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Full Length Article

Toxicological Evaluation of Sodium Benzoate on Hematological and Serological Parameters of Wistar Rats

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Abstract

Sodium benzoate is extensively used for the preservation of huge number of food items and medicines at commercial scale. Present study was carried out to investigate *in vivo* potential toxic effects of sodium benzoate in male wistar rats. For the purpose, 60 sexually mature male wistar rats without any clinical and behavioral abnormalities were randomly allocated to five different groups (A–E). After 5 days of acclimatization, various doses of sodium benzoate were given orally to groups (B–E) of experimental animals for a period of sixty days. The rats kept in group A served as control. The experimental rats were sacrificed at 20, 40 and 60 days of the study for collection of blood samples. Statistical analysis indicates that different hematological parameters including red blood cell counts, hemoglobin concentration and hematocrit values decreased significantly as compared with control group of animals while total white blood cell counts increased significantly at higher concentrations of sodium benzoate. Results on serum biochemical analysis showed elevated concentrations of different liver function tests (total bilirubin, AST and ALT), kidney function tests (urea and creatinine), cardiac enzymes (LDH, CPK and CKMB), serum malondialdehyde (MDA) while reduced concentrations of different parameters of lipid profile (cholesterol, triglycerides, LDL and HDL) and serum proteins (albumin, total protein) as compared to control group. In conclusion, the findings of current experimental study suggest that sodium benzoate exerted adverse effects on different blood biochemical parameters of experimental rats. Furthermore, these effects aggravated with increasing dose levels and length of study period.

Keywords: Serum biochemistry; AST; ALT; LDH; Cholesterol; LDL

Introduction

Sodium benzoate is frequently used as food additive to prevent the microbial growth including bacteria and fungi. Among different food preservatives sodium benzoate holds an important and significant status in food processing industries throughout the world. It is extensively used in a variety of food stuff including carbonated drinks, jams, jellies, fruit juices, beer, margarine, bakery items, cheeses, various pickles and sauces (Zengin *et al*., 2011) and for the preservation of liquid medicines as well (Oyewole *et al*., 2012; Shahmihammadi *et al*., 2016).

Although sodium benzoate is in the category of safe additives, yet its disadvantages on human health has been reported (Yolmeh *et al*., 2014) and it is associated with adverse health effects in consumers (Oyewole *et al*., 2012). It is associated with antagonistic health effects such as liver dysfunction and gastrointestinal irritation (Gao *et al*., 2017). According to world health organization, the acceptable daily intake of sodium benzoate is 5 mg/kg.bw/day; however, it is being used in higher concentrations in many food items (Yadav *et al*., 2016).

Sodium benzoate forms benzene as result of reaction of benzoic acid and ascorbic acid in soft drinks and fruit juices (Gardner and Lawrence, 1993). It can trigger skin rashes, asthma and believed to be causing brain damage. It is associated with *in vitro* clastogenic, mutagenic and cytotoxic effects to human lymphocytes (Zengin *et al*.,

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2011). The metabolism of this compound can ultimately lead to the formation of compounds that interact with DNA, change the genetic structure of cells and has adverse effects on cell division (Afshar *et al*., 2013). It causes high blood pressure, eventually tearing the blood cells of the rats (Eberechukwu *et al*., 2007) and kidney malfunctioning (Bakar and Aktac, 2014). Sodium benzoate has also been known to increase the levels of serum creatinine, urea and uric acid in experimental mice (Na and Minghao, 2006).

As this food additive is being extensively used in numerous food items and medicines; therefore, determination and evaluation of its possible toxic effects is of vital importance to minimize its adverse role in public health. Moreover, in previously published literature no report is available about the long term toxic effects of sodium benzoate at low concentrations. Therefore, the present study was designed to determine the adverse effects of sodium benzoate at low concentration in rats.

Materials and Methods

Study Animals

Mature, healthy, albino rats of Wistar strain having age of 7–8 weeks were obtained from Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture Faisalabad. All the study animals were transported carefully in wire cages and kept under standard laboratory conditions at the animal room of Department of Food Science and Technology, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur. Initially for acclimatization, a basal diet was given to experimental animals for a week. After seven days of acclimatization, all rats were randomly classified into five groups (A–E) with twelve rats in each group with four replicates. Various doses of sodium benzoate (Table 1) were administered orally (in the form of water solution) to the animals under study for sixty days. The animals were kept in wire cages with accessibility of 12 h light/dark cycles and free availability of feed and water during the period of trial. All the experimental rats were carefully monitored twice a day for any behavioral and clinical ailments.

