Short Communication

Duygu Aydemir, Burcu Oztasci, Nurhayat Barlas and Nuriye Nuray Ulusu*

Influence of the butylparaben administration on the oxidative stress metabolism of liver, kidney and spleen

[Bütilparabenin karaciğer, böbrek ve dalağın oksidatif stres metabolizmalarına etkisi]

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Abstract

Objectives: Butylparaben is widely used synthetic polymer as preservative in the food, pharmaceutical and cosmetic industries. Although butylparaben is metabolized in the detoxification organs including liver and kidney, some parts of it can retain and accumulate in the body. Parabens can impair developmental and reproductive health, though there is not any published data related with the influence of the butylparaben on the oxidative stress metabolism in the detoxification organs. Therefore, we aimed to evaluate antioxidant enzyme activities in the liver, kidney and spleen of butylparaben-treated rat.

Methods: Prepubertal Wistar albino male rats were administered with 0, 100, 200, 400 mg/kg/day butylparaben for 28 days. After treatment, enzyme activities were evaluated as the biomarkers of the oxidative stress.

Results: Enzyme activities including glucose-phosphate dehydrogenase (G6PD), 6-phosphoglucanate dehydrogenase (6-PGD), glutathione reductase (GR), glutathione s-transferase (GST) and glutathione peroxidase (GPx) were impaired upon butylparaben treatment in the liver, kidney and spleen tissues.

Conclusions: Exposure to endocrine disruptors may affect enzyme activities of the detoxification organs and change the pentose phosphate glutathione (GSH) metabolisms. According to our data oxidative stress metabolism is impaired in the spleen, kidney and liver tissue upon butylparaben treatment that has been indicated first time in the literature.

Keywords: antioxidant enzymes; butlyparaben; kidney; liver; oxidative stress; spleen.

Öz

Amaç: Bütilparaben yiyecek, ilaç ve kozmetik endüstrilerinde yaygın olarak kullanılan sentetik bir polimerdir. Bütilparaben kısmen karaciğer ve böbrek gibi detoksifikasyon organları tarafından metabolize edilirler, ancak bir kısım metabolize edilmeden kalır ve vücutta birikir.

Parabenlerin gelişim ve üreme sağlığına zarar verdiği bilinmektedir, ancak bütilparabenin detoksifikasyon organlarının oksidatif stres metabolizmasına etkileri henüz çalışılmamıştır. Bu nedenle çalışmamızda bütilparabene maruz kalmış sıçanların karaciğer, böbrek ve dalaklarında antioksidan enzim aktivitelerini değerlendirmeyi amaçladık.

Gereçveyöntem: Prepubertal Wistar albino erkek sıçanlara 0, 100, 200, 400 mg/kg/gün butilparaben oral gavaj ile

***Corresponding author: Nuriye Nuray Ulusu,** Professor of Biochemistry, School of Medicine, Department of Medical Biochemistry, Koc University, Rumelifeneri Yolu, Sariyer, 34450, Istanbul, Turkey; and Koc University Research Center for Translational Medicine (KUTTAM), Sariyer, 34450, Istanbul, Turkey, Phone: +90 (212) 338 11 60, Fax: +90 (212) 338 11 68, E-mail: nulusu@ku.edu.tr. https://orcid.org/0000-0002-3173-1389
Duygu Aydemir: School of Medicine, Department of Medical Biochemistry, Koc University, Istanbul, Turkey; Koc University Research Center for Translational Medicine (KUTTAM), Istanbul, Turkey
Burcu Oztasci and Nurhayat Barlas: Hacettepe University, Science Faculty, Department of Biology, Ankara, Turkey

28 gün boyunca uygulandı. Uygulamadan sonra antioksidan enzim aktiviteleri oksidatif stres biyo-belirteçleri olarak değerlendirildi.

Bulgular: Dalak, karaciğer ve böbrek dokularında glikoz 6-fosfat dehidrogenaz, 6-fosfoglukanat dehidrogenaz, glutatyon reduktaz, glutatyon s-transferaz ve glutatyon peroksidaz enzimlerinin kontrol grubuna göre bozulduğu gözlemlenmiştir.

