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# Investigation of the Combined Effects of Propylparaben and Methylparaben on Biochemical and Histological Parameters in Male Rats

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## ABSTRACT

**Objective:** This research aimed to study the effects of co-exposure to methylparaben and propylparaben (MeP+PrP) on renal and hepatic parameters in male rats.

**Materials and Methods:** MeP and PrP were mixed at a ratio of 1:1. MeP+PrP doses were administered to 42-day-old male rats on a daily basis for 30 days. Each of the five groups consisted of six male rats, including three MeP+PrP dose groups with daily combination doses of 10 mg/kg bw, 100 mg/kg bw, and 500 mg/kg bw. The other two groups included the positive control group exposed to a daily dose of 50 mg/kg bw Bisphenol-A (BPA), and the corn oil control group used as a vehicle. At the end of the procedure period, liver and kidney tissues were obtained from histopathological analysis, morphometric analysis was performed on kidney tissue, and hematological and biochemical parameters were examined.

**Results:** Histopathological changes, such as tubular degeneration, edema, fibrous tissue formation, and congestion, were observed in the kidney tissue, while degeneration, edema, and congestion were observed in the liver tissue. Statistical analysis comparing the administered groups to the corn oil control group revealed significant variations in biochemistry and hematological markers.

**Conclusion:** These findings demonstrate that co-exposure to MeP and PrP alters biochemical and hematological parameters and negatively affects the liver and kidney tissues of male rats.

**Keywords:** Methylparaben, propylparaben, male rat, liver, kidney, toxicity.

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## INTRODUCTION

Endocrine-disrupting chemicals are natural or man-made substances that interfere with the body's production, secretion, or elimination of endocrine hormones.[1] The term "paraben" refers to p-hydroxybenzoic acid esters. Parabens are widely used as preservatives in cosmetics, medicines, food, and industrial products due to their broad spectrum of activity against microorganisms and thermal stability.[2]

The liver and kidney play crucial roles in the detoxification of endocrine-disrupting chemicals.[3] Consequently, these organs are susceptible to damage from parabens and other endocrine-disrupting chemicals.[4] Previous studies on parabens have shown their toxic effects on liver and kidney health.[2,5] However, the full extent of these impacts remains unclear.

In our research, we aimed to investigate the negative effects of co-exposure to methylparaben (MeP) and propylparaben (PrP) on the liver and kidney. Many studies have reported that these chemicals are commonly used in combination in various products, resulting in simultaneous exposure to both substances.[6,7] Therefore, in our study, we used a mixture of MeP and PrP, which are frequently used parabens.[8]

## MATERIALS AND METHODS

### Chemicals

The following chemicals were purchased from Sigma-Aldrich (USA): Bisphenol-A (CAS No. 80-05-7), Methylparaben (CAS No. 99-76-3, 99%), and Propylparaben (CAS No. 94-13-3). Enzyme-Linked Immunosorbent Assay (ELISA) kits for Aspartate Aminotransferase (Cat. No. E-BC-K236), Alanine Aminotransferase (Cat. No. E-BC-K235), triglyceride (Cat. No. E-BC-K238), albumin (Cat. No. E-BC-K058), creatinine (Cat. No. E-BC-K186), and urea (Cat. No. E-BC-K183) were obtained from Elabscience-Biotechnology (China).

### Animals and Housing

Thirty male Wistar albino rats were obtained from the Experimental Animals Production Center at Hacettepe University (Ankara, Türkiye). Permission for the research was obtained from the Hacettepe University Experimental Animals Ethics Committee under the number 2018/06-01. The average weight of the rats was 150 g, and their age was 42 days. Throughout the experiment, the photoperiod (12 hours light/dark), humidity (50±5%), and temperature (21±2°C) were maintained constant. Standard rat feed pellets were obtained from Korkutelim Feed Factory (Afyon, Türkiye). Rats had ad libitum access to drinking water and feed.

### Experimental Protocol

This research was conducted according to Test Guideline 407 from the Organization for Economic Cooperation and Development.[9] The objective of this study was to assess the potential negative effects of MeP+PrP mixture exposure during the peripubertal period on liver and kidney tissues. Therefore, MeP+PrP was administered to male rats via oral gavage daily from postnatal day 42 to 72.

In our research, we used a 1:1 ratio of MeP and PrP based on the preferred evaluation of toxicity for chemical mixtures.[10] Five groups of six rats each were randomly selected. The two control groups included the corn oil control group (where corn oil was used as the vehicle) and the positive control group (Bisphenol-A (BPA), 50 mg/kg bw/day). The three dose groups received MeP+PrP at doses 10, 100, and 500 mg/kg bw/day.

