*Proceedings of the Pakistan Academy of Sciences: B Life and Environmental Sciences* 59(3): 31-37 (2022)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-3)719

Pakistan Academy of Sciences

Research Article

# Hepatic and Renal Histopathological Effects of Local Fruit Juices containing Sodium Benzoate as Preservative

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**Abstract:** Packed fruit juices are commonly consumed on daily basis around the globe which usually contain sodium benzoate as a food preservative to enhance their shelf life and its amount may vary from defined food standards among brands. This mammalian model-based research was designed to investigate the impact of locally packed fruit juices' regular intake on consumers. For this purpose, albino mice (Mus musculus) were acclimatized in the laboratory environment for ten days and then categorized into control and two experimental groups. The selected local brands were Murree brewery lemon malt and Shezan mango juice and on their bottles and tetrapacks, it was already mentioned that they contain sodium benzoate as a preservative. For further confirmation, the titration method was used to detect the presence of sodium benzoate in selected locally packed juice samples. After dose preparation and optimization, 0.1 ml of the prepared dose was given to each experimental group I (treated with lemon malt) and II (treated with mango juice), whereas the control group was treated with the equivalent amount of distilled water. The obtained histopathological results emphasized that regular intake of sodium benzoate having fruit juices may cause severe damage to hepatic and renal tissues, usually in the form of necrosis, vascular congestion sometimes dilation and other cellular alterations i.e., in glomeruli and bile duct. Moreover, it may result in the onset of tumorogenesis. The conclusion of this study is local food authorities should ensure the addition of a defined amount of sodium benzoate as a juice preservative in locally packed juices to provide healthy products to consumers.

**Keywords:** Fruit juices, food preservative, sodium benzoate, necrosis, tumorogenesis.

## 1. INTRODUCTION

Fruit juices are the products of daily consumption. They may be prepared either through fresh extraction or supplied to markets in tetra packs which contain different types of food preservatives to avoid microbial growth and to improve their shelf life. The amount of these food preservatives varies from brand to brand and it is also an important parameter to compare the quality of locally packed fruit juices with international food standards. In this regard, reported data also highlight that the appliance of diverse synthetic compounds as food preservatives may cause detrimental health effects on consumers [1].

The commonly used juice preservatives are benzoate (SB), sodium metabisulphite (SM), potassium sorbate (PS), and their compositional mixtures but none of them is side effect free [11]. Among them, sodium benzoate is considered less harmful or sometimes safe, if added according to internationally defined standards by food and health organization [12]. But in general practice, especially in the case of local less renowned brands, such food standards are not uniformly followed which often alters the attributes of juices and beverages and indirectly results in adverse health effects.

Reported data also supports that if a higher concentration of sodium benzoate enters in living body, which is often used as a food preservative in fruit juices too, has been reported to affect renal function and as result may elevate the blood plasma level and disturbs cellular carbohydrate metabolism which can be observed in histological sections of

Received: May 2022; Accepted: September 2022
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kidneys [2]. This compound has also been found to induce immunosuppressive effects and disrupts the process of blood filtration and detoxification in the liver and kidney [3]. Recent mammalian model-based studies have also supported these above-mentioned side effects of sodium benzoate present in different food items as a preservative [4]. That is why; this research was designed to estimate the possible side effects of sodium benzoate as a preservative of locally packed fruit juices.

## 2. MATERIALS AND METHODS

### 2.1 Animal Collection and Habituation

Thirty female albino mice (Mus musculus) of 6 weeks of age were purchased from the University of Veterinary and Animal Sciences, Lahore. The mice were placed in an animal house, School of Zoology, Minhaj University, Lahore, at room temperature (25 °C ± 2 °C) and after habituation of 10 days, they were divided into 3 groups: control group, experimental group I (treated with Murree Brewery lemon malt) and experimental group II (treated with Shezan mango juice).

### 2.2 Detection of Sodium Benzoate

For detection and confirmation of sodium benzoate in selected juice samples, the acidimetry-alkalimetry titration method was used and the percentage of sodium benzoate was calculated [13].

