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Research Article

The Effects of Propetamphos, Cypermethrin and Propetamphos-Cypermethrin Combination on Some Biochemical and Histopathological Parameters in Mice [1]

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Abstract

This study was aimed at investigating the toxic effects of subchronic and chronic exposure to propetamphos (PRO) and cypermethrin (CYP) combination in mice. Seventy male Swiss albino mice were used for this study. Group 1 was maintained for control and given insecticide-free feed for 60 days. Group 2 was administered with 5.0 mg/kg/bw/day of PRO (LD50/20); Group 3 with 10.0 mg/kg/BW/day of CYP (LD50/20), Group 4 with PRO and CYP combination at the same doses in feed for 60 days. Blood samples, liver and kidney were taken on days 45 and 60 from 10 animals. Serum samples were analysed for biochemical parameters, and liver and kidney tissues were examined histopathologically. When compared to the control group with insecticide-treated groups, it was determined that cholesterol, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) levels increased and albumin levels decreased ($P<0.05$), bilirubin levels increased ($P<0.05$) in Group 4. It was seen that triglyceride and bilirubine levels and ALP and GGT activities were more high ($P<0.05$) in Group 4 than other groups; also ALT activity and bilirubine level were high ($P<0.05$) in Group 4 at 60 day. In all treatment groups, necrosis of hepatocytes, cytoplasmic vacuolation, bile duct hyperplasia and mononuclear cellular infiltration were presented in liver. In Group 4 at 60 day, tigroid basophilic cytoplasm and hepatocellular hypertrophy of liver; necrosis of the tubular epithelial cells, cytoplasmic vacuolation, cellular infiltration and glomerular atrophy of kidney were seen. As a result, it was concluded that the exposure of mice to PRO and CYP for periods of 45 and 60 days cause to adverse effects on both liver and kidney functions and protein and lipid metabolism. These effects were observed to be more severe in the case of PRO-CYP exposure for 60 days.

Keywords: *Cypermethrin, Propetamphos, Toxicity, Mice, Serum biochemical parameter, Histopathology*

Sipermetrin, Propetamfos ve Sipermetrin + Propetamfos Karışımının Farelerde Bazı Biyokimyasal ve Histopatolojik Parametreler Üzerine Etkileri

Özet

Bu çalışmada propetamfos (PRO), sipermetrin (CYP) ve bunların karışımlarının farelerde subkronik ve kronik toksik etkilerinin incelenmesi amaçlandı. Çalışmada 70 adet Swiss albino ırkı erkek fare kullanıldı. Grup 1 kontrol olarak tutuldu ve gruptaki hayvanlara 60 gün boyunca insektisit içermeyen yem verildi. Grup 2'ye 5 mg/kg/ca/gün PRO (LD50/20), Grup 3'e 10 mg/kg/ca/gün CYP (LD50/20), Grup 4'e aynı dozda PRO-CYP karışımı 60 gün boyunca yem içinde verildi. Çalışmanın 45 ve 60. günlerinde 10'ar farenin kan örnekleri ile karaciğer ve böbrekleri alındı. Serum örneklerinde biyokimyasal analizler, karaciğer ve böbrekte histopatolojik incelemeler yapıldı. İnsektisit uygulanan gruplar kontrol grubu ile karşılaştırıldığında, serum kolesterol ve trigliserit düzeyleri ile aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), alkalin fosfataz (ALP) ve gama glutamil transferaz (GGT) aktivitelerinde yükselme ($P<0.05$), albumin düzeyinde azalma ($P<0.05$), grup 4'te bilirubin düzeyinde yükselme ($P<0.05$) olduğu belirlendi. Grup 4'de trigliserit ve bilirubin düzeyleri ile ALP ve GGT aktivitelerinin diğer gruplara göre daha yüksek olduğu ($P<0.05$); ayrıca 4. grupta 60. gün ALT aktivitesi ve bilirubin düzeylerinin yüksek seyrettiği ($P<0.05$) gözlendi. İnsektisit uygulanan tüm gruplarda karaciğerde hepatositlerde nekroz, sitoplazmik vakuolleşme, safra kanallarında hiperplazi ve hücre infiltrasyonu olduğu gözlendi. Bunlara ilaveten grup 4'te 60 gün insektisit uygulaması sonrasında karaciğerde tigroid bazofilik sitoplazma ve hepatoseluler hipertrofi; böbrekte tubuler epitelyal hücrelerde nekroz, sitoplazmik vakuolleşme, hücre infiltrasyonu ve glomerular atrofi gözlendi. Sonuç olarak farelerde 45 ve 60 gün boyunca PRO, CYP ve PRO-CYP karışımı verilmesinin karaciğer ve böbrek fonksiyonları ile protein ve lipid metabolizmasını olumsuz yönde etkilediği; bu etkilerin 60 gün PRO-CYP verilen hayvanlarda daha şiddetli olduğu gözlendi.

