Effects of butylparaben on antioxidant enzyme activities and histopathological changes in rat tissues

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Butyl p-hydroxybenzoic acid, also known as butylparaben (BP), is one of the most common parabens absorbed by the skin and gastrointestinal tract and metabolised in the liver and kidney. Recent in vivo and in vitro studies have raised concern that BP causes reproductive, development, and teratogenic toxicity. However, BP-induced oxidative stress and its relation to tissue damage has not been widely investigated before. Therefore, we aimed to investigate the effects of butyl 4-hydroxybenzoate on enzyme activities related to the pentose phosphate pathway and on glutathione-dependent enzymes such as glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6-PGD), glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in kidney, liver, brain, and testis tissues. Male rats were randomly divided into four groups to orally receive corn oil (control) or 200, 400, or 800 mg/kg/day of BP for 14 days. Then we measured G6PD, GR, GST, 6-PGD, and GPx enzyme activities in these tissues and studied histopathological changes. BP treatment caused imbalance in antioxidant enzyme activities and tissue damage in the liver, kidney, brain, and testis. These findings are the first to show the degenerative role of BP on the cellular level. The observed impairment of equivalent homeostasis and antioxidant defence points to oxidative stress as a mechanism behind tissue damage caused by BP.

KEY WORDS: endocrine disrupting chemicals, glucose 6-phosphate dehydrogenase; 6-phosphogluconate dehydrogenase; glutathione reductase; glutathione peroxidase; glutathione-S-transferase

Butylparaben (butyl 4-hydroxybenzoate, BP) from the paraben family has widely been used as antimicrobial/antifungal preservative in cosmetic products, deodorants, toiletries, face creams, pharmaceuticals, toys, food, and beverages for more than 50 years (1, 2). Parabens are endocrine disruptors which impair natural metabolism of hormones by mimicking, interfering, and/or blocking them (3–6).

After exposure through skin, ingestion, or inhalation, BP is metabolised by the liver, kidney, and skin esterases. Conjugated forms of BP are excreted in the urine and skin, but some of it remains unchanged and accumulates in the body over time (2, 7–9). This accumulation raises health concerns, as paraben has been reported for oestrogen-like activity, endocrine disruption, and impairment of the reproductive system. Parabens are also closely associated with carcinogenesis, obesity, diabetes, inflammatory diseases, and neurodegenerative disorders (10–15).

Molecular mechanisms behind this association remain unclear, but some studies have shown that endocrine disrupting chemicals can damage tissue through oxidative imbalance that results in the damage of lipids, DNA, and proteins, which in turn triggers the development of a number of diseases (16, 17).

Antioxidant enzymes 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6-PGD), glutathione-S-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR) have a vital role in reductive biosynthesis and cellular detoxification from free radicals and chemicals to help the organism deal with internal and external stress (4, 18). GST, GR, and GPx are glutathione-dependent antioxidant enzymes, whereas G6PD and 6-PGD are glutathione-independent. G6PD is the rate-limiting enzyme in the pentose phosphate pathway (PPP) that maintains the cytosolic NADPH pool involved in cellular redox balance (19, 20). 6-PGD is another enzyme in the PPP pathway responsible for NADPH production. Their impairment is closely associated with elevated reactive oxygen species (ROS) in several diseases, including cancer, diabetes, inflammatory diseases, and obesity (16, 17, 21, 22). Reduced glutathione (GSH) is mainly responsible for the protection of cells against ROS and it is required as cofactor for glutathione dependent enzymes (23, 24).
MATERIALS AND METHODS

Materials

Oxidised glutathione (GSSG), 6-phosphogluconate (6-PG), glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide phosphate (NADP\(^+\)), reduced nicotinamide adenine dinucleotide phosphate (NADPH + H\(^+\)), magnesium chloride (MgCl\(_2\)), butylparaben (butyl 4-hydroxybenzoate, >99 % purity), sodium phosphate monobasic and dibasic, (6-PG), glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide phosphate (NADPH + H\(^+\)), magnesium chloride (MgCl\(_2\)), butylparaben (butyl 4-hydroxybenzoate, >99 % purity), sodium phosphate monobasic and dibasic, tris, glutathione reductase (GR), hydrogen peroxide (H\(_2\)O\(_2\)), ethylenediaminetetraacetic acid (EDTA), cOmplete™ Protease Inhibitor Cocktail, and sodium azide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, albumin, triglyceride, creatinine, and glucose kits for biochemical analysis were obtained from Audit Diagnostics (Cork, Ireland).

