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Protective effects of resveratrol on permethrin-induced fetotoxicity in rats

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Sıçanlarda permetrin kaynaklı fetotoksisite üzerine resveratrolün koruyucu etkileri

Abstract: Synthetic pyrethroid insecticides have been widely used for years to prevent harmful effects of insects and control disease vectors. In this study, the effects of resveratrol against the potential toxicity of permethrin, an effective pyrethroid derivative, on the fetus were investigated. Accordingly, *Wistar* female rats were divided into four groups as Control, Sham, Permethrin, and Permethrin + Resveratrol. Lung, liver, kidney and small intestine of developing fetuses were evaluated histopathologically. Also, Bone Morphogenetic Protein-4 (BMP-4) in bone tissue development and Fibroblast Growth Factor-1 (FGF-1) expressions in lung were examined immunohistochemically. All structures in the Control and Sham groups were normal. Permethrin caused epithelial damage, regression in bronchial and primitive alveolar development in the lung; congestion, edema and sinusoidal dilatation around the central vein in the liver; tubular epithelial degeneration, regression in glomeruli and tubule formation in the kidney; epithelial degeneration and irregularity in the villus structure in the small intestine. Immunohistochemical results indicated that permethrin administration decreased BMP-4 levels in bone tissue and FGF-1 levels in lung. Resveratrol application was found to greatly alleviate histopathological and immunohistopathological variability in all tissues. Oral consumption of permethrin by pregnant rats caused growth retardation and tissue damage in many different tissues in offspring. Intake of resveratrol during pregnancy showed protective effects against fetotoxicity caused by permethrin.

Key words: Permethrin, Resveratrol, Fetotoxicity, FGF-1, BMP-4

Özet: Sentetik piretroid insektisitler, yıllardır böceklerin zararlı etkilerinden korunmak ve hastalık vektörlerinin kontrolü için yaygın olarak kullanılmaktadır. Bu çalışmada, etkili bir piretroid türevi olan permetrinin fetüs üzerindeki olası toksisitesine karşı polifenolik bir bileşik olan resveratrolün potansiyel etkileri araştırılmıştır. Çalışmada *Wistar* dişi sıçanlar Kontrol, Sham, Permetrin ve Permetrin+Resveratrol olmak üzere dört gruba ayrıldı. Histopatolojik olarak fetüslerin akciğer, karaciğer, böbrek ve ince bağırsak gelişimleri değerlendirildi. Ayrıca immunohistokimyasal olarak kemik doku gelişiminde Bone Morphogenetic Protein 4 (BMP-4) ve akciğerde Fibroblast Growth Factor-1 (FGF-1) ekspresyonlarına bakıldı. Kontrol ve Sham grubunda tüm yapılar normal görünümde izlendi. Permetrin; akciğerde epitelyal hasara, bronşiyal ve primitif alveolar gelişimde gerilemeye; karaciğerde santral ven çevresinde konjesyon, ödem ve sinüzoidal dilatasyona; böbrekte tübül epitelinde dejenerasyona, glomerül ve tübül oluşumunda gerilemeye; ince bağırsakta da epitelyal dejenerasyona ve villüs yapısında düzensizliğe neden olduğu saptanmıştır. İmmünohistokimyasal sonuçlarda permetrin uygulaması ile kemik dokuda BMP-4 ve akciğerde FGF-1 ekspresyonlarında azalma saptanmıştır. Resveratrol uygulaması sonrasında tüm dokularda histopatolojik ve immünohistokimyasal değişiklikler büyük oranda hafiflemiştir. Gebe sıçanların oral yolla permetrin tüketimi, yavrularda birçok farklı dokuda gelişme geriliği ve doku hasarına neden olmaktadır. Gebelik sürecinde resveratrol alımı ise permetrinin neden olduğu fetotoksisiteye karşı koruyucu etkinlik göstermektedir.

Anahtar Kelimeler: Permetrin, Resveratrol, Fetotoksisite, FGF-1, BMP-4

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1. Introduction

Synthetic pyrethroids constitute 30% of the insecticides used worldwide (Elser et al., 2022). However, these products pose a threat to public health by spreading to the environment outside of their intended targets and accumulating in drinking water and agricultural products. Depending on the ingestion of insecticide residues in foods, toxic effects can be observed in the central nervous system, cardiovascular system, urogenital system, metabolic and endocrine systems in humans and animals (Mossa et al., 2018). Permethrin (Perm), is a pyrethroid-derived insecticide that is frequently used in agriculture, veterinary, forestry, public and environmental health spraying, the protection of stored products, and livestock (Nguyen et al.,

