

## The protective and therapeutic effect of phenoxy-2-methyl-2-propionic acid on experimental fatty liver in rats

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**Abstract:** In this study, the potential protective and therapeutic effect of phenoxy-2-methyl-2-propionic acid (PMPA) was investigated in rats with experimental hepatic steatosis induced by a carbon tetrachloride and paraffin liquid mixture (1:1). Forty Wistar albino rats were allocated to 4 groups with 10 rats in each. Group 1 was administered a saline solution; group 2 was administered the carbon tetrachloride and paraffin liquid mixture; group 3 was administered the carbon tetrachloride and paraffin liquid mixture and PMPA for 7 days; and in group 4, hepatic steatosis was created with the carbon tetrachloride and paraffin liquid mixture during the first 7 days, after which the rats were administered only PMPA during the last 7 days. All injections were done intraperitoneally. Blood samples were obtained from all rats and livers were extirpated before euthanasia. Blood parameters were found to be significantly different between the groups ( $P < 0.05$ ). On histopathologic examination, grade 1 hepatic steatosis was observed in group 1; grade 3 hepatic steatosis and grade 2 portal fibrosis were observed in group 2; grade 2 hepatic fibrosis and grade 1 portal fibrosis were observed in group 3; and grade 1 hepatic steatosis was observed in group 4. Hepatic fibrosis was induced in the rats using a carbon tetrachloride and paraffin liquid mixture. Results indicated that PMPA has a limited protective effect and a superior therapeutic effect on hepatic steatosis.

**Key words:** Hepatic steatosis, rat, phenoxy-2-methyl-2-propionic acid, histopathologic examination, blood parameters

### 1. Introduction

Excessive triglyceride accumulation, which largely affects liver parenchyma, is defined as hepatic steatosis (1). Factors like fasting, obesity, hormonal changes, toxins, drugs, pancreatectomy, and alcohol use play a role in the etiology of the disease in humans and animals (2–4). Hepatic steatosis is particularly common in cats among animals (3). Clinical findings include depression, anorexia, vomiting, icterus, diarrhea, cervical ventroflexion, weight loss, hepatomegaly, ptyalism, and dehydration (1–4). Biochemical analysis of blood, ultrasonography, Doppler ultrasonography, computed tomography, magnetic resonance imaging, and biopsy are recommended for diagnosis (5,6). Treatment includes supportive therapy; rehydration; consumption of nonlactose, nonglucose liquids and electrolyte-balancing liquids; and supplementation with L-carnitine, taurine, and vitamins B, E, and K (1–4,7,8).

In this study, we aimed to investigate whether phenoxy-2-methyl-2-propionic acid (PMPA) has a protective and/or therapeutic effect on hepatic steatosis in rats.

### 2. Materials and methods

#### 2.1. Animals

A total of 40 adult Wistar albino rats constituted the study material. Humane rules prepared for the care and use of laboratory animals as reported by the US National Research Council in 1996 were applied to the animals ([http://www.nap.edu/openbook.php?record\\_id=5140](http://www.nap.edu/openbook.php?record_id=5140)). Forty Wistar albino rats were bought from the İstanbul University Experimental Medicine Institute (İstanbul, Turkey). Rats were kept at 22–25 °C with artificial light from 0700 to 1900 hours for 1 week and adaptation was provided. Animals were continuously fed with clean water and pellet feed during the experiment process. Rats were allocated to 4 groups with 10 rats in each after 1 week of adaptation.

The first (negative control) group was created by giving 0.2 mL of 0.9% NaCl; the second (positive control) group was given 0.8 mg/kg  $\text{CCl}_4$  and paraffin liquid fluid (1:1) to induce a fatty liver model; the third (protecting) group was given 0.8 mg/kg  $\text{CCl}_4$  + paraffin liquid fluid (1:1) with 10 mg/kg PMPA; and the fourth (treatment) group was given

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0.8 mg/kg CCl<sub>4</sub> + paraffin liquid fluid (1:1) in the first week and 10 mg/kg PMPA in the second week. All injections were given intraperitoneally.

All rats received water and feed ad libitum. The feed was commercial pellet feed. The experiment took 1 week in Groups 1, 2, and 3 and 2 weeks in Group 4. Two milliliters of blood samples were taken from the heart on day 8 (Groups 1, 2, 3) and day 15 (Group 4). Blood was taken into tubes without an anticoagulant and clotting was allowed. Sera were separated by centrifugation at 3500 rpm for 15 min thereafter, and these sera were stored in Eppendorf tubes at -20 °C in a freezer until the analysis was done. Rats were euthanized with 150 mg/kg pentobarbital and the livers were removed for histopathologic examination.