Animal care and dose administration

Male Wistar albino rats (Rattus norvegicus) were purchased from the Experimental Animals Production Center of Hacettepe University (Ankara, Turkey) at six weeks of age. We used 24 rats weighing 140–180 g. All were housed in polycarbonate cages with stainless steel covers in an air-conditioned room (12 h light/dark cycle with a temperature of 22 ± 2 °C and relative humidity of 50±5 %). The animals were acclimated to the laboratory for one week before the experiments. During the 14-day BP administration, they had free access to standard rat pellet food (Korkutelim Feed Factory, Afyon, Turkey). All experimental procedures and animal use were approved by the Ethics Committee of Hacettepe University (B.30.2.HAC.0.05.0.6.00/132).

The value of low observed toxic effects (LOEL) for BP administration was fixed in the Bouin’s solution for 8 h before histopathological examination. All tissues were embedded in the paraffin, and 4 µm thick sections stained with Harris haematoxylin and eosin (H&E). All slides were inspected with an Olympus BX51 light microscope (200x magnification, (Tokyo, Japan). The photographs were processed with a Bs200prop software (BAB Imaging System, Ankara, Turkey).

Tissue preparation and evaluation of enzyme activity

All tissues were placed in ice-cold sterile physiological saline solution to wash out blood and then placed in liquid nitrogen.

Samples for determination of enzyme activity and protein concentrations were homogenised with an Ultra Turrax homogeniser with a S18N-10G probe (KA, Königswinter, Germany) and mixed in a 1:3 volume with 50 mmol/L potassium phosphate buffer (pH 7.4) containing protease inhibitors. The homogenate was first centrifuged in a Beckman Coulter ultracentrifuge (Fullerton, CA, USA) at 105,000×g for 60 min, the pellet was removed, and the supernatants used to measure anti-oxidant enzyme activities with a LKB Ultraspec Plus spectrophotometer (4054 UV/visible, Biochrom Ltd., Cambridge, UK).

G6PD activity was measured according to the method described by Betke et al. (29) in a reaction mixture containing 0.6 mmol/L G6P and 100 mmol/L of Tris-HCl buffer (pH 8.0) containing 10 mmol/L MgCl\(_2\) and 0.2 mmol/L NADP\(^+\). NADPH production was monitored at 340 nm at 37 °C for 60 s. Measurements were performed in duplicate, and one unit (U) of activity was defined as the amount of enzyme required to reduce one mmol NADP\(^+\)/min under the assay conditions. 6-phosphogluconate dehydrogenase activity (6PGD) was determined in the same way as above except that the mixture contained 0.6 mmol/L 6PG as substrate instead of G6P (30).

Glutathione reductase activity was determined according to a modified Staal method (31). Decrease in the absorbance of NADPH at 340 nm was monitored.
spectrophotometrically at 37 °C for 60 s. Unit of activity (U) was defined as the amount of enzyme needed to catalyse the oxidation of 1 µmol of NADPH in 1 min. Glutathione-S-transferase (GST) activity was evaluated by the determining the conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) (32). The assay mixture consisted of 0.2 mol/L sodium phosphate buffer (pH 6.5), 20 mmol/L GSH, 20 mmol/L CDNB, 5 µL tissue homogenate, and double distilled water added to fill up the volume to 500 µL. Experiments were carried out in the duplicate, and activities followed for 30 s. Glutathione peroxidase (GPx) activity was measured according to Beutler et al (33). The assay mixture contained 100 mM potassium phosphate buffer (pH 7.0), 200 mmol/L EDTA, GR enzyme (10 U/mL), 400 mmol/L sodium azide, 100 mmol/L GSH, 2 mmol/L NADPH, 5 µL tissue lysate, and distilled water to fill up the volume to 500 µL. The mixture was incubated at 37 °C for 10 min and then 5 µL of 10 mmol/L H₂O₂ was added into the reaction mixture. The decrease in optical density (OD) of the system was measured at 340 nm at room temperature for 30 s. One unit of activity (U) was defined the same as above.

Protein concentration of tissue homogenates was determined with the bicinchoninic (BCA) method in 96-well plates using Spectramax M2 microplate reader (BioTek, Winooski, VT, USA) (34).

**RESULTS AND DISCUSSION**

**Animal weight, food and water consumption**

Table 1 shows the differences in food and water consumption and weight gain between control and BP-treated animal groups. Lower food consumption and correspondingly lower weight gain in BP-treated animals may point to adverse health effects of BP.

