Effect of Sodium Benzoate on Corticosterone Hormone Level, Oxidative Stress Indicators and Electrolytes in Immature Male Rats

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ABSTRACT

Objectives: This study was aimed to determining the effects of sodium benzoate in the levels of corticosterone hormone, oxidative stress indicators and electrolytes in immature albino male rats in different durations.

Methods: 60 immature male rats were used in the main experiment that included three secondary experiments each one of these was included four groups of immature albino male rats, 5 male for each group. In the first secondary experiment G1,G2, and G3 groups were orally given sodium benzoate daily in concentrations of 50,100,200 mg/kg of body weight for one week, whereas in the second and the third secondary experiments G1,G2, and G3 groups were orally given sodium benzoate in the same concentrations daily for two and three weeks respectively. The control groups of the three secondary experiments were given distilled water throughout experiments. After the end of treatments, the level of corticosterone hormone and the indices of the oxidation glutathione and malondialdehyde, as well as levels of sodium and potassium were measured.

Results: The results showed a significant increase (P<0.05) in the level of corticosterone hormone in both G2 and G3 groups during one week of treatment and in G1,G2, and G3 groups during two and three weeks of each treatment in comparison with control groups. Also there was a significant decrease (P<0.05) in the glutathione level accompanied by a significant increase in the malondialdehyde level in both G2 and G3 groups during one week of treatment and also in the G1,G2, and G3 groups during two and three weeks of treatment in comparison with control groups. A significant increase (P<0.05) in the sodium level in G1,G2, and G3 groups during each duration of experiment in comparison with control groups. A significant increase (P<0.05) in the potassium level in both G2 and G3 groups during one week of treatment and in G1,G2, and G3 groups during two and three weeks of treatment in comparison with control groups.

Conclusion: Sodium benzoate caused oxidative stress and have negative effect on corticosterone and electrolytes levels.
INTRODUCTION
People have been interested in keeping food for later consumption. Over time, many methods of food preservation have been tried including heating, Freezing, Drying, fermenting and adding chemical preservatives, which have been used in recent years as a result of developments in food marketing and wide variety of food offered for consumption food was produced and consumed locally and needed a short time to conserve. At present, food is produced locally and can be processed elsewhere and distributed later to many other places around the world. That the period between food production and consumption has become longer and that it is necessary to conserve food for a long time to prevent damage and unwanted changes in taste and color. To support this new trend in food processing, distribution and consumption, Chemical preservatives are more important and provide a wide variety of food for longer periods of time and for a larger number of people. Chemical preservatives are used to prevent chemical and biological spoilage of food. One of these, Antimicrobial preservatives, which inhibit the growth of bacteria and fungi (yeasts and molds) which can produce undesirable effects in both the appearance and taste of foods, as well as their nutritional value, and can produce toxicity pose a major threat to human health examples of these substances are Benzoates, Sorbates, Propionate, Sulfate, Sulfates and Sodium Nitrate. Benzoic acid is one of the weak organic acids, as well as sodium benzoate, a commonly used substance as a preservative for many products consumed by humans. While in other countries of the world it is between 0.15 - 0.25%. In the European Union countries the permissible limits range from 0.015% to 0.5% . Due to increased production of processed and fast food, sodium benzoate has become a dietary supplement of increasing importance in modern food technology and can be responsible for many health problems of the consumer, Therefore the current study was designed to determine the effect of oral dosage of sodium benzoate at three concentrations (50,100,200) mg/kg bw and for different periods on immature male rats.

MATERIALS AND METHODS
In this study, three concentrations of sodium benzoate (50, 100, 200) mg/kg bw were prepared and the animals were vaccinated orally by 0.5 ml per animal using a gavage tube . In this study 60 of immature male rats aged 4 weeks at a weight of 37gm were used. This study included three secondary experiments depending on the duration of the administration as follows:
First Secondary Experiment: Twenty immature male rats were randomly divided into four groups. Each group included five animals as follows:
1. Control group (C): Animals of this group were orally administered with distilled water throughout the experiment.
2. The first treatment group (G1) were orally administered with sodium benzoate at a concentration of 50 mg/kg/bw per day for one week.
3. The second treatment group (G2): were orally administered with sodium benzoate at concentration of 100 mg/kg bw of sodium benzoate per day for one week.
4. The third treatment group (G3) was orally injected with a concentration of 200 mg/kg bw of sodium benzoate per day for one week.