2023). Although they are considered to be safer in terms of use and environmental impact than Perm's other insecticide types (organophosphate derivative, carbamate), there is no clear reliability about long-term exposure. Perm and its metabolites can be found indoors and outdoors, in soil and dust. Exposure of humans to perm can occur through inhalation, consumption of contaminated food, or through the skin. Perm is hydrolyzed to a significant extent in the gastrointestinal tract after it is taken into the body, and 40-60% of the amount taken orally passes into the systemic circulation. Perm, due to its lipophilic properties, accumulates in tissues with high lipid content such as liver, kidney and breast, as well as fat and nervous system, and is metabolized in the liver and excreted through the kidneys (Ding et al., 2013). Experimental studies have shown that Perm adversely affects the peripheral and central nervous system in mammals, causes neurotoxic effects by inducing oxidative stress in the neonatal rat brain, increases malondialdehyde levels by causing lipid peroxidation, has advers effects on the liver and endocrine system, and causes degenerative and necrotic damage in the kidney, increases apoptotic cell death, decreases sperm concentration in rats, and causes heart diseases (Wang et al., 2016; Romero et al., 2017; Curtis et al., 2021). These undesirable effects of Perm can cause fetal toxicity and developmental delay in offspring, especially during pregnancy. The bone morphogenetic proteins (BMPs) have been described as a potent inducer of bone and cartilage tissue formation (Tateiwa and Kaito, 2023). In this context, especially BMP-4 is considered as an important factor in the formation and repair of endochondral bone (Ye et al., 2022). Moreover, fibroblast growth factor-1 (FGF-1) belongs to the FGF family and has been shown to inhibit fibroblast collagen production and differentiation into myofibroblasts, and reverse the epithelial-mesenchymal transition by inhibiting TGF-β1 signaling pathways (Gasser et al., 2022). FGF-1 participates in many vital functions in the cell, such as the axial structuring of embryonic tissues, identification and differentiation of cell types, morphogenesis of organs and systems such as the vascular system, and regulation of cell proliferation and movement (Xiao et al., 2021). In addition, it has a role in many biological events from tissue repair to cancer cell growth and spread (Coleman et al., 2014). In addition to being an angiogenic factor, it is involved in endothelial cell migration and proliferation, and also contributes to the development of mesenchymal and neuroectodermal cells (Fernandes-Freitas and Owen, 2015).

Resveratrol (Res), is a polyphenolic compound of the phytoalexin group (Chen et al., 2023). Polyphenolic compounds show antioxidant properties by destroying free radicals and minimizing the harmful effects of oxidative stress (Borsoi et al., 2023). Res has anti-inflammatory, antioxidant, and anticarcinogenic properties (Chen et al., 2023). Discovered in grape seeds and peel, Res is especially found in dark fruits. Res, which has a role in the defense mechanism of plants against fungicides, has been found to have many different properties in experimental studies (Bohara et al., 2022). In a cell culture study, Res was found to suppress the epithelial-mesenchymal transition in the YKG1 glioblastoma cell line (Pektas et al., 2021). Its protective efficacy against spinal cord ischemia-reperfusion injury has been demonstrated in rats (Aslan et al., 2021). Its reparative efficacy against ovarian deformation induced by metabolic syndrome has been reported (Pektaş et al., 2014). Other studies have reported anti-inflammatory effects in the heart (Pektas et al., 2017), vasodilation due to eNOS induction in vascular smooth muscles (Pektas et al., 2018), and protective effects against diabetes-induced kidney damage (Koca et al., 2016). In another study, apoptotic effects of Res metabolites were detected on *Ehrlich Ascites Carcinoma*, a resistant rodent cancer type (Bozkurt et al., 2020).

To date, there are very few animal studies in the literature showing the toxic effects of Perm on the fetus by crossing the placenta. On the other hand, the benefits of Res, which can be taken with natural foods and have epigenetic protective effects as well as antioxidant effects, during pregnancy are not known. This study compiled clues to fetotoxicity and potential effects of Res in rats given Perm on days 7 and 14 of gestation.

2. Materials and Method

2.1. Animals and the experimental procedures

This study was approved by Afyon Kocatepe University Animal Ethics Committee (49533702/58). In the study, 24 adult female *Wistar* Albino rats with a weight of 220 ± 20 *g* were used. During the study period, rats were housed in temperature and humidity-controlled rooms (20 – 22°C) with a 12-hour light-dark cycle in polycarbonate cages with standard pellet food and regular water provided. After ovulation times were determined in order to achieve pregnancy in the rats, they were caged with male rats by the one-to-two method between 17:00 and 21:00 p.m. when they were in the proestrus stage. The next day, vaginal smears were taken from female rats, sperm (+) in the smear and those showing diestrus phase in the following tests were considered pregnant and included in the study. Embryo development is mostly affected by teratogenic agents during the organogenesis period. Therefore, our applications were performed between the 6th and 17th days, which is the organogenesis stage. Pregnant (+) rats were divided into 4 groups with 6 rats in each group. No application was made to the Control group of pregnant rats during the 18-day of experimental period (Group 1). In the Sham group, on the 7^{th} and 14^{th} days of pregnancy, 0.5 cc - 5% alcohol + corn oil mixture at a ratio of 1/1, additionally only 0.5 cc - 5% alcohol application on the 7^{th}, 10^{th}, 13^{th}, and 16^{th} days of pregnancy were given with gavage (Group 2). Perm (300 mg/kg) dissolved in a 1/1 ratio of corn oil + alcohol solution was applied by gavage to the Perm group on the 7^{th} and 14^{th} days of pregnancy (Group 3). In the Perm + Res group, on the 7^{th} and 14^{th} days of pregnancy, Perm (300 mg/kg) was dissolved in alcohol and corn oil solution prepared at a ratio of 1/1, in addition, on the 7^{th}, 10^{th}, 13^{th}, and 16^{th} days of pregnancy resveratrol (50 mg/kg) dissolved in %5 alcohols were given with gavage. On the 18^{th} day of pregnancy, fetuses and placentas were removed from pregnant rats via cesarean-section under CO_2 anesthesia. Afterward, the rats were sacrificed by taking blood from their hearts. Removed baby rats were fixed in a 10% neutral buffered formaldehyde solution.