Anahtar sözcükler: *Sipermetrin, Propetamfos, Zehirlenme, Fare, Serum biyokimya, Histopatoloji*

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INTRODUCTION

PRO is an organic phosphorus insecticide of a phosphoroimidate group, which has a strong insecticidal effect and weak anticholinesterase activity [1]. Similar to other organophosphate insecticides, PRO irreversibly blocks the activity of acetyl cholinesterase (AChE), which breaks down acetylcholine (ACh), and thereby, leads to the accumulation of ACh at the neuromuscular junctions, postganglionic nerve terminals in smooth muscle, the myocardium, etc., and in all autonomic ganglia and the cholinergic synapses of the central nervous system, causing intoxication [2].

Owing to its strong insecticidal activity, broad spectrum, and low toxicity in mammals, CYP has commonly used in treatment of parasitic infestations [3]. The LD_{50} of CYP in mice is 82-779 mg/kg [4]. The major target area of CYP and other pyrethroids in the body is the voltage-dependent sodium channels located in the membrane of nerve cells. Pyrethroids affect the opening and closure of these channels, resulting in their remaining open for an extended time period, the acceleration of Na^+ transport, and eventually nervous discharge and blockage. Apart from the Na^+ channels, pyrethroids also affect voltage-sensitive Ca^{+2} channels, by inducing the secretion of neurotransmitters and inhibiting Ca^{+2}-ATPases and Mg^{+2}-ATPases [2,3].

Insecticides are widely used in agriculture, environmental health, human and animal health and many insecticide formulations contain two or more active ingredient. Because of this reason, humans and animals are constantly exposed to multi-insecticide residues. Chronic exposure to insecticides has been associated with many hazardous effect on nervous, endocrine, reproductive and immune system [5-7]. It has been showed that chronic exposure of PRO, CYP and PRO-CYP combination cause lipid peroxidation in mice [5]. There are several insecticide formulations containing CYP and PRO for veterinary medicine in Turkey. PRO and CYP poisonings have been reported for human by Turkey Public Health Corporation [8]. There is not any study about effects of chronic administration to PRO, CYP and PRO-CYP combination on serum biochemical parameters, and liver and kidney in mice. With this study, subchronic and chronic effects of PRO, CYP and their mixtures on serum biochemical parameters and, liver and kidney histopathology in mice to understand the possible health effects to animals and human was investigated.

MATERIAL and METHODS

The trial was performed in compliance with the instructions of Local Ethical Board of Erciyes University Veterinary Faculty (040/051). Forty-day old male Swiss albino mice, each weighing 35-40 g and the progeny of parent mice, constituted the material of the study. The mice were raised under a daily 12-h light and 12-h dark regime, in a heat- (20-22°C) and ventilation-regulated room, in polyethylene cages, each containing a maximum of 10 animals. Feed and water were provided as *ad libitum* [5]. For this research, technical standards of cypermethrin (70%) and propetamphos (90%) obtained by Topkim Drug Corporation, Istanbul, Turkey.

Four groups were established. While the control group comprised 10 animals, each treatment group was composed of 20 animals. The control group (Group 1) was provided *ad libitum* with insecticide-free feed for 60 days. The mice in Groups 2, 3 and 4 were administered with 5.0 mg/kg/BW/day of PRO, 10.0 mg/kg/BW/day of CYP, and 5.0 mg/kg/BW/day of PRO in combination with 10.0 mg kg/BW/ day of CYP, respectively, in feed for a period of 60 days [5].

Blood samples were collected by cardiac puncture on day 60 from the control group and on days 45 and 60 from 10 mice included in each of the treatment groups. The blood samples were centrifuged at 3.000 rpm for 10 min by using Sigma 3K-30 and serum was transferred in eppendorph tubes. Serum samples were maintained at - 80°C in a deep freezer until analysed. The serum samples were analysed for cholesterol, triglyceride, total protein, albumin, and bilirubin levels, and AST, ALT, ALP and GGT activities. The analyses were performed using Johnson & Johnson kits in a Vitros 750 model auto-analyser. AST, ALT, ALP and GGT were given in U/L, while the results of the other analyses were given in mg/dL.

