Original Article

Effect of Sodium Benzoate on Liver and Kidney Lipid Peroxidation and Antioxidant Enzymes in Mice

Abstract

Introduction: Sodium benzoate (SB), as a chemical preservative, is used in many kinds of foodstuff. Some studies reported toxicity effects of SB in food products and suggested to limit its usage. The aim of this study was to evaluate the effects of oral administration of SB on antioxidant enzymes and lipid peroxidation in the liver and kidney of mice. **Materials and Methods:** A total of 24 animals were divided into four groups: Control group and three treated groups that received 150, 300, and 600 mg/kg/day of SB, respectively, in drinking water for 4 weeks. The malondialdehyde level, glutathione (GSH) content, superoxide dismutase (SOD), and catalase (CAT) activities of the liver and kidney were measured and the results of the treated groups were compared with those of the control group (one-way analysis of variance). **Results:** Results showed that SB caused histological alterations in the liver and kidney tissues. Moreover, SB significantly increased lipid peroxidation and GSH content in the kidney tissues ($P < 0.05$). Also, CAT activity significantly declined in the kidney ($P < 0.05$), without changing the SOD activity, but SB did not have any effect on the biochemical parameter of the liver tissue. **Conclusion:** The results of this study showed that SB caused kidney injury more than liver injury, but as a food preservative, which is consumed for a long period of life, it may cause liver damage additionally. For that reason, the excessive SB intake in the food is disturbing.

Keywords: *Antioxidant enzymes, kidney, lipid peroxidation, liver, sodium benzoate*

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Introduction

Sodium benzoate (SB) is a chemical preservative, which inhibits the activity of the microorganisms in very low concentration, and has been generally recognized as a safe food preservative.[1,2] SB is used in carbonated beverages, syrups, salted margarine, olives, sauces, relishes, jellies, jams, preserves, pastry and pie fillings, low-fat salad dressing, fruit salads, prepared salads, and in the storage of vegetables.[3,4] The current maximum usage level permitted is 0.1% in food.[5] SB inhibits the growth of bacteria, yeast, and mold.[6]

Some studies reported toxicity effects of SB in food products and suggested that its usage should be limited.[7] SB reacts with ascorbic acid and produces benzene that is a carcinogen although SB carcinogenicity is ruled out.[8,9] Other researchers showed that benzoate metabolizes in the liver and conjugates with glycine and converts into hippuric acid.[10,11] In fact, glycine acts as a detoxifying agent, and glycine deficiency in the body causes a decrease in creatinine, glutamine, urea, and uric acid level.[11,12] Also, there is some evidence showing that SB may metabolize to benzene, which leads to mitochondrial DNA destruction.[2,13]

Some reports from animal models indicated that short-term administration of SB caused a significant elevation in remarkable serum enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase,[14] suggesting the harmful effects of SB on the hepatic and renal functions.[13]

Oxidative stress is a term in biology, indicating that imbalance in the system of peroxide/antioxidant leads to cell damage.[15] In natural conditions, aerobic metabolism produces peroxide such as reactive oxygen species (ROS), which is scavenged by antioxidants.[16-18] The toxic effects of ROS lead to lipid peroxidation (LPO) and production of 4-hydroxynonenal and malondialdehyde (MDA). The results of LPO induce oxidative damage in the tissues.[19,20] The antioxidant defense system of the cells that protect against oxidative stress, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), eliminates the excessive ROS.[21]

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On the basis of the studies about the toxic effects of SB on the liver and kidney and also the essential role of the cellular antioxidant defense system in the function of important organs such as the liver and kidney, the aim of this study was to figure out the effect of short-term oral exposure of SB on tissue antioxidant indices, including GSH and LPO levels and the SOD and CAT enzyme activity in the liver and kidney of mice.

Materials and Methods

Chemicals

5,5'-dithiobis 2-nitrobenzoic acid (DTNB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pyrogallol, and ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) were purchased from Sigma-Aldrich (St. Louis, Missouri). Thiobarbituric acid (TBA), SB, trichloroacetic acid (TCA), and hydroxymethyl aminomethane (Tris) were obtained from Merck (Darmstadt, Germany).

Animal treatment

In our procedure, male BALB/c mice weighing approximately 25–30 g were provided from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. Also, the animal care and use were performed in accordance with the institution guidelines approved by the Shiraz University of Medical Sciences Animal Care and Use Committee (95-01-21-11700). Animals were kept in standard cages with a 12-h darkness interval, temperature (23 ± 2°C), and free access to food and water.