### Liver and Kidney Organ Weights

To calculate organ/body weight ratios, the tissues were dissected and weighed. Relative organ weights were determined in two ways. Firstly, the organ weights of the rats were divided by the terminal body weight. Secondly, the organ weights of the rats were divided by the brain weight to support the results.

### Hematological Analysis

Blood collected from the rats' hearts was transferred into tubes containing Dipotassium Ethylenediaminetetraacetic Acid (K2-EDTA). Hematological analysis was performed using the MELET SCHLOESING MS9-5 (France) veterinary blood count device without delay.

### Biochemical Analysis

The blood tubes were centrifuged at +4 °C, 2608 g for 30 minutes to obtain serums. After centrifugation, serum samples were transferred into Eppendorf tubes in specific amounts and stored at -80°C. Analyses were conducted using the BIOTEK µ-Quant (USA) biochemistry device.

### Histopathological Analysis

Liver and kidney tissues were immersed in 10% neutral formalin fixation solutions for 12 hours. After fixation, the tissues were embedded in paraffin. Thin sections (4 µm) were obtained from the blocks using a microtome (Leica-Germany). The sections were stained with Hematoxylin and Eosin (H&E) and examined under the Olympus BX51 light microscope (Germany). Histopathological findings were photographed using Bs200prop program (Türkiye).

### Histomorphometric Measurement of Kidney Tissue

In all groups, glomeruli in the kidney tissue were histomorphometrically measured. One hundred glomeruli were randomly selected from each experimental group, and the shortest and longest diameters of the selected glomeruli were measured. The measurements were performed using an Olympus BX51 light microscope (Germany) and the Bs200prop program (Türkiye). The glomerular volume was calculated using the formula 4 (d(G)/2)3/3, where d(G) represents the arithmetic mean of glomerular diameters.[11]

### Statistical Analysis

The data analysis was conducted using the statistical program SPSS IBM-23 (version 23, Chicago, Illinois, USA). The SPSS IBM-23 program was licensed by Hacettepe University. The normality of the data was assessed using the Kolmogorov-Smirnov test, which indicated that the data followed a normal distribution. The homogeneity of variances was evaluated using the Levene test. When the variances were homogeneous, Analysis

of Variance (ANOVA) was performed. In cases where the variances were not homogeneous, the Welch test was utilized. Post-hoc tests included Tukey for ANOVA and Games-Howell for Welch. Fisher's Exact Test was employed for evaluating the histopathological findings. All tests were two-tailed, and the results were reported as mean±standard deviation. A significance level of $p<0.05$ was considered statistically significant.

## RESULTS

The study included a control group that did not receive any treatment. Comparison between the control and corn oil control groups revealed no statistically significant differences, as their values were nearly identical. Therefore, all results are presented based on the corn oil control group.

### Liver and Kidney Organ Weights

Table 1 presents the liver and kidney final body weights, absolute organ weights, and relative organ weights of rats in the corn oil control and administered groups. There was a significant increase in the relative left kidney weight when calculated according to body weight. However, apart from this, there were no significant changes in the relative and absolute organ weights of the kidney and liver when comparing the corn oil control and administered groups (Table 1).

### Hematological Analysis

Table 2 presents the hematological values of the control groups and administered groups. The %lymphocyte values decreased in the BPA group and all administered groups compared to the corn oil group. This decrease was statistically significant in the BPA group and the 10 and 100 mg/kg bw/day MeP+PrP administered groups. Mean Platelet Volume (MPV) values significantly decreased in the BPA group and all administered groups compared to the corn oil control group. %Granulocyte and Mean Corpuscular Hemoglobin (MCH) showed a statistically significant increase in the BPA group and all administered groups compared to the corn oil control group. Mean Corpuscular Volume (MCV) values increased in the BPA group and all administered groups (10, 100, and 500 mg/kg bw/day MeP+PrP). This increase was statistically significant in the BPA group and the 10 and 500 mg/kg bw/day MeP+PrP administered groups. Mean Corpuscular Hemoglobin Concentration (MCHC) values increased in the BPA group and all administered groups compared to the corn oil control group. However, this