The mice, which were sampled for blood, were euthanized by cervical dislocation. After extracted, the liver and kidneys were examined for macroscopic lesions. Subsequently, samples taken from the hepatic and renal tissues were fixed in neutral formalin for 24 h, subjected to routine processing, and embedded in paraffin. The paraffin blocks were cut into sections of 5-6 µm thickness and stained with haematoxylene and eosin (H&E) for microscopic examination.

Statistical analysis of biochemical parameters were made with SPSS 16.0 software. Data were given as arithmetic means and standard deviation. Significance of among groups were employed with one-way variance analysis and Tukey test.

RESULTS

When compared to the control group, in the treatment groups it was observed that, the cholesterol and triglyceride levels, and AST, ALT, ALP and GGT activities had increased ($P<0.05$) and the albumin levels had decreased ($P<0.05$); also the bilirubin level had increased ($P<0.05$) in PRO-CYP treated group (Group 4) *(Table 1)*. It was seen that triglyceride and bilirubine levels and ALP and GGT activities were more high ($P<0.05$) in PRO-CYP treated group (Group 4) than other groups; also ALT activity and bilirubine level were high ($P<0.05$) in PRO-CYP treated group (Group 4)

at 60 day *(Table 1)*. When compared to the PRO treated (Group 2) and CYP treated (Group 3) groups, triglyceride level and AST activity were high (P<0.05) in CYP treated group (Group 3) *(Table 1)*. When compared to the PRO-CYP treated group (Group 4), cholesterol, triglyceride and bilirubin levels and AST, ALT and GGT activities decreased (P<0.05) in PRO treated group (Group 2); also triglyceride and bilirubin levels and ALP and GGT activities decreased (P<0.05) in CYP treated group (Group 3); also triglyceride and bilirubin levels and GGT activities were low (P<0.05) both PRO treated group (Group 2) and CYP treated group (Group 3) *(Table 1)*.

Degenerative changes were present in all of the treatment groups and were classified as severe (+++), moderate (++), and mild (+), according to their severity *(Table 2)*. Microscopically, severe lesions were observed in the kidney and liver samples of Group 4 on days 45 and 60 *(Table 2)*.

The presence of many focal inflammatory cells and degeneration were observed in the hepatic tissues. Degenerative changes were characterized by parenchymatous degeneration and the disintegration of hepatocytic nuclei along with the hyperplasia of the bile

Table 1. *Biochemical parameters of groups (mean±sd)*
Tablo 1. *Gruplardaki biyokimyasal parametreler (ort±ss)*

Parameter	Group I (Control)	Group II (PRO)		Group III (CYP)		Group IV (PRO-CYP)	
		45 DAY	60 DAY	45 DAY	60 DAY	45 DAY	60 DAY
Cholesterol (mg/dL)	88.2±8,1^{d}	116.3±12.2^{c}	118.7±13.1^{c}	127.3±11.9^{bc}	129.3±11.9^{bc}	142.2±4.0^{ab}	147.7±5.7^{a}
Triglyceride (mg/dL)	107.7±8.2^{d}	160.0±19.5^{c}	161.0±14.8^{c}	207.8±27.1^{b}	210.8±26.6^{b}	260.5±38.6^{a}	265.0±26.9^{a}
Total protein (mg/dL)	7.6±0.6	7.3±0.5	7.2±0.2	7.1±0.5	7.2±0.4	7.4±0.3	7.5±0.2
Albumin (mg/dL)	4.6±0.2^{a}	4.0±0.2^{b}	3.7±0.2^{bcd}	4.0±0.4^{bc}	3.5±0.2^{bcd}	3.5±0.2^{bcd}	3.4±0.1^{bcd}
AST (U/L)	106.7±16.0^{e}	225.3±42.6^{d}	242.2±30.8^{d}	300.3±8.5^{c}	311.5±10.2^{bc}	343.8±21.0^{ab}	362.3±19.5^{a}
ALT (U/L)	21.3±2.9^{d}	36.2±6.7^{c}	36.7±2.9^{c}	40.7±8.2^{bc}	47.8±5.4^{b}	50.2±6.2^{b}	69.3±6.7^{a}
ALP (U/L)	49.7±1.0^{d}	77.2±8.5^{c}	80.0±7.3^{bc}	76.0±5.4^{c}	78.8±8.8^{c}	94.0±7.7^{ab}	97.3±5.6^{a}
GGT (U/L)	1.7±0.8^{c}	14.7±2.1^{b}	15.3±2.0^{b}	15.3±3.2^{b}	16.0±3.3^{b}	25.2±4.8^{a}	27.2±1.0^{a}
Bilirubin (mg/dL)	2.4±0.2^{c}	2.6±0.2^{c}	2.8±0.1^{c}	2.8±0.3^{c}	2.9±0.1^{c}	3.4±0.5^{b}	4.0±0.3^{a}