Second Secondary Experiment: Included The same procedures and subdivisions applied in the first secondary experiment, except for the duration of the dosage, were two weeks instead of one week.
Third Secondary Experiment: The design was similar to the first and second secondary experiment and differed with them in the duration of the dosage, where it was three weeks.

After the end of each experiment, the animals were anesthetized using Chloroform and the blood was pulled directly from the heart by using a 5 mL sterile syringe. The blood was placed in a clean test tube free of anticoagulants and left for 15-20 minutes at laboratory temperature. Centrifuge samples were placed at 3000 cycles / min for 15 minutes to separate the serum. Serum was isolated with a fine pipette and put in new plastic tubes for the purpose of conducting serum biochemical tests. The serum was kept at -20 ° C until use.

Estimation of serum corticosterone level
The serum corticosterone level was measured using the enzyme-linked immune sorbent assay (ELISA) and by the equipment manufactured by ABO (Switzerland).

Estimation of serum glutathione level
The level of glutathione in the serum was estimated using the modified method of Sedlak and Lindsay which depend on the use of Ellman’s reagent DTNP [5,5- dithio bis (2-Nitrobenzoic acid)] which reacts rapidly with glutathione and is reduced by The sulfate group (SH group) of the glutathione forming a colored product whose absorption is read at 412 nanometers. The concentration of the resulting product depends on the concentration of the glutathione found in the serum.

Estimation of serum MDA level
The level of MDA in the serum was estimated using the modified method of . The serum lipid peroxide level was estimated by measuring the amount of MDA, which is one of the main products of lipid peroxidation. The method is based on the interaction between lipid peroxides (mainly malondialdehyde) and Thiobarbituric acid (TBA). This reaction is done in acidic medium and produce a color product . Absorption strength is measured at 532 nanometers.

Estimation of serum Na level
The level of serum sodium was measured using several ready-made laboratories equipped by German company Human. The basis of the test depends on the deposition of sodium with Uranium-magnesium acetate, and the
remaining Uranium ions in suspension formed with the thioglycolic acid-amber color complex. The difference between Reagent Blank (without sodium deposition) and the analysis sample is proportional to the sodium level.

Estimation of serum K level

Potassium ions interact with sodium tetr Phenylboron sodium TPB-Na in the protein-free alkaline medium to produce a turbid suspension of tetr Phenylboron potassium. The resulting turbidity is proportional to the concentration of potassium, and spectrometry is read spectroscopy.

RESULTS

Effect on Corticosterone level

Treatment for one week: The results shown in Table 1 showed no significant difference (P > 0.05) in the level of corticosterone in the serum of immature male rats in group G1 treated with sodium levels at 50 mg / kg body weight for one week compared to control group. While the G2 and G3 groups treated with Sodium benzoate concentrations of 100 and 200 mg/kg respectively for one week showed a significant increase (P < 0.05) in serum corticosteroids compared to control group. The results showed that G1 showed a significant difference (P <0.05) in corticosteroids compared with both G2 and G3 which were not significantly different (P > 0.05).

Treatment for two and three weeks: The results of the statistical analysis shown in Table 1 showed a significant increase (P <0.05) in the serum corticosterone level for immature male rats in groups G1, G2 and G3 treated with Sodium benzoate concentrations of 50, 100 and 200 mg / kg bw respectively for two and three weeks compared to control group. The results showed that G1 group differed significantly (P <0.05) in GSH compared to G2 and G3, which did not differ significantly (P > 0.05) in GSH level.

Treatment for two and three weeks: The results of the statistical analysis shown in Table 2 showed a significant decrease (P <0.05) in the serum GSH level of the immature male rats in groups G1, G2 and G3 treated with Sodium benzoate of 50, 100 and 200 mg/kg bw respectively. For two and three weeks compared to the control group. The results showed significant differences (P <0.05) in the GSH level between groups treated with sodium benzoate when compared with each other.