Twenty-four animals were randomly divided into four groups: Control mice received tap water and three treated groups received various concentrations of SB (0.1125%, 0.225%, and 0.56%) in drinking water for 4 weeks. The SB doses were in the experimental groups considering the doses mentioned in the literature[13,22] and our previous study.[23] The animals' body weight and water consumption were recorded before and after the animal treatment every 3 days. SB was administered considering the average of body weight and water consumption of each group; every mouse used approximately 150, 300, or 600 mg/kg/day SB in three groups of treatment.

After 4 weeks of treatment, the mice were anesthetized and killed by decapitation; also, their liver and kidney were removed rapidly and washed in a cold saline solution.

Histopathological examination

The kidney and a sample of liver were fixed in a formalin solution (10%) for tissue histopathological evaluation, and also the rest of the tissues were frozen in liquid nitrogen and stored at −80°C until assayed. Paraffin-embedded sections (5 µm) of the liver and kidney were prepared and stained with hematoxylin and eosin (H&E).

Malondialdehyde measuring method

MDA is a LPO index. MDA reacts with TBA in acidic pH to form a pink-colored complex and this complex has maximum absorption in 532 nm.[24] A total of 200 µL of 10% TCA was added to 200 µL of 10% homogenate tissue; then, 100 µL HCl (0.5 N) and 200 µL of 0.6% TBA aqueous solution were mixed and shaken, and the mixture was heated in boiling water bath for 45 min. After cooling, the mixture was centrifuged and the absorbance of supernatant was read in 532 nm. MDA levels were expressed as nmol/g tissue.

Glutathione measuring method

The tissue GSH content reacts with 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) reagent to form a compound that is absorbed in 412 nm.[25] A total of 100 µL of supernatant of homogenated tissue was added to 500 µL of sodium phosphate buffer with pH, 8; next, 100 µL of DTNB was added and its absorbance was measured in 412 nm. The level of GSH in the samples was reported as µmol/g tissue.

Catalase activity measuring method

For measuring the activity of CAT enzyme according to a previous study,[26] 0.2 mL supernatant of the homogenated tissue was incubated in 1.0 mL substrate (65 µmol/mL H_2O_2 in 60 mmol/L sodium–potassium phosphate buffer, pH 7.4) at 37°C for 60 s. The enzymatic reaction was stopped with 1.0 mL of 32.4 mmol/L $(NH_4)_6 Mo_7O_{24}.4H_2O$, and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm. The enzyme activity was reported as the percentage of the control group.

Superoxide dismutase activity measuring method

For measuring the activity of SOD enzyme, we used the Madesh and Balasubramanin method.[27] The reaction mixture contained 325 µL phosphate buffered saline (0.1M, pH 8.5), 30 µL 3-(4-5-diethylthiazol 2-xl 2,5 diphenyltetrazolium bromide (MTT; 1.25 mM), 75 µL pyrogallol (100 µM), and 10 µL tissue supernatant, which was incubated at room temperature for 5 min and the reaction was stopped by adding 375 µL of dimethyl sulfoxide. The absorbance was read at 570 nm, and the enzyme activity was reported as the percentage of the control group.

Statistical analysis

Results are shown as mean ± standard error of mean (SEM) for six animals in each group. Comparisons between multiple groups were made by a one-way analysis of variance followed by Fisher's least significant difference test. Differences were considered significant when $P < 0.05$. GraphPad Prism 6 software (GraphPad Software, San Diego, California) was used for the statistical analysis.

Results

The Food and Drug Administration (FDA) acceptable concentration of SB in food is 0.1%. In this study, the concentration of SB in water equaled to 0.1125%, 0.225%, and 0.56%, which was close to the FDA-permitted concentration in the food. Therefore, according to the volume of water consumption, the daily intake of SB was approximately 150,

300, and 600 mg/kg in the mice. The result showed treatment with SB had no effects on the water consumption and the body weight, as previously observed.[23]

Figure 1 presents morphological changes of the liver and kidney tissues in the SB treatment groups (150, 300, and 600 mg/kg) by H&E staining.

It was observed that 300 and 600 mg/kg of SB caused histological alterations in the liver, such as focal acute inflammation and moderate portal inflammation in the hepatocytes, respectively. Also, SB at a dose of 600 mg/kg caused mild interstitial inflammation.