PRO: *Propetamphos,* ***CYP:*** *Cypermethrin,* ***PRO-CYP:*** *Propetamphos and cypermethrin combination,* ***AST:*** *Aspartate aminotransferase,* ***ALT:*** *Alanine aminotransferase,* ***ALP:*** *Alkaline phosphatase,* ***GGT:*** *Gamma-glutamyl transferase;* ***a,b,c,d,e:*** *Different characters indicate statistically significant differences in the same line (P<0.05)*

Table 2. *Histopathological findings of groups*
Tablo 2. *Gruplardaki histopatolojik bulgular*

Organ /Lesion	Group II		Group III		Group IV	
	PRO-45	PRO-60	CYP-45	CYP-60	CYP-PRO-45	CYP-PRO-60
Liver						
Cytoplasmic vacuolation					+++	++++
Condensed/pyknotic nuclei				+++		
Necrosis		+++		+++	+++	++++
Cellular infiltration		+++	++	+++	++	++++
Hyperplasia of bile duct						
Passive congestion		++	++		++	++++
Kidney						
Pyknotic nuclei						
Necrosis						++++
Deposition of casts						++++
Epithelium cytoplasmic vacuolation				+++		++++
Glomerulus atrophy						++++
Cellular infiltration	+++					++++

CYP: *Cypermethrin,* ***PRO:*** *propetamfos,* ***CYP-PRO:*** *Cypermethrin and propetamfos combination*

