Blood indices of sodium-benzoate-administrated albino rats: effect of olive oil and/or time-dependent recovery

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Abstract

The study aims to determine hematological and biochemical blood indices of male albino rats administered sodium benzoate for different periods of time, and to investigate the effects of olive oil and/or recovery time on these blood parameters. There were seven groups of six rats: the control group had distilled water; of the remaining six groups, three had olive oil and three not; in each group of three, all were administered the same amount of sodium benzoate, one for 12 days, and the other two for 26 days, but one of these was left for four weeks before blood measurements were taken to study the possibility of time dependent recovery. At the end of the experiment, blood collection and examination were carried out. Sodium benzoate increased white blood cells and lymphocytes, and decreased hematocrit, hemoglobin, red blood cell counts and platelets. Olive oil and four weeks of recovery time reduced the effects of sodium benzoate. Similar effects were evident for glucose and urea, and levels of creatinine, urea, uric acid, total protein, albumin, globulin cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase. Olive oil had some significant effects on enzyme levels.

Keywords: hematological & biochemical blood indices, olive oil, recovery time.

Introduction

Assessment of blood constituents of experimental animals as changes from the normal levels due to administration of different products consumed by humans have been continued to play valuable method in studying effects of these products on human health. The wide use of a great number of food additives has caused adverse effects on human health that require continuous evaluation. In evaluating additives one needs, therefore, to establish a benefit-to-risk relationship. Some food additives such as sodium benzoate (C6H5COONa, food additive E211) are added routinely for preserving food from deterioration by various microbes.

Sodium benzoate is a very stable solid material, soluble in water at room temperature. It has antimicrobial activity against bacteria, fungi and yeasts, and shows most activity at pH below 4.5. It is recommended as a preservative for a number of food products consumed by humans at an optimum level of 0.1 % (Chipley 1983; Baldwin et al. 1995). The recommended limits in foods are 0.1 to 0.5 % for different countries (Chipley 1983; European Commission 1995). However, sodium benzoate has already been the subject of concern about cancer because when mixed with another additive, vitamin C, in soft drinks, it forms benzene, a carcinogenic substance. It also may damage mitochondrial DNA (Kubota & Ishizaki 1991; Fujitani 1993). Both short- and long-term studies of the effects of sodium benzoate in vivo have investigated various enzymes (Ibekwe et al. 2007), and suggested adverse effects of both chronic and subchronic intake (Fujitani 1993; Vogt 1999) or the absence of negative effects (Sodemoto & Enomoto 1980; Toth 1984).

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 (Kubota & Ishizaki 1991). Since the liver is the principle organ for various metabolic and detoxification reactions, it is important to continue to study the adverse effects that sodium benzoate may exert on this vital organ.

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Blood urea is the principal end product of protein catabolism and a good indicator for kidney function, while uric acid is the end product of the catabolism of the purine bases. An increase in uric-acid concentrations in the blood over the normal range might be due to extra degradation of purines in the liver, or an inability to excrete uric acid by the kidneys. Creatinine appears in the serum in amounts proportional to the body’s muscle mass, and is more readily excreted by the kidneys than urea and uric acid.

Blood enzymes are normally found in circulation in small amounts because of normal tissue turnover. Alanine aminotransferase as a liver enzyme is elevated significantly in hepatobiliary disease, but this can can also occur in connection with damage to the heart or skeletal muscle as well as the liver parenchyma. Alkaline phosphatase is present on the cell surface in most human tissues, and belongs to a group of enzymes that catalyzes the hydrolysis of phosphomonoesters at alkaline pH. High alkaline phosphatase activity is observed especially when the liver and bones are damaged.

Although many studies have been carried out to look for toxic effects of the benzoate anion in laboratory animals, making considerable progress in understanding its in vivo effects, information about the effects of olive oil and time-dependent recovery are still controversial (Sodemento & Enomoto 1980; Toth 1984; Kubota & Ishizaki 1991; Fujitani 1993). The present study, therefore, aims to investigate the effects of sodium benzoate on various blood indices of albino rats, and the subsequent response of rat tissues to the actions of olive oil and recovery time.

Materials & Methods

The design was a case control. It involves one control animal group and six animal case groups, with a total of 42 adult male albino rats weighing 180-200 g. The control group was administered orally 2 ml dH2O by means of a stomach tube. The case groups were all treated every day with 500 mg per kg-body-weight of sodium benzoate, groups one and two for 12 days and groups three to six for 26 days. Half of the groups (two, four and six) were treated every day with olive oil as well (4 ml per kg-body-weight), again for 12 (group two) or 26 (groups four and six) days. After 26 days, groups one to four were sampled for blood and measurements taken (see below): groups five and six were left for four weeks to study the possibility of recovery after a period of time.

