parenchyma and amyloid formation, b) A significant thickened splenic capsule (black arrow) and congestion (asterisk) in the splenic parenchyma, c) Congestion (asterisk) in dilated sinusoid (arrow) and karyolysis (arrowhead) in megakaryocyte d) Congestion (asterisk) in enlarged arteriole. Stain: H&E.

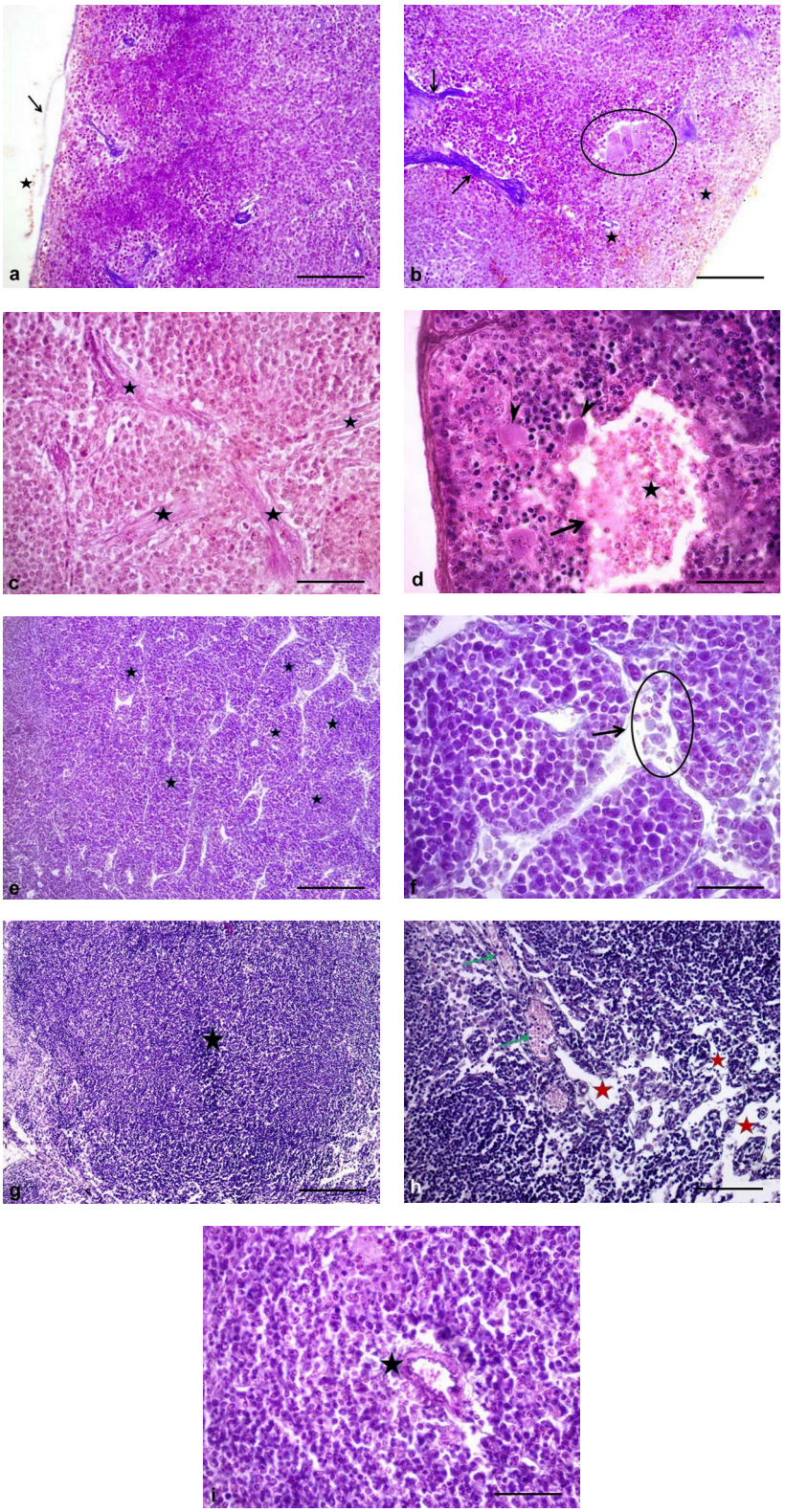

Figure 3. Histological section of medium dose propanil-treated group, a) Hemorrhage (asterisk) and capsule separation (arrow) from splenic tissue, Stain: MT Scale bar = 100 µm b) Amorphous megakaryocytes (encircled), fibrosis (arrow) and congestion (asterisk), Stain: MT Scale bar = 100 µm c) Fibrosis (asterisk) Stain: PAS Scale bar = 50 µm d), Karyolysis in megakaryocytes (arrowheads), and congestion (asterisk) in enlarged sinusoids (arrow), Stain: H&E Scale bar = 50 µm e) Lobule formation (asterisk), Stain: MT Scale bar = 100 µm f) Passing cells (encircled) into enlarged sinusoids (arrow) located between lobule formation, Stain: MT Scale bar = 50 µm g) Enlargement of white pulp (asterisk), Stain: H&E Scale bar = 200 µm h) Enlargement of sinusoids (asterisk) and congestion (green arrow), Stain: H&E Scale bar = 100 µm i) Deformation of the arteriole (asterisk), Stain: H&E Scale bar = 50 µm.