### 2.3 Dose Optimization

For dose optimization, 0.5 ml of each selected packed juice was diluted in 9.5 ml of distilled water and 0.1 ml of this prepared dilution was given to both experimental groups whereas control group mice were given 0.1 ml of distilled water orally during 4 weeks of experimentation.

### 2.4 Histopathology & Microscopy

The mice were dissected weekly and kidney and liver tissues were collected and preserved in 10 % formalin solution for further tissue processing through an automatic tissue processor. Their histological sections were prepared by microtomy and the prepared slides were stained by using Hematoxylin and Eosin staining. The microscopic observations and micrometry of prepared slides were done at 40X and their photomicrographs were recorded at 10X with the help of PixelPro software.

### 2.5 Statistical Analysis

Single-factor ANOVA was applied for statistical data analysis [5].

## 3. RESULTS AND DISCUSSION

First of all, the detected percentage of sodium benzoate in the experimental group I sample was 0.096 % and in the experimental group II sample was 0.11 % which was higher than the defined amount of sodium benzoate 0.1% used as food preservative according to the food authorities and especially the formulation of lemon malt should be revised [14]. Moreover, the weekly observations of body weight (Table 1) indicated considerable variations in both experimental groups after i.e. at the end weight gain was observed at 37.33 g of 1$^{st}$ week group II but at the end of 2$^{nd}$ week weight reduction was noticed while in group I body weight increased up to 34.33 g. Similarly, as compared to the previous week though body weight was reduced in group II (30 g) it was still significantly higher than group I (27 g) on completion of 3$^{rd}$ week. Whereas, 24.67 g and 36.67 g body weights were noted in groups I and II, respectively, at the end of the experimentation [15]. Dermal tumors were also observed in mice of experimental groups. These side effects occurred in presence of sodium benzoate which may induce toxicological and adverse changes in the biochemical markers and physiological processes of consumers [6].

For histopathological analyses, first of all, the variations in blood vessel diameter were observed. The diameter of renal arteries was significantly changed during 2$^{nd}$ week, in group I vasoconstriction (50.70 µm) and group II vasodilation (84.53 µm) were noted (Table 2). The reduction in arterial diameter was noticed in both experimental groups I and II, 48.65 µm and 75.02 µm, respectively and same pattern continued till the end of 4$^{th}$ week (Figure 1). Whereas in the case of renal veins diameter, at the end of the 4$^{th}$ week, vasoconstriction was observed in group I (61.29 µm) while vasodilation was noted in group II (3.48 µm). The histopathological observations regarding glomerular diameter

(Table 3) indicated the considerable shrinkage of glomeruli at the end of 2nd week in both groups I and II, 46.49 µm and 35.93 µm, respectively, in comparison to the control group because exposure to sodium benzoate as preservative damage structure of kidney and disturbs its functions [15]. Moreover, the results related to the diameter of necrotic renal tissue presented maximum damage in groups I and II of 83.48 µm and 81.36 µm, respectively, at the end of 3rd week. It indicates that regular use of sodium benzoate-containing food items may lead to serious vascular complications like calcification and congestion of renal blood vessels [7].

In addition to this, histopathological changes related to the diameter of liver blood vessels were examined and among them, hepatic arteries showed prominent vasoconstriction in groups I and II of 46.49 µm and 85.92 µm, respectively, at the end of 2nd week but later on the trend was found opposite till the end of experimentation (Table 4). Similarly, vasodilation was also noticed in diameter of hepatic veins, 117.23 µm (group II) and 54.96 µm (group I) after 2nd week. Then till the end of the experimentation, a gradual reduction in the diameter of the vein was noticed in both experimental groups (Figure 2) due to biochemical alterations [8].

For the measurements of the diameter of bile duct, highlighted the gradual increase, from 1st to 4th week (Table 5). But the striking size of the damaged and necrotic area of hepatic tissue was observed at 86.65 µm and 110.95 µm, in experimental groups I and II respectively, at the end of 3rd week [9]. Thus, locally available juice products are not reliable and their components may induce broad spectrum health side effects [10].