Hypertrophy of the hepatocytes in the periportal area was a characteristic feature in the liver of treated mice with SB at doses of 300 and 600 mg/kg. The histology of the liver, in 150 mg/kg of SB, was the same as the control groups; the central vein and normal hepatocyte were evident. In addition, the kidney of the mice receiving 150 and 300 mg/kg SB was similar to that of the control group, with no change in the glomeruli and tubes, vessels, and interstitium. However, SB 600 mg/kg induced mild to moderate inflammation in the kidney tissues.

As shown in Figure 2, all doses of SB increased the LPO significantly in the kidney tissue in comparison with the control group (P <0.05). However, no significant difference was observed in the liver of all SB groups compared to that of the control group. In Figure 3, the level of GSH in the liver and kidney of mice exposed to different doses of SB (150, 300, and 600 mg/kg) are compared with the control group. It showed that, in all of SB dosages, the GSH content of the kidney was increased. However, none of the doses showed significant differences in the liver tissue.

In Figure 4, the antioxidant activity of CAT in the liver and kidney of mice exposed to different doses of 150, 300, and 600 mg/kg is compared with the control group. The activity of CAT enzyme in the kidney of the mice at doses of 150, 300, and 600 mg/kg was 85.03, 70.86, and 75.39%, respectively, which showed a significant decrease compared to is compared with the control group (P <0.05). As indicated in Figure 4, with increasing SB dosage, no change in the liver CAT activity was seen.

In Figure 5, the antioxidant enzyme activity of SOD in the liver and kidneys of the mice exposed to different doses of 150, 300, and 600 mg/kg is compared with the control group. As indicated in the figure, with the increase in SB doses, the activity of the SOD enzyme in the liver and kidney showed no significant changes.

Discussion

According to the mentioned studies about the toxic effects of SB and also the widespread use of this food additive in drinks, pharmaceutical and cosmetics products, the aim of this study was to investigate the effect of SB on MDA and GSH levels, SOD, and CAT activities as antioxidant indicators in the mice kidney and liver.

Results showed that SB caused histological alterations in the liver and kidney tissues. Moreover, SB significantly increased LPO and GSH content in the kidney tissues. Also, CAT activity significantly declined in the kidney. Moreover, SB significantly increased LPO and GSH content, declined CAT activity, Without changing the SOD activity in the kidney tissues. but SB did not have any effect on the biochemical parameters of the liver tissue.

Although there are some short-term studies on subacute and high-dose toxicity of SB in rats and mice, they described lethality or change in the organs and body weight,[28-30] but the mechanism or cause of SB toxicity is not well known.