Low-dose group

In the low-dose group, the separation of the capsule from the splenic parenchyma and amyloid formation were determined (Figure 2a). Significant splenic capsule thickening and congestion in the splenic parenchyma were also examined (Figure 2b). Congestion in dilated sinusoids and karyolysis in megakaryocytes were observed (Figure 2c). Additionally, congestion in enlarged arterioles was determined (Figure 2d).

Medium-dose group

Hemorrhage and the separation of the capsule from the splenic parenchyma were examined in the medium-dose group (Figure 3a). Amorphous megakaryocytes, fibrosis, and congestion were clearly seen (Figure 3b). Intensive fibrosis was determined (Figure 3c). Karyolysis in megakaryocytes and congestion in enlarged sinusoids were also detected (Figure 3d). On the other hand, lobule formation (Figure 3e) and some passing cells from splenic cords into enlarged sinusoids (Figure 3f) were detected. Enlargement of the white pulp was also determined (Figure 3g). Sinusoidal dilatation and some enlarged sinusoids with congestion were observed (Figure 3h). Moreover, the deformation of the arteriole wall was detected (Figure 3i).

High-dose group

In the high dose group, hemorrhage and the separation of the capsule from the splenic parenchyma were detected (Figure 4a). Karyolysis in megakaryocytes and congestion in the splenic parenchyma were also observed (Figure 4b). In addition to the enlargement of white pulp (Figure 4c) fibrosis and congestion (Figure 4d) were determined. Necrosis in the white pulp and congestion in the red pulp were examined (Figure 4e). Congestion in enlarged sinusoids was detected (Figure 4f). Cellular swelling in the splenic red pulp was clearly seen (Figure 4g). Histopathological results were also summarized in Table 1.

DISCUSSION

The spleen has the ability to filter pathogens and antigens carried by blood. It also acts as an important organ in maintaining iron metabolism and erythrocyte homeostasis. The spleen red pulp contains macrophages which mostly act in filtering of blood and recycling iron from old red blood cells. The white pulp is structurally similar to the lymph node. It allows the production of antigen-specific immune responses that protect the body from diseases caused by blood-borne bacterial, viral, and fungal infections [16]. In the white pulp, three (sub)compartments can readily be defined. One of these compartments is called periarteriolar lymphatic sheaths (PALS) closely related to the splenic artery' branches. The second structure is known as follicles surrounding PALS. The third one is the marginal zone, encircling PALS and follicles. There are many immunocompetent cells in several regions of the white pulp. PALS, a thymus-dependent area, mainly contains T-lymphocytes. On the other hand, follicles and the marginal zone are occupied by B-lymphocytes derived from the bone marrow [17].

The spleen has a major role in hematopoiesis especially during fetal life in humans [12, 13]. The bone marrow alone is an essential lifelong blood-forming organ in humans. However, both the spleen and bone marrow have function in the blood cell production during the postnatal life in mice [15]. Therefore, many megakaryocytes were observed in the histological section of the spleen. Megakaryocytes are very specialized precursor cells forming and releasing platelets in circulation [18]. These polyploid cells have granular cytoplasm as they mature. Platelets originate from the megakaryocyte cytoplasm. There is an uncertainty about which platelets are released from their parent cells, but this occurs via cytoplasmic fragmentation and may take place in an extramedullary place (e.g. lung, spleen) [19].

Laboratory studies related to environmental pollutants are more useful when performed using concentrations close to approximate levels found in the environment [20]. The spleen is an organ in which direct and indirect toxicity can occur. It serves as a target for carcinogens and metastasis of malignant neoplasms coming from other sites [21]. With this perspective in mind, this study aimed to investigate the histopathological effects of propanil on the spleen tissue of Swiss albino mice. Erosions in the histological architecture of splenic tissue may disturb spleen' functionality. Therefore, it is essential to research the potential negative effects of pesticides on this organ. Given the neglect of these types of studies, the results of this study can only be discussed within the context of limited research.