## 2.2. Biochemical analysis

Analysis was done using an autoanalyzer (TMS-1024, Boeki, Japan) at the İstanbul University Veterinary Faculty Central Laboratory. Measurements were done using commercial kits (Spinreact, Spain, and Cormay, Poland). Enzymes were examined in the blood. Lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total protein, albumin, glucose, bilirubin, urea, triglyceride, and cholesterol values were determined in serum samples.

In our study, CCl<sub>4</sub> (Merck, Germany), PL/BP/USP (Kimetsan, Turkey), PMPA (Hepagen®, Fatro, Italy), 0.9% NaCl (Isotonic®, Eczacıbaşı, Turkey), and hematoxylin and eosin (H&E) and Oil Red O (Bio Optica, Italy) preparations were used.

PMPA (Hepagen®) is a specific peroxisome proliferator-activated receptor alpha agonist. It is effective for treatment or protection of hepatic steatosis of cattle and plays an important role in hepatic steatosis and metabolic diseases. It is nontoxic, has a choleric effect, and is recommended for protection and support in patients with liver diseases accompanied by hepatic failure (7).

## 2.3. Histopathologic examination

Histopathologic specimens were embedded into paraffin and stained with H&E. Some parts of tissues were stored at -80 °C cryostat cutting for Oil Red O application. Histologic grading of the fatty changes in liver tissues was adapted from the classification system previously defining classification for humans (9). The main criteria of this system are fatty change, vacuolated degeneration, lobular inflammation, portal inflammation, and fibrosis.

## 2.4. Statistical analysis

All results were taken as means. Intergroup and in-group statistical significance of mean and standard error values were evaluated with one-way ANOVA and Duncan tests using SPSS 13.0 (10). P < 0.05 was taken as statistically significant.

## 3. Results

Statistical significance of in-group variables was detected as P < 0.001. Intergroup statistical significance was found as P < 0.05. During the studying process, 2 rats died in both the positive control and treatment groups, and 1 rat from the protection group died. Protective and therapeutic effects of PMPA were found to be similar for LDH, AST, GGT, total protein, albumin, glucose, bilirubin, and triglyceride levels. In addition, the therapeutic effect of PMPA gives better results than its protective effect for ALT, AST, ALP, GGT, total protein, albumin, glucose, urea, and cholesterol values (Table).

Liver parenchyma cell arrangement was normal in the negative control group. Degenerative or necrotic reaction or inflammatory infiltration was not detected in hepatocytes (Figures 1A and 1B). Hemorrhagic, wide, and bridging coagulation necrosis areas were observed in the positive control group. Grade 3 macrovesicular fatty changes were observed in degenerative hepatocytes around necrotic areas, while limited mononuclear and polymorphonuclear inflammatory cellular infiltration, portal fibrosis (grade 2), and little fatty cysts were seen in portal and lobular areas. Hepatocytes had vacuolar degeneration and lost their hexagonal shapes, their cytoplasm became round or fusiform, nuclei were in the center or partially pushed to the cellular margin, and karyopyknosis occurred. Hyperemia and hepatocellular cholestasis were observed in general. Perihepatitis, which consisted of fibrin and inflammatory cells, was also seen (Figures 2A–2C). In the protection group, macro- and microvesicular fatty changes were seen in cytoplasm of the hepatocytes (usually grade 2), and vacuolar degeneration, polymorphonuclear inflammatory infiltrations in portal and lobular areas, mild portal fibrosis (grade 1), hemorrhagic local coagulation necrosis areas, and hyperemia were observed (Figures 3A and 3B). In the treatment group, local vacuolar degeneration was seen in some hepatocytes, while mild microvesicular fatty change was seen in cytoplasm and mononuclear inflammatory cellular infiltration was detected in the lobular areas. In addition, thickening was seen in capsules due to inflammatory infiltration and vascularization; subcapsular fat tissue infiltration was also seen (Figures 4A and 4B).

## 4. Discussion

Levels of some liver enzymes showed alterations in some studies investigating hepatic steatosis (11,12), and these changes were also found in animals (13,14). As mentioned below, we compared parameters in experimental hepatic steatosis in rats.