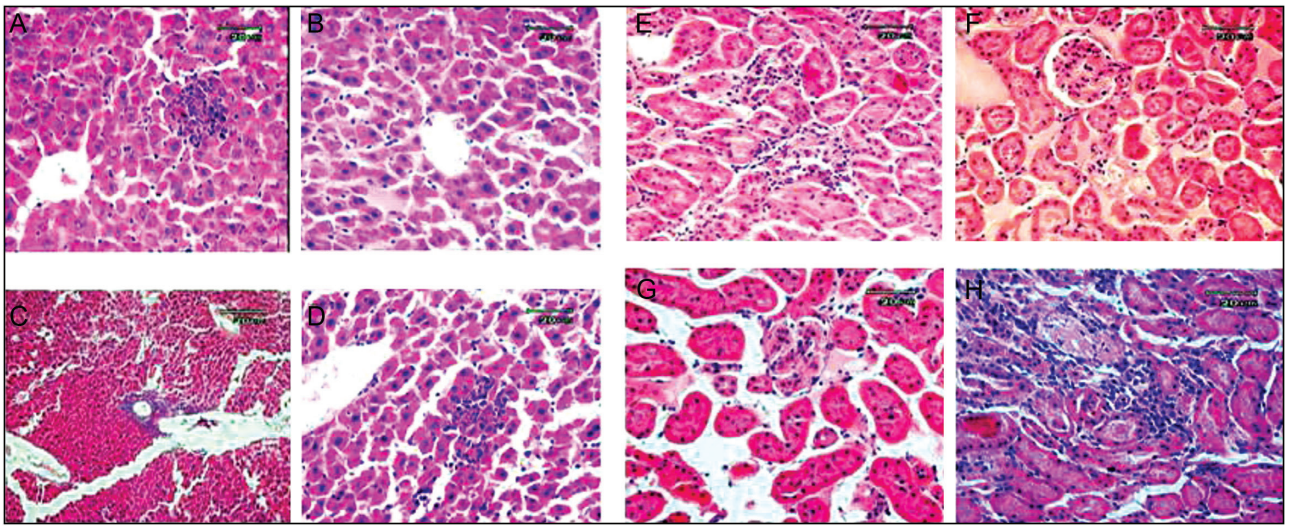

Figure 1: Morphological changes of the liver tissue. (A) Control group. (B) 150 mg/kg Sodium benzoate. (C) 300 mg/kg Sodium benzoate. (D) 600 mg/kg Sodium benzoate. Morphological changes of the kidney tissues. (E) Control group. (F) 150 mg/kg Sodium benzoate. (G) 300 mg/kg Sodium benzoate. (H) 600 mg/kg Sodium benzoate. After 4 weeks of treatment. Hematoxylin and eosin staining, ×400 magnification

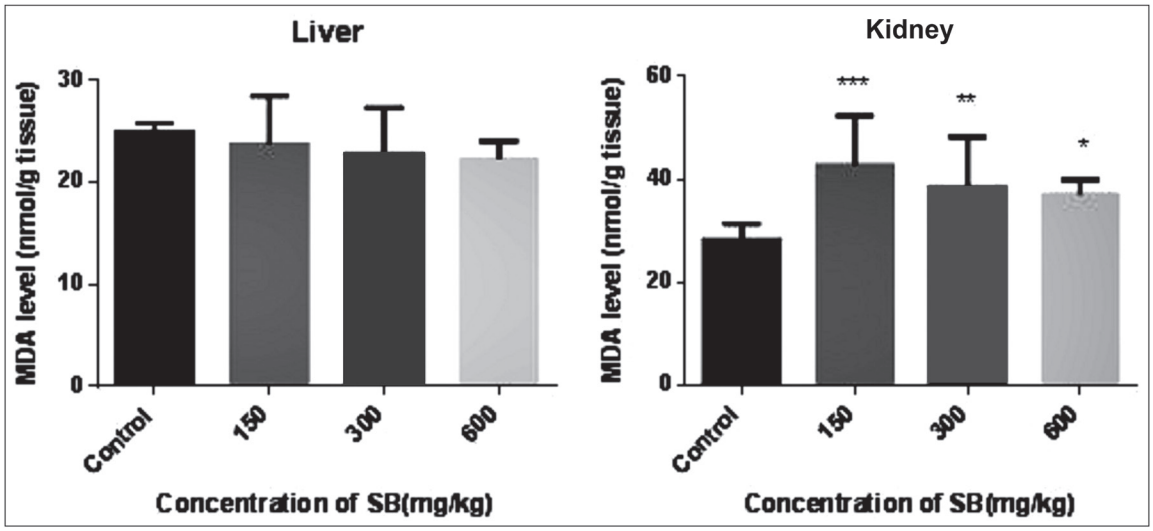

Figure 2: Malondialdehyde (MDA) level in the kidney and liver of animals in the control and experimental groups. Values are mean ± standard error of mean (SEM) (*n* = 6). SB = sodium benzoate. *Significant difference (*P* < 0.05), **significant difference (*P* < 0.01), ***significant difference (*P* < 0.001) compared with the control group