Effect on MDA level

Treatment for one week: The results shown in Table 2 showed that there was no significant difference (P > 0.05) in GSH level in the serum of G1 male rats treated with Sodium benzoate of 50 mg/kg bw for one week compared to control group. The GSH level was significantly reduced (P <0.05) in the serum of immature male rats in groups G2 and G3 compared to control group.

On the other hand, the results showed that the G1 group differed significantly (P <0.05) in GSH compared to G2 and G3, which did not differ significantly (P > 0.05) in the GSH level.

Treatment for two and three weeks: The results of the statistical analysis shown in Table 2 showed a significant decrease (P <0.05) in the serum GSH level of the immature male rats in groups G1, G2 and G3 treated with Sodium benzoate of 50, 100 and 200 mg/kg bw respectively. For two and three weeks compared to the control group. The results showed significant differences (P <0.05) in the GSH level between groups treated with sodium benzoate when compared with each other.

Effect on MDA level

Treatment for one week: The results of the statistical analysis shown in Table 3 showed that there was no significant difference (P > 0.05) in the MDA level of immature male rats in group G1 compared with control group. While there was a significant increase (P <0.05) in the MDA serum of immature male rats in groups G2 and G3 compared with control group. The results of the comparison between the groups treated with the three concentrations of sodium benzoate showed significant differences (P <0.05) in the level of corticosterone hormone between these groups.

Effect of different concentrations of sodium benzoate at different durations on corticosterone hormone level (nmol/L) in immature white male rats.

Table 1: Effect of different concentrations of sodium benzoate at different durations on corticosterone hormone level (ng/ml) in immature white male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>One Week</th>
<th>Two Weeks</th>
<th>Three Weeks</th>
<th>LSD&lt;sub&gt;α&lt;/sub&gt; For Durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A 0.94± 0.114  a</td>
<td>B 0.92 ± 0.09 b</td>
<td>C 0.93 ± 0.09 d</td>
<td>0.084</td>
</tr>
<tr>
<td>G1</td>
<td>A 1.23 ± 0.089 a</td>
<td>B 1.37 ± 0.05 b</td>
<td>A 1.33 ± 0.025 c</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>C 1.02 ± 0.09 a</td>
<td>B 1.37 ± 0.05 c</td>
<td>A 1.48 ± 0.049 a</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>C 1.04 ± 0.09 a</td>
<td>B 1.47 ± 0.034 a</td>
<td>A 1.57 ± 0.032 a</td>
<td></td>
</tr>
</tbody>
</table>

LSD<sub>α</sub> for Concentration 0.097

Table 2: Effect of different concentrations of sodium benzoate at different durations on GSH level (snmol/L) in immature white male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>One Week</th>
<th>Two Weeks</th>
<th>Three Weeks</th>
<th>LSD&lt;sub&gt;α&lt;/sub&gt; For Durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A 2.82± 0.071 a</td>
<td>B 2.12 ± 0.073 b</td>
<td>A 3.08 ± 0.075 a</td>
<td>0.280</td>
</tr>
<tr>
<td>G1</td>
<td>A 2.73± 0.089 a</td>
<td>B 1.75 ± 0.076 c</td>
<td>C 1.35 ± 0.065 c</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>A 2.22 ± 0.132 b</td>
<td>B 1.38 ± 0.084 d</td>
<td>B 1.23 ± 0.089 e</td>
<td></td>
</tr>
</tbody>
</table>

LSD<sub>α</sub> for Concentration 0.323

Table 3: Effect of different concentrations of sodium benzoate at different durations on MDA level (μmol/L) in immature white male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>One Week</th>
<th>Two Weeks</th>
<th>Three Weeks</th>
<th>LSD&lt;sub&gt;α&lt;/sub&gt; For Durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A 2.23± 0.071 a</td>
<td>B 1.75 ± 0.076 c</td>
<td>C 1.35 ± 0.065 c</td>
<td>0.280</td>
</tr>
<tr>
<td>G1</td>
<td>A 2.73± 0.089 a</td>
<td>B 1.75 ± 0.076 c</td>
<td>C 1.35 ± 0.065 c</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>A 2.22 ± 0.132 b</td>
<td>B 1.38 ± 0.084 d</td>
<td>B 1.23 ± 0.089 e</td>
<td></td>
</tr>
</tbody>
</table>

LSD<sub>α</sub> for Concentration 0.323

The results shown in Table 2 and Table 3 showed a significant increase in MDA level.
(P<0.05) in the serum MDA level of immature male rats in groups G1, G2 and G3 treated with sodium benzoate at concentrations of 50, 100 and 200 mg/kg bw respectively for two and three weeks. The results also showed a significant differences (P<0.05) in the MDA level between the groups treated with sodium benzoate when compared between them.