Increased LDH, ALT, and AST values were detected in studies in which a high cholesterol diet and CCl<sub>4</sub> were administered; high ALT and AST values were also detected in doxorubicin (DOX) studies (11,13,15,16). No change

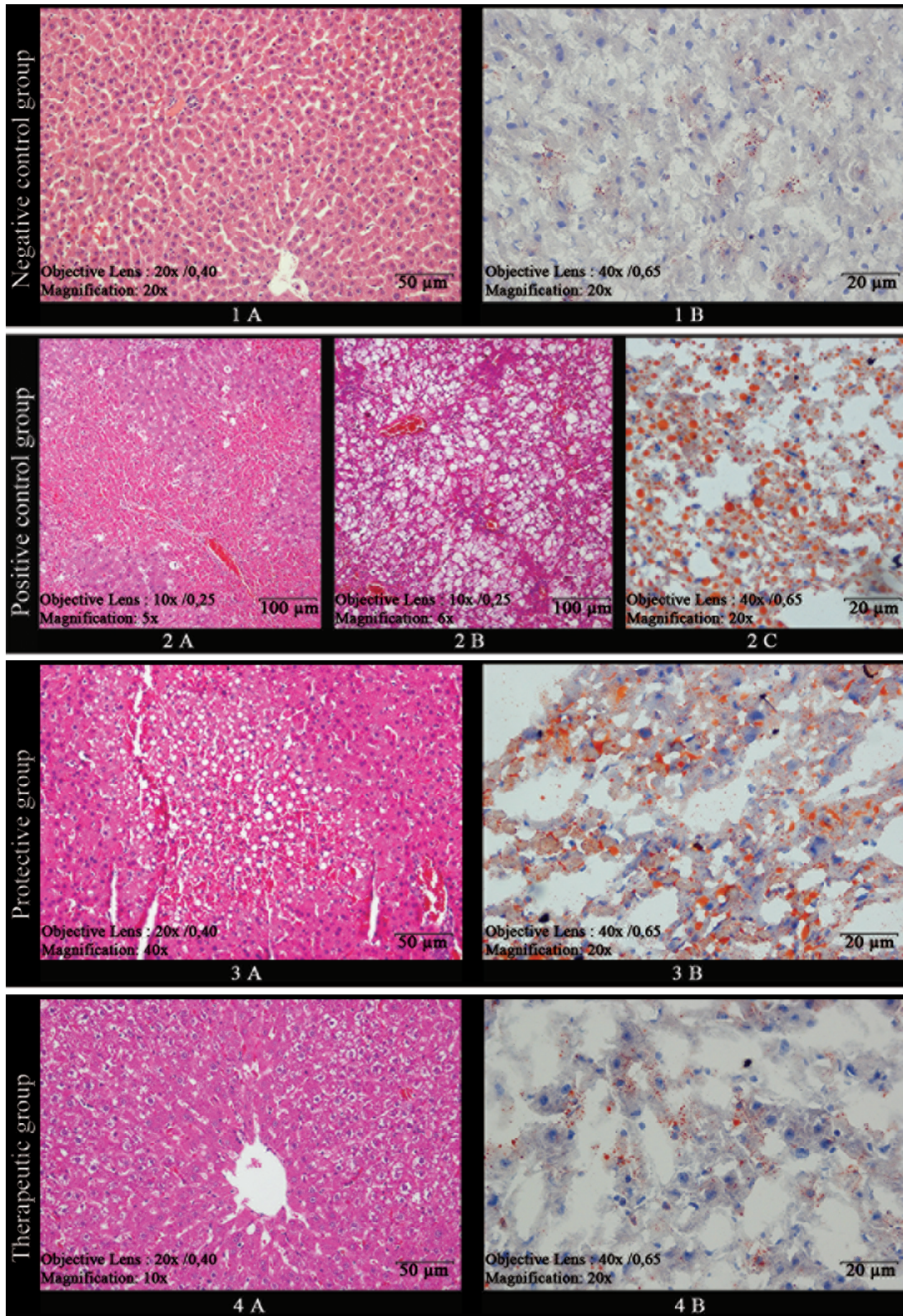
**Table.** Blood parameters. a, b, c: There is statistical significance between groups shown with different letters in the same row ( $P < 0.05$ ).