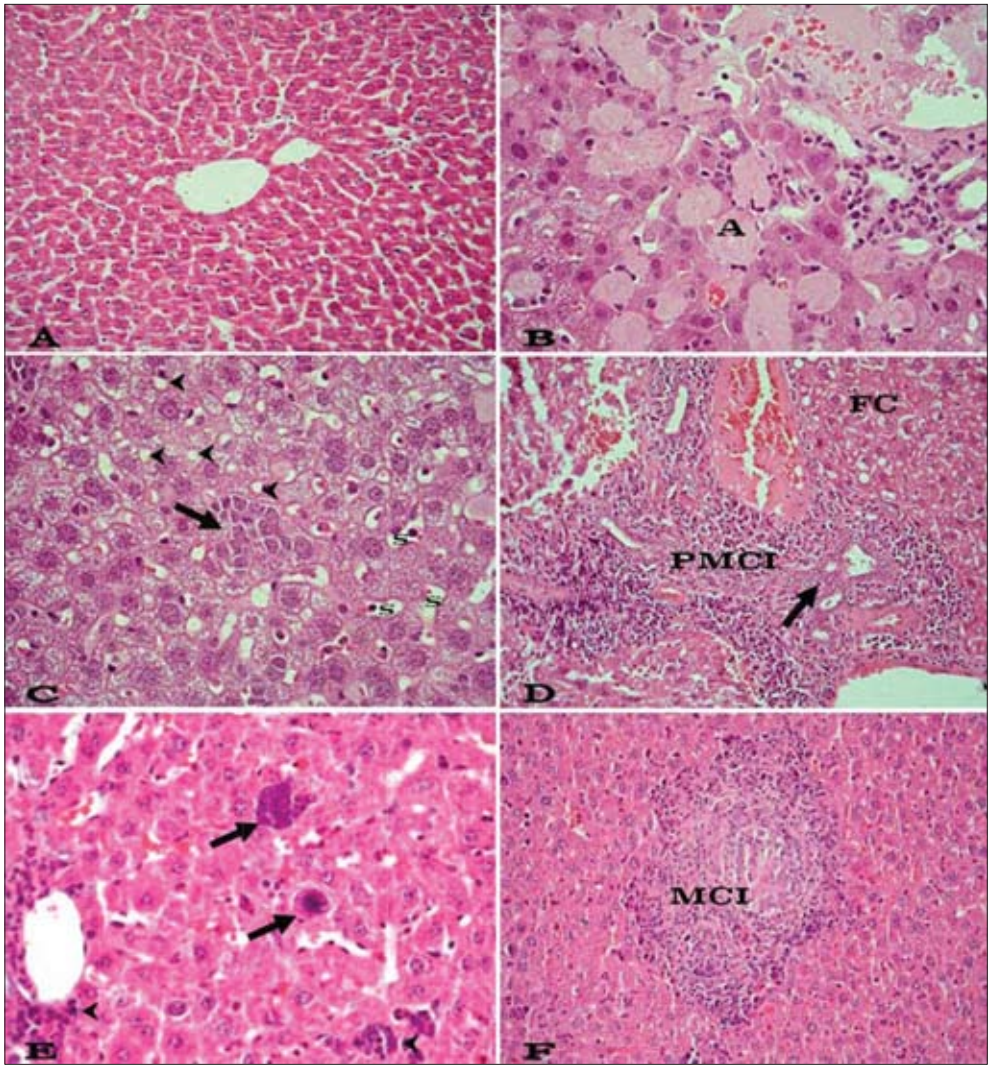

Fig 1. Histopathological findings of liver in Group 4. **A:** Histology of control group liver, **B-F:** Pronounced histopathological abnormalities observed in Group 4, **B:** Perisinusoidal amyloidosis (A), **C:** Hepatocellular hypertrophy with cytoplasmic basophilia, Fatty changes *(arrowheads)* and dilated sinusoids (S), **D:** Perivascular cell infiltration and fatty changes, **E:** Parenchymatous degeneration of hepatocytes with mild necrosis. Mononuclear cell infiltration and multinucleation of hepatocytes *(arrow),* **F:** Focal cellular granulomatous lesions. PMCI: Perivasküler round cell infiltration; FC: Fatty change; MCI: Mononuclear cell infiltration. Magnification: A&D&F, x200; B-C-E, x400

Şekil 1. Gruplarda karaciğere ait histopatolojik bulgular. **A:** Kontrol grubuna ait karaciğerlerin histolojisi, **B-F:** Grup 4'te gözlenen belirgin histopatolojik bulgular, **B:** Perisinuzoidal amiloidler (A), **C:** Sitoplazmik bazofiler içeren hepatoselüler hipertorifi. Yağlı değişimler *(ok uçları)* ve genişlemiş sinüzoidler (S), **D:** Perivasküler hücre infiltrasyonu ve yağlı değişimler, **E:** Hepatositlerde orta şiddetli nekrozla karakterize paranşimatöz dejenerasyon. Mononuklear hücre infiltrasyonu ve hepatositlerde multinükleasyon *(oklar)*, **F:** Fokal hücresel granülomatöz lezyonlar. PMCI: Perivasculer hücre infiltrasyonu; FC: Yağlı değişimler; MCI: Mononüklear hücre infiltrasyonu. Büyütme: A&D&F, x200; B-C-E, x400

ducts. The cytoplasm of the hepatocytes was of a light colour and foamy appearance and was filled with vacuoles. The cell size had increased, the nuclear chromatin was more compact, and the slightly smaller nucleoli were not conspicuous. The necrotic hepatocytic nuclei were contracted, pyknotic, filled with condensed chromatin, and the nuclear cytoplasm was strongly acidophilic. The accumulation of mononuclear cells was observed in the vicinity of sinusoids. Histopathological alterations included perivascular round cell infiltration, marked degeneration of the hepatic cords, and increased incidence of vacuolar degeneration *(Fig. 1, Table 2)*. Hepatocellular hypertrophy, multinucleated hepatocytes and the deposition of pale, homogeneous and amorphous eosinophilic material in the periportal and perisinusoidal areas and the blood vessel wall were observed on day 60 in only Group 4. Microscopically, the kidneys showed toxic tubular necrosis characterized by pyknotic cells, sloughing of the tubular epithelium and epithelial casts in the tubular lumen associated with the atrophy of the glomeruli in Group