Table 3: Effect of different concentrations of sodium benzoate at different durations on MDA level (μmol/L) in immature white male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>One Week</th>
<th>Two Weeks</th>
<th>Three Weeks</th>
<th>LSD&lt;sub&gt;0.370&lt;/sub&gt; For Durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td>1.23±0.089</td>
<td>1.07±0.025</td>
<td>1.25±0.105</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.52±0.13</td>
<td>2.22±0.152</td>
<td>2.30±0.047</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.10±0.71</td>
<td>1.32±0.084</td>
<td>3.65±1.26</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.15±0.063</td>
<td>3.51±0.11</td>
<td>3.87±0.085</td>
<td></td>
</tr>
</tbody>
</table>

Effect on Na level

The results shown in Table 4 showed a significant increase (P<0.05) in the level of sodium in the serum of immature male rats in groups G1, G2 and G3 treated with sodium benzoate at concentrations of 50, 100 and 200 mg/kg bw respectively for one, two and three weeks compared to control group. The results showed significant differences (P<0.05) in the GSH level between groups treated with sodium benzoate when compared with each other.

Table 4: Effect of different concentrations of sodium benzoate at different durations on Na + level (mmol/L) in immature white male rats.

<table>
<thead>
<tr>
<th>Durations</th>
<th>Groups</th>
<th>One Week</th>
<th>Two Weeks</th>
<th>Three Weeks</th>
<th>LSD&lt;sub&gt;2.08&lt;/sub&gt; For Durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.894±0.128</td>
<td>0.701±0.130</td>
<td>0.718±0.130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.707±0.132</td>
<td>0.707±0.133</td>
<td>1.414±0.136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.837±0.134</td>
<td>0.632±0.137</td>
<td>1.265±0.137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.882±0.135</td>
<td>0.712±0.138</td>
<td>0.891±0.140</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effect on K level

Treatment for one week: The results indicated in Table 5 showed that there was no significant difference (P>0.05) in serum potassium level for immature male rats in groups G1, G2 and G3 treated with sodium benzoate at 50, 100 and 200 mg/kg bw respectively for one week and no significant differences (P>0.05) were observed in the potassium level when comparing the treated groups.

Treatment for two and three weeks: The results showed that the treatment of immature male rats in groups G1, G2 and G3 with sodium benzoates at concentrations of 50, 100 and 200 mg/kg bw respectively for two and three weeks resulted in a significant increase (P<0.05) in the serum potassium level of the above groups compared to the control group. The results also showed a significant differences (P<0.05) in the potassium level between the groups treated with sodium benzoate when compared between them.

Table 5: Effect of different concentrations of sodium benzoate at different durations on K + level (mmol/L) in immature white male rats.

<table>
<thead>
<tr>
<th>Durations</th>
<th>Groups</th>
<th>One Week</th>
<th>Two Weeks</th>
<th>Three Weeks</th>
<th>LSD&lt;sub&gt;0.216&lt;/sub&gt; For Durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.02±0.081</td>
<td>4.07±0.073</td>
<td>4.1±0.096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.20±0.242</td>
<td>4.55±0.085</td>
<td>4.72±0.086</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.58±0.16</td>
<td>4.77±0.141</td>
<td>4.93±0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.60±0.137</td>
<td>5.02±0.09</td>
<td>5.25±0.147</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The results of the present study showed a significant increase (P<0.05) in the serum corticosterone level in males of immature rats treated with different concentrations of sodium benzoate compared to control groups. This was agreed with Sinha and D’Souza suggesting that sodium benzoate can cause chemical stress, which has been suggested by several studies to increase the levels of glyocorticoids such as cortisol and corticosteroids. The types of stress generally activate the nerve pathways contained within the central nervous system, which extend to the centers of the metencephalon where the response arises. The essential endocrine system activated in the mammals is the hypothalamus-pituitary-adrenal (HPA) axis. The response to stress types includes the release of the hypothalamic hormone corticotropin releasing hormone (CRH), which stimulates the anterior pituitary to release
adrenocorticotropic hormone (ACTH), which in turn stimulates secretion of cortisol in humans and corticosteroids in rats from the adrenal cortex 11, 13, 14. The results of this study agree with the results obtained by Bojanovic and his group 15 when they observed that the treatment of non-adult mice with monosodium glutamate (one of the chemical preservatives) resulted in stimulation of the HPA axis and a significant increase in corticosteroid level.