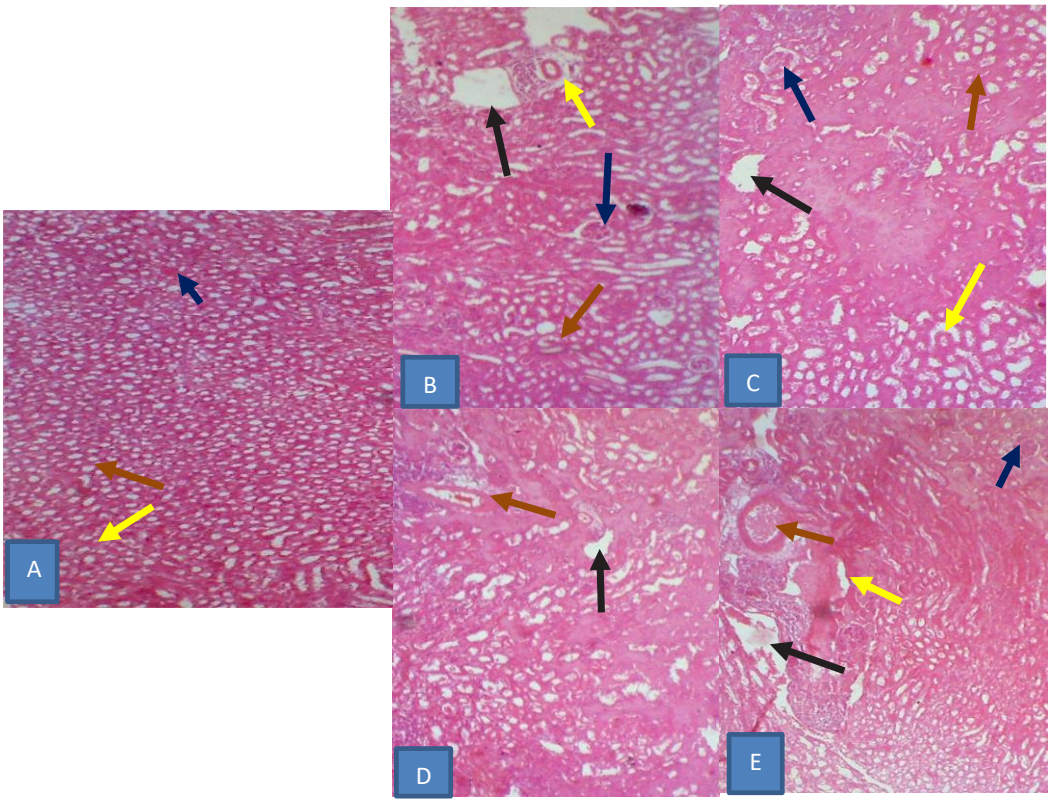


**Fig. 1.** Comparative histopathological renal observations of (A) control group, (B) 1st week of experimental group I, (C) 1st week of experimental group II, (D) 4th week of experimental group I, (E) 4th week of experimental group II. Black arrows show damaged area diameter (µm), blue arrows show glomerulus diameter (µm), yellow arrows show artery (µm) and red arrow show vein diameter (µm). All microphotographs are of 100X magnification.