Commercial balanced diet and water were continuously and regularly supplied ad libitum to the animals all over the experimental period. The duration of the experiment was 8 weeks. At the end of the experiment, a blood sample was collected from the jugular vein of each rat for haematological and biochemical examination.

Routine haematological parameters were measured and a complete blood count made using an 18-automated-parameter hematology analyzer (ABX, Micros 60 from Horiba ABX, France). Clear serum samples were separated by centrifugation at 3000 rpm for 20 min, collected and stored in a deep freeze at -20 °C. Biochemical analysis of glucose, triglycerides and cholesterol were determined using methods described by Trinder (1969), Fossati & Precipce (1982) and Allain et al. (1974), respectively. Serum urea measurement was based on cleavage of urea with urease, following Fawcett & Scott (1960). Serum uric acid was determined according to Fossatti et al. (1980). Serum creatinine was measured without protein precipitation (Bartels et al. 1972). Activities of serum aspartate aminotransferase and alanine aminotransferase were determined according to the method of Reitman & Frankel (1957). Measurement of serum alkaline phosphatase activity was based on Bessey et al. (1946) method. Serum total blood protein was determined according to the method described by Weichselbaum (1946). Serum albumin was determined using bromocresol green method.
according to Doumas et al. (1971). Serum globulin was calculated by subtracting serum albumin from serum total protein.

Data were analyzed using SPSS 13 for Windows and differences were considered significant if p<0.05. The one-way ANOVA test was used to compare treated groups (1-6) with the control. In a further analysis, groups 1-6 were analyzed by two-way ANOVA in order to investigate the effects of olive oil across different treatment times.

**Results**

Table 1 represents blood indices of albino rats in the various groups. The more obvious changes that resulted from the administration of sodium benzoate to the rats were significant increments in white-blood-cell and lymphocyte counts. In contrast, there were decreases in the other parameters caused by sodium benzoate administration. From the pattern of letters across the rows, which give the results of the multiple-range test on each parameter, it is obvious that olive oil had no effect, while and extended recovery time allowed blood parameters to change back towards the control values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>control</th>
<th>1 12 doses of benzoate</th>
<th>2 26 doses of benzoate</th>
<th>3 no recovery</th>
<th>4 no recovery</th>
<th>5 4 weeks recovery</th>
<th>6 olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>white blood cells (count)</td>
<td>c</td>
<td>7.10 ± 0.21</td>
<td>7.40 ± 0.24</td>
<td>7.73 ± 0.25</td>
<td>8.70 ± 0.29</td>
<td>9.03 ± 0.30</td>
<td>8.61 ± 0.81</td>
</tr>
<tr>
<td>lymphocytes (count)</td>
<td>c</td>
<td>3.90 ± 0.11</td>
<td>5.70 ± 0.20</td>
<td>5.43 ± 0.26</td>
<td>6.85 ± 0.33</td>
<td>6.78 ± 0.12</td>
<td>6.30 ± 0.21</td>
</tr>
<tr>
<td>red blood cells (count)</td>
<td>a</td>
<td>7.28 ± 0.19</td>
<td>6.60 ± 0.17</td>
<td>6.67 ± 0.22</td>
<td>6.56 ± 0.31</td>
<td>6.72 ± 0.27</td>
<td>6.68 ± 0.12</td>
</tr>
<tr>
<td>haemoglobin (g dl⁻¹)</td>
<td>a</td>
<td>14.12 ± 0.65</td>
<td>13.15 ± 0.41</td>
<td>13.27 ± 0.35</td>
<td>13.19 ± 0.27</td>
<td>13.26 ± 0.25</td>
<td>13.30 ± 0.33</td>
</tr>
<tr>
<td>haematocrit (%)</td>
<td>a</td>
<td>37.70 ± 1.20</td>
<td>34.37 ± 0.91</td>
<td>34.40 ± 1.50</td>
<td>33.91 ± 2.19</td>
<td>34.11 ± 0.73</td>
<td>34.86 ± 1.30</td>
</tr>
<tr>
<td>mean cell volume (fl)</td>
<td>a</td>
<td>52.36 ± 0.60</td>
<td>52.08 ± 0.80</td>
<td>51.57 ± 1.10</td>
<td>51.69 ± 1.60</td>
<td>50.76 ± 0.88</td>
<td>52.19 ± 0.48</td>
</tr>
<tr>
<td>mean cell haemoglobin (pg)</td>
<td>a</td>
<td>19.40 ± 0.30</td>
<td>19.92 ± 0.80</td>
<td>19.89 ± 0.45</td>
<td>19.97 ± 0.27</td>
<td>19.73 ± 0.38</td>
<td>19.91 ± 0.51</td>
</tr>
<tr>
<td>mean cell haemoglobin concentration (g dl⁻¹)</td>
<td>a</td>
<td>37.45 ± 0.38</td>
<td>38.26 ± 0.27</td>
<td>38.57 ± 0.36</td>
<td>38.63 ± 0.22</td>
<td>38.70 ± 0.25</td>
<td>37.72 ± 0.40</td>
</tr>
<tr>
<td>platelet count (count)</td>
<td>a</td>
<td>690.3 ± 50.2</td>
<td>601.7 ± 28.9</td>
<td>667.5 ± 40.4</td>
<td>580.5 ± 29.3</td>
<td>594.3 ± 45.7</td>
<td>610.0 ± 22.3</td>
</tr>
</tbody>
</table>