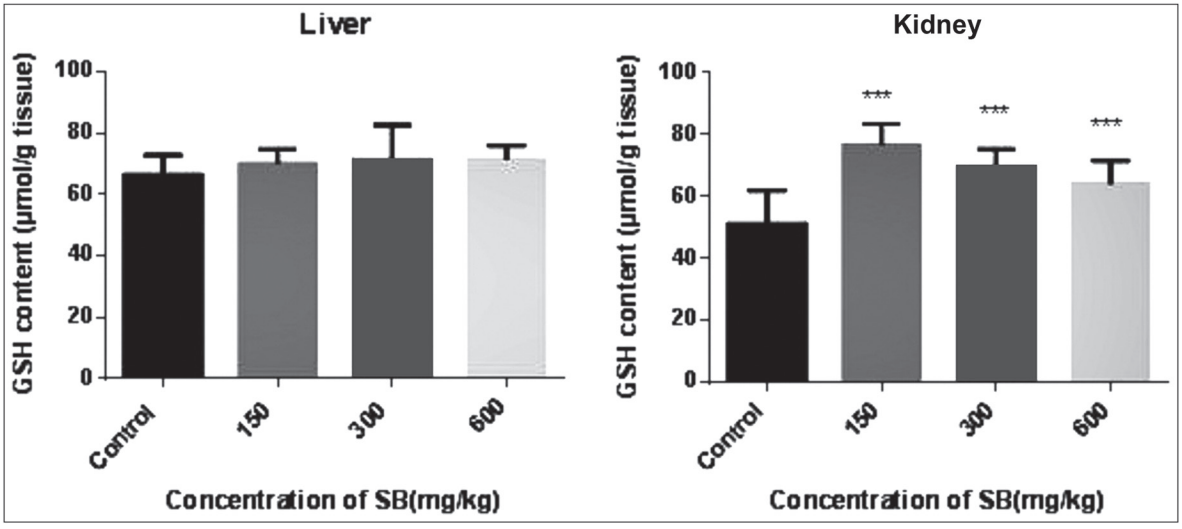

Figure 3: Glutathione (GSH) content in the kidney and liver of animals in the control and experimental groups. Values are mean ± standard error of mean (SEM) (*n* = 6). SB = sodium benzoate. ***Significant difference (*P* < 0.001) compared with the control group

This study revealed that the intake of SB in drinking water for 4 weeks produced histological alterations in the liver and kidney tissues. Most of the previous studies reported histological damages to the liver. Swelling, hemorrhage, and syncytium formation of some hepatocytes were similarly stated by Fujitani.[29] Changes in the tissue and accumulation of cell debris induced inflammatory cell responses to injuries, whereas studies on the renal effects of SB have been less considered. We also observed histopathological changes in the kidney sections. Furthermore, SB significantly increased the LPO and GSH content in the kidney tissues. Moreover, CAT activity significantly declined in the kidney, without any changes in the SOD activity. However, SB did not have any effect on biochemical parameter of the liver tissue.

Although increase of oxidative damage in the liver of treated animals was not significant, examination by light microscopy revealed histological changes in the highest treatment group. The results suggested that the hepatotoxic effects of SB in exposure for a long time may induce severe damage in the liver.

Similar to these results, Bakar and Aktac[30] found the nuclei and basal membrane injury of the tubular cells, degeneration in the glomerular structure, and visceral epithelial injury in the renal tissues by light microscopy. Electron microscopy showed hepatocytes membrane injury, degradation in nuclear membrane and also crista loss in the mitochondria, and fusion in outer membranes of mitochondria vacuolization, which were the result of administration of SB in organs and body (2442 mg/kg body weight) for 10 days in rats.[30]

The kidney is a highly vulnerable organ to ROS injury, probably due to the abundance of long-chain polyunsaturated fatty acids in the composition of the lipids of the renal cells.[31] The results