	Group 1	Group 1	Group 2	Group 2	Group 3	Group 3	Group 4	Group 4
	(Negative control)	(Negative control)	(Positive control)	(Positive control)	(Protective group)	(Protective group)	(Treatment group)	(Treatment group)
	(n = 10)	(n = 10)	(n = 8)	(n = 8)	(n = 9)	(n = 9)	(n = 8)	(n = 8)
	Mean	Std. error	Mean	Std. error	Mean	Std. error	Mean	Std. error
LDH (IU/L)	1413.80 c	148.335	5386.75 a	358.707	2415.11 b	91.932	2193.00 b	155.441
ALT (IU/L)	61.50 b	10.459	1282.38 a	132.977	1262.56 a	38.748	92.25 b	4.963
AST (IU/L)	181.40 c	26.187	2702.0 a	152.731	2326.56 b	53.16	175.63 c	10.672
ALP (IU/L)	197.60 c	26.871	510.25 b	51.025	1051.89 a	61.029	414.13 b	33.244
GGT (IU/L)	1.10 c	0.1	34.88 b	9.261	91.11 a	7.545	3.00 c	0.627
T. protein (g/dL)	7.380 a	0.2065	6.175 b	0.3057	5.333 c	0.0667	6.825 a	0.2328
Albumin (g/dL)	3.690 b	0.1027	4.788 a	0.3393	2.578 c	0.0795	3.238 b	0.0778
Glucose (mg/dL)	138.60 ab	9.188	117.75 b	3.668	83.00 c	5.462	140.25 a	8.006
Bilirubin (mg/dL)	0.1420 b	0.01474	6.7456 a	0.29369	1.3913 b	1.3576	0.1675 b	0.0225
Urea (mg/dL)	30.30 c	2.996	67.00 ab	11.397	84.56 a	4.1	57.13 b	3.671
Triglyceride (mg/dL)	36.40 c	6.665	240.88 a	19.015	93.44 b	11.542	74.75 b	2.403
Cholesterol (mg/dL)	58.90 b	2.483	44.88 b	4.45	120.67 a	14.3	67.75 b	4.3

was reported in AST values in a rabbit study when a high cholesterol diet was given (15). A significant increase was reported in ALP values in a rat study when  $\text{CCl}_4$  was given (16). A significant elevation was observed in GGT levels in rabbit studies when hepatic injury was created (17,18). In a study that was carried out in rats, when hepatic steatosis was created with  $\text{CCl}_4$ , an insignificant reduction was detected in protein level and a significant reduction was detected in albumin value (16). Albumin level was seen to be elevated by PMPA administration in a study conducted with lactating cows in the postpartum period (19). Serum glucose levels were reported to be elevated in rabbit studies (20,21). In studies where rats were given high carbohydrate diets, the glucose level in serum was insignificant (22). Bilirubin was seen to increase in experimental studies done with rabbits (23). A significant elevation was reported in serum urea levels in a DOX study in rabbits (11). Insignificant changes were seen in serum triglyceride levels in both rat and rabbit studies (22,24). Increased serum triglyceride levels were detected in rabbit and rat studies in which  $\text{CCl}_4$  and a high fat diet were used (14,17). Triglyceride levels were significantly higher in PMPA-administered Holstein cows compared to those not given PMPA (8). Cholesterol level was insignificant in an experimental rat study (24). High cholesterol levels were found to be significant in diet studies of rats and rabbits (15,22). Cholesterol level was significantly higher in PMPA-administered Holstein cows compared to those not given PMPA (8). An elevation was observed in cholesterol

levels in a study conducted with PMPA-administered cows in the postpartum period (19).

In our study, the statistical difference in serum LDH level between the other 3 groups as compared to the negative control group was significant ( $P < 0.05$ ). On the other hand, changes in LDH levels between the protection and treatment groups were found to be statistically insignificant. Serum ALT levels of the positive control and protection groups were found to have higher statistical significance than those of the negative control and treatment groups ( $P < 0.05$ ). ALT value was lower in the treatment group compared to the protection group ( $P < 0.05$ ). The reduction of ALT, a specific liver enzyme, in the treatment group compared to the positive control and protection groups reveals the therapeutic effect of PMPA. Serum AST levels were significantly higher in the positive control and protection groups as compared to the negative control and treatment groups ( $P < 0.05$ ). ALP level was significantly higher in the positive control, protection, and treatment groups compared to the negative control group ( $P < 0.05$ ), and it was lower in the treatment group compared to the protection group ( $P < 0.05$ ). GGT level was significantly higher in the positive control and protection groups compared to the negative control and treatment groups ( $P < 0.05$ ), and it was lower in the treatment group compared to the protection group ( $P < 0.05$ ). Total protein values were lower in the other groups compared to the negative control group. These values were lower in the positive control and protection groups compared to the negative control group



**Figure 1.** Histopathological images of liver for group 1, the negative control group: A) H&E staining, B) Oil Red staining.

