

Effect of Sodium Benzoate on DNA Breakage and Antioxidant Enzymes in Male Rats

By

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ABSTRACT

The present study was carried out to examine the effects of a food preservative sodium benzoate (SB) on DNA and some biochemical parameters. Because there is a controversy concerning the use of food preservatives, especially as they may have a carcinogenic effect. An adult fifteen males Sprague Dawley rats, were used in the present experiment. The rats were randomly divided into three groups, Group I: normal control group, Group II & III: treated groups were received SB at concentration of 2.5% and 4% for four weeks. At the end of the experiment the animals were sacrificed and samples were taken for biochemical analysis and Quantitative measure of DNA.

The aim of this study was to assess the effect of different concentrations of SB on the DNA breakage in liver cells and on some biochemical parameters in male rats. The obtained result showed that, quantitative measure of DNA significantly decreased in the amount of DNA/1g of liver when two concentrations of SB were added as compared with the control group.

Serum biochemical analysis, revealed significant elevation in total protein, albumin, SOD, GPX and AST at both doses 2.5% and 4% comparing to the control group. ALP was increased in 2.5% dose and decreased in 4% dose, ALT was increased in 4% dose. On the other hand, the body weight was significantly decreased in both treated groups as compared to the control group. Liver weight showed a significant increase in 2.5% dose and decrease in 4% dose.

INTRODUCTION

Sodium benzoate (SB) is one of the most widely used additives in food products in the world. It is the sodium salt of benzoic acid with formula (C_6H_5COONa) which is a well stable and water-soluble food preservative with bacteriostatic and fungistatic properties. SB inhibits the activity of the microorganisms in a very low concentration, and has been recognized as a safe food preservative (1, 2), It is used as pickling agent and used in processed foods and beverages to extend shelf life. FAO/WHO expert

committee on food additives recommend acceptable daily intake levels of SB as 5 mg/kg b .wt. (3) Excessive intake of these preservatives might be potentially harmful to the consumers. Studies have suggested that excessive use of SB induced changes in serum clinical parameters, showing hepatocellular damage (4). A large concern over the use of sodium benzoate is its ability to convert to benzene, a known carcinogen when mixed with another additive, vitamin C, in soft drinks, it forms benzene, a carcinogenic substance. It also may damage mitochondrial DNA.

Notably, diet beverages are more prone to benzene formation, as the sugar in regular sodas and fruit drinks may reduce its formation (5)

Other factors, including exposure to heat and light, as well as longer storage periods, can increase benzene levels (6)

Both short- and long-term studies of the effects of sodium benzoate in vivo have investigated various enzymes and suggested adverse effects of both chronic and sub - chronic intake or the absence of negative effects (7).

Preliminary studies have evaluated other possible risks of sodium benzoate, which include:

Inflammation: Animal studies suggest that sodium benzoate can activate inflammatory pathways in the body in direct proportion to the amount consumed. This includes inflammation promoting cancer development (8)

Attention Deficit Hyperactivity Disorder (ADHD): A study of college students linked ADHD with higher intake of sodium benzoate in beverages. The additive has also been linked to ADHD in children in some studies (9&10)

Thus, the aim of this study was to evaluate the effects of different concentrations of SB (1) on the weight gain, food and water intake of rats; (2) on DNA formation in liver and (3) on antioxidant enzymes and (4) some biochemical parameters in rats.

MATERIALS AND METHODS:

5,5-'dithiobis 2-nitrobenzoic acid (DTNB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pyrogallol, and ammonium molybdate ((NH₄)₆Mo₇O₂₄.4H₂O) were purchased from Sigma-Aldrich (St. Louis, Missouri). Thiobarbituric acid (TBA), SB,

Experimental animals:

Adult males Sprague Dawley white albino rats (*Rattus norvegicus*), weighting about 130 to140 g obtained from The National Research Centre in Egypt, were housed in well ventilated room with under controlled laboratory conditions of temperature (25oC), 12h light/12h dark cycle, and humid conditions. The animals were housed in plastic cages under standard hygienic conditions, supplied with enough standard rat chow pellets and drinking tap water ad libitum. All the animals received human care throughout the duration of the experiment.

Experimental Design:

Fifteen animals were kept in plastic cages with upper steel mesh and randomly divided into three groups:

Group I: (Normal control group), rats of this group were normal healthy rats and received a daily received tap water.

Group II and III: (treated groups): received two concentrations of SB which 2.5% and 4% as reported by previous authors (11)

At the end of the experiment the animal were sacrificed and samples were taken.

DNA Extraction

The extraction of DNA from liver was carried out as explained by (12)& (13)

Blood Samples Collection

At the end of the experiment period (30 days), rats were deprived of food but not water over night.

Blood samples were collected on the day of sacrifice in glass tubes without anticoagulant for the separation of serum. The blood samples were allowed to coagulate at room temperature and centrifuged at 3000 rpm for 10min, then serum was stored at -80 0C.

Biochemical Parameters:

Total serum protein was determined according to Biuret method of Reinhold (14). Serum albumin was determined by the bromocresol green dye-binding technique (15). Globulin concentration was obtained by subtracting albumin from total protein (16).

Serum ALT and AST were determined by a previous method (17). Serum Alkaline phosphatase (ALP) activity was determined by (18)

Glutathione peroxidase activity was determined according to the method of (19)

Superoxide dismutase (SOD)enzyme activity was determined according to the method of (20)

Statistical Analysis

Results obtained from this experiment were analyzed by comparing values for different groups with the values for controls. Results were expressed as means \pm S.E. The significant differences among values were analyzed by using analysis of variance ANOVA one-way test with the statistical package for social sciences (SPSS) for windows version 10.0. Differences were considered significant at $P \leq 0.05$ level of significance.