**Tissue weight**

Table 2 shows differences in tissue weights between animal groups. Liver weight in the 200 mg/kg/day dose group was significantly lower and in the 400 and 800 mg/kg/day dose groups significantly higher than in controls. The last two groups also had significantly higher kidney weight than controls. These increases are indicative of tissue damage (35–37), which was confirmed by histopathological findings below. However, BP treatment did not affect the weights of the brain and testis.

**Serum biochemical parameters**

Table 3 shows that BP treatment significantly affected only ALT and AST levels. Low ALT and AST levels indicate impaired liver function (36, 37), which was confirmed by our histopathology and antioxidant enzyme findings below (Figure 2, Table 5).

**Antioxidant enzyme activities and histopathological confirmation**

Tables 4–7 clearly show that BP significantly affected anti-oxidant enzyme activities in rat liver, kidney, brain, and testis compared to control. This is in line with the few

| Table 1 Food and water consumption of rats in the control and butylparaben-treated groups |
|-----------------------------------------------|
| Control | Butylparaben |
|        | 200 mg/kg/day | 400 mg/kg/day | 800 mg/kg/day |
| Food consumption (g) | 14.61±2.82 | 13.68±2.79 | 12.51±1.43 | 13.88±1.66 |
| Water consumption (ml) | 30.3±6.82 | 28.07±5.36 | 32.24±3.63 | 38.88±5.97 |
| Initial body weight (g) | 141.83±19.67 | 147.83±10.14 | 183.83±13.59 | 188.83±7.29 |
| Final body weight (g) | 187±25.66 | 182.67±17.75 | 224.67±20.27 | 232±17.81 |
| Weight gain (%) | 31.39±11.83 | 23.52±7.63 | 21.93±4.31 | 22.74±6.1 |

All results are given as mean±SD of six animals. *significantly different from control (p<0.05)

| Table 2 Organ weight of rats in control and butylparaben-treated groups |
|-----------------------------|
| Organ | Control | Butylparaben |
|      |        | 200 mg/kg/day | 400 mg/kg/day | 800 mg/kg/day |
| Brain (g) | 1.50±0.06 | 1.59±0.08 | 1.73±0.04 | 1.72±0.05 |
| Liver (g) | 7.5±1.02 | 6.08±0.84 | 8.02±1.13 | 8.37±1.37 |
| Kidney (g) | 1.44±0.22 | 1.31±0.18 | 1.56±0.19 | 1.70±0.22 |
| Testis (g) | 1.28±0.12 | 1.22±0.15 | 1.40±0.14 | 1.47±0.09 |

All results are given as mean±SD of six animals. *significantly different from control; **significantly different from the 200 mg/kg/day dose group (p<0.05)
reports there are about BP-induced oxidative stress. The one by Shah and Verma (25) reported higher levels of oxidative stress enzymes GPx, GST, catalase (CAT), and superoxide dismutase (SOD) in mice liver. Samarasinghe et al. (26) have shown that parabens can induce oxidative stress in human spermatozoa. Schreiber et al. (27) reported that BP can impair oxidative stress metabolism in rat testis.

Furthermore, we have supported our results by histopathological findings indicating tissue damage (Figures 1–4).

Consistent changes have also been reported earlier by Zhang et al. (38), who found that n-butylparaben induced male reproductive disorders via deregulation of oestradiol and oestrogen receptors, reduced the layers of germinal epithelium, and changed the structure of the seminiferous tubule. Other authors reported that BP administration led to the apoptosis of spermatogenic cells in prepubertal rats and to the impairment of the Sertoli cell vimentin filaments (38–40). Aubert et al. (41) suggested that tubular degeneration, tubular dilatation, and dead cells in the tubules found at the highest BP dose may have resulted from apoptosis to avoid irreversible DNA damage.

CONCLUSION

This is the first study to have investigated and clearly shown that BP can impair oxidative balance and cause damage across rat brain, kidney, and liver (in addition to the testis). These results are consistent with the degenerative role of butylparaben on the cellular level, as it impairs homeostasis and antioxidant defence.