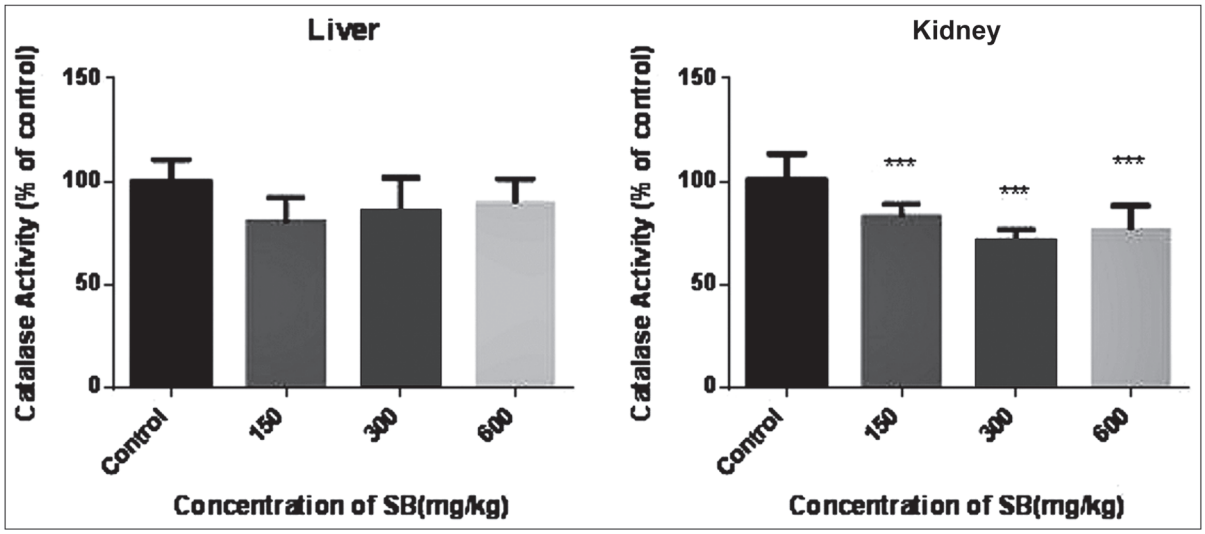

Figure 4: Catalase activity in the kidney and liver of animals in the control and experimental groups. Values are mean ± standard error of mean (SEM) (*n* = 6). SB = sodium benzoate. ***Significant difference (*P* < 0.001) compared to the control group

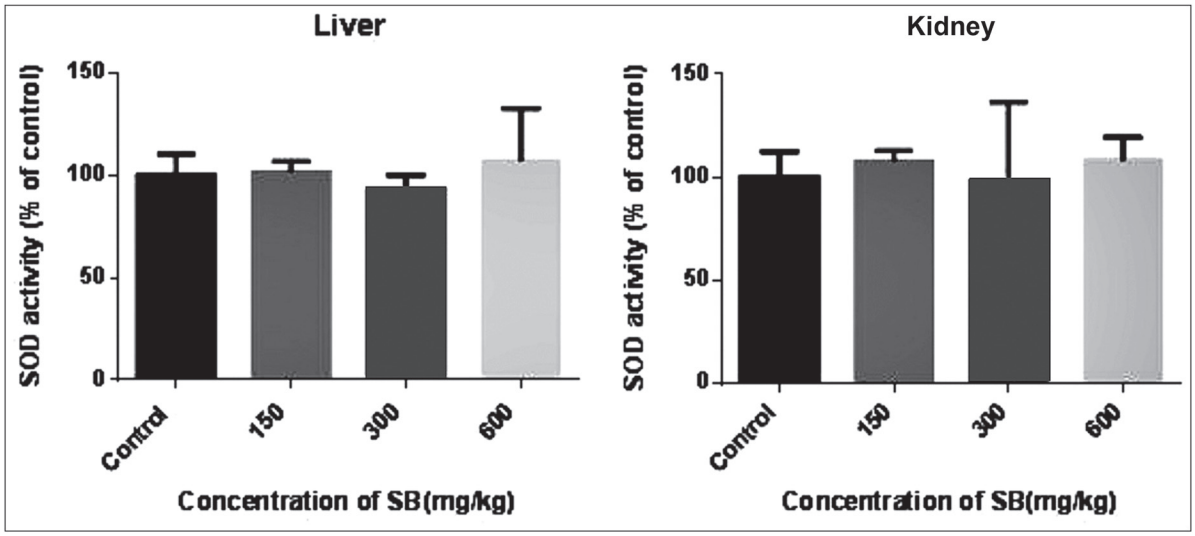

Figure 5: Superoxide dismutase (SOD) activity in the kidney and liver of animals in the control and experimental groups. Values are mean ± standard error of mean (SEM) (*n* = 6). SB = sodium benzoate

of our study showed that kidney damage was greater than the liver damage in the mice treated with SB because the level of LPO significantly increased in the kidney.

However, the effects of SB on the level of liver LPO did not show any significant change, which may be due to the high level of antioxidant defense system in the liver that prevents damage to this tissue.

The kidney GSH content, parallel to the kidney oxidative damage, significantly increased; probably, it is a compensatory mechanism to prevent the kidney damage. SB in all various doses (150, 300, and 600 mg/kg) did not change the liver GSH content because it did not make significant oxidative damage in the liver. Therefore, there was no need to increase the hepatic GSH content or other antioxidant enzyme activity in the liver.