Sonuç: Endokrin bozuculara maruz kalmak detoksifikasyon organlarında glutatyon ve pentoz fosfat metabolizması enzim aktivitelerini bozmaktadır. Literatürde ilk defa, sonuçlarımız oksidatif stres metabolizmasının karaciğer, dalak ve böbrek dokularında bütilparaben uygulaması nedeniyle bozulduğunu göstermiştir.

Anahtar Kelimeler: antioksidan enzimler; böbrek; bütilparaben; dalak; karaciğer; oksidatif stres.

Introduction

Butylparaben is an alkyl ester compound and widely used synthetic polymer as preservative in the various industrial products including food, personal care products, cosmetics and pharmaceuticals [1]. Total daily exposure to the parabens is 76 mg, although the Acceptable Daily Intake (ADI) for them is 0–10 mg/kg Bw/day for human [2, 3]. After absorption from the skin and gastrointestinal system, butylparaben is metabolized in the detoxification organs including liver and kidney. However, some part of the parabens remains and accumulates in the body over time. There is a growing concern about adverse health effects of the butylparaben, since it has been reported that parabens can impair reproductive, developmental and redox systems by acting endocrine disrupting chemical (EDC) [4–7].

Liver, kidney and spleen are responsible for the detoxification of the xenobiotics including butylparaben and other EDCs which interfere with hormones. Because of the detoxification reactions, oxidative stress increases in these organs that may cause imbalance in the cellular processes leading to the tissue damage and diseases [7–11]. Therefore, in this study we designed our experiments to evaluate impacts of the butylparaben on the detoxification organs which have not been studied before in the literature. Our findings may reveal butylparaben-induced impairments in the oxidative branch of pentose phosphate metabolism and glutathione dependent enzyme activities which were both tightly correlated with oxidative stress metabolisms of the detoxification organs.

Material & Method

Materials

Butylparaben (Butyl 4-hydroxybenzoate, >99% purity) and all other chemicals were purchased from Sigma–Aldrich (USA).

Methods

Animals: Wistar albino male rats (*Rattus norvegicus*) were obtained from Hacettepe University, Ankara, Turkey. The rats were kept in a room maintained 12 h light/dark cycle and at a temperature of 22 ± 2 °C and relative humidity 50 ± 5. Standard rat diet was administered to the animals and tap water *ad libitum*. Ethics Committee of Hacettepe University (B.30.2.HAC.0.05.0.6.00/132) approved all experimental procedures.

Dosages and butylparaben administration: Twenty four rats were randomly divided into four groups after one week of acclimatization. Three groups served as the treatment groups (100, 200 and 400 mg/kg/day), where one group was used as the oil-control group. Butylparaben was administrated by oral gavage daily for 28 consecutive days. Corn oil was prepared weekly depending on the body weight of the rats. The rats in the oil-control group were additionally given corn oil orally in amounts equal to those used in the dosed groups. The dosages were selected by considering the toxicokinetic properties of the butylparaben and similar previous studies performed to show the degree of effect in the increasing dosages. The maximum dose was defined at as high a dose as possible, without causing any quantifiable effect at physiological effects without causing mortality. In this study, maximum tolerated dose (MTD) was estimated according to Rothfuss et al. and Garcia et al. (acute oral LD_{50}; acute MTD) [5, 12]. Also, the investigation of estrogenic activity, highest administration dose level of butylparaben was considered as the LD_{50} value. After the treatment period all the tissues of all animals were immediately removed.

Evaluation of the antioxidant enzyme activities: We have used UV/visible spectrophotometry for the quantitative determination of glucose-phosphate dehydrogenase (G6PD), 6-phosphoglucanate dehydrogenase (6-PGD), glutathione reductase (GR), glutathione s-transferase (GST) and glutathione peroxidase (GPx) enzyme activities. Enzyme activities were measured two times and activities were followed for 1 min. G6PD and 6-PDG enzyme activities were evaluated as described previously by Aydemir et al. [9–11]. GR enzyme activity is evaluated by the modified Staal method as described by Aydemir et al. [9–11]. GST belongs to the family of eukaryotic and prokaryotic phase II metabolic enzyme and activity of GST was determined using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as described by Aydemir et al. [13].