RESULTS and DISCUSSIONS:

Table (1): oral administration of SB on amount of DNA mg/ 1gm of liver in rats

Groups	Concentration	Amount of DNA/1gm of liver
Control	-	0.0408±0.009
Standard	10mg	0.043±0.0001
Treated group	2.5%	0.0093±0.0001***
Treated group	4%	0.00562±0.0002***

*** P<0.001

Table (2): Total protein, albumin, globulin and A/G ratio mean ± SD in control and treated groups

Groups	T .protein	Albumin	Globulin	A/G ratio
Control	5.82 ^a ±0.286	3.90 ^{ab} ±0.158±	1.92 ^a 0.179±	2.04 ^{ab} 0.174±
Group I	6.04 ^a 0.297±	4.26 ^a 0.114 ±	1.78 ^a ±0.259	2.43 ^a 0.343±
Group II	5.66 ^a 0.27±	3.56 ^{ac} ±0.422	2.43 ^a 0.255±	1.74 ^{bc} 0.449±

There are significance differences (P<0.05) between means having different letters in the same column

Table (3): oral administration of SB on Antioxidant & liver enzymes mean ± SD

Groups	SOD	GPX	ALP	AST	ALT
Control	116.47 ^a ±15.546	33.80 ^a ±3.196	205.40 ^{ab} ±33.813	158.70 ^a ±7.244	55.40 ^b ±4.099
Group I	244.41 ^b ±40.427	44.88 ^b ±8.259	245.60 ^a ±4.722	197.62 ^{ab} ±26.969	54.16 ^b ±8.914
Group II	233.14 ^b ±34.370	43.12 ^b ±3.968	188.60 ^b ±38.312	233.62 ^b ±68.348	94.94 ^a ±32.123

There are significance differences (P<0.05) between means having different letters in the same column.

Table (4): oral administration of SB on Bodyt & liver weight mean ± SD

Groups	B. WT 1st day	B. WT 20th day	Total liver W
Control	154.0 ^b ±4.183	170.0 ^b ±7.071	5.18 ^b ±1.298
Group I	146.0 ^b ±11.402	160.2 ^b ±12.357	7.94 ^a ±1.057
Group II	127.0 ^a ±5.70	106.0 ^a ±9.618	4.08 ^b ±1.171

There are significance differences (P<0.05) between means having different letters in the same column.

Results of sodium benzoate after 30 days of treatment was shown in the previous tables.

In our study, the qualitative measure of DNA showed significantly decrease in the amount of DNA / 1g of liver in both concentrations of SB as compared with control group. This coincident with (21&22).

ALT, AST ALP, total protein, albumin and globulin are serum biochemical markers whose concentration reflects the status of many internal organs especially liver. The degree of tissue damage is examined by calculating the amount of biochemical markers released in circulation (23)

The obtained results revealed insignificant hyperproteinemia and hyperalbuminemia in group I ,while in group II there is no change in protein level comparing with control. This result is coincide with the result obtained by(24)who found that high doses of sodium benzoate produce significant increase in total protein, albumin and globulin serum levels, but the change in these parameters was found insignificant in comparison to control rats. The increase in total protein may be due to activation of protein synthesis during liver damage, inflammation or may be due to chronic kidney disease (25).

The elevated levels of albumin noticed in the present study may be indicative of the toxic effect of sodium benzoate on hepatic and renal tissues. In addition, exposure to sodium benzoate may cause an adverse effect on the renal function due to oxidative stress induced by SB on the renal tissue (26).

Slight elevation in serum globulin in group II may be due to protective mechanism of body to protect from the toxic effects of SB.

Antioxidants are agents that substantially decrease the oxidation of the substrate when present in a low or high concentrations (27). Superoxide dismutase (SOD)and, glutathione peroxidase (GPx) increase significantly in both groups comparing with control. The increased activities of GPx and SOD in this study could be attributed to their increased synthesis resulted from the induction, as antioxidant enzymes are induced in response to oxidative stress (28). GPx play an important role as a protective agent, where it is significantly delay irreversible oxidative. The result of GPx and SODin this study in contrast with the result obtained with (29) who mentioned that, that rats treated with high concentrations of sodium benzoate in high concentration produce a significant depletion in hepatic level of GPx, and SOD. Also, the decrement in the activities of antioxidant enzymes (GPx, & SOD) was also reported when human lymphocytes were treated with different concentrations of SB under in vitro conditions (30).

Sodium benzoate caused derangement of liver function as revealed by significant elevation of serum liver enzymes (ALT, AST and ALP).In the present study ,serum AST exhibited significant increase in both treated groups comparing with control

group. ALP showed a significant increase in second treated group which has SB with concentration 4%.

ALT enzyme is a sensitive marker of liver damage (31). Therefore, the significant increase in high dose in the present study may be due to effect in high dose.

In blood plasma, sodium benzoate has a binding affinity for plasma proteins where it is carried out to different tissues. In the liver, it is metabolized by conjugation with glycine, resulting in the formation of hippuric acid (32).

Observed elevation in the activities of serum enzymes as ALT, AST and ALP in response to sodium benzoate (33). This finding is in alignment with our results.

Alkaline phosphatase is present on cell surfaces in most human tissues especially liver and kidneys. The specific location of the enzyme within sinusoidal and bile canalicular membranes could account for its serum elevation in the current study in response to sodium benzoate administration.

The decrease in body weight seen with SB was similar to a finding in which rats and mice were treated with different concentrations of SB for 10 days (34). The decrease in body weight due to SB may be due to poor digestion and absorption and interference with the overall general metabolism of the body.

various studies using experimental animals and human subjects revealed some adverse effects due to both chronic and sub-chronic intake of benzoate. For example, changes in serum parameters and an increased relative liver weight (35).

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