Butyl 4-hydroxybenzoate may not be as safe as initially thought, as this chemical affects enzyme activity, cellular homeostasis, and key tissues. Our findings have pointed toward oxidative stress as a likely mechanism behind BP-induced damage, which calls for further investigation.
Figure 2 Photomicrographs showing congestion (*), necrosis (→), mononuclear cell infiltration (↔), and vacuolization (v) of male rat liver tissue of the BP-treated groups (B – 200 mg/kg/day; C and D – 400 mg/kg/day; E and F – 800 mg/kg/day) compared to control (A) (stained with H&E, 200x magnification)

Table 3 Serum biochemistry of control and butylparaben-treated groups

| Serum biochemical parameters | Control | Butylparaben |
|------------------------------|---------|--------------|
|                              | 200 mg/kg/day | 400 mg/kg/day | 800 mg/kg/day |
| ALT (UI/I)                   | 19.31±2.74     | 17.1±0.85    | 15.91±0.93*   | 17.05±1.05     |
| AST (UI/I)                   | 101.13±2.77    | 83.52±3.76   | 59.43±0.58*   | 60.8±0.42*     |
| Glucose (mg/dL)              | 179.82±16.04   | 169.57±6.39  | 166.28±17.49  | 165.85±26.23   |
| Albumin (g/dL)               | 2.78±0.14      | 2.75±0.23    | 2.9±0.17      | 2.96±0.11      |
| Creatinine (mg/dL)           | 0.15±0.04      | 0.16±0.01    | 0.15±0.02     | 0.17±0.02      |
| Triglycerides(mg/dL)         | 0.2±0.04       | 0.21±0.05    | 0.21±0.05     | 0.2±0.04       |
| Urea (mg/dL)                 | 7.37±1.32      | 7.68±1.43    | 8.01±1.39     | 8.07±1.39      |

All results are given as mean±SD of six animals. *significantly different from control (p≤0.05)
Figure 3 Photomicrographs showing atrophic tubules (at), congestion (c), germinal cell debris (gcd), and damaged tubules (dt) in testis tissues of the BP-treated groups (B – 200 mg/kg/day; C and D – 400 mg/kg/day; E and F – 800 mg/kg/day) compared to control (A) (stained with H&E, 200x magnification)

Table 4 Brain antioxidant enzyme activities in control and butylparaben-treated rats

| Brain Enzyme                        | Control              | Butylparaben 200 mg/kg/day | Butylparaben 400 mg/kg/day | Butylparaben 800 mg/kg/day |
|-------------------------------------|----------------------|-----------------------------|-----------------------------|-----------------------------|
| Glucose-6-phosphate dehydrogenase   | 0.0561±0.008         | 0.0771±0.012                | 0.1551±0.022                | 0.0968±0.010                |
| 6-phosphogluconate dehydrogenase   | 0.0235±0.007         | 0.0325±0.004                | 0.0654±0.012                | 0.0415±0.003                |
| Glutathione-S-transferase (GST)     | 2.066±0.323          | 0.9858±0.219                | 3.427±0.876                 | 1.699±0.22                  |
| Glutathione reductase (GR)          | 0.0394±0.007         | 0.1297±0.023                | 0.1460±0.019                | 0.0670±0.007                |
| Glutathione peroxidase (GPx)        | 0.0725±0.018         | 0.2066±0.083                | 0.6133±0.138                | 0.2755±0.065                |

All results are given as mean±SD of n=6 animals. * 200 mg/kg/day butylparaben dose group is different from control group (p=0.013); † 200 mg/kg/day dose group is different from 400 mg/kg/day dose group (p=0.024); ‡ 400 mg/kg/day dose group is different from control group (p=0.0001); ‡‡ 400 mg/kg/day dose group is different from 800 mg/kg/day dose group (p=0.0001); †† 200 mg/kg/day dose group is different from 400 mg/kg/day dose group (p=0.0001); †‡ 800 mg/kg/day dose group is different from control group (p=0.0001); ††† 400 mg/kg/day dose group is different from 800 mg/kg/day dose group (p=0.0001); †‡‡ 800 mg/kg/day dose group is different from control group (p=0.0001); †††† 200 mg/kg/day dose group is different from control group (p=0.0001); †††‡ 400 mg/kg/day dose group is different from control group (p=0.0001); †††‡‡ 800 mg/kg/day dose group is different from control group (p=0.0001); ††††† 200 mg/kg/day dose group is different from control group (p=0.0001); †††‡‡‡ 400 mg/kg/day dose group is different from control group (p=0.0001); †††‡‡‡‡ 800 mg/kg/day dose group is different from control group (p=0.0001); †††††† 200 mg/kg/day dose group is different from control group (p=0.0001); ††††‡‡ 400 mg/kg/day dose group is different from control group (p=0.0001); ††††‡‡‡ 800 mg/kg/day dose group is different from control group (p=0.0001); ††††††† 200 mg/kg/day dose group is different from control group (p=0.0001).
Figure 4 Photomicrographs of atrophic glomerulus (ag), congestion (c), enlargement of glomerular capsule (egc), tubular degeneration (td), and mononuclear cell infiltration (mci) in the kidney tissue the BP-treated groups (B – 200 mg/kg/day; C and D – 400 mg/kg/day; E and F – 800 mg/kg/day) compared to control (A) (stained with H&E, 200x magnification)