GPx activity measured at 37 °C in the 100 mmol/L potassium phosphate buffer contained GR (10 U/mL), 100 mmol/L GSH, 400 mmol/L sodium azide, 200 mmol/L EDTA, 2 mmol/L reduced NADP and 5 µL tissue supernatant [14]. Samples were incubated for 600 s then the reaction was initiated by the addition of tissue supernatant and hydrogen peroxide. This assay is based on coupled enzyme activity by GR and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The pH of the buffer was 7.0 and the concentration was 0.1 mol/L.

Evaluation of protein determination: The protein concentration of liver, kidney and spleen tissue supernatant was evaluated by the bicinchoninic acid (BCA) method using Spectramax M2 microplate reader (Pierce™ BCA Protein Assay Kit).

Statistical analysis: We have analyzed our data by GraphPad Prism by one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test for multiple comparison. Our results were presented as the mean ± standard deviation (SD).

Results

Anti-oxidant enzyme activities were impaired upon butylparaben administration in the liver, kidney and spleen tissues.

GST enzyme activity meaningfully decreased in the all butylparaben administrated groups compared to the control group, however GPx activity significantly increased in the 200 mg/kg/day dose group compared to the all other groups in the liver (Figure 1). G6PD, 6PGD and GR activities were fluctuated upon butylparaben administration in the liver (Figure 1).

G6PD and GR activities significantly increased in the butylparaben treatment groups compared to the control in the kidney (Figure 2). GST activity meaningfully increased in 200 mg/kg/day butylparaben treatment group compared to the control group and other dose groups; however GPx activity significantly decreased in 200 and 400 mg/kg/day dose group compared to the other groups in the kidney samples (Figure 2).

G6PD, 6-PGD, GR and GPx activities increased in butylparaben dose groups compared to the control, however this change was significant for G6PD and GR activities in the spleen tissue (Figure 3). On the other hand, GST activity significantly decreased in all dose groups, but only significant change was observed in highest butylparaben treatment groups compared to the control in the spleen tissue (Figure 3).

Discussion

Effects of parabens and other EDCs have been widely studied on the reproductive and developmental systems, however there is no detailed data about the butylparaben-enhance oxidative stress in the detoxification organs including liver, kidney and spleen [1–8]. Since parabens