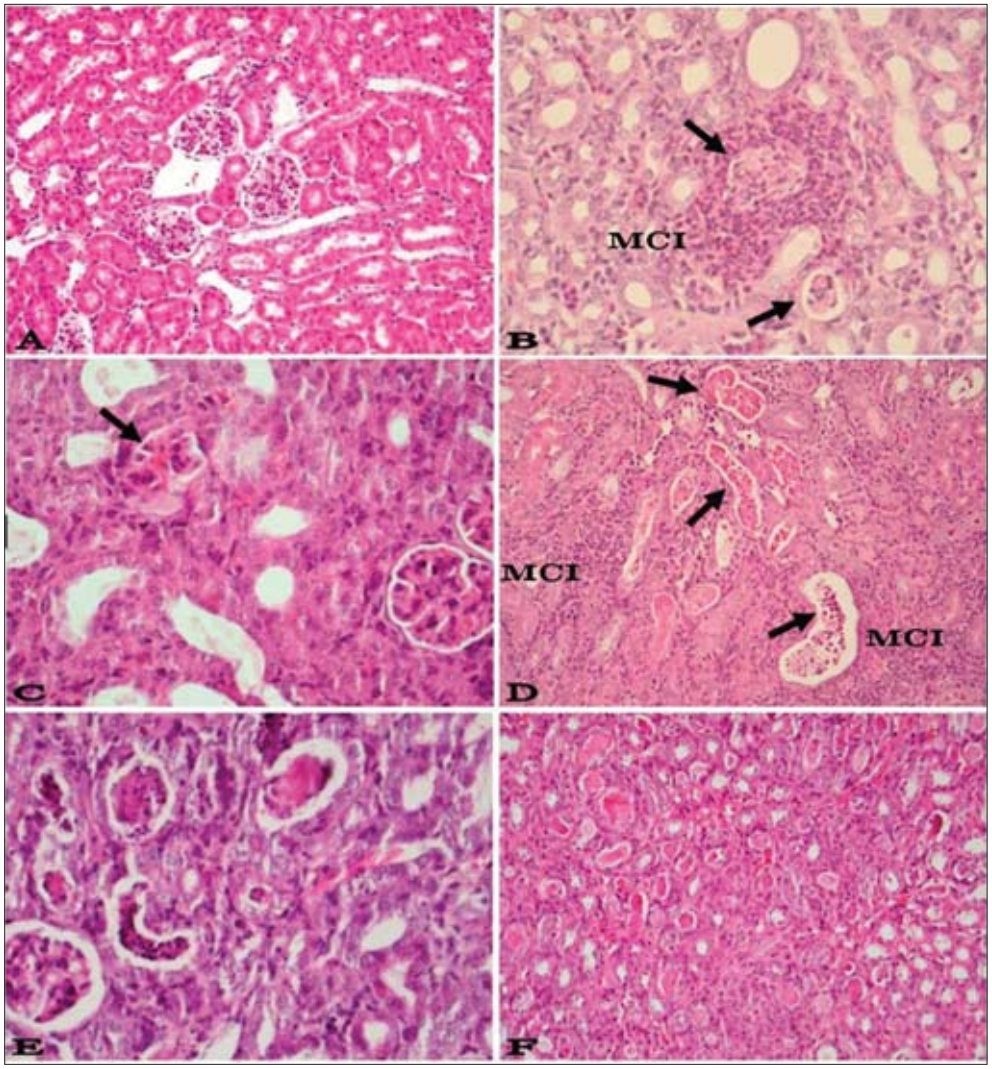

Fig 2. Histopathological findings of kidney on Group 4. **A:** Histology of control group kidney, **B-F:** Pronounced histopathological abnormalities in Group 4 on day 60, **B:** Tubular necrosis *(arrows)* and focal mononuclear cell infiltration, **C:** Glomerular necrosis *(arrow)* and tubular dilatation, **D:** Tubules containing necrotic cells *(arrows)* and mononuclear cell infiltration, **E:** Tubular necrosis, **F:** Hyaline casts in tubule lumens. MCI: Mononuclear cell infiltration. Magnification: A&D&F, x200; B-C-E, x400

Şekil 2. Gruplarda böbreklere ait histopatolojik bulgular. **A:** Kontrol grubuna ait böbreklerin histolojisi, **B-F:** Grup 4'de 60. günde gözlenen belirgin histopatolojik değişimler, **B:** Tubuler nekroz *(oklar)* ve fokal mononuclear hücre infiltrasyonu, **C:** Glomeruler nekroz *(oklar)* ve tubuler genişleme, **D:** Tubuler nekrotik hücreler ve mononuclear hücre infiltrasyonu *(oklar),* **E:** Tubuler nekroz, **F:** Tubul lümenlerinde hiyalinize bölgeler. MCI: Mononüklear hücre infiltrasyonu. Büyütme: A&D&F, x200; B-C-E, x400

4. Karyorrhexis, and in some cells karyolysis and cellular infiltration, were also observed in Group 4 *(Fig. 2, Table 2)*.