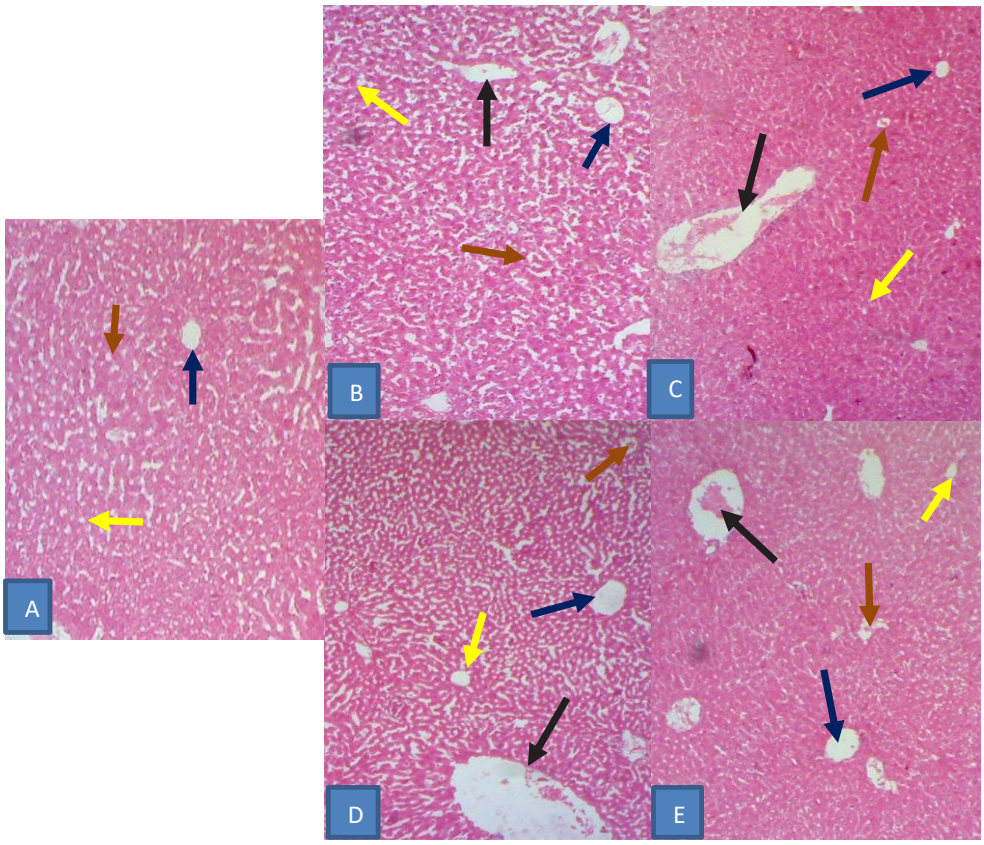


**Fig. 2.** Comparative histological hepatic observations of (A) control group (B) 1st week of experimntal group I (C) 1st week of experimental group II, (D) 4th week of experimntal group I, (E) 4th week of experimntal group II. Black arrows show the damaged area diameter (µm), blue arrows show the diameter of bile duct (µm), yellow arrows show the artery (µm) and red arrow show vein diameter (µm). All microphotographs are of 100X magnification.

**Table 1.** Effect of sodium benzoate as juice preservative on body weight (g)

| Week | Control group | Experimental Group I | Experimental Group II |
|---|---|---|---|
| 1 | 35±2.52 (3) | 31.33±1.33 (3) | *37.33±2.33 (3) |
| 2 | | *34.33±4.81 (3) | 31.67±3.53 (3) |
| 3 | | 27±2.65 (3) | *30±2.31 (3) |
| 4 | | 24.67±1.45 (3) | *36.67±4.41 (3) |

*Values represent Mean ± S.E.M. (n). Data was compared by employing single factor analysis of variance and results were found significant at a 5% level (*).

**Table 2.** Effect of sodium benzoate as juice preservative on renal artery and vein diameter (µm)

| Week | For renal artery | | | For renal vein | | |
|---|---|---|---|---|---|---|
| | Control group | Experimental Group I | Experimental Group II | Control group | Experimental Group I | Experimental Group II |
| 1 | 85.60± 4.84 (3) | 57.06 ± 12.81 (3) | 49.66 ± 10.08 (3) | 102.50 ± 2.80 (3) | 72.91 ± 3.17 (3) | 70.80 ± 2.80 (3) |
| 2 | | 50.70 ± 10.21 (3) | **84.53 ± 7.40 (3) | | 53.89 ± 5.50 (3) | 59.17 ± 2.80 (3) |
| 3 | | 48.61 ± 9.40 (3) | ** 75.02 ± 9.212 (3) | | 71.85 ± 2.11 (3) | 47.56 ± 5.48 (3) |
| 4 | | *57.06 ± 5.50 (3) | 53.89 ± 11.13 (3) | | 61.29 ± 8.25 (3) | 83.48 ± 8.65 (3) |

*Values represent Mean ± S.E.M.(n). Data was compared by employing single factor analysis of variance and results were found significant at 5% (*) and 1% (**) levels.