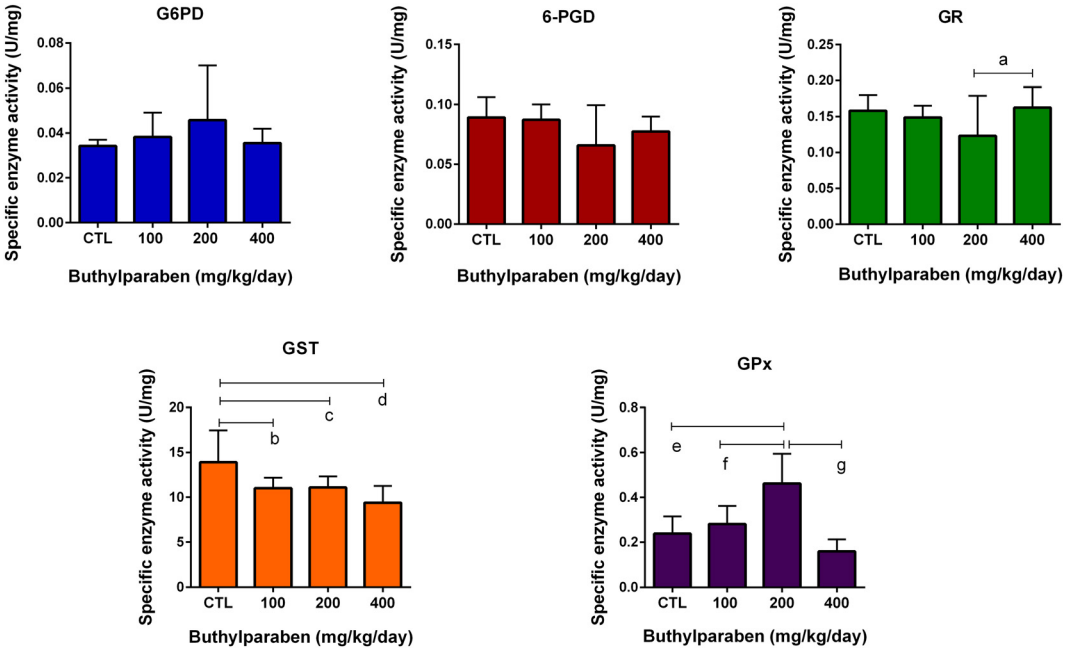


Figure 1: Anti-oxidant enzyme activities in the liver samples of male rats in control and butylparaben treatment groups. All groups are compared to CTL group and each other. All results were given as the mean ± SD of n=6 animals.
[a] 200 mg/kg/day butylparaben dose group is different 400 mg/kg/day dose group (p=0.0488), [b] 100 mg/kg/day butylparaben dose group is different from CTL group (p=0.0109), [c] 200 mg/kg/day butylparaben dose group is different from CTL group (p=0.0416), [d] 400 mg/kg/day butylparaben dose group is different from CTL group (p=0.0002), [e] 200 mg/kg/day butylparaben dose group is different from CTL group (p=0.0028), [f] 200 mg/kg/day butylparaben dose group is different from 100 mg/kg/day dose group (p=0.0126), [g] 200 mg/kg/day butylparaben dose group is different from 400 mg/kg/day dose group (p=0.0002).