2.2. Chemicals, immunostaining and the histochemical staining

In experiments, Perm (Permethrin, 45614, Sigma), Res (Resveratrol, 51852282, Molecula), BMP-4 primary antibody (PA5-19683, Thermo Scientific), FGF-1 primary

antibody (sc-7910, Santa Cruz), and Secondary antibody kit (Thermo, Cat. No: 85-9043) were used.

Sections taken on adhesive slides were stained immunohistochemically using appropriate primary antibodies to evaluate BMP-4 and FGF-1 expressions in the samples after deparaffinization and rehydration processes. Expressions of BMP-4 in the bone and FGF-1 in the lung tissues were evaluated. The prepared preparations were examined under a light microscope (Nicon-Eclipse E600, Tokyo, Japan) and their images were taken.

After the excised fetuses were cut transversely and horizontally, they were left in 10% neutral formaldehyde solution for fixation. Three fetuses from each mother rat were included in the study, and the fetal samples were embedded in paraffin blocks after passing through the tissue follow-up method stages described. Sections of 5-micron thickness were taken from paraffin blocks. Sections were stained with hematoxylin-eosin (HE) for histopathological examinations. The images of lung, liver, kidney, and small intestine (jejunum) tissues in the sections stained with HE were examined under the light microscope (Nicon Eclipse E600, Tokyo, Japan), and the histopathological changes were recorded and visualized.

Histopathological changes in the lung, liver, kidney and small intestine (jejunum) were evaluated semi-quantitatively (−, no lesions; +, mild; ++, moderate; +++, severe) according to the Gibson-Corley scoring (Gibson-Corley et al., 2013).

After the experiment, bronchoalveolar development and infiltration scoring were performed in the lungs of the fetuses. In the 18th day of rat fetuses, terminal and respiratory bronchiole development; precursors of alveolar canals and sacs were observed to have developed in the lung.

In the lung, bronchoalveolar development and infiltration scoring were performed. In the evaluation of lung maturation, there are glandular stage, canalicular stage and saccular stage, and glandular-canalicular stage and canalicular-saccular stage as intermediate stages, according to the developmental order. Pseudo-glandular stage is observed on the 18th day of pregnancy in the lung maturation of rat fetuses, and canalicular stage is observed on the 19th and 20th days of pregnancy. Since the fetuses were removed on the 18th day in our study, the pseudo-glandular stage in lung development begins to end and the transition to the canalicular stage begins as of the developmental stage. At this stage, the major ducts have branched and started to develop, terminal bronchioles have formed, and respiratory bronchioles will begin to form. In the light of this information, lung development scoring was done by modifying the scoring in previous studies. In three lung sections belonging to each group, 10 different areas were scanned and the lung development level was scored (1: 25-40%, 2: 40-60%, 3:60-80%, 4:80-100%) as a percentage (Burri, 1984).

Congestion, infiltration and parenchymal damage in the liver were evaluated. In the kidney, the developmental stage was evaluated by looking at the parameters of cortex-medulla differentiation, glomerular development and tubule development. The kidney development was scored (1: 25-40%, 2: 40-60%, 3: 60-80%, 4: 80-100%) as a percentage (Simsek et al., 2009) by examining ten different areas in 3 different fetal kidney sections and also tubular damage was graded semi-quantitatively.

In the intestine tissue samples, changes in the villus epithelium of the jejunum region were evaluated and scored semi-quantitatively.

2.3. Statistical analysis

Immunohistochemical data were represented as mean ± standard error of the mean (SEM) throughout the study. Student's t test for unpaired data or one-way ANOVA followed by the *Bonferroni* post hoc analysis were performed for statistical comparisons in which p value less than 0.05 was considered significant. *$p<0.05$ significantly different from the Sham; #$p<0.05$, significantly different from the Perm-treated rats.

3. Results

3.1. Histopathological modifications in the lung, liver, kidney, and the small ıntestine tissues

In the Control and Sham groups, bronchiole structures and alveolar canal sacs and precursors of these structures were formed; these structures were observed to be regular and normal. Vena centralis, portal area, and sinusoidal structures of liver were evaluated as normal. In kidney tissue examination, primitive glomerular capillary network, *Bowman's space*, and tubule structures were observed. Villi and crypt structures were observed regularly in small intestine sections (Fig. 1). No significant difference was found in the scoring of the Control and Sham groups (Table 1).