Body Weight

Body weight of animals from experimental groups was recorded at 20, 40 and 60 days of study.

Blood Sampling

Blood samples were collected from rats at different intervals (20, 40 and 60 days) with and without anticoagulant in sterile test tubes. For the purpose, four rats from each group were euthanized and blood was collected from jugular vein. All the experimental animals were examined for any physical and clinical responses throughout the experiment. Serum was separated from all the blood samples collected without anticoagulant and subjected to estimation of different serological parameters.

Hematological Studies

Blood samples with anticoagulant were used for determination of different hematological parameters like red blood cell count, white blood cell count, hemoglobin and hematocrit values (Sharma *et al*., 2010).

Serum Biochemical Analysis

Various parameters of serum like bilirubin total, AST and ALT (liver function tests), urea and creatinine (renal function tests), LDH, CPK and CKMB (cardiac enzymes), cholesterol, triglycerides, HDL and LDL (lipid profile), protein total and albumin (serum proteins) were measured using chemistry analyzer employing commercially available kits (Ahmad *et al*., 2013; Hussain *et al*., 2017). Serum malondialdehyde (MDA) a lipid peroxidation product and biomarker for oxidative stress was determined using the method described by Hussain *et al*. (2014) and Ghaffar *et al*. (2017).

Statistical Analysis

The data collected from the current experimental study (Complete Randomized Design-CRD with four replicates) was processed statistically and the techniques described by Steel and Dickey (1997) were used for the determination of analysis of variance. DMR (Duncan's Multiple Range) Test was used for the comparison of means utilizing CoStat Statistical Software (2003).

Results

Physically all the rats in groups A, B, C, D and E did not show any behavioral and clinical alterations throughout the experiment. All the rats in these groups were found active. Results exhibited non-significant differences in body weight of all the experimental rats kept in different treatment groups (Table 2).

The results on different blood parameters showed significant changes (Table 3) in response to various doses of sodium benzoate. The red blood cells exhibited significant reductions in the groups D–E at 40 and 60 days of trial. The white blood cell counts increased significantly at 20 and 40 days in groups D–E; however, at 60 days of the study, significant elevations in white blood cell counts were recorded in the groups C–E. In case of hemoglobin, at 20 days of study, significant reduction was noted only in group E. However, in groups D and E momentous reductions were noted at 40 and 60 days of experiment. Regarding hematocrit, non-significant decrease was noted in this parameter during 20 days of trial; whereas, at 40 and 60 days of study remarkably significant reductions were found in groups D and E.

Table 1: Different doses of sodium benzoate administered to various groups of wistar rats

Groups	Treatments
Group A (Control)	0 mg/kg.bw/day
Group B	2 mg/kg.bw/day
Group C	4 mg/kg.bw/day
Group D	6 mg/kg.bw/day
Group E	8 mg/kg.bw/day

Table 2: Effect of different doses of sodium benzoate on body weight (g) of rats

Experimental days	Groups				
	A	B	C	D	E
20	137.5 ± 6.5	136.7 ± 4.8	135.3 ± 2.6	135.7 ± 3.8	133.5 ± 2.8
40	170.2 ± 2.5	168.4 ± 3.5	166.5 ± 4.5	165.2 ± 3.2	164.2 ± 2.7
60	221.4 ± 5.4	220.4 ± 8.4	218.5 ± 6.4	217.2 ± 6.5	216.2 ± 3.9

Values (mean ±SE) in different groups in rows have no significant difference ($P \leq 0.05$) from control group

Table 3: Effect of different doses of sodium benzoate on various blood parameters of wistar rats