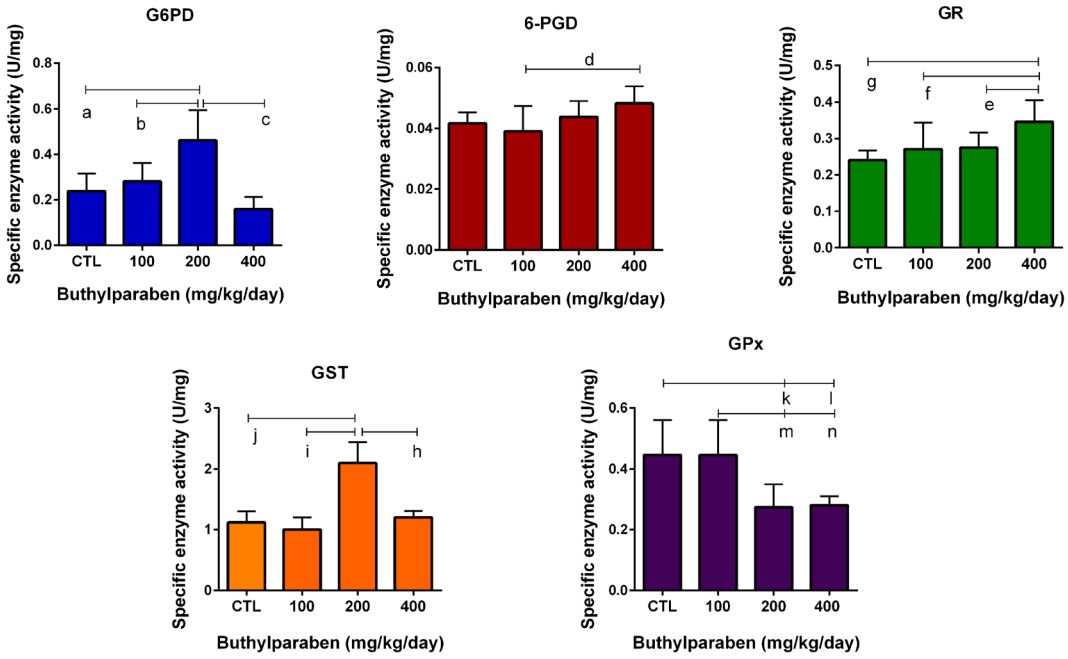


Figure 2: Anti-oxidant enzyme activities in the kidney samples of male rats in control and butylparaben treatment groups. All groups are compared to CTL group and each other. All results were given as the mean ± SD of n=6 animals.
[a] 200 mg/kg/day butylparaben dose group is different from CTL group (p=0.0028), [b] 200 mg/kg/day butylparaben dose group is different from 100 mg/kg/day dose group (p=0.0126), [c] 200 mg/kg/day butylparaben dose group is different from 400 mg/kg/day dose group (p=0.0002), [d] 100 mg/kg/day butylparaben dose group is different from 400 mg/kg/day dose group (p= 0.0039), [e] 400 mg/kg/day butylparaben dose group is different from 200 mg/kg/day dose group (p=0.0003), [f] 400 mg/kg/day butylparaben dose group is different from 100 mg/kg/day dose group (p=0.0125), [g] 400 mg/kg/day butylparaben dose group is different from CTL group (p=0.0187), [h] 200 mg/kg/day butylparaben dose group is different from 400 mg/kg/day dose group (p<00001), [i] 200 mg/kg/day butylparaben dose group is different from 100 mg/kg/day dose group (p<0.0001), [j] 200 mg/kg/day butylparaben dose group is different from CTL group (p<00001), [k] 200 mg/kg/day butylparaben dose group is different from CTL group (p=0.0212), [l] 400 mg/kg/day butylparaben dose group is different from 100 mg/kg/day dose group (p=0.0370), [m] 200 mg/kg/day butylparaben dose group is different from 100 mg/kg/day dose group (p=0.0212), [n] 400 mg/kg/day butylparaben dose group is different from 100 mg/kg/day dose group (p=0.0370).

and other EDCs can induce oxidative stress resulting in the tissue damage in human and wildlife, cause formation of the various diseases [4, 9–11, 15, 16].

Pentose phosphate pathway (PPP) play vital role in xenobiotic metabolism. Endocrine disruptors or any other molecules, chemicals that affects the PPP enzyme activities especially G6PD and 6-PGD will results in fail of metabolic network of the cell and the organism [11, 17–20].

The reduced form of GSH is one of the cell's most significant antioxidants and reduction of this molecule is directly associated with PPP's product NADPH and the enzyme GR [9, 11, 13, 21]. Moreover, Zhang et al. reported that GSH depletion or increased oxidized GSH concentration can increase oxidative phase of PPP and cells with low levels of GSH are susceptible hemolysis in erythrocytes [22]. Thus, we evaluated antioxidant enzyme activities including G6PD, 6-PGD, GR, GST and GPx in the detoxification after butylparaben treatment as the oxidative stress biomarkers in our study. G-6-PD and GR activities significantly increased upon butylparaben treatment in the main xenobiotic detoxification tissues (Figures 1–3). GST activity meaningfully reduced in the butylparaben treated groups compared to the control in the liver and spleen (Figures 1, 3). However, GPx activity significantly increased in the liver, where significantly decreased in the kidney tissue (Figures 1, 2).

GST, GR and GPx are involving in the GSH metabolism responsible for the detoxification and oxidative stress metabolisms of xenobiotics including EDCs such as butylparaben and phthalates. Since these enzymes keep balance of the oxidized glutathione (GSSG)/GSH ratio, impairment of this ratio causes central redox homeostasis in the organisms [9, 16, 17, 23].

G6PD and 6-PGD play key role in the PPP and responsible to produce NADPH required for the function of