DISCUSSION

In the present study, when compared to the control group, it was observed that in the PRO treated group (Group 2) for 45 and 60 days, the serum cholesterol and triglyceride levels and AST, ALT, ALP and GGT activities had increased, while the albumin levels had decreased. In particular, the increases observed in the activity of ALT, an enzyme specific to hepatic damage [9], as well as in the activities of AST, ALP and GGT demonstrated that PRO caused hepatic damage in mice. It has been reported that PRO undergoes desulphuration by the liver P-450 enzymes and is converted into oxygen-derived metabolites, while by hydrolytic reactions it is converted into acetoacetate, acetone and CO_2 in rats and mice [10]. In the present study, increasing of serum cholesterol and triglyceride levels in PRO treated group (Group 2) demonstrate that PRO have adverse effects on the lipid metabolism. It is reported that serum ALP activity, and cholesterol and triglyceride levels also increase in cases of cholestasis [10]. In addition to increase of ALP activity and cholesterol and triglyceride levels in the PRO treated group (Group 2) suggest that subchronic exposure to PRO causes cholestasis in mice.

Albumin is an enzyme synthesized in the liver and found at a high level in the serum [10]. This study showed that the administration of PRO decreased serum albumin levels, and this decrease may be associated with hepatic damage.

Cetin et al.[11] reported that, following the administration of 15 mg/kg of PRO for 28 days to rats, serum triglyceride levels, and AST, ALP, and ALT activities increased, while total protein levels decreased. Upon administering propetamphos at doses of 7.5 and 15 mg/kg to rats for 28 days, Kanbur et al.[12] reported that plasma MDA levels had increased while erythrocyte SOD, CAT and GSH-Px activities had decreased. It is suggested that the adverse effects of PRO on the liver could be related to the active metabolites of the drug, which are generated in the liver, and to the lipid peroxidation induced by these metabolites.

The present study demonstrated that, when compared to the control group, the administration of CYP at a dose of 10 mg/kg to mice for periods of 45 and 60 days led to increased serum cholesterol and triglyceride levels, and AST, ALT, ALP and GGT activities and decreased albumin levels. Bhushan et al.[13] reported that the acute (300 mg/kg) and subchronic (10.7 mg/kg) exposure of rats to CYP caused increases in serum AST, ALT, ALP, and LDH activities, and total lipid, triglyceride, total protein, cholesterol, and bilirubin levels particularly on day 28. Gomaa et al.[14] indicated that the administration of rats with 14.5 mg/kg of CYP for 30 days elevated serum liver enzymes, cholesterol and MDA levels, and reduced total protein, albumin, triglyceride and LDL levels as well as antioxidant enzyme (SOD, CAT, GSH-Px) activities. It has been reported that, in rats, exposure to CYP causes haemolytic anaemia and hyperbilirubinaemia due to erythrocytic membrane disease [13]. In a study conducted by Khan [15], in which mice were given 20 mg/kg of CYP by gavage for 15 days, serum ALP, AST and ALT activities were determined to have increased, while liver SOD, CAT, GSH-Px and GSH-S-transferase activities were ascertained to have decreased. Manna et al.[16] determined that, when rats were administered with alpha-CYP at a dose of 14.5 mg/kg for 30 days, serum AST, ALT, and ALP activities, and blood glucose and liver MDA levels increased, and CAT and SOD activities decreased. ALT activity is considered as an indicator of general hepatocellular damage, whilst AST activity is used as an indicator of mitochondrial damage. On the other hand, the increase of blood ALP activity indicates the presence of cholestasis in the liver. Furthermore, increased serum LDH levels are considered to be an indicator of hepatic necrosis [17]. Both the results of the present study and those reported in previous research suggest that CYP leads to hepatic damage and induces cholestasis. The results of this study also show that the damaging effect of CYP on the liver is more severe than that of PRO. Xenobiotics activate the sympathetic nervous system, inducing the release of adrenaline from the adrenal medulla, thus increasing both the mobilisation of lipids from tissues and serum triglyceride levels [13]. The triglyceride-increasing effects of CYP may also be related to this mechanism.

Manna et al.[18] reported that CYP caused moderate histopathological alterations in the liver and kidneys. Gomaa et al.[14] observed congestion of the central and portal veins and hydropic degeneration of the hepatocytes in the liver, and suggested hepatic damage to be associated with free radicals generated as a result of the oxidation of CYP by the CYP-450 enzyme system. In various research, rats administered with CYP were observed to present with the enlargement of the hepatic sinusoids, degeneration of the hepatic cords and hepatocytes in the centrilobular areas, dilatation and desquamation, cytoplasmic accumulation of eosinophils, and erythrocyte accumulation in the cerebellar and meningeal blood vessels of the brain, and these effects were considered to be associated with the generation of free radicals [19-21]. Apart from causing free radical generation in the liver, CYP also inhibits the activity of hepatic ATPase, resulting in the necrosis, inflammation and cytoplasmic hypertrophy of hepatocytes. It has been reported that synthetic pyrethroids lead to the formation of unstable cyanides and aldehydes in the liver [22]. Thus, these metabolites may be responsible for the cellular damage caused by CYP in the liver. The hepatic damage caused by cypermethrin in the present study may be related to these metabolites as well as to free radical-induced lipid peroxidation and reduced ATPase activity.