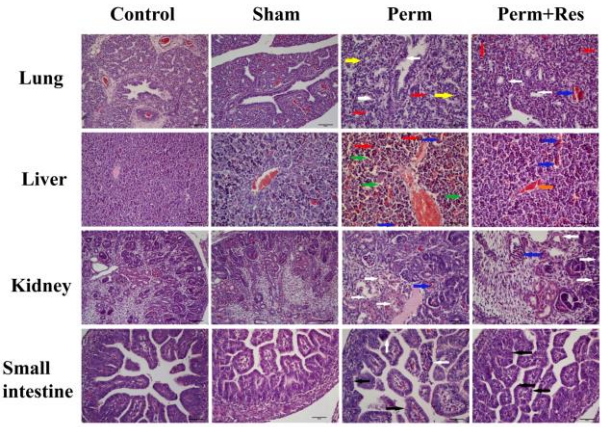

Figure 1. Representative H-E stained sections from the experimental groups of fetal tissues. Control and Sham groups have normal morphology in all tissues. In perm group histopathological changes were seen in all tissues. Histopathological changes in all tissues were greatly alleviated after resveratrol administration. Infiltrative cells (red arrow), epithelial degeneration (white arrow) and areas of delayed alveolar structure development (yellow arrow) congestion (blue arrow), dilatation in sinusoidal areas (green arrow), apoptotic changes (black arrow), vena centralis dilatation (orange arrow). It was observed that congestion and epithelial degeneration decreased in the Perm + Res group.

HE evaluations of Perm group revealed degenerative and infiltrative findings in lung parenchymal structure and deterioration in bronchiolar / alveolar epithelium. It was observed that the development of the primitive alveolar structure was slower in the samples of Perm group than in the Control and Sham groups. In liver samples, there was significant venous edema and congestion, and dilatation in

sinusoidal areas next to the infiltrative areas in the parenchyma. When kidney sections were examined, retardation was detected in the development of glomerular and tubular structures. Degenerative and vacuolar changes were observed in the tubular epithelium, and there was also congestion in the capillary area. Looking at the villi and crypt structures; irregular, degenerative and apoptotic changes were seen in some villi epithelium (Fig. 1). In the scoring of the Perm group, tissue growth retardation, congestion, edema, and degenerative changes were found to be higher compared to the Control and Sham groups (Table 1). In the examinations of the Perm + Res group, congestion in the lung and degenerative changes in the epithelial region were observed. Infiltrative cells were observed intensively in the parenchymal area. In the examination of the liver tissue, there were signs of congestion in the venous areas and dilatation of the vena centralis, but these findings were observed to be milder than the damage findings in the Perm group. A decrease in glomerular congestion was observed in kidney sections. It was observed that the degenerative appearance in the tubules was less than in the Perm group. In the small intestine sections, mild signs of damage and apoptotic cells were observed in the villi (Fig. 1). In the scoring of the Perm + Res group, it was determined that the variables seen in the Perm group were alleviated (Table 1).

Table 1. Scoring of histopathological changes of lung, liver, kidney, and small intestine.

		Control	Sham	Perm	Perm+Res
		(+) Scores			
Lung	Bronchoalveolar growth	4	4	2.67	3
	Infiltration	0	0	2	1.5
Liver	Congestion	0	0	2.17	1.83
	Parenchymal damage	0	0	2.33	1.5
Kidney	Kidney growth	4	4	3	3.33
	Tubular damage	0	0	2.16	1.33
Small intestine	Epithelial damage	0	0	1.5	1

3.2. Immunohistochemical changes of BMP-4 in the bone and FGF-1 in the lung tissues

BMP-4 expressions in the bone tissue were expressed as a percentage. Intense BMP-4 expression was observed in the area of chondrogenesis of the Control and Sham groups. Evidently, there was a significant decrease in BMP-4 expression in the sections belonging to the Perm group compared to Control. In the Res-treated group, BMP-4 levels were found to be significantly increased compared to the Perm group (Figure 2 and Figure 3a).

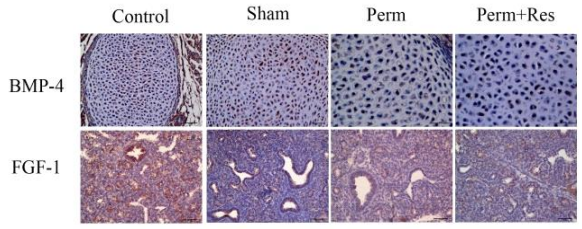

Figure 2. Representative photomicrographs of immuno-histochemical staining of fetal tissues. Immunohistochemical staining for BMP-4 in fetal bone and FGF-1 in fetal lung. In the Perm group, deterioration in the structure of the lacunae and decrease in the staining intensity with BMP-4 were evident. Staining with FGF-1 showed decrease in staining in the Perm group.

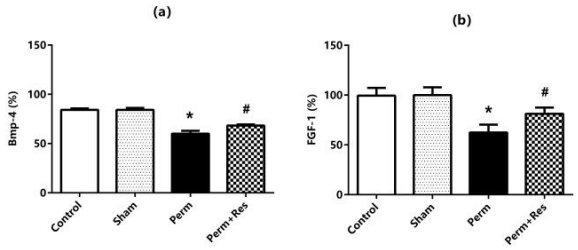

Figure 3. Relative expressions of BMP-4 (a) and FGF-1 (b). *p<0.05 significantly different from the Control; #p<0.05, significantly different from the Perm-treated rats.