**Table 3.** Effect of sodium benzoate as juice preservative on diameter of glomerulus and renal necrotic area (µm)

| Week | Glomerular diameter | | | Diameter of renal necrotic area | |
|---|---|---|---|---|---|
| | Control group | Experimental Group I | Experimental Group II | Experimental Group I | Experimental Group II |
| **1** | 86.65 ± 9.21 (3) | 102.50 ± 32.35(3) | 45.44 ± 7.40(3) | 68.68 ± 15.35 (3) | 67.63 ± 20.08 (3) |
| **2** | | **46.49 ± 5.60 (3) | 35.93 ± 2.80 (3) | 54.95 ± 6.93 (3) | 116.23 ± 8.65 (3) |
| **3** | | **67.63 ± 2.11 (3) | 62.34 ± 11.20 (3) | 83.48 ± 4.61 (3) | 81.36 ± 17.01 (3) |
| **4** | | 66.57 ± 12.001 (3) | 67.63 ± 4.6 (3) | 62.34 ± 62.34 (3) | 63.40 ± 9.69 (3) |

*Values represent Mean ± S.E.M.(n). Data was compared by employing single factor analysis of variance and results were found significant at 1 % level (*).

**Table 4.** Effect of sodium benzoate juice as juice preservative on diameter of liver artery and vein (µm)

| Week | For hepatic artery | | | For hepatic vein | | |
|---|---|---|---|---|---|---|
| | Control group | Experimental Group I | Experimental Group II | Control group | Experimental Group I | Experimental Group II |
| 1 | 73.97 ± 9.21 (3) | 102.50 ± 32.35 (3) | 45.44 ± 7.40 (3) | 126.8± 30 (3) | 68.68 ±15.35 (3) | 67.63±20.08 (3) |
| 2 | | **46.49 ± 5.60 (3) | 35.93 ± 2.80 (3) | | 54.95 ± 6.93 (3) | *116.23±8.65 (3) |
| 3 | | 67.63 ± 2.11 (3) | 62.3433 ± 11.2 (3) | | 83.48 ± 4.61 (3) | 81.36±17.01 (3) |
| 4 | | 66.57 ± 12.001 (3) | 67.63 ± 4.61 (3) | | 62.34 ± 3.81 (3) | 63.4±9.69 (3) |

*Values represent Mean ± S.E.M.(n). Data was compared by employing single factor analysis of variance and results were found significant at 5% (*) and 1% (**) levels.

**Table 5.** Effect of sodium benzoate as juice preservative on bile duct and necrotic area diameter of liver (µm)

| Week | For diameter of bile duct | | | For diameter of hepatic necrotic area | |
|---|---|---|---|---|---|
| | Control group | Experimental Group I | Experimental Group II | Experimental Group I | Experimental Group II |
| 1 | | 48.61 ±14.80 (3) | 42.27±2.11 (3) | 69.74 ± 12.81 (3) | 83.48 ± 10.41 (3) |
| 2 | | 62.34 ± 13.74 (3) | 60.23±3.66 (3) | 77.14 ± 19.74 (3) | 85.59±12.68 (3) |
| 3 | 65.51±9.21 (3) | 58.12 ±10.72 (3) | 68.68 ±14.68 (3) | 86.65 ± 8.6492 (3) | 110.95±8.39 (3) |
| 4 | | 78.19 ± 13.86 (3) | 57.06±6.34 (3) | 71.85 ± 3.81 (3) | 100.4 ± 16.51(3) |

*Values represent Mean ± S.E.M. (n). Data was compared by employing single factor analysis of variance and no significant result was found.

## 4. CONCLUSION

It can be concluded that sodium benzoate is not a suitable juice preservative and the consumption of products having this food preservative may cause hepatic and renal physiological disturbances and their long term consumption may lead to adverse health complications. The current study showed that it affected the functioning of the liver and kidney. In this regard, local food authorities should take appropriate action to secure the health of consumers.

## 5. ACKNOWLEDGEMENTS

We are obliged to Dr. Hafza Zainub, Head of the Anatomy & Histology Department, University of Veterinary and Animal Sciences, Lahore, Pakistan for providing us with Laboratory facilities.