**Table 1:** Hematological parameters of sodium benzoate administered rats and effect of olive oil and/or recovery time; n=6 in all groups; all counts have units of x10³ μl⁻¹. All values represent mean ± SE; those with different letters in the same row differ significantly (Duncan’s multiple-range test, p<0.05).

Table 2 shows the blood biochemical parameters measured for the experimental groups. Blood glucose levels and urea significantly increased in all groups given benzoate over the longer period of time (26 doses: groups three to six), but neither olive oil nor recovery time had any effect. Many parameters showed increases in all the case groups over the control, but few if any differences among case groups (e.g. uric acid, triglycerides). In many cases the olive-oil treated groups had lower mean values, but these were not often significant differences. Olive
oil and long recovery times appeared to reduce levels of cholesterol. Changes in urea were not very consistent.

<table>
<thead>
<tr>
<th>Group</th>
<th>control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td>glucose (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>105.4 ± 3.2</td>
<td>109.0 ± 4.2</td>
<td>109.2 ± 5.1</td>
<td>160.8 ± 4.2</td>
<td>140.2 ± 3.3</td>
<td>134.7 ± 4.2</td>
<td>130.2 ± 3.2</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.45 ± 0.09</td>
<td>1.13 ± 0.05</td>
<td>1.00 ± 0.04</td>
<td>2.1 ± 0.06</td>
<td>1.08 ± 0.03</td>
<td>1.05 ± 0.07</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>c</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>40.7 ± 0.9</td>
<td>43.9 ± 0.8</td>
<td>41.2 ± 0.6</td>
<td>59.0 ± 0.5</td>
<td>56.9 ± 0.7</td>
<td>52.9 ± 0.6</td>
<td>51.0 ± 0.7</td>
</tr>
<tr>
<td>ALP (IU/ml)</td>
<td>215.1 ± 4.1</td>
<td>248.8 ± 3.2</td>
<td>231.7 ± 7.9</td>
<td>236.5 ± 8.1</td>
<td>228.7 ± 4.1</td>
<td>252.5 ± 5.1</td>
<td>241.6 ± 7.7</td>
</tr>
<tr>
<td>AST (IU/ml)</td>
<td>47.3 ± 2.1</td>
<td>55.5 ± 3.1</td>
<td>53.5 ± 4.1</td>
<td>63.7 ± 1.9</td>
<td>62.2 ± 3.2</td>
<td>59.3 ± 3.6</td>
<td>58.6 ± 2.2</td>
</tr>
<tr>
<td>ALT (IU/ml)</td>
<td>31.9 ± 3.1</td>
<td>36.2 ± 4.1</td>
<td>32.6 ± 3.1</td>
<td>39.5 ± 2.2</td>
<td>37.4 ± 3.5</td>
<td>36.3 ± 4.2</td>
<td>35.5 ± 3.2</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>5.30 ± 0.18</td>
<td>8.40 ± 0.19</td>
<td>8.00 ± 0.16</td>
<td>7.77 ± 2.15</td>
<td>7.10 ± 0.11</td>
<td>6.65 ± 0.17</td>
<td>6.30 ± 0.20</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>2.98 ± 0.13</td>
<td>4.65 ± 0.15</td>
<td>4.45 ± 0.21</td>
<td>4.30 ± 0.22</td>
<td>4.20 ± 0.14</td>
<td>3.73 ± 0.10</td>
<td>3.60 ± 0.17</td>
</tr>
<tr>
<td>Globulin (gm/dl)</td>
<td>2.32 ± 0.11</td>
<td>3.75 ± 0.16</td>
<td>3.55 ± 0.13</td>
<td>3.47 ± 0.21</td>
<td>2.90 ± 0.20</td>
<td>2.87 ± 0.16</td>
<td>2.70 ± 0.12</td>
</tr>
<tr>
<td>Cholesterol (gm/dl)</td>
<td>70.8 ± 2.2</td>
<td>82.4 ± 3.2</td>
<td>78.1 ± 2.7</td>
<td>90.1 ± 3.1</td>
<td>86.2 ± 2.2</td>
<td>83.3 ± 3.1</td>
<td>76.7 ± 2.3</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>60.6 ± 3.1</td>
<td>66.5 ± 2.2</td>
<td>68.2 ± 3.2</td>
<td>69.1 ± 3.1</td>
<td>70.2 ± 2.2</td>
<td>67.5 ± 3.1</td>
<td>68.9 ± 2.1</td>
</tr>
</tbody>
</table>