**Table 1.** Absolute and relative organ weights of BPA, corn oil control, and MeP+PrP dose groups

| | Control | | MeP+PrP | | | p |
|---|---|---|---|---|---|---|
| | Corn oil control Mean±SD | BPA (50 mg/kg bw/day) Mean±SD | 10 (mg/kg bw/day) Mean±SD | 100 (mg/kg bw/day) Mean±SD | 500 (mg/kg bw/day) Mean±SD | |
| Initial body weights (g) | 146±23 | 152±19 | 160±10 | 133±27 | 146±27 | 0.426 |
| Terminal body weights (g) | 252±37 | 245±26 | 247±27 | 244±27 | 226±41 | 0.508 |
| Brain weights (g) | 1.72±0.16 | 1.73±0.20 | 1.77±0.11 | 1.84±0.12 | 1.73±0.13 | 0.691 |
| Right kidney weights (g) | 0.98±0.21 | 0.92±0.09 | 0.96±0.17 | 1.02±0.11 | 0.94±0.24 | 0.776 |
| Left kidney weights (g) | 0.98±0.23 | 0.87±0.06 | 0.95±0.6 | 1.02±0.10 | 0.87±0.16 | 0.103 |
| Liver weights (g) | 10.23±2.21 | 9.8±1.47 | 9.88±2.10 | 10.42±1.60 | 9.23±1.88 | 0.888 |
| Relative right kidney weights (g/body weight kg) | 3.88±0.26 | 3.75±0.18 | 3.87±0.38 | 4.17 ±0.27 | 4.13±0.38 | 0.065 |
| Relative left kidney weights (g/body weight kg) | 3.85±0.37 | 3.58±0.21 | 3.83±0.41 | **4.18±0.23*** | 3.87±0.14 | **0.042** |
| Relative liver weights (g/body weight kg) | 40.38±4.35 | 39.88±2.60 | 39.62±4.40 | 42.62±3.95 | 40.72±1.95 | 0.183 |
| Relative right kidney weights (g/brain g) | 0.58±0.12 | 0.52±0.04 | 0.55±0.05 | 0.55± 0.05 | 0.55± 0.10 | 0.808 |
| Relative left kidney weights (g/brain g) | 0.58±0.12 | 0.52±0.04 | 0.50±0.06 | 0.57±0.05 | 0.52±0.08 | 0.350 |
| Relative liver weights (g/brain g) | 5.95±1.03 | 5.68±0.70 | 5.53±0.82 | 5.68±0.92 | 5.3±0.70 | 0.824 |

*: Statistically different from the corn oil control group (significance level $p<0.05$). MeP: Methylparaben; PrP: Propylparaben; BPA: Bisphenol-A; SD: Standard deviation.

**Table 2.** Results of hematologic analysis in BPA, corn oil control, and MeP+PrP dose groups

| | Control | | MeP+PrP | | | p |
|---|---|---|---|---|---|---|
| | Corn oil control Mean±SD | BPA (50 mg/kg bw/day) Mean±SD | 10 (mg/kg bw/day) Mean±SD | 100 (mg/kg bw/day) Mean±SD | 500 (mg/kg bw/day) Mean±SD | |
| WBC ($mm^3$) | 4.25±3.69 | 5.08±3.61 | 5.54±5.98 | 3.39±2.81 | 5.02±3 | 0.922 |
| RBC ($mm^3$) | 11.02±1.54 | 5.96±0.74 | 7.14±2.04 | 8.09±2.49 | 7.25±2.62 | 0.103 |
| Mon# | 0.2±0.14 | 0.21±0.12 | 0.29±0.26 | 0.21±0.17 | 0.18±0.11 | 0.741 |
| Lenf# | 4±3.39 | 4.29±2.67 | 4.36±3.95 | 3.09±2.03 | 4.26±2.11 | 0.938 |
| Gran# | 0.15±0.07 | 1.56±0.97 | 1.86±1.87 | 1.07±0.75 | 1.39±0.66 | 0.536 |
| Mon% | 4.25±0.92 | 2.67±0.52 | 4±2.10 | 7.33±7.92 | 3.18±0.40 | 0.332 |
| Lenf% | 93.55±1.34 | **70±5.40*** | **66.67±15.49*** | **67.5±8.60*** | 71.12±4.24 | **0.033** |
| Gran% | 2.2±0.42 | **24.5±5.82*** | **26.33±13.26*** | **22.5±3.94*** | **23.7±3.47*** | **0.014** |
| Hct% | 38.55±6.29 | 41.5±4.93 | 50±13.99 | 42.67±14.49 | 43.97±13.51 | 0.796 |
| RDW% | 24.1±0.85 | **9.5±1.05*** | **16±3.49*** | 16±7.07 | **13±2.10*** | **0.004** |
| PDW% | 8.7±0.07 | **16.83±1.33*** | 24.83±9.37 | 13.33±4.18 | **15.93±1.14*** | **0.004** |
| Hb (g/dl) | 12.35±1.06 | 11.83±1.53 | 14.87±3.82 | 14.25±4.72 | 14.03±4.81 | 0.765 |
| MPV (fl) | 19.3±0.14 | **5.72±0.34*** | **6.57±1.77*** | **8.67±4.74*** | **6±0.65*** | **<0.001** |
| MCV (fl) | 34.7±0.57 | **59.68±1.36*** | **61.47±1.78*** | 48.28±12.74 | **54.22±1.53*** | **0.001** |
| MCH (pg) | 9.7±0.14 | **17.38±0.49*** | **18.57±1.38*** | **15.92±2.94*** | **17.37±0.83*** | **0.002** |
| MCHC (g/dl) | 27.45±0.64 | 29.3±0.32 | 30.4±2.58 | 34.22±4.16 | **32.17±0.98*** | **0.014** |