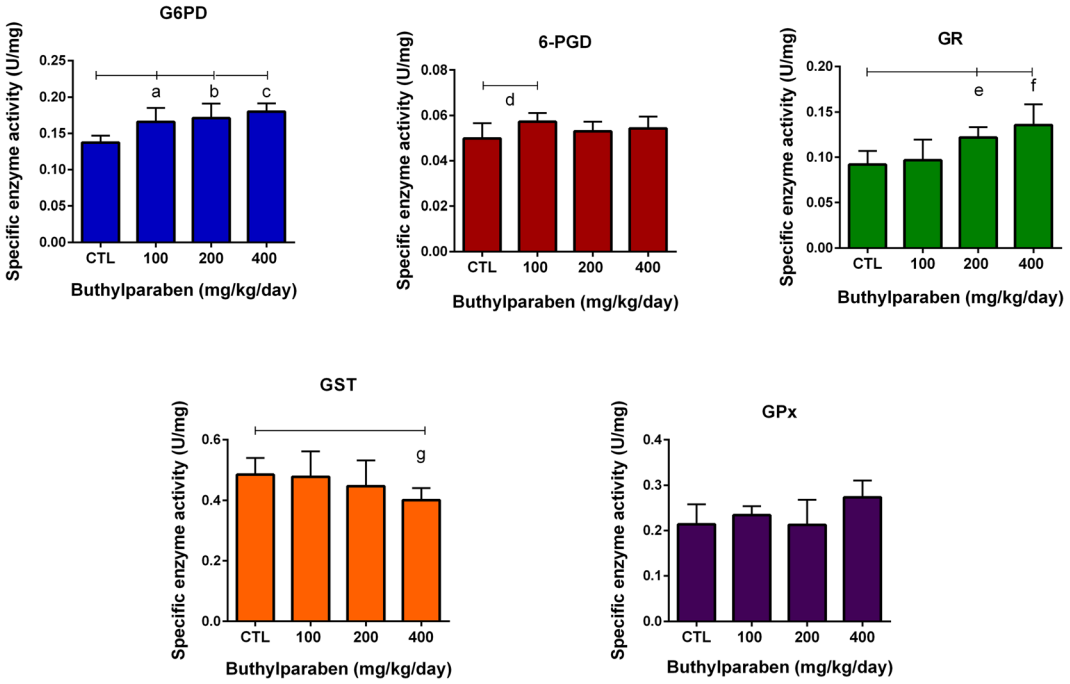


Figure 3: Anti-oxidant enzyme activities in the spleen samples of male rats in control and butylparaben treatment groups. All groups are compared to CTL group and each other. All results were given as the mean ± SD of n=6 animals.
[a] 100 mg/kg/day butylparaben dose group is different from CTL group ($p<0.0004$), [b] 200 mg/kg/day butylparaben dose group is different from CTL group ($p<0.0001$), [c] 400 mg/kg/day butylparaben dose group is different from CTL group ($p<00001$), [d] 100 mg/kg/day butylparaben dose group is different from CTL group ($p< 00058$), [e] 200 mg/kg/day butylparaben dose group is different from CTL group ($p=0.0037$), [f] 400 mg/kg/day butylparaben dose group is different from CTL group ($p<0.0001$), [g] 400 mg/kg/day butylparaben dose group is different from CTL group ($p=0.0348$).

GR, GST and GPx. Increased levels of oxidative stress may result from impairment in the antioxidant enzyme activities such as G6PD, 6-PGD, GR, GST and GPX. Elevated levels of ROS attack lipids, proteins and DNA may end up tissue injury [4, 15–17, 19, 20, 23].

Liver and kidney are the major detoxification organs, where spleen is mainly responsible for the immune defense system of the organism. Butylparaben-induced impairments have been reported previously in liver, kidney and spleen. For instance, butylparaben may cause high incidence of amyloidosis and significant atrophy in the spleen [15–19, 23]. Moreover, Aydemir et al. reported EDC DEHP can induce tissue injury in the liver, kidney, brain and testis via elevated levels of oxidative stress [9, 10].

Overall, our data showed that oxidative stress metabolism is activated to fight against butylparaben in the liver, kidney and spleen tissues because of fluctuated antioxidant enzyme activities. Impairment in the indicated enzyme activities may damage tissue function and cause initiation of different types of diseases.

Conclusion

There is an increasing concern and attention about adverse health effects of EDCs including butylparaben.We showed first time in the literature that oxidative stress metabolism is impaired in spleen, kidney and liver tissue upon butylparaben treatment. Our results also highlighted the importance of endocrine disruptor, butylparaben on the oxidative part of PPP enzymes as an important component in the response to xenobiotic metabolism and various anabolic reactions. The present study concludes that realistic exposure to endocrine disruptors can increase the spleen enzymes and even more hazardous and may induce immunosuppression of the target organism.

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Competing interests: All authors declare that there is no conflict of interest.

Ethical approval: The study was approved by Ethics Committee of Hacettepe University (B.30.2.HAC.0.05.0.6.00 /132).

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