Parameters/days	Groups				
	A	B	C	D	E
Red blood cell count (10^{12}/L)					
20	6.88 ± 0.42	6.71 ± 0.47	6.55 ± 0.09	6.22 ± 0.33	5.51 ± 0.14
40	6.99 ± 0.31	6.61 ± 0.29	6.39 ± 0.41	5.06 ± 0.13*	4.91 ± 0.10*
60	6.83 ± 0.22	6.42 ± 0.11	6.21 ± 0.19	4.97 ± 0.17*	4.69 ± 0.25*
White blood cell counts (10^{9}/L)					
20	8.19 ± 0.55	8.33 ± 0.19	8.88 ± 0.91	10.55 ± 0.67*	10.88 ± 0.49*
40	8.21 ± 0.31	8.55 ± 0.67	9.11 ± 0.68	10.91 ± 0.53*	11.01 ± 0.11*
60	8.25 ± 0.11	8.77 ± 0.77	10.92 ± 0.88*	10.98 ± 0.43*	11.23 ± 0.34*
Hemoglobin (g/dL)					
20	14.03 ± 0.73	13.90 ± 0.33	13.50 ± 0.48	13.23 ± 0.61	11.18 ± 0.64*
40	14.28 ± 0.77	13.78 ± 0.6	13.38 ± 0.72	11.12 ± 0.36*	10.30 ± 0.27*
60	13.98 ± 0.78	13.43 ± 0.46	12.98 ± 0.68	10.15 ± 0.37*	9.90 ± 0.47*
Hematocrit (%)					
20	40.58 ± 2.01	42.05 ± 1.92	39.98 ± 2.26	39.45 ± 1.73	38.98 ± 2.19
40	41.70 ± 1.41	41.13 ± 2.00	39.23 ± 1.67	37.33 ± 1.01*	36.38 ± 1.15*
60	41.15 ± 1.88	40.23 ± 2.23	38.88 ± 2.01	36.03 ± 1.96*	35.80 ± 2.05*

Values having asterisk (mean ±SE) in different groups in rows have significant difference ($P \leq 0.05$) from control group

Results showed that the values of various serological parameters like liver function tests and kidney function tests increased significantly in rats exposed to higher concentrations of sodium benzoate (Table 4). The data exhibited that at 20 days of experiment, the levels of AST and ALT increased significantly in groups D and E while significant elevations in values of these parameters were recorded in groups C–E at 40 and 60 days as compared to control group. In case of bilirubin and creatinine at 20 and 40 days, only the groups D and E exhibited significant rise while at 60 days of study the groups C–E showed significant increase in their concentration. On the other hand, the concentration of urea increased significantly in groups D–E at 20 days of study while at 40 & 60 days momentous increase was found in its concentration in groups C–E.

The cardiac enzymes like LDH, CPK and CKMB (Table 5) showed significant elevations in their concentrations when compared with the control group (A). LDH exhibited significant rise in its levels in the group E only at 20 and 40 days of experiment while at 60 days its values increased significantly in groups D–E. The enzymes CPK and CKMB showed significantly increased concentrations at 20, 40 and 60 days of trial in the groups D–E; however, the concentrations went on increasing with increasing dose levels of sodium benzoate and length of study period. Regarding various parameters of blood lipid profile (cholesterol, triglycerides, LDL, HDL), the data (Table 5) shows that cholesterol, triglycerides, LDL and HDL exhibited significant reductions in the concentration of these parameters during the trial. However, at 20 days of trial, significant reductions (in cholesterol, triglycerides, LDL) were noted in the group E only. Whereas, at 40 and 60 days of study significant reductions were observed in groups D–E and C–E respectively. On the other hand, HDL expressed significant lower levels in groups D–E at 20 and 40 days of trial while at 60 days of experiment the significant reductions were observed in the groups C–E.