*: Statistically different from the corn oil control group (significance level $p<0.05$). The measured hematological parameters are: leukocyte amount - White Blood Cell (WBC), erythrocyte amount - Red Blood Cell (RBC), monocyte-granulocyte-lymphocyte amount (Mon#, Gran#, Lymph#), monocyte-granulocyte-lymphocyte percentages (%Mon, %Gran, %Lymph), hemoglobin amount in the blood (Hb), hematocrit value (Hct), Mean Corpuscular Volume (MCV), hemoglobin amount in erythrocytes - Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW). MeP: Methylparaben; PrP: Propylparaben; BPA: Bisphenol-A; SD: Standard deviation.

increase was statistically significant only in the 500 mg/kg bw/day MeP+PrP administered group. Red cell Distribution Width (% RDW) values decreased in the BPA group and all administered groups compared to the corn oil control group, with a statistically significant decrease observed in the BPA group and the 10 and 500 mg/kg bw/day MeP+PrP administered groups. Platelet Distribution Width (% PDW) values increased in the BPA group and all administered groups compared to the corn oil control group. This increase was statistically significant in the BPA group and the 500 mg/kg bw/day MeP+PrP administered group (Table 2).

## Biochemical Analysis

Table 3 presents the biochemistry values of the corn oil control and administered groups. According to the results, the BPA and MeP+PrP administered groups showed higher Alanine Aminotransferase (ALT) values than the corn oil control group, although this difference was not statistically significant. Serum Aspartate Aminotransferase (AST) levels were significantly higher in the BPA group and the 10 and 500 mg/kg bw/day MeP+PrP administered groups compared to the corn oil control group. However, we found that serum AST levels decreased in the 100 mg/kg bw/day group, although this decrease was not statistically significant. Additionally, we evaluated the AST/ALT ratio as an indicator of liver and kidney function. The AST/ALT ratio significantly increased in the BPA group and the 10 and 500 mg/kg bw/day MeP+PrP administered groups compared to the corn oil control group. In contrast, we observed a decrease in the 100 mg/kg bw/day MeP+PrP administered group, but this decrease was not significant. A statistically significant decrease was observed in albumin values in the BPA group and all MeP+PrP administered groups compared to the corn oil group. Serum creatinine values significantly decreased in the 100 mg/kg bw/day MeP+PrP administered group. There was an increase in serum triglyceride levels in the BPA group and all MeP+PrP dose groups compared to the corn oil group. However, this increase was statistically significant only in the 100 mg/kg bw/day MeP+PrP dose group. The BPA positive

**Table 3.** Results of biochemical analysis in BPA, corn oil control, and MeP+PrP dose groups

| | Control | | MeP+PrP | | | p |
|---|---|---|---|---|---|---|
| | Oil control Mean±SD | BPA (50 mg/kg bw/day) Mean±SD | 10 (mg/kg bw/day) Mean±SD | 100 (mg/kg bw/day) Mean±SD | 500 (mg/kg bw/day) Mean±SD | |
| ALT/GPT(IU/L) | 23.75±5.85 | 38.75±19.62 | 52±18.79 | 34.25±21.48 | 35±4.24 | 0.104 |
| AST/GOT(IU/L) | 21.50±6.36 | **144.50±19.09*** | **73.0±5.66*** | 19.5±0.71 | **109.50±9.19*** | **0.018** |
| AST/ALT ratio | 1.05±0.12 | **2.23±0.41*** | **2.03±0.08*** | 0.74±0.08 | **2.59±0.17*** | **0.001** |
| Albumin (g/L) | 31.75±1.34 | **29.13±0.25*** | **28.78±1.44*** | **29.27±1.40*** | **29.21±0.74*** | **0.010** |
| Creatinine (µmol/L) | 44±4.69 | 42.5±8.35 | 52±8.04 | **23±4.24*** | 37.5±9.40 | **0.001** |
| Triglyceride (mmol/L) | 0.18±0.07 | 0.25±0.07 | 0.24±0.06 | **0.35 ±0.02*** | 0.23±0.06 | **0.028** |
| Urea (mmol/L) | 2.49±0.15 | 1.98±0.43 | **2.06±0.07*** | 2.46±0.15 | 2.11±0.20 | **0.001** |

*: Statistically different from the corn oil control group (significance level p<0.05). MeP: Methylparaben; PrP: Propylparaben; BPA: Bisphenol-A; SD: Standard deviation; ALT: Alanine aminotransferase; GPT: Glutamic pyruvic transaminase; AST: Aspartate aminotransferase; GOT: Glutamic oxaloacetic transaminase.

control group had a reduced serum urea level compared to the corn oil control group, although this difference was not statistically significant. The 10 mg/kg bw/day MeP+PrP dose group had significantly lower serum urea levels (Table 3).