Al-Bader et al. [22] reported that after exposure to thioacetamide, there was a general hyperplasia of the white pulp in rat spleen and an increase in the enlargement of white pulp until the end of the experiment. This re-

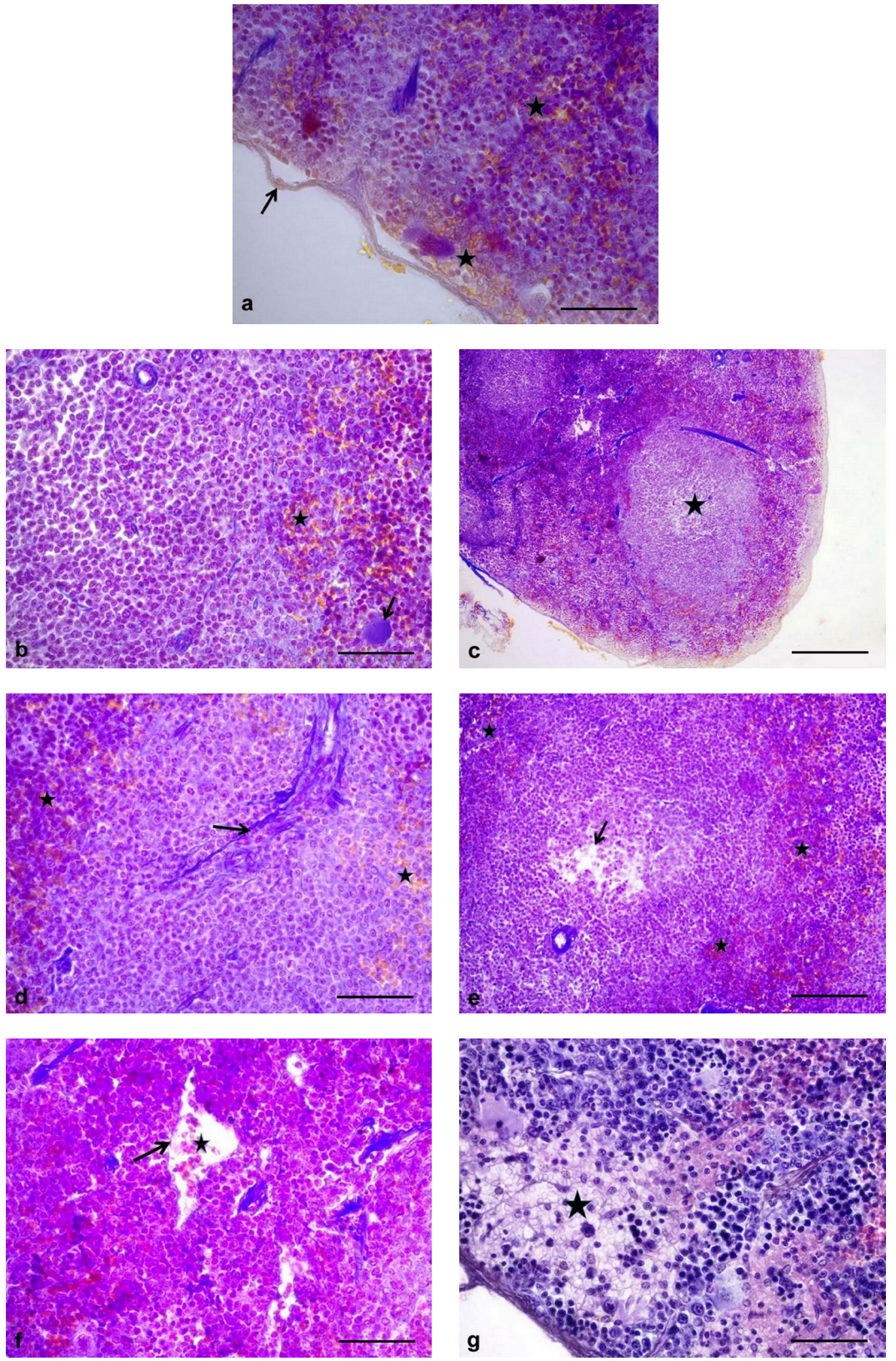

Figure 4. Histological section of high dose propanil-treated group a) Hemorrhage (asterisk) and capsule separation (arrow) from splenic tissue, Stain: MT Scale bar = 50 µm b) Karyolysis in megakaryocyte (arrow) and congestion (asterisk), Stain: MT Scale bar = 50 µm c) Enlarged white pulp (asterisk), Stain: MT Scale bar = 200 µm d) Fibrosis (arrow) and congestion (asterisk), Stain: MT Scale bar = 50 µm e) Necrosis (arrow) in white pulp and congestion (asterisk) in red pulp, Stain: MT Scale bar = 100 µm f) Congestion (asterisk) in enlarged sinusoid (arrow), Stain: MT Scale bar = 50 µm g) Cellular swelling in splenic red pulp (asterisk), Stain: H&E Scale bar = 50 µm.