Table 5 Liver antioxidant enzyme activities in control and butylparaben-treated rats

| Liver                          | Control          | Butylparaben 200 mg/kg/day | Butylparaben 400 mg/kg/day | Butylparaben 800 mg/kg/day |
|-------------------------------|------------------|-----------------------------|-----------------------------|-----------------------------|
| Glucose-6-phosphate dehydrogenase (G6PD) | 0.0342±0.002     | 0.0338±0.011                | 0.0475±0.001a               | 0.0375±0.007                |
| 6-phosphogluconate dehydrogenase (6PGD) | 0.0891±0.017     | 0.0629±0.014b               | 0.0755±0.009c               | 0.0668±0.012c               |
| Glutathione-S-transferase (GST)  | 13.91±3.554      | 7.927±0.671c               | 8.481±2.602c               | 7.280±3.342e               |
| Glutathione reductase (GR)    | 0.1583±0.021     | 0.0954±0.008e               | 0.1371±0.023f               | 0.1527±0.028g               |
| Glutathione peroxidase (GPx)  | 0.2382±0.076     | 0.2222±0.070                | 0.3933±0.117c               | 0.3566±0.102                |

All results were given as mean ± SD of n=6 animals. * 400 mg/kg/day butylparaben dose group is different from 200 mg/kg/day group (p=0.038); # 200 mg/kg/day butylparaben dose group is different control group (p<0.0001); $ 400 mg/kg/day dose group is different from control group (p=0.047); @ 800 mg/kg/day dose group is different control group (p=0.015), (p=0.021); © 200 mg/kg/day butylparaben dose group is different control group (p=0.0001); † 400 mg/kg/day dose group is different from control group (p=0.0001); ¥ 800 mg/kg/day dose group is different from control group (p=0.0001); ² 200 mg/kg/day butylparaben dose group is different control group (p=0.0001); ^ 400 mg/kg/day dose group is different control group (p=0.0001); $ 400 mg/kg/day dose group is different from 200 mg/kg/day dose group (p<0.0001); ° 800 mg/kg/day dose group is different from 200 mg/kg/day dose group (p=0.0001); † 400 mg/kg/day dose group is different control group (p=0.031); ¥ 400 mg/kg/day dose group is different from 200 mg/kg/day dose group (p=0.015)
Table 6  Testis antioxidant enzyme activities in control and butylparaben-treated rats

| Testis                        | Control | Butylparaben |
|-------------------------------|---------|--------------|
| Glucose-6-phosphate dehydrogenase (G6PD) | 0.0316±0.006 | 0.0623±0.010<,c,d | 0.0868±0.009<,e | 0.0293±0.002 |
| 6-phosphogluconate dehydrogenase (6PGD) | 0.0378±0.011 | 0.0915±0.014<,g,h | 0.0516±0.008<,j | 0.0351±0.004 |
| Glutathione-S-transferase (GST) | 12.69±2.043 | 15.30±3.561<,k | 11.9±1.853 | 13.42±2.314 |
| Glutathione reductase (GR)     | 0.0215±0.003 | 0.0445±0.009<,m,n,x | 0.0243±0.004 | 0.0355±0.005<,p,s |
| Glutathione peroxidase (GPx)   | 0.0764±0.007 | 0.0208±0.008<,s,t | 0.0332±0.006<,s,t | 0.0778±0.013 |