FGF-1 expressions in lung sections were scored by evaluating the staining intensity in the epithelial area. According to the results, intense FGF-1 expression was observed in the Control and Sham groups. In cross-sections of Perm-treated rats, a significant decrease in FGF-1 expression was observed, especially in regions with intense degenerative changes in the bronchiole epithelial structure. In FGF-1 expressions, a partial increase was detected in the Perm + Res group compared to Per group (Fig. 2, 3b).

4. Discussions

Perm is one of the first insecticides with limited persistence in soil with its high insecticidal activity and low mammalian toxicity (Wang et al., 2016). They are widely used because they are considered to be extremely safe in terms of use and effects on the environment compared to other insecticide groups. Due to its widespread use in cities and environmental settlements, it also affects non-target organisms as well as target organisms. Although it is claimed that synthetic pyrethroid insecticides, including Perm, are much safer than other agents, it has been revealed that they can cause damage to many organs and may have teratogenic effects, especially if exposed during pregnancy. Perm frequently targets the nervous system in target organisms (López-Aceves et al., 2021). It can cause neurotoxicity, paralysis and even death in the organism by damaging voltage-dependent sodium channels in neurons. The liver is an organ that plays a vital role in the detoxification of xenobiotics. Perm is a potent inhibitor of cytochrome P4501A, which causes significant accumulation of some chemicals associated with fatal toxicity (Ding et al., 2013). In a study, it was shown that Perm destabilizes and damages the redox system of the liver in neonatal and adult rats. In addition, Perm disrupts the antioxidant balance, causes lipid peroxidation, protein oxidation and increases apoptotic cell death (Gabbianelli et al., 2013). Since exogenous chemicals and their metabolites are removed from the body via kidney tissues, they are target organs for many toxic agents. Prethyroid insecticides containing Perm are also among these agents. There are three main mechanisms responsible for Perm-induced nephrotoxicity; accumulation of xenobiotics and macromolecules induced by xenobiotics in renal tissue; accumulation in renal tissue of toxic metabolites biosynthesized in other organs; accumulation in the kidney due to bioactivation of xenobiotics into reactive metabolites. Particularly, the pars recta of the proximal tubules are more sensitive to chemicals since it is the site of enzymes that metabolize xenobiotics, especially P450 (Dekant and Vamvakas, 1996). Reactive oxygen radicals