The effect of increasing the duration of treatment of each group with sodium benzoate was observed in the high level of hormone in immature male rats, indicating that the duration of exposure to benzoate had a significant effect in increasing hormone level. This may be due to the fact that sodium benzoate is a substance whose effects appear cumulatively in the body and their effect increases with increased concentration and duration of dosage. The reduction in the level of glutathione may be due to the occurrence of oxidative stress caused by continuous treatment of sodium benzoate 16 due to the active participation of glutathione in the prevention of oxidation in oxidative stress either through the direct removal of free radicals or by the enzymes such as glutathione peroxidase, leading to an increase in the consumption of glutathione and its transformation into an ineffective form of disulfate glutathione. In the synthesis of glutathione, the thiol group is a well-recognized agent that easily blows the hydrogen atom because of the weak bond between sulfur and hydrogen (SH) and the kinetic strength of CH in the free radicals. Therefore, it protects membranes from oxidative damage and is consumed when combined with free radicals 17. Benzoic acid and sodium benzoate have the ability to interfere with the thiol aggregates of different compounds, including glutathione 18, 19.

MDA is one of the most important end products of lipid peroxidation resulting from free radical interactions with the molecules of biochemical compounds. The polyunsaturated fatty acids of cellular membranes are the most susceptible to free-radical reactions because they have double bonds which represent the primary target of free radicals, oxidation of these fatty acids through free radicals reactions produced MDA by lipid peroxidation. The increase in lipid peroxidation is not only a clear sign of stress and oxidative damage, but a sign of abnormal regulation of antioxidants 20, 21, 22. The results of the current study agree with Shu 23 that both benzoic acid and sodium benzoate stimulate lipid peroxidation and lead to destruction of liver cells by observing elevated levels of MDA in rat liver treated with different concentrations of these substances. The results of this study were consistent with the results of studies carried out outside the body, that the treatment of human red blood cells with different concentrations of sodium benzoate caused significantly increased in MDA and significantly decreased in the level of efficacy of both glutathione and catalase enzymes, glutathione peroxidase and glutathione transports 16, 24.

The results of the present study agreed with Ibekwe and his group 25 that there was a significant increase in sodium and potassium levels in rats treated with sodium benzoate at concentrations of 60 and 120 mg/kg bw for two weeks. The disturbance in the equilibrium of these electrolytes can be attributed to the effect of oxidative stress on the effectiveness of Na+/K+ ATPase 26, an enzyme that plays an important role in regulating electrolytes within and outside the cell. The enzyme Na+/K+ ATPase causes disturbance in the balance of these ions, leading to a change in cell metabolism, a change in the cell membrane fluid and then disturbances in cell function.

The generation of active roots can cause severe destruction of membrane-related enzymes such as Na+/K+ ATPase, Mg+2 ATPase and Ca+2 ATPase 27. Gubdjorson and his group 28 noted that free radicals can show their cytotoxicity effects by caused peroxidation of phosphate lipids in the cell membrane. The lipid peroxidation of biological membranes can adversely affect many functionally important parameters such as membrane fluid, permeability, electrical voltage and control of transport through the membrane, leading to a decrease in membrane fluid, disruption of cell structure regulation, transport potential and energy production. In turn, it affects the efficacy of Na+/K+ ATPase, and free radicals can target this enzyme directly 29, 30, 31.

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