### Histopathological Analysis

Table 4 presents the frequency of histopathological findings of the liver tissues of rats. Microscopic evaluation of the liver sections of the corn oil control group, BPA, and MeP+PrP groups is shown in Figure 1. Liver sections from the corn oil control group exhibited the characteristic appearance of normal hepatic tissue. However, the BPA group, and the 10, 100, and 500 mg/kg bw/day MeP+PrP administered groups showed necrotic lesions, degenerations in hepatic parenchyma cells, well-developed fibrosis, and congestion compared to the corn oil control group. Cytoplasmic vacuolization in hepatocytes and edema were observed in the 100 and 500 mg/kg bw/day MeP+PrP administered groups.

The frequency of histopathological findings in the kidney tissues of rats is demonstrated in Table 4. Microscopic evaluation of the kidneys in the corn oil control, BPA, and MeP+PrP groups is shown in Figure 1. The kidneys of was the corn oil control group exhibited a normal histological structure. However, histopathological examination of kidneys from rats treated with BPA and MeP+PrP revealed necrotic lesions, tubular degenerations, congestion areas, and fibrosis. Additionally, the 500 mg/kg bw/day MeP+PrP administered group showed separation between the parietal and visceral layers in the glomerulus, atrophic changes in several glomeruli, and widening of Bowman's space with evident cell degeneration in the kidney (Fig. 1 and Table 4).

### Histomorphometric Measurement of Kidney Tissue

The results of kidney morphometric analysis in the corn oil control, BPA, and administered groups are presented in Table 5. We observed a significant decrease in all parameters in the BPA positive control group compared to the corn oil group (p<0.05) (Table 5).

## DISCUSSION

The harmful effects of parabens and endocrine disrupting compounds on liver and kidney weights are still unclear. While one study reported a significant difference in organ weights,[3] another study stated that no changes were observed.[12] It is evident that the effects of these chemicals on organ weights depend on various factors.[13]

Impairment in kidney and liver function can result in the leakage of certain enzymes into the bloodstream. Consequently, an elevation in these enzymes in the blood is indicative of abnormal liver and kidney function. When hepatocytes are damaged, AST and ALT are often released into the blood, indicating liver damage.[14] Studies on parabens and endocrine-disrupting chemicals have reported increased serum ALT and AST levels.[3,15–17] The most common reason for elevated serum ALT levels is likely plasma membrane injury, with ALT activity increasing significantly during hepatocyte damage and necrosis.[17] In our research, necrotic areas were observed in the dose groups. The AST/ALT ratio, also known as the De Ritis ratio, is used as a marker of liver damage.[18] Therefore, we believe that the increase in serum AST levels and the AST/ALT ratio is a result of cellular damage and necrosis caused by MeP+PrP in the liver. These changes in serum ALT and AST levels confirm previous studies on the toxicity of parabens and chemical mixtures.[5,15]

**Table 4.** The incidence of histopathological findings detected in the liver and kidney tissues of the control and dose groups