Table 1. Histopathological lesions of Swiss albino mice after exposure to propanil. Low-dose group 75 mg/kg, medium-dose group 150 mg/kg and high-dose group 300 mg/kg.

Tissue	Histopathological defects	Control	75 mg/kg	150 mg/kg	300 mg/kg
Spleen	The separation in capsule	0	2	2	1
	Amyloid formation (Occurrence of amorphous material)	0	1	0	0
	Congestion (A local increased volume of blood)	0	2	2	3
	Karyolysis in megakaryocytes (Dissolution of megakaryocyte nucleus)	0	1	1	1
	Fibrosis (Connective tissue replaces parenchyma)	0	0	2	1
	Enlarged white pulp (Expansion in white pulp)	0	0	2	2
	Dilated sinusoid (Enlarged sinusoid)	0	2	3	2
	Lobule formation (Occurrence of small divisions in spleen)	0	0	3	0
	Cellular swelling in red pulp (Cellular edema in red pulp)	0	0	0	2

Note: Histopathological defects were presented based on their severity (0, none; 1, mild; 2, moderate; 3, severe)

action was considered a normal condition because the immune response of the spleen begins here. T and B lymphocytes in the white pulp capture and process the antigens, promoting the immune response [23, 24]. The enlargement of white pulp was determined in both the medium and high dose groups in our study. Enlarged white pulp was reported in mice spleen due to exposure to prothor (imidacloprid) insecticide 0.03mg/kg.bw for 90 days [25]. We also observed enlarged white pulp with slight necrosis in the high-dose group. This response can be related to the high dose being more toxic than other doses, and as a result, causing more degenerative damage. Similarly, fish spleens showed prominent destruction of the white pulp area after exposure to lindane (high dose). The red pulp was minimally affected by lindane [26]. Petrovova et al. [27] observed an increased number of lymphocytes in the splenic parenchyma of rabbits after exposure to bendiocarb. The authors stated that the antigen leads to the accumulation of lymphocytes without leaving the spleen. Due to the antigenic exposure and stimulation accumulated with age, the ratio of white pulp to the red pulp rises [28].

The red pulp includes venous cavities in which arteriolar blood is discharged, along with the structural frame of the spleen containing macrophage populations. In addition to macrophages, the red pulp has an increased number of lymphocytes, monocytes, granulocytes, platelets, erythrocytes, and megakaryocytes, which complement the protection function of the organ against harmful agents [24, 29]. In our study, prominent results were detected in all exposed groups such as congestion in the splenic parenchyma and sinusoidal dilatation. Severe congestion was also determined in rats exposed to the pyrethroid insecticide [30] and an organo-pesticides mixture [31].

Our other result observed in all exposed groups was the separation of the capsule from the splenic parenchyma. Similarly, pesticide exposure resulted in the separation of the capsule from the splenic parenchyma in rats. The authors stated that this defect partially occurred due to the atrophied parenchyma and destructive alterations in the extracellular space, leading to complete narrowing of parenchyma and hypocellularity [31].

In this study, karyolysis in megakaryocytes was determined in all experimental groups, and the number of these types of megakaryocytes were especially increased in the high-dose group. It is evident that this toxic chemical caused degeneration and disruption in function of megakaryocytes. On the other hand, endosulfan exposure resulted in karyorrhexis and karyopyknosis in megakaryocytes of the spleen in rabbits [32].

Fibrosis is known as an end result of chronic inflammatory responses triggered by various stimuli, including persistent chemical insults, tissue injury, radiation, infections, autoimmune reactions, and allergic responses [33]. In this study, fibrosis was observed in both the medium-and high-dose groups. Similar results were also determined in the spleen of male mice after exposure to imidacloprid [25] and in the splenic parenchyma of *Pelophylax bedriagae*, Levantine frog, after exposure to carbaryl [34].

Cell swelling is the first stage of alteration occurring in most types of acute injuries and can be the starting point for more severe changes. Cells of the affected tissue are characteristically swollen, and their staining affinity generally decreases, giving the cells a pale or cloudy appearance [35]. In our study, we observed cellular swelling in some regions of the red pulp of the spleen, especially in the high-dose group.

CONCLUSION

As discussed above, it is evident that pesticides caused various histological changes in the splenic parenchyma. Similarly, propanil induced significant histological damages in the splenic parenchyma of Swiss albino mice, and these damages may result in organ failure. Due to the limited research on the effects of pesticides on the mammalian spleen, this study will be helpful for toxicology research on other mammals.

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