Serum albumin (Table 5) showed significant decrease in the group E at 20 days of study; although at 40 and 60 days of trial, significantly lower concentrations were found in the animals of groups C–E. Total protein (Table 5) showed reduction in its concentration during the study

Table 4: Effect of different doses of sodium benzoate on liver and kidney function tests in wistar rats

Parameters/days	Groups				
	A	B	C	D	E
AST (U/L)					
20	39.43 ± 1.73	41.10 ± 2.17	43.33 ± 2.03	45.03 ± 0.80*	46.40 ± 0.96*
40	38.33 ± 2.10	42.60 ± 2.11	44.33 ± 2.39*	47.03 ± 2.08*	49.05 ± 2.14*
60	40.03 ± 2.19	43.85 ± 1.45	45.23 ± 2.32*	50.55 ± 2.03*	53.98 ± 1.26*
ALT (U/L)					
20	51.48 ± 1.71	52.60 ± 1.31	54.25 ± 1.68	58.78 ± 2.72*	59.70 ± 1.53*
40	52.35 ± 2.10	55.55 ± 2.54	59.78 ± 1.87*	60.28 ± 3.10*	62.13 ± 1.81*
60	53.03 ± 2.71	57.73 ± 3.11	60.30 ± 3.38*	64.60 ± 3.72*	69.75 ± 3.04*
Bilirubin (mg/dL)					
20	0.44 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.55 ± 0.02*	0.57 ± 0.02*
40	0.46 ± 0.02	0.48 ± 0.02	0.54 ± 0.02	0.58 ± 0.02*	0.61 ± 0.02*
60	0.48 ± 0.02	0.52 ± 0.02	0.55 ± 0.03*	0.61 ± 0.02*	0.65 ± 0.02*
Urea (mg/dL)					
20	28.78 ± 1.27	29.65 ± 1.66	32.88 ± 1.49	33.68 ± 1.34*	35.13 ± 1.86*
40	29.88 ± 1.21	31.28 ± 1.68	35.48 ± 1.83*	36.70 ± 1.91*	38.40 ± 1.63*
60	30.25 ± 1.58	32.83 ± 1.37	39.78 ± 1.83*	41.08 ± 2.18*	43.88 ± 1.69*
Creatinine (mg/dL)					
20	0.61 ± 0.01	0.63 ± 0.04	0.65 ± 0.02	0.69 ± 0.03*	0.73 ± 0.01*
40	0.63 ± 0.02	0.65 ± 0.02	0.68 ± 0.03	0.72 ± 0.02*	0.75 ± 0.02*
60	0.62 ± 0.02	0.68 ± 0.04	0.70 ± 0.03*	0.75 ± 0.04*	0.81 ± 0.02*

Values having asterisk (mean ±SE) in different groups in rows have significant difference ($P \leq 0.05$) from control group

Table 5: Effect of different doses of sodium benzoate on cardiac, lipid profile and serum biochemical parameters of wistar rats