| Histopathological findings | Control | | MeP+PrP | | |
|---|---|---|---|---|---|
| | Corn oil control | BPA (50 mg/kg bw/day) | 10 (mg/kg bw/day) | 100 (mg/kg bw/day) | 500 (mg/kg bw/day) |
| Liver | | | | | |
| Congestion | 0/6 | 0/6 | 2/6 | 3/6 | 2/6 |
| Edema | 0/6 | 0/6 | 1/6 | 0/6 | 2/6 |
| Mononuclear cell infiltration | 0/6 | 1/6 | 1/6 | 2/6 | 1/6 |
| Sinusoidal expansion | 0/6 | **4/6*** | **4/6*** | **5/6*** | 3/6 |
| Degeneration in hepatic parenchyma | 0/6 | 3/6 | 2/6 | 3/6 | 2/6 |
| Lipid accumulation in cells | 0/6 | 2/6 | 0/6 | 1/6 | 0/6 |
| Picnotic cells | 0/6 | 1/6 | 1/6 | 0/6 | 0/6 |
| Necrotic lesions | 0/6 | 1/6 | 1/6 | 1/6 | 0/6 |
| Cytoplasmic vacuolization | 0/6 | 1/6 | 1/6 | 1/6 | 0/6 |
| Fibrous tissue formation | 0/6 | 1/6 | 1/6 | 1/6 | 0/6 |
| Cytoplasmic meltdown | 1/6 | 0/6 | 1/6 | 3/6 | 2/6 |
| Kidney | | | | | |
| Congestion | 1/6 | 3/6 | 4/6 | **5/6*** | 4/6 |
| Edema | 0/6 | 3/6 | 1/6 | 0/6 | 0/6 |
| Mononuclear cell infiltration | 0/6 | 2/6 | 1/6 | 2/6 | 1/6 |
| Glomerular atrophy | 1/6 | **6/6*** | 3/6 | **6/6*** | 3/6 |
| Degeneration in kidney structure | 0/6 | 5/6 | 1/6 | 1/6 | 0/6 |
| Tubular degeneration | 0/6 | **4/6*** | 3/6 | **5/6*** | 3/6 |
| Cell expulsion into the lumen | 0/6 | **6/6*** | 3/6 | **5/6*** | 3/6 |
| Necrotic lesions | 0/6 | **5/6*** | 4/6 | 1/6 | 0/6 |
| Fibrous tissue formation | 0/6 | **4/6*** | 0/6 | 3/6 | 2/6 |
| Hyperplasia | 0/6 | 3/6 | 2/6 | 1/6 | 1/6 |

Values are given as the number of rats with histopathological findings/number of rats examined in the group. *: Statistically different from the corn oil control group. Represents significance generated by Fisher Exact Test (significance level p<0.05). MeP: Methylparaben; PrP: Propylparaben; BPA: Bisphenol-A; SD: Standard deviation.

**Table 5.** Histomorphometric measurements of glomeruli in BPA, corn oil control, and MeP+PrP dose groups

| | Control | | MeP+PrP | | | p |
|---|---|---|---|---|---|---|
| | Corn oil control Mean±SD | BPA (50 mg/kg bw/day) Mean±SD | 10 (mg/kg bw/day) Mean±SD | 100 (mg/kg bw/day) Mean±SD | 500 (mg/kg bw/day) Mean±SD | |
| Long diameter (µm) | 75.64±13.43 | **56.71±19.29*** | 74.17±11.46 | 76.49±1.16 | 71.98±12.35 | **0.021** |
| Short diameter (µm) | 64.25±11.83 | **44.55±16.64*** | 63.90±8.42 | 57.89±10.98 | 64.05±10.30 | **0.003** |
| Glomerular diameter (µm) | 68.95±10.44 | **50.63±17.63*** | 62.67±9.05 | 67.19±9.54 | 68.02±13.81 | **0.010** |
| Glomerular volume (x$10^6$ $\mu m^3$) | 0.18±0.07 | **0.09±0.07*** | 0.14±0.07 | 0.17±0.06 | 0.17±0.07 | **0.024** |

*: Statistically different from the corn oil control group (significance level p<0.05). MeP: Methylparaben; PrP: Propylparaben; BPA: Bisphenol-A; SD: Standard deviation.