All results were given as mean ± SD of n=6 animals. * 200 mg/kg/day butylparaben dose group is different from control group (p<0.0001); † 400 mg/kg/day dose group is different from control group (p<0.0001); ‡ 400 mg/kg/day dose group is different from 800 mg/kg/day dose group (p<0.0001); ¶ 400 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.005); †† 200 mg/kg/day dose group is different from control group (p<0.0001); ‡‡ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p<0.0001); ††† 200 mg/kg/day butylparaben dose group is different from 400 mg/kg/day dose group (p<0.0001); ††‡ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.06); ††§ 400 mg/kg/day dose group is different from control group (p<0.0001); ††‖ 400 mg/kg/day butylparaben dose group is different from control group (p<0.0001); †‡ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.02); †‡† 200 mg/kg/day dose group is different from control group (p<0.0001); †‡‡ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.06); †‡§ 800 mg/kg/day dose group is different from control group (p<0.0001); †‡‖ 800 mg/kg/day dose group is different from 400 mg/kg/day dose group (p=0.005); †‡‖† 400 mg/kg/day butylparaben dose group is different from control group (p<0.0001); †‡‖‡ 400 mg/kg/day dose group is different from 800 mg/kg/day dose group (p=0.0013); †‡‖§ 200 mg/kg/day dose group is different from 800 mg/kg/day dose group (p=0.025); †‡‖‖ 400 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.0013); †‡‖‖† 200 mg/kg/day butylparaben dose group is different from 400 mg/kg/day dose group (p=0.240); †‡‖‖‡ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p<0.0001); †‡‖‖§ 800 mg/kg/day dose group is different from control group (p<0.0001); †‡‖‖‖ 800 mg/kg/day dose group is different from 400 mg/kg/day dose group (p=0.005); †‡‖‖‖† 400 mg/kg/day butylparaben dose group is different from control group (p<0.0001); †‡‖‖‖‡ 400 mg/kg/day dose group is different from 800 mg/kg/day dose group (p=0.0013).

Table 7  Kidney antioxidant enzyme activities in control and butylparaben-treated rats

| Kidney                        | Control | Butylparaben |
|-------------------------------|---------|--------------|
| Glucose-6-phosphate dehydrogenase (G6PD) | 0.0316±0.006 | 0.0623±0.010<,c,d | 0.0868±0.009<,e | 0.0293±0.002 |
| 6-phosphogluconate dehydrogenase (6PGD) | 0.0378±0.011 | 0.0915±0.014<,g,h | 0.0516±0.008<,j | 0.0351±0.004 |
| Glutathione-S-transferase (GST) | 12.69±2.043 | 15.30±3.561<,k | 11.9±1.853 | 13.42±2.314 |
| Glutathione reductase (GR)     | 0.0215±0.003 | 0.0445±0.009<,m,n,x | 0.0243±0.004 | 0.0355±0.005<,p,s |
| Glutathione peroxidase (GPx)   | 0.0764±0.007 | 0.0208±0.008<,s,t | 0.0332±0.006<,s,t | 0.0778±0.013 |

All results were given as mean ± SD of n=6 animals. * 200 mg/kg/day butylparaben dose group is different from control group (p<0.0001); † 400 mg/kg/day dose group is different from control group (p<0.0001); ‡ 400 mg/kg/day dose group is different from 800 mg/kg/day dose group (p<0.0001); ¶ 400 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.005); †† 200 mg/kg/day dose group is different from control group (p<0.0001); ‡‡ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p<0.0001); ††† 200 mg/kg/day butylparaben dose group is different from 400 mg/kg/day dose group (p<0.0001); ††‡ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.06); ††§ 800 mg/kg/day dose group is different from control group (p<0.0001); ††‖ 800 mg/kg/day dose group is different from 400 mg/kg/day dose group (p=0.005); †‡ 200 mg/kg/day butylparaben dose group is different from control group (p<0.0001); †‡† 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.02); †‡‡ 200 mg/kg/day dose group is different from control group (p<0.0001); †‡§ 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.025); †‡‖ 400 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p<0.0001); †‡‖† 200 mg/kg/day butylparaben dose group is different from 800 mg/kg/day dose group (p=0.06); †‡‖‡ 800 mg/kg/day dose group is different from control group (p<0.0001); †‡‖§ 800 mg/kg/day dose group is different from 400 mg/kg/day dose group (p=0.005); †‡‖‖ 400 mg/kg/day butylparaben dose group is different from control group (p<0.0001); †‡‖‖† 400 mg/kg/day dose group is different from 800 mg/kg/day dose group (p=0.0013).
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**Conflict of interests**

None to declare.

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Djelovanje butilparabena na aktivnost antioksidacijskih enzima i histopatološke promjene u tkivima štakora

Butil p-hidroksibenzoična kiselina, poznata i pod nazivom butilparaben (BP), najčešće je oblik parabena, koji se apsorbira putem kože i probavnog sustava te razgrađuje u jetri i bubrezima. Najnovija istraživanja Butilparabena na aktivnost antioksidacijskih enzima u podlozi oštećenja tkiva izazvanog BP-om. Food Chem Toxicol 2019;131:110573. doi: 10.1016/j.fct.2019.110573

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