Compared to the PRO and CYP treated group (Group 2 and Group 3), cholesterol, triglyceride, and bilirubin levels, and AST, ALT and GGT activities increased ($P<0.05$) in PRO-CYP treated group particularly in 60 days (Group 4). The results of the present study demonstrated that the combined administration of CYP and PRO caused synergistic interaction in mice and aggravated hepatic damage. The results of the present study also showed that while the administration of PRO and CYP alone did not cause any statistically significant difference in serum bilirubin levels, the combined use of PRO and CYP led to a statistically significant increase in serum bilirubin levels. Increased bilirubin levels are considered an indicator of hepatobiliary disorders [9]. This data confirms PRO-CYP treatment aggravate the liver damage.

In a study in which mice were administered with CYP (10 mg/kg), PRO (5 mg/kg) and a combination of CYP and PRO [5] it has been reported that MDA and NO significantly increase, SOD, CAT and GSH-Px activities significantly decrease in all groups; CYP, PRO and CYP+PRO combination induce lipid peroxidation. In our study, triglyceride and bilirubine levels and ALP and GGT activities were more high in PRO-CYP treated group (Group 4) than other groups; also ALT activity and bilirubine level were high in PRO-CYP treated group (Group 4) at 60 day. Results of

this study show that PRO-CYP treatment exacerbates the liver degeneration. PRO-CYP induced hepatic damage could be related to lipid peroxidation as mentioned in previous studies.

In the present study, the combined administration of PRO and CYP, particularly for a period of 60 days, was observed to be associated with passive congestion, necrosis, mononuclear cell infiltration and hyperplasia of the bile ducts in the liver, and with cellular infiltration and degenerative alterations in the kidneys. Bile duct hyperplasia may result from hepatic injury and repair, and is often associated with evidence of these phenomena. Mononuclear cells with areas of hepatocyte necrosis can be considered as an indication of the chronic inflammation of the liver. The infiltration of inflammatory cells is a typical response to parenchymal cell death following exposure to toxicants and their toxic metabolites [23]. Similar but more severe lesions were observed in the Group 4.

In the Group 4, degenerative and aggressive lesions including necrosis, multinucleated and disarranged hepatocytes and amyloid degeneration, were characteristic pathological changes. Hepatocellular hypertrophy following enzyme induction was considered to be an adaptive response to chemical stress. Tubular necrosis and focal mononuclear cell infiltration, glomerular necrosis and hyaline casts in tubule lumens with tubular dilatation, and perivascular round cell infiltration were observed in the Group 4. The histopathological changes show consistency with our biochemical results *(Table 1)* and previous studies [24,25].

Enzymes responsible for metabolic interactions (biotransformation), which are one of the major mechanisms of drug interactions, are in general enzymes belonging to the cytochrome P-450 group. When drugs, which are the substrate of the same enzyme group, are administered in combination, there is a possibility of metabolic interactions occurring [26,27]. It has been reported that pyrethroids were rapidly metabolized by esterases in the body and that their metabolic rate could be reduced by esterase inhibitors, such as organic phosphorous insecticides, increasing the toxicity of pyrethroids [5,27]. Similarly, the present study demonstrated that the combined administration of PRO and CYP to mice for 60 days caused to synergistic effect on biochemical parameters and at histopathological findings. The aggravation of adverse effects with combined use may not only be related to metabolic interactions, but also to the two pesticides prolonging the elimination of each other from the body.

In the present study, it was concluded that the exposure of mice to PRO, CYP and PRO-CYP combinations for subchronic and chronic periods caused adverse effects on liver and kidney functions as well as on protein and lipid metabolism; PRO-CYP combination aggravated these adverse effects; chronic exposure of PRO, CYP and their mixture are more toxic than subchronic exposure. The results show that usage of insecticide combinations can increase insecticide induced toxic effects on mammals and duration time affects toxicity of insecticides. So, *in vivo* and *in vitro* researches are required to understand toxicities of insecticides and insecticide mixtures on mammals.

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