consist primarily of arachidonic acid metabolism, the xanthine oxidase system, and activated neutrophils. Reactive free oxygen radicals (mainly hydroxyl radical and superoxide anion) cause oxidative stress in the cell by causing oxidation of membrane lipids, protein denaturation, DNA degradation, and sulfhydryl enzyme inactivation. This increases membrane permeability, leading to protein and nucleic acid degradation and ultimately to aging, cell damage, and death in cells. Under normal conditions, antioxidant enzymes render the reactive oxygen radicals formed in the organism harmless. However, agents such as Perm can inhibit antioxidant enzymes with different mechanisms, causing an increase in reactive oxygen radicals in the environment and damage to the cell. Pesticide-induced oxidative stress has been the focus of toxicological research in recent years as a possible mechanism of toxicity. Various studies have been conducted to determine whether oxidative stress in humans or animals is caused by various agents in this group and is associated with their toxic effects (Agrawal and Sharma, 2010; Gabbianelli et al., 2013; López-Aceves et al., 2021). It has been shown that oxidative stress increases in tissues after exposure to prethyroid insecticides such as Perm and that increased oxidative stress plays a fundamental role in the pathogenesis of many organ damage (Wang et al., 2016). Romero and his team showed that Cypermethrin, one of the prethyroid insecticides, causes apoptotic, necrotic and autophagic cell death by inducing oxidative stress that causes DNA damage in neuroblastoma cells (Romero et al., 2017). Although the target organs of prethyroids are different, Perm-mediated oxidative stress has been shown in humans and animals caused neurotoxicity (Nasuti et al., 2014), hepatotoxicity (Roma et al., 2012), nephrotoxicity (Guvenc et al., 2013), immunotoxicity (Jin et al., 2010), cardiotoxicity (Dhivya Vadhana et al., 2010). However, Res is a molecule that shows antioxidant potential by destroying free radicals, binding metal ions, decreasing the activity of enzymes involved in the formation of reactive oxygen species, and increasing antioxidant enzyme activities (Chen et al., 2023). In this study, we aimed to investigate the damage of Perm, which is used in many different areas and to which we are unintentionally exposed, on different organs that will be caused by exposure in the prenatal period, and the experimental model in which we planned the possible effectiveness of Res. When the lung findings were evaluated in our study, it was found that the bronchoalveolar development of the Control and Sham groups was normal and no infiltration was observed. The bronchoalveolar development of the entire Sham group was determined as stage 4. While growth stage 2 and severe infiltration were observed in the Perm group, decreased infiltration and growth were evaluated as stage 3 in the Res-treated group. In a similar study, it was shown that infiltration of the chick embryo increased with Perm injection and tissue development decreased (Curtis et al., 2021). However, Res is known to be a powerful antioxidant and tissue-protector (Aslan et al., 2021; Bohara et al., 2022). In this context, it can be said that the effects of Perm and Res are compatible with the literature. Although the protective effects of Res do not show high potency, this may be dose related. In liver sections, parenchymal damage and congestion were not observed in the Control and Sham groups, while severe congestion and parenchymal damage were observed in the Perm group. Furthermore, Res treatment was found to reduce Perm-induced congestion and parenchymal damage. In studies investigating the effects of Perm on the liver, it was shown that Perm causes hepatotoxicity by increasing lipid peroxidation (Roma et al., 2012; Gabbianelli et al., 2013). From this point of view, this may be the reason for Perm-dependent congestion and parenchymal damage in our study. However, the free radical scavenging activity of Res, whose hepatoprotective activity has been shown in many studies (Pektas et al., 2017; Aslan et al., 2021), may have suppressed Perm-dependent variables. In another similar study, the effects of Perm on the kidney were examined histopathologically, and it was shown that Perm causes necrotic and degenerative changes in the tubule epithelium and also increases apoptotic cell death (Guvenc et al., 2013). Liang et al. (2013) also found that a metabolite that causes nephrotoxicity and hepatotoxicity in rats after 60 days of Perm administration increased in urine. When kidney sections were evaluated in our study, it was found that tubular development was sufficient (Stage 4) and no damage occurred in the Control and Sham groups; tubular damage and growth retardation (Stage 2) were observed in the sections of Perm-treated rats. Similarly, our results indicate that Perm-derived metabolites may be associated with kidney damage. On the other hand, our results show that Res treatment reduces Perm-induced changes. This may be related to the free oxygen radical scavenging activity of Res and facilitating the excretion of Perm-derived metabolites. When the intestine results were evaluated, epithelial damage was observed in the sections of Perm-treated rats, while no epithelial damage was observed in the Control and Sham groups. In parallel with the findings of our study, degeneration, necrosis, and cell infiltrations of epithelial and cartilage tissues in the gills of Deltamethrin, one of the prethyroid insecticides, were detected in a study conducted in a fish of the Carassius gibelio species. In the same study, degenerative and necrotic changes with edema between the submucosa and mucosa in the intestine, enlargement of the blood vessels of the serosa and atrophy of the muscularis and submucosa, shortening and thickening of the villi were observed (Gey and Ersan, 2020). Moreover, it has been reported that Res protects intestinal barrier integrity, improves antioxidant capacity, and alleviates inflammation in the jejunum of ducks exposed to acute heat stress (Yang et al., 2021). In general, both the effects of Perm on tissues and the influences of Res on the Perm-derived variants are in line with similar studies. BMP-4 is a protein found in demineralized bone and cartilage tissue, involved in growth and differentiation. In our study, it was shown that BMP-4 levels decreased in Per group compared to the Control group and were normalized with Res supplementation. It has been reported in many studies that pesticides reduce BMP-4 levels in bone tissue (Feng et al., 2016). Supportingly, chlorpyrifos exposure has been shown to reduce BMP-4 expression, which is essential for cranial neural crest morphogenesis and chondrogenesis in *Xenopus laevis* embryos (Tussellino et al., 2016). In the same study, it was reported that chlorpyrifos inhibited development by affecting the bone formation signaling pathway. A similar activity may be valid for Perm in our study as well. However, Res is known to regulate BMP-4 levels (Min et al., 2020). Therefore, we can say that the influences of Perm and Res on BMP-4 levels in the bone tissues are also compatible with previous studies. FGF-1 protein; it is a

myth of the FGF family, which is responsible for many biological procedures such as embryo development, cell growth, morphogenesis, organogenesis, and tissue repair (Coleman et al., 2014). A decrease in FGF-1 expressions in proportion to the damage to the lung tissues with Perm was observed. In rats with Res added to the treatment, FGF-1 expressions increased compared to Perm groups according to our study. In a similar study, it was reported that Paraquat which is known as a pesticide, increases connective tissue growth factor expression and impairs lung fibroblast proliferation and viscoelasticity (Zhang et al., 2014). It has also been reported that Res has a synergistic protective effect against cardiotoxicity and hepatotoxicity in experimental studies (Lu et al., 2022; Xu et al., 2022). Thus, it is seen that the immuno-histochemical results of FGF-1 in lung sections are very similar to the literature. Histological observations in our study revealed that Perm caused histopathological changes in lung, liver, kidney and small intestine structures in rat fetuses. It has been determined that Res partially reduces or prevents these developmental and toxicological changes caused by Perm. Thanks to the antioxidant properties of Res, it can be said that it plays a role in alleviating the toxicity on the organs by reducing the oxidative damage caused by Perm. In this study, besides examining the possible side effects of Perm, an insecticide that is widely used in daily life, and revealing its fetotoxic effects, it is aimed to reveal the therapeutic and/or protective effects of foods that are widely recommended in terms of epigenetic effects and taken from natural sources on the side effects of this agent. In this study, the effects of Perm and Res on the organogenesis period were examined in many organs - there is no previous study on this subject in the literature. We think that it can contribute to some extent as a guide both in the prevention of developmental abnormalities due to the use of Perm and in the protection from these possible toxic effects. However, due to the scarcity of studies on Perm-related organ damage and the antioxidant activity of Res on this damage, there is a need for experimental studies planned in larger series on this subject.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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References

Agrawal A, Sharma B (2010). Pesticides induced oxidative stress in mammalian systems: A review. International Journal of Biological and Medical Research 1(3): 90-104.