**Figure 2.** Histopathological images of liver for group 2, the positive control group: A and B) H&E staining, C) Oil Red staining.

**Figure 3.** Histopathological images of liver for group 2, the protective group: A) H&E staining, B) Oil Red staining.

**Figure 4.** Histopathological images of liver for group 4, the therapeutic group: A) H&E staining, B) Oil Red staining.

( $P < 0.05$ ). For total protein values, the positive control group was significantly lower than the negative control group but was significantly higher than the protection group ( $P < 0.05$ ). Protection and treatment groups showed a significant reduction compared to the positive control group ( $P < 0.05$ ). Albumin levels were significantly higher in the treatment group compared to the protection group ( $P < 0.05$ ). Glucose levels were significantly lower in the protection group compared to the negative control group ( $P < 0.05$ ), and the glucose levels of the positive control group were significantly higher than those of the protection group and lower than those of the treatment group ( $P < 0.05$ ). Total bilirubin level was significantly higher in the positive control group compared to the negative control, protection, and treatment groups ( $P < 0.05$ ). Urea level was significantly higher in the positive control, protection, and treatment groups compared to the negative control group ( $P < 0.05$ ). While urea levels were high in the protection group, they were found to be low in the treatment group ( $P < 0.05$ ). Triglyceride levels were found to be significantly higher in the positive control, protection, and treatment groups compared to the negative control group ( $P < 0.05$ ). While triglyceride levels were found to be significantly lower in the protection and treatment groups compared to the positive control group ( $P < 0.05$ ), the values of the protection and treatment groups were insignificant in intergroup comparison ( $P < 0.05$ ). Cholesterol levels were significantly higher in the protection group compared to the other groups ( $P < 0.05$ ). Cholesterol levels were significantly lower in the treatment group compared to the protection group ( $P < 0.05$ ). Reduction of cholesterol levels in the positive control group was found to be insignificant ( $P < 0.05$ ). Cholesterol levels were higher in PMPA-treated groups compared to the groups in which PMPA was not used (Table).

The livers of the rats in which experimental hepatic injury was induced were stained with H&E and vacuolar degeneration, single cell necrosis, mitosis, centrilobular necrosis, bridging necrosis, fatty liver, inflammation, oxidative stress, and fibrosis were reported (12,25,26). In experimental hepatic steatosis and injury studies carried out with the administration of high fat and low carbohydrate diets, DOX, cholesterol-containing diets, or parenteral nutrition, evidence of grade 1 and 2 steatosis, vacuolar, necrotic areas, fibrosis, diffuse steatosis in hepatocytes, fat infiltration, and focal vacuolation was reported using H&E, Sudan, Azan–Mallory, and Mason stainings (11,18,21,27,28). Fat deposition was reported in hepatocytes using H&E and Oil Red O staining in hepatic steatosis induced in rats with a high fat diet (14).

In this study, grade 1 fat infiltration was detected in the negative control group (Figure 1B), and grade 3 fat infiltration and grade 2 portal fibrosis were detected in the positive control group (Figure 2C). Grade 2 fat infiltration and grade 1 portal fibrosis were observed in the protection group (Figure 3B), and grade 1 fat infiltration was observed in the treatment group (Figure 4B) on histopathologic examination. While a significant reduction was not seen in steatosis with PMPA administration in the protection group, similar findings were detected in the livers of rats in the treatment group and negative control group. According to these results, it was considered that the therapeutic effect of PMPA was superior to its protective effect in experimentally induced hepatic steatosis created with a carbon tetrachloride and paraffin liquid mixture in rats. In light of these data, it is concluded that PMPA use would be beneficial for the treatment of hepatic steatosis, which is common among cats and frequent among dogs; further clinical studies investigating the treatment of hepatic steatosis, which is naturally seen in cats and dogs, must be done.

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