Parameters/days	Groups				
	A	B	C	D	E
LDH (U/L)					
20	151.60 ± 6.35	154.35 ± 7.81	156.88 ± 7.33	162.38 ± 9.24	169.05 ± 8.67*
40	149.83 ± 6.26	157.40 ± 5.79	161.08 ± 8.19	166.28 ± 7.38	173.40 ± 4.52*
60	152.93 ± 8.55c	162.23 ± 8.29	166.83 ± 5.48	171.58 ± 9.50*	180.35 ± 7.21*
CPK (U/L)					
20	31.98 ± 1.73	33.13 ± 1.83	34.88 ± 1.75	39.75 ± 1.52*	48.40 ± 2.36*
40	32.83 ± 1.29	34.10 ± 1.38	35.25 ± 1.68	45.85 ± 2.61*	51.50 ± 2.78*
60	31.35 ± 1.58	34.14 ± 1.76	35.35 ± 1.74	50.48 ± 2.46*	55.48 ± 1.99*
CKMB (U/L)					
20	81.65 ± 4.47	84.88 ± 3.96	85.60 ± 2.63	97.95 ± 3.56*	100.75 ± 2.95*
40	83.25 ± 4.11	86.90 ± 4.86	86.40 ± 3.82	101.33 ± 3.92*	104.95 ± 4.67*
60	82.95 ± 4.36	87.18 ± 5.07	88.90 ± 4.74	108.50 ± 2.96*	113.45 ± 5.22*
Cholesterol (mg/dL)					
20	92.58 ± 4.76	88.13 ± 3.65	86.35 ± 4.28	83.23 ± 4.38	81.60 ± 4.42*
40	90.75 ± 3.52	85.30 ± 4.77	83.10 ± 4.35	81.30 ± 3.69*	78.80 ± 4.06*
60	91.95 ± 4.73	82.68 ± 3.46	80.75 ± 3.64*	78.05 ± 4.39*	75.25 ± 2.82*
Triglyceride (mg/dL)					
20	82.45 ± 4.2	81.95 ± 3.76	78.65 ± 3.61	76.15 ± 3.23	72.48 ± 3.98*
40	81.30 ± 3.79	79.28 ± 2.85	76.95 ± 3.75	73.45 ± 2.46*	71.13 ± 3.79*
60	83.55 ± 4.30	77.23 ± 3.77	73.65 ± 4.25*	71.73 ± 3.75*	68.80 ± 3.47*
LDL (mg/dL)					
20	39.50 ± 1.45	38.38 ± 2.11	37.35 ± 1.50	35.7 ± 1.79	32.43 ± 1.81*
40	40.20 ± 2.08	38.30 ± 1.40	37.95 ± 1.60	34.40 ± 1.39*	31.20 ± 1.76*
60	42.53 ± 2.39	37.25 ± 2.04	32.35 ± 1.89*	31.78 ± 1.64*	30.23 ± 1.21*
HDL (mg/dL)					
20	42.05 ± 1.76	41.45 ± 1.83	39.95 ± 2.11	35.18 ± 2.01*	34.13 ± 1.45*
40	41.78 ± 2.12	39.85 ± 1.99	38.68 ± 1.56	36.23 ± 1.29*	33.70 ± 1.55*
60	40.48 ± 2.08	38.85 ± 1.84	35.98 ± 1.87*	34.48 ± 1.84*	32.30 ± 1.51*
Albumin (g/dL)					
20	2.75 ± 0.06	2.72 ± 0.05	2.57 ± 0.08	2.37 ± 0.15	2.10 ± 0.11*
40	2.67 ± 0.13	2.63 ± 0.15	2.45 ± 0.15*	2.05 ± 0.17*	2.04 ± 0.08*
60	2.72 ± 0.12	2.59 ± 0.17	2.41 ± 0.19*	2.0 ± 0.18*	2.01 ± 0.15*
Total Protein (g/dL)					
20	6.65 ± 0.36	6.61 ± 0.26	6.49 ± 0.20	6.25 ± 0.27	5.10 ± 0.39
40	6.80 ± 0.17	6.55 ± 0.31	6.38 ± 0.39	5.73 ± 0.38	4.63 ± 0.13*
60	6.53 ± 0.36	6.52 ± 0.26	6.03 ± 0.40	4.77 ± 0.46*	4.49 ± 0.41*
MDA (nmol/g)					
20	3.28 ± 0.17	3.40 ± 0.14	3.45 ± 0.13	3.53 ± 0.19	3.76 ± 0.21*
40	3.38 ± 0.13	3.49 ± 0.18	3.60 ± 0.16	3.95 ± 0.20*	4.20 ± 0.33*
60	3.43 ± 0.24	3.84 ± 0.14	4.01 ± 0.15*	4.24 ± 0.22*	4.67 ± 0.10*

Values having asterisk (mean ±SE) in different groups in rows have significant difference ($P \leq 0.05$) from control group

period; however significant reductions were noted at 40 and 60 days of trial. The levels of MDA (Table 5) expressed significant elevations in its concentration in group E at 20 days of trial; whereas at 40 and 60 days of

study, significantly higher levels were found in the groups D–E and C–E respectively.

Discussion

Monitoring and evaluation of different frequently used synthetic compounds as food additive, to prevent the microbial growth like bacteria and fungi is crucial to minimize their adverse effects in public health. Generally, it is tempting to speculate that variety of synthetic compounds may not exert immediate deleterious and notable adverse effects when they are present in low levels in food; however, they can cause countless abnormalities in consumers. Sodium benzoate is frequently used in a variety of food stuff such as carbonated drinks, fruit juices, jams, jellies, beer, margarine, bakery items, various pickles and sauces (Zengin *et al*., 2011) and for the preservation of liquid medicines (Oyewole *et al*., 2012; Shahmihammadi *et al*., 2016). However, there is little information about hemato-biochemical effects of sodium benzoate in experimental animals in long term studies.