The results of CAT enzyme activity in the kidney tissues showed that all doses of SB significantly decreased its activity in comparison to the control group. This is the same as the results of the previous study about the effect of SB on the CAT activity of the erythrocytes.[32] They have suggested that the decrease in SOD, CAT, Glutathione peroxidase, and GST activities in the erythrocytes by SB might be due to the damage in these antioxidant enzymes by superoxide anions.[32]

No statistically significant changes in SOD activities were observed in the liver and kidney after SB administration. Also, it was found that other detoxification systems and enzymes such as thioredoxin and GSH peroxidase have important roles in redox homoeostasis in the mammals.[33,34] These results suggest that perhaps the mentioned system is more important than SOD for detoxifying SB-induced oxidative stress in the

liver and kidney. In addition, different organ tissues have various antioxidant capacities to cope with oxidative stress, as previously we observed a reduction in the GSH content and an increase in the MDA level in the brain of the mouse that had used SB. Also, SB treatment showed learning and memory deficits in the behavioral tests.[23]

Based on Oyewolee *et al*.'s study (2012), two weeks of oral administration of 200 mg/kg SB caused some harmful effects on the rat liver and kidney functions. Also, administration of the *Azadirachta indica* leaves extract could act as a prophylactic agent against adverse effects of SB on considerably increasing serum level of ALT, AST, creatinine, urea, and uric acid.[13] Although this study evaluated the hepatorenal toxicity of SB, they only evaluated the serum biomarkers of the liver and kidney injury of one dose of SB, so other parameters such as liver and kidney oxidative stress damages in different doses were not considered.

However, there were some limitations in our study and further examination is required to provide an insight into specific hepatic and renal effects of SB. The mitochondria is an important organelle in the control of cell survival and death and plays a role in the oxidative stress, which could be evaluated. Also, the level of ROS in the cytosolic and mitochondrial fractions of the hepatic and renal cells could be measured.

Conclusion

SB after oral administration in drinking water causes kidney damage more than liver injury. In the liver, further active antioxidant system could protect the cells against oxidative stress; however, in the kidney, in spite of the increasing antioxidant GSH to fight oxidative stress, LPO is induced by SB. However, the consumption of SB for a long period may also cause liver damage. As SB is a preservative used in various food products for a long time, renal damage has possibly resulted from the excessive intake of high dose or chronic consumption of this food additive.

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Conflicts of interest

There are no conflicts of interest.

References

1. Heydaryinia A, Veissi M, Sadadi A. A comparative study of the effects of the two preservatives, sodium benzoate and potassium sorbate on *Aspergillus niger* and *Penicillium notatum*. Jundishapur J Microbiol 2011;4:301.
2. Shahmohammadi M, Javadi M, Nassiri-Asl M. An overview on the effects of sodium benzoate as a preservative in food products. Biotech Health Sci 2016;3:e35084.
3. Kregiel D. Health safety of soft drinks: Contents, containers, and microorganisms. BioMed Res Int 2015;2015:128697.
4. Lennerz BS, Vafai SB, Delaney NF, Clish CB, Deik AA, Pierce KA, *et al*. Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. Mol Genet Metab 2015;114:73-9.
5. Misel ML, Gish RG, Patton H, Mendler M. Sodium benzoate for treatment of hepatic encephalopathy. Gastroenterol Hepatol (N Y) 2013;9:219-27.
6. Ostergaard E. Evaluation of the antimicrobial effects of sodium benzoate and dichlorobenzyl alcohol against dental plaque microorganisms: An *in vitro* study. Acta Odontologica Scandinavica 1994;52:335-45.
7. Salviano dos Santos V, Paula N, Medeiros Salgado A, Guedes Torres A, Signori Pereira K. Benzene as a chemical hazard in processed foods. Int J Food Sci 2015;2015:1-7.
8. Hoang YT, Vu AT. Sodium benzoate and potassium sorbate in processed meat products collected in Ho Chi Minh City, Vietnam. Int J Adv Sci Eng Inf Technol 2016;6:477-82.
9. Law S. The effect of incubation time on the generation of benzene in Sierra Mist Free. J South Carolina Acad Sci 2009;7:2.
10. Bray H, Thorpe W, White K. Kinetic studies of the metabolism of foreign organic compounds. 1. The formation of benzoic acid from benzamide, toluene, benzyl alcohol and benzaldehyde and its conjugation with glycine and glucuronic acid in the rabbit. Biochem J 1951;48:88.
11. Irwin C, van Reenen M, Mason S, Mienie LJ, Westerhuis JA, Reinecke CJ. Contribution towards a metabolite profile of the detoxification of benzoic acid through glycine conjugation: An intervention study. PLoS One 2016;11:e0167309.
12. Mizutani N, Maehara M, Hayakawa C, Kato T, Watanabe K, Suzuki S. Hyperargininemia: Clinical course and treatment with sodium benzoate and phenylacetic acid. Brain Dev 1983;5:555-63.
13. Oyewole OI, Dere FA, Okoro OE. Sodium benzoate mediated hepatorenal toxicity in Wistar rat: Modulatory effects of *Azadirachta indica* (neem) leaf. Eur J Med Plants 2012;2:11.
14. Hennigan SL, Driskell JD, Ferguson-Noel N, Dluhy RA, Zhao Y, Tripp RA, *et al*. Detection and differentiation of avian mycoplasmas by surface-enhanced Raman spectroscopy based on a silver nanorod array. Appl Environ Microbiol 2012;78:1930-5.
15. Kelly F. Oxidative stress: Its role in air pollution and adverse health effects. Occup Environ Med 2003;60:612-6.
16. Mittler R. ROS are good. Trends Plant Sci 2017;22:11-9.
17. Zhang H, Tsao R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr Opin Food Sci 2016;8:33-42.
18. Heidari R, Jamshidzadeh A, Niknahad H, Mardani E, Ommati MM, Azarpira N, *et al*. Effect of taurine on chronic and acute liver injury: Focus on blood and brain ammonia. Toxicol Rep 2016;3:870-9.
19. Wells PG, Bhatia S, Drake DM, Miller-Pinsler L. Fetal oxidative stress mechanisms of neurodevelopmental deficits and exacerbation by ethanol and methamphetamine. Birth Defects Res C Embryo Today 2016;108:108-30.
20. Jamshidzadeh A, Heidari R, Abasvali M, Zarei M, Ommati MM, Abdoli N, *et al*. Taurine treatment preserves brain and liver mitochondrial function in a rat model of fulminant hepatic failure and hyperammonemia. Biomed Pharmacother 2017;86:514-20.
21. Atli G, Grosell M. Characterization and response of antioxidant systems in the tissues of the freshwater pond snail (*Lymnaea stagnalis*)