## 6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

## 7. ETHICS APPROVAL

The experimental protocols and procedures used in this study were approved by the Ethical Committee of the Directorate of Academics, Minhaj University Lahore, Pakistan with reference number: MUL/DA/11356.

## 8. REFERENCES

1. B. Prakash, A. Kedia, P. K. Mishra, A. K. Dwivedy, and N. K. Dubey. Assessment of chemically characterized Rosmarinus officinalis L. essential oil and its major compounds as plant based preservative in food system based on their efficacy against foodborne moulds and aflatoxin secretion and as antioxidant. I*nternational Journal of Food Science & Technology* 50(8): 1792-1798 (2015).
2. B.S. Lennerz, S.B. Vafai, N.F. Delaney, C.B. Clish, A.A. Deik, K.A. Pierce, and V.K. Mootha. Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. *Molecular Genetics and Metabolism* 114(1): 73-79 (2015).
3. A. Yadav, A. Kumar, M. Das, and A. Tripathi. Sodium benzoate, a food preservative, affects the functional and activation status of splenocytes at non cytotoxic dose. Food and Chemical Toxicology, 88: 40-47(2016). H. C. Han. Twisted blood vessels: symptoms, etiology and biomechanical mechanisms. *Journal of Vascular Research* 49(3): 185-197 (2012).
4. F. Khodaei, H. Kholghipour, M. Hosseinzadeh, and M. Rashedinia. Effect of sodium benzoate on liver and kidney lipid peroxidation and antioxidant enzymes in mice. *Journal of Reports in Pharmaceutical Sciences* 8(2): 217-19 (2019).
5. D. Matheoud, T. Cannon, A. Voisin, A.M. Penttinen, L. Ramet, A.M. Fahmy, and M. Desjardins.

Intestinal infection triggers Parkinson's disease-like symptoms in Pink1−/− mice. *Nature* 571 (7766): 565-569 (2019).

6. I.S. Khan, K.B. Dar, S.A. Ganie, and M.N. Ali. Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats. *Drug and Chemical Toxicology* 45(3): 1345-1354 (2022).
7. M. Hasson, E.M. Majhwol, and H.J.N. Almuoswi. Evaluation of some kidney functions of rates treated with sodium benzoate. *Annals of the Romanian Society for Cell Biology* 4859–4866 (2021).
8. A. Javed, and J. I. Qazi. Testosterone propionate promotes angiogenesis and nerve regeneration in extensor digitorrum longus muscle grafts. *Sindh University Research Journal-SURJ (Science Series)* 50(01): 59-64 (2018).
9. H. Erfan, S.M. Yousef, K. A. R. I. M. A. El-Sayed, and A. Z. Mohamed. Assessment of the effect of concomitant use of sodium benzoate and fructose on the liver structure and function in young albino rats. *The Medical Journal of Cairo University* 89(June): 761-767 (2021).
10. M. Sen. Food chemistry: role of additives, preservatives, and adulteration. *Food Chemistry: The Role of Additives, Preservatives and Adulteration* 1-42 (2021).
11. World Health Organization. Understanding the Codex Alimentarius. *Food and Agriculture Organization* 247: 1-19 (2018).
12. H. Chen, M.M. Brashears, and Q. Zhong. Sodium benzoate and sodium bisulfate as preservatives in apple juice and alternative sanitizers for washing cherry tomatoes. *International Journal of Food Microbiology* 109697 (2022).
13. T. Alawiyah, and I. Yuwindry. Analysis of sodium benzoate levels in drink samples carbonized with brand X. *Proceedings of the First National Seminar Universitas Sari Mulia* 1-6 (2020).
14. M. Aslam, S. Hamid, S. Khalid, H. Kamran, and S. Azhar. Determination of preservative sodium benzoate in selected samples of fruit juices and squashes. *Asian Journal of Allied Health Sciences* 27-32 (2020).
15. K. Zeghib, and D.A. Boutlelis. Food additive (sodium benzoate)-induced damage on renal function and glomerular cells in rats; modulating effect of aqueous extract of Atriplex halimus L. *Iranian Journal of Pharmaceutical Research* 20(1), 296 (2021).