Aslan E, Boyacı MG, Güzel H, Pektas MB (2021). Better neuroprotective profile of caffeic acid phenyl ester over resveratrol in non-traumatic ischemia-reperfusion injury of the spinal cord. British Journal of Neurosurgery 9: 1-7.

Bohara RA, Tabassum N, Singh MP, Gigli G, Ragusa A, Leporatti S (2022). Recent overview of resveratrol's beneficial effects and its nano-delivery systems. Molecules 27(16): 5154.

Borsoi FT, Neri-Numa IA, de Oliveira WQ, de Araújo FF, Pastore GM (2023). Dietary polyphenols and their relationship to the modulation of non-communicable chronic diseases and epigenetic mechanisms: A mini-review. Food Chemistry: Molecular Sciences. 6: 100155.

Bozkurt E, Atay E, Pektas G, Ertekin A, Vurmaz A, Korkmaz OA, et al. (2020). Potential anti-tumor activity of kefir-induced juglone and resveratrol fractions against erhrlich ascites carcinoma-bearing BALB/C mice. Iranian Journal of Pharmaceutical Research 19(3): 358-369.

Burri PH (1984). Fetal and postnatal development of the lung. Annual Review of Physiology 46(1): 617-628.

Chen X, Zhang J, Yin N, Wele P, Li F, Dave S, et al. (2023). Resveratrol in disease prevention and health promotion: A role of the gut microbiome. Critical Reviews in Food Science and Nutrition 1(1): 1-18.

Coleman SJ, Bruce C, Chioni AM, Kocher HM, Grose RP (2014). The ins and outs of fibroblast growth factor receptor signalling. Clinical Science 127(4): 217-231.

Curtis GH, Nogueiro S, Schneider S, Bernhofer M, McDermott M, Nixon E, et al. (2021). Trans-ovo permethrin exposure affects growth, brain morphology and cardiac development in quail. Environmental Toxicology 36(7): 1447-1456.

Dekant W, Vamvakas S (1996). Biotransformation and membrane transport in nephrotoxicity. Critical Reviews in Toxicology 26(3): 309-334.

Dhivya Vadhana MS, Nasuti C, Gabbianelli R (2010). Purine bases oxidation and repair following permethrin insecticide treatment in rat heart cells. Cardiovascular Toxicology 10(3): 199-207.

Ding Y, Landrum PF, You J, Lydy MJ (2013). Assessing bioavailability and toxicity of permethrin and DDT in sediment using matrix solid phase microextraction. Ecotoxicology 22(1): 109-117.

Elser BA, Hing B, Stevens HE (2022). A narrative review of converging evidence addressing developmental toxicity of pyrethroid insecticides. Critical Reviews in Toxicology 52(5): 371-388.

Feng L, Cook B, Tsai SY, Zhou T, LaFlamme B, Evans T, et al. (2016). Discovery of a small-molecule BMP sensitizer for human embryonic stem cell differentiation. Cell Reports 15(9): 2063-2075.

Fernandes-Freitas I, Owen BM (2015). Metabolic roles of endocrine fibroblast growth factors. Current Opinion in Pharmacology 25: 30-35.

Gabbianelli R, Palan M, Flis DJ, Fedeli D, Nasuti C, Skarydova L, et al. (2013). Imbalance in redox system of rat liver following permethrin treatment in adolescence and neonatal age. Xenobiotica 43(12): 1103-1110.

Gasser E, Sancar G, Downes M, Evans RM (2022). Metabolic messengers: Fibroblast growth factor 1. Nature Metabolism 4(6): 663-671.

Gey N, Ersan Y (2020). Investigation of histopathological effects of deltamethrin on gills, liver and intestinal tissues of Carassius gibelio (Bloch, 1782). Halic University Journal of Science 3(2): 189-210.

Gibson-Corley KN, Olivier AK, Meyerholz DK (2013). Principles for valid histopathologic scoring in research. Veterinary Pathology 50(6): 1007-1015.

Guvenc D, Kabak Y, Atmaca E, Aksoy A, Guvenc T (2013). Examination of caspase-dependent apoptotic and necrotic changes in rat kidney exposed to different doses of permethrin. Biotechnic and Histochemistry. 88(2): 76–85.

Jin M, Li L, Xu C, Wen Y, Zhao M (2010). Estrogenic activities of two synthetic pyrethroids and their metabolites. Journal of Environmental Sciences 22(2): 290-296.

Koca HB, Pektas MB, Koca S, Pektas G, Sadi G (2016). Diabetes-induced renal failure is associated with tissue inflammation and neutrophil gelatinase-associated lipocalin: Effects of resveratrol. Archives of Biological Sciences 68(4): 747-752.

Liang YJ, Wang HP, Long DX, Li W, Wu YJ (2013). A metabonomic investigation of the effects of 60days exposure of rats to two types of pyrethroid insecticides. Chemico-Biological Interactions 206(2): 302-308.

López-Aceves TG, Coballase-Urrutia E, Estrada-Rojo F, Vanoye-Carlo A, Carmona-Aparicio L, Hernández ME, et al. (2021). Exposure to sub-lethal doses of permethrin is associated with neurotoxicity: Changes in bioenergetics, redox markers, neuroinflammation and morphology. Toxics 9(12): 337.