during acute copper exposure. Aquat Toxicol 2016;176:38-44.
22. Noorafshan A, Erfanizadeh M, Karbalay-Doust S. Sodium benzoate, a food preservative, induces anxiety and motor impairment in rats. Neurosciences (Riyadh) 2014;19:24-8.
23. Khoshnoud MJ, Siavashpour A, Bakhshizadeh M, Rashedinia M. Effects of sodium benzoate, a commonly used food preservative, on learning, memory, and oxidative stress in brain of mice. J Biochem Mol Toxicol 2018;32:e22022.
24. Heidari R, Jafari F, Khodaei F, Shirazi Yeganeh B, Niknahad H. The mechanism of valproic acid-induced Fanconi syndrome involves mitochondrial dysfunction and oxidative stress in rat kidney. Nephrology (Carlton, Vic) 2018;23:351-61.
25. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
26. Goth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta Int J Clin Chem 1991;196:143-51.
27. Madesh M, Balasubramanian K. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem Biophys 1998;35:184-8.
28. Kaboglu A, Aktac T. A study of the effects of sodium benzoate on the mouse liver. Biologia 2002;57:375-82.
29. Fujitani T. Short-term effect of sodium benzoate in F344 rats and B6C3F1 mice. Toxicol Lett 1993;69:171-9.
30. Bakar E, Aktac T. Effects of sodium benzoate and citric acid on serum, liver and kidney tissue total sialic acid levels: An ultrastructural study. J Appl Biol Sci 2014;8:9-15.
31. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol 2012;2012:465897.
32. Yetuk G, Pandir D, Bas H. Protective role of catechin and quercetin in sodium benzoate-induced lipid peroxidation and the antioxidant system in human erythrocytes *in vitro*. ScientificWorldJournal 2014;2014:874824.
33. Jones DP, Eklöw L, Thor H, Orrenius S. Metabolism of hydrogen peroxide in isolated hepatocytes: Relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H_2O_2. Arch Biochem Biophys 1981;210:505-16.
34. Turanov AA, Kehr S, Marino SM, Yoo MH, Carlson BA, Hatfield DL, *et al*. Mammalian thioredoxin reductase 1: Roles in redox homoeostasis and characterization of cellular targets. Biochem J 2010;430:285-93.