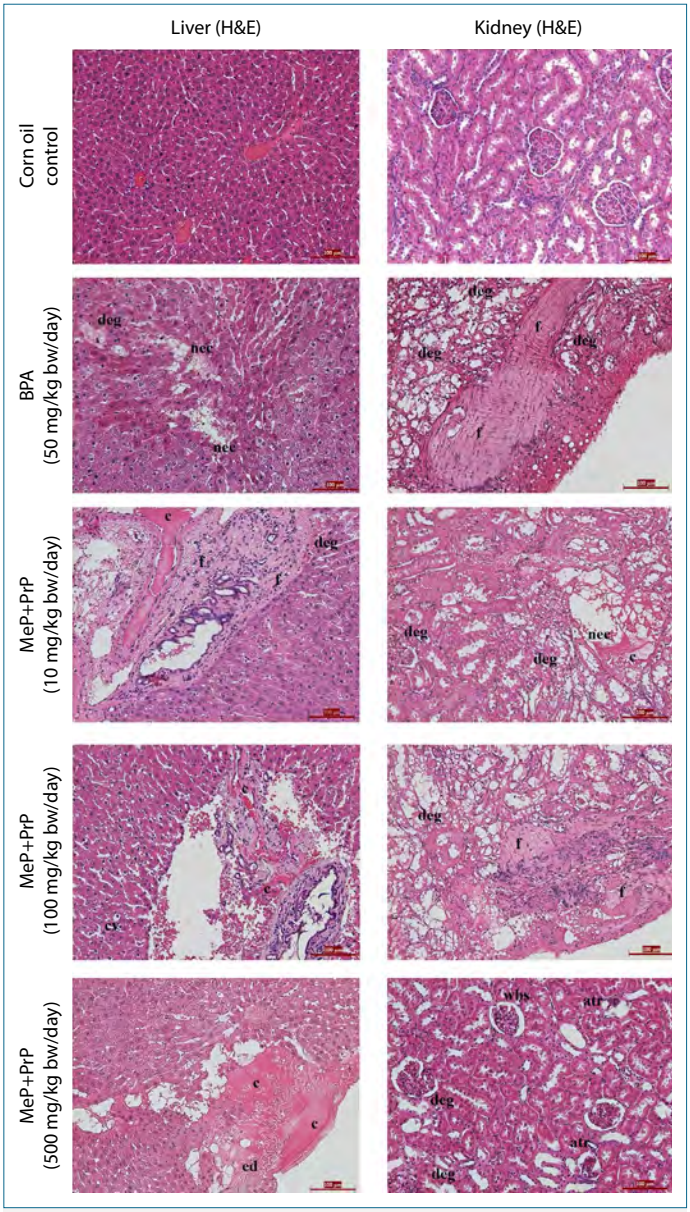


**Figure 3.** Representative photomicrographs of liver and kidney tissues from the corn oil control, BPA, and MeP+PrP dose groups. In the livers of male rats, the images depict congestion (c), degenerations in hepatic parenchyma (deg), well-developed fibrosis (f), and cell necrosis (nec) in the BPA, 10, 100 and 500 mg/kg bw/day MeP+PrP administered groups. Additionally, cytoplasmic vacuolization in hepatocytes (cv) and edema (ed) are observed in the 100 and 500 mg/kg bw/day MeP+PrP administered groups (stained with H&E, 200X). In the kidneys of male rats, the images show necrotic lesions (nec), tubular degenerations (deg), congestion (c), and fibrosis (f) in the BPA, 10, 100, and 500 mg/kg bw/day MeP+PrP administered groups. Furthermore, atrophic changes in glomeruli (atr) and widening of Bowman's space with obvious degeneration of cells (wbs) are observed in the 500 mg/kg bw/day MeP+PrP administered group (stained with H&E, 200X).

The decrease in serum albumin levels in our research may be attributed to liver damage. Additionally, albumin may have been excreted in the urine due to kidney impairment. This finding is consistent with previous studies conducted on parabens, which also reported a decrease in serum albumin levels.[16]

Disorders in kidney function can lead to alterations in blood urea levels, resulting in either an increase or decrease. Changes in kidney structure can influence urea levels.[16] In our research, various findings were observed in the examined kidney tissues, including glomerular atrophy, tubular degeneration, and structural deterioration. Therefore, it is speculated that the decreased serum urea value is a result of kidney impairment.

It is known that serum creatinine levels increase in cases of insufficient glomerular filtration, making it a biomarker for kidney damage.[14] Additionally, creatinine is synthesized by the liver, making it an important biomarker for liver health. Parabens and other endocrine-disrupting chemicals have been found to increase serum creatinine levels.[16] In a study examining the consequences of kidney failure in rodents, an increase in serum creatinine levels was observed.[19] However, in contrast to these studies, our research showed a decrease in serum creatinine levels, indicating toxicity.[20] The decrease in serum creatinine levels is consistent with the histopathological findings of kidney damage.

Studies have shown a link between MeP and PrP excretion in urine and reduced levels of circulating triglycerides.[21] Recent molecular studies have provided further insight into this phenomenon. For instance, a study with zebrafish reported down-regulation of transcription factors involved in lipid homeostasis, including triglycerides, in the livers of female fish exposed to MeP. These findings suggest that parabens disrupt lipid metabolism.[5] Another study conducted on zebrafish demonstrated that parabens induce oxidative stress, apoptosis, and alterations in fatty acid metabolism, leading to damage.[22] Although the exact mechanisms through which parabens affect lipid metabolism are not fully understood, it is evident that they disrupt the metabolic process. Numerous histopathological findings have been observed in studies on parabens and other endocrine-disrupting chemicals.[2,4,16] However, there are also studies where no damage could be detected, depending on the exposure route and dose of these chemicals. For example, rats treated with parabens topically on the skin did not exhibit notable histopathological findings in their liver or kidney tissues.[12] Liver injuries often exhibit various histopathological findings, including congestion, degeneration, and necrosis.[23] In our research, we observed several findings such as hepatic parenchyma degeneration, cellular degeneration, and necrotic areas in the histopathological examination of liver tissues. Similar outcomes were obtained in another study that evaluated the antiandrogenic effects of Propylparaben (PrP).[2]