Lu G, Liu Q, Gao T, Li J, Zhang J, Chen O, et al. (2022). Resveratrol and FGF1 synergistically ameliorates doxorubicin-induced cardiotoxicity via activation of SIRT1-NRF2 pathway. Nutrients 14(19): 4017.

Min KK, Neupane S, Adhikari N, Sohn W, An S, Kim J, et al. (2020). Effects of resveratrol on bone-healing capacity in the mouse tooth extraction socket. Journal of Periodontal Research 55(2): 247-257.

Mossa ATH, Mohafrash SMM, Chandrasekaran N (2018). Safety of natural insecticides: Toxic effects on experimental animals. BioMed Research International 2018: 1-17.

Nasuti C, Fattoretti P, Carloni M, Fedeli D, Ubaldi M, Ciccocioppo R, et al. (2014). Neonatal exposure to permethrin pesticide causes lifelong fear and spatial learning deficits and alters hippocampal morphology of synapses. Journal of Neurodevelopmental Disorders 6(1): 7.

Nguyen, QBD, Vu MA, Hebert AA (2023). Insect repellents: An updated review for the clinician. Journal of the American Academy of Dermatology 88(1): 123-130.

Pektas A, Pektas MB, Koca HB, Tosun M, Aslan E, Koca S, et al. (2017). Effects of resveratrol on diabetes-induced vascular tissue damage and inflammation in male rats. Turkish Journal of Biochemistry 42(4): 451-458.

Pektas MK, Pektas MB, Surmen A, Karatepe F, Akar F (2014). Can resveratrol supplementation reduce adverse effects of metabolic syndrome on ovaries? Turkiye Klinikleri Journal of Gynecology Obstetrics 24(2): 98-103.

Pektas MB, Turan O, Ozturk G, Sumlu E, Sadi G, Akar F (2018). High glucose causes vascular dysfunction through Akt/eNOS pathway: reciprocal modulation by juglone and resveratrol. Canadian Journal of Physiology and Pharmacology. 96(8): 757–764.

Pektas MB, Aslan E, Firat F, Karaca C, Guzel H, Yildizhan S (2021). Suppressive effects of resveratrol on epithelial-mesenchymal transition in the YKG1 glioblastoma cell-line. Medicine Science 10(4): 1293-1298.

Roma GC, Oliveira PR, De Bechara GH, Mathias MIC (2012). Cytotoxic effects of permethrin on mouse liver and spleen cells. Microscopy Research and Technique 75(2): 229-238.

Romero A, Ramos E, Ares I, Castellano V, Martínez M, Martínez-Larrañaga MR, et al. (2017). Oxidative stress and gene expression profiling of cell death pathways in alpha-cypermethrin-treated SH-SY5Y cells. Archives of Toxicology 91(5): 2151-2164.

Simsek N, Altunkaynak BZ, Unal D, Can S, Malkoc I, Unal B (2009). A stereological and electron microscopic study of the development of the nephron in prenatal and postnatal rats. The Eurasian Journal of Medicine 41(2): 84-90.

Tateiwa D, Kaito T (2023). Advances in bone regeneration with growth factors for spinal fusion: A literature review. North American Spine Society Journal 13: 100193.

Tussellino M, Ronca R, Carotenuto R, Pallotta MM, Furia M, Capriglione T (2016). Chlorpyrifos exposure affects fgf8, sox9, and bmp4 expression required for cranial neural crest morphogenesis and chondrogenesis in Xenopus laevis embryos. Environmental and Molecular Mutagenesis 57(8): 630-640.

Wang X, Martínez MA, Dai M, Chen D, Ares I, Romero A, et al. (2016). Permethrin-induced oxidative stress and toxicity and metabolism. A review. Environmental Research 149(1): 86-104.

Xiao M, Tang Y, Wang AJ, Wang BJ, Lu G, Guo Y, et al. (2021). Regulatory role of endogenous and exogenous fibroblast growth factor 1 in the cardiovascular system and related diseases. Pharmacological Research 169: 105596.

Xu X, Liu Q, Li J, Xiao M, Gao T, Zhang X, et al. (2022). Co-treatment with resveratrol and FGF1 protects against acute liver toxicity after doxorubicin treatment via the AMPK/NRF2 pathway. Frontiers in Pharmacology 13: 940406.

Yang C, Luo P, Chen S, Deng Z, Fu X, Xu D, et al. (2021). Resveratrol sustains intestinal barrier integrity, improves antioxidant capacity, and alleviates inflammation in the jejunum of ducks exposed to acute heat stress. Poultry Science 100(11): 101459.

Ye Y, Jiang Z, Pan Y, Yang G, Wang Y (2022). Role and mechanism of BMP4 in bone, craniofacial, and tooth development. Archives of Oral Biology 140: 105465.

Zhang N, Xie YP, Pang L, Zang XX, Wang J, Shi D, et al. (2014). Paraquat increases connective tissue growth factor expression and impairs lung fibroblast proliferation and viscoelasticity. Human and Experimental Toxicology 33(12): 1232-1240.