The results revealed significantly lower values of various hematological parameters including red blood cell counts, hemoglobin concentrations and hematocrit while significantly increased values of white blood cell counts. The significantly lower hematological values in rats might be due to adverse effects of sodium benzoate on hematopoietic tissue. Previously, decreased values of different hematological parameters including red blood cell counts, hemoglobin concentration and increased white blood cell counts in rats due to sodium benzoate have been reported by Aziz and Zabut (2012). Moreover, the lower values of hemoglobin in treated rats may also be due to impaired oxygen supply to hematopoietic tissues (Ilyas *et al*., 2016). The lower hematological values (red blood cells and hemoglobin concentrations) might be due to oxidative stress to blood forming tissues (Hussain *et al*., 2014). The higher values of white blood cell count in rats in present experimental study are suggestive of injurious stimulus induced by sodium benzoate.

In current study the results showed, the values of different parameters including liver function tests (ALT, AST and bilirubin) and kidney function tests (urea and creatinine) increased significantly in exposed rats. These findings are in line with the results reported by Oyewole *et al*. (2012) and Tawfek *et al*. (2015) who reported significantly higher values of these parameters in response to different treatments of sodium benzoate. The significantly increased values of liver function and kidney function tests might be due to hapto-cellular and kidney damage caused by food additive sodium benzoate (Mekkawy *et al*., 1998) leading to higher levels of intracellular enzymes into the blood stream (Amin *et al.,* 2010). Previously, it is reported that sodium benzoate induces different histopathological changes including swelling, necrosis, vacuolation and pyknosis of the liver cells (Mehedi *et al.,* 2013). Necrosis results because of toxicity to the cells by harmful compounds (Javed *et al.,* 2013). The higher concentrations of liver function tests and kidney function tests in rats in present study might be due to increased oxido-nitrosative stress leading to abnormal up regulation of TGF-β, Caspase-3, KIM-1 and expression of TNF-α mRNA in kidneys and liver of treated rats (Adil *et al*., 2015). Previously, different studies have also reported the increased concentrations of liver function tests, kidney biomarkers, lipid peroxidation products in rats in association to oxidative stress, dyslipidemia and hepatic mitochondrial toxicity (Elshenawy and El-Kadi, 2015; Sener *et al*., 2016).

The results revealed significantly increased concentrations of different cardiac biomarkers in sodium benzoate treated rats. The increased concentrations of these cardiac enzymes could be due to the toxic effects of sodium benzoate on cardiac cells. The increased concentrations of cardiac enzymes in exposed rats might be due to down-regulation of protein expressions Nrf2 and HO-1 and up-regulation of myocardial NADPH sub units (NOX2 and NOX4) and Keap-1 (Ghaffar *et al*., 2017). Previously, higher concentrations of these enzymes have also been reported in sodium benzoate treated animals (Adesokan and Akanji, 2003).

The values of different lipid profile parameters such as cholesterol, triglycerides, LDL and HDL decreased significantly in treated rats. These findings are in agreement with the previous study by Brahmachari *et al*. (2009) who reported a decrease in the concentration of serum lipids profile in response to sodium benzoate treatments in mice. The decreased concentrations of these parameters might be associated with damage to the liver leading to abnormal liver functions and reduced biosynthetic ability resulting in hypolipidemia (Cicognani *et al*., 1997). In present experimental study the lower concentrations of serum albumin and serum total proteins can be related to increased oxidative stress leading to poor protein synthesis in different tissues of the treated rats (Amin *et al*., 2010). A lipid peroxidation product (biomarker of oxidative stress) such as serum malondialdehyde (MDA) exhibited pronounced increase in sodium benzoate treated rats. Similar results were also reported by Tawfek *et al*. (2015) in sodium benzoate treated animals. The elevated levels of MDA may be attributed with the action of reactive oxygen species on cell membrane lipids (Amin *et al*., 2010), development of products of lipid peroxidation and inhibition to the activity of antioxidants (Mehedi *et al*., 2013).

Conclusion

From the results of present study, it is concluded that administration of higher doses (6–8 mg/kg bw/day) of sodium benzoate for periods of 40 and 60 days causes adverse health effects in experimental animals. Thus, this food additive has the potential to cause toxicity in consumers; hence, the level of sodium benzoate should be

reduced in food items to minimize its intake.

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