Many histopathological findings were observed in the examined kidney tissues, including glomerular atrophy, deterioration of kidney structure, and tubular damage. Similar findings have been reported in studies investigating the histopathological effects of parabens on the kidney.[16] Survey-based studies on the impact of endocrine-disrupting chemicals on renal function also support the detrimental effects of these chemicals on kidney function. Furthermore, the kidney and liver mitochondria undergo numerous oxidation reactions, making these organs highly susceptible to damage from oxidative stress.[24] Studies have demonstrated that oxidative stress can have adverse effects on kidney and liver function.[25] P-hydroxybenzoic acid, a metabolite of parabens, has been shown to cause mitochondrial dysfunction. Therefore, it is believed that parabens may induce oxidative stress and related hepatotoxicity and nephrotoxicity.[15] In conclusion, it can be stated that endocrine-disrupting chemicals, including parabens, can negatively affect kidney and liver function and damage to these organs.[24] Overall, our histopathological findings support existing literature on the harmful effects of MeP and PrP on kidney and liver tissues.

Morphometric data were utilized in this study to evaluate the detrimental effects of MeP and PrP on the kidney. In a previous study where we examined the impact of octylphenol, an endocrine-disrupting chemical, on 13-week-old male rats, kidney morphometry measurements were recorded. A slight decrease was observed in the short diameter, long diameter, glomerular diameter, and glomerular volume. However, statistical significance could not be determined.[4] The results of our current study align with these findings. However, in the positive control group, a statistically significant decrease was observed. It is speculated that the significant difference observed in the BPA group may be attributed to the fact that the rats in our study were in the pre-pubertal period, which is a more sensitive period compared to the 13-week-old rats. When considering the histological and morphometric analyses together, it becomes evident that parabens, like other xenoestrogens, have detrimental effects on the kidney.[24]

The pathophysiological condition within the organism is reflected in blood parameters.[26] Similar to the results of another study we conducted with PrP, a decrease in % Lymphocyte values was detected.[27] An *in vitro* study examined the effect of paraben on human lymphocytes. The findings demonstrated that paraben directly affects DNA and chromosomes, leading to potential genotoxic consequences. Paraben also exhibits cytotoxic effects on human lymphocyte cells.[28] Therefore, the combination of MeP and PrP directly and/or indirectly has negative effects on lymphocytes. The decrease in Red Blood Cells (RBC) count may be attributed to cell membrane breakdown or irregularities in hemopoiesis due to spleen and kidney impairment caused by toxic substances. White Blood Cells (WBC) count serves to protect the body against foreign substances and stimulates antibody production in the fight against infections.[26] We believe that the stimulation of the immune system in response to tissue damage induced by MeP+PrP is the underlying cause of the increased WBC count observed in this study. Decreased blood Mean Platelet Volume (MPV) values may indicate a slowing of platelet production. Multiple factors, such as reduced platelet production in the bone marrow and increased platelet destruction, can contribute to a decrease in MPV values in the blood. The findings from studies examining hematological data on parabens and endocrine-disrupting chemicals vary significantly. While some studies have reported statistically significant changes in hematological parameters, others have found no significant alterations.[12,26] This discrepancy can likely be attributed to factors such as the specific structure of the endocrine-disrupting chemicals used, the species, sex, and age of the experimental animals, the route and duration of exposure, among other variables.

In a study, it was suggested that the combination of paraben exposure may have a synergistic estrogenic effect.[29] However, another study indicated that the use of parabens in combination does not exhibit a synergistic effect.[30] Based on our research findings, we emphasize the importance of examining the toxicity of chemical co-exposure since individuals are exposed to endocrine-disrupting chemicals in mixtures. Other studies evaluating mixture toxicity also support this perspective.[12,28]

This research demonstrated that MeP+PrP induces toxic effects on liver and kidney functions. The investigation of mixture toxicity of endocrine-disrupting chemicals is a recent area of study. Consequently, the available literature on the effects of mixture toxicity remains limited. Therefore, we believe that the histopathological, hematological, and biochemical data obtained from this research contribute to the existing toxicological studies on paraben mixtures. However, this study did not provide detailed information on the exact mechanisms underlying the harmful effects of MeP+PrP exposure on the liver and kidney. Further studies involving molecular analyses are necessary to elucidate the toxic mechanisms of MeP+PrP. We have planned such studies for the future. Although the impacts of these chemicals and the processes involved are not fully understood, it is evident that a lifestyle characterized by frequent consumption of products containing these chemicals is unhealthy.[24]

## CONCLUSION

In conclusion, our research revealed that co-exposure to MeP and PrP has detrimental effects on liver and kidney tissues and the related biochemical parameters. Considering that we are exposed to these chemicals as mixtures on a daily basis, it is crucial to determine their adverse effects through further studies. More experimental research is needed, particularly to elucidate the molecular mechanisms involved.

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