Table 2: Blood biochemical parameters of sodium benzoate administered rats and effect of olive oil and/or time dependent recovery. Means with difference superscripts in the same row differ significantly (P< 0.05). All values represented as mean ± S.E and % of change from the control. ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Total protein concentration in rat serum was strongly affected by sodium benzoate, increasing levels by about 50%, but over time the levels reduced whether or not more sodium benzoate was administered; olive oil appeared to reduce mean values, but this effect was not significant. Albumin and globulin concentration increased significantly in response to sodium benzoate, and olive oil reduced levels, significantly for the 26-dose groups. The activities of the enzymes were increased in response to sodium benzoate treatment, and were reduced after treatment with olive oil and longer recovery time. The effect of olive oil across different time groups was found to be significant (p < 0.05).

**Discussion**

During the current study, different doses of sodium benzoate were administrated to albino rats to determine its effects on their different blood indices and to investigate the effect of olive oil treatment and long recovery time. The significant increase in white blood cell counts and lymphocytes caused by the administration of sodium benzoate is possibly due to the infection response because these cells perform an important role in defending the organism (Schalm et
al. 1975). The observed decreased haemoglobin content could be attributed to depressed erythropoesis by direct action of sodium benzoate on haemopoietic tissues. These findings suggested the occurrence of anemia among the rats (Schalm et al. 1975; Cheeseborough 1991).

The normal value of blood glucose of the rats was significantly increased in groups three to six. Sodium benzoate may play a role in enhancing pancreatic secretions, glycogen metabolism or gluconeogenesis, and hence glucose mobilization to the blood (Papadopoulos & Boakou 1991). Blood lipids values were also significantly increased in all case groups. These effects of sodium benzoate on the lipid profile may due to imbalances between normal rates of fat metabolism and secretion (Glaser & Mayer 1972). The possible explanation of these observed increments may reside in the direct or indirect action of sodium benzoate on lipid metabolism or lipid peroxidation (Berne & Levy 1998).

Albumin and globulin concentrations increased significantly in response to sodium benzoate. The observed increase in total protein may be attributed to activation of enzymes of anabolism of protein (Fujitani 1993). The significant increases in the levels of some blood parameters contrasts with the work of Bedford & Clark (1972), which indicated no adverse effects of short-term administration of sodium benzoate on blood constituents of cats. Fujitani (1993) also reported changes in the serum levels of albumin and total protein among rats administered sodium benzoate.

Enhanced protein catabolism and accelerated amino-acid deamination for gluconeogenesis is an acceptable postulate to interpret the elevated levels of urea (Bishop et al. 2005). The presence of toxic compounds may increase blood urea and decrease plasma proteins (Varely 1987). Increases in uric acid concentration might be due to degradation of purines, or inability of the kidneys to excrete it. Elevated creatinine in the blood serum is associated with abnormal glomerular filtration and diminishing of body muscles.

The observed elevation in the activities of serum enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in response to sodium benzoate are similar to results from rats treated with N-nitrosodiethylyamine (Bansal et al. 2005) or N-nitrosoamines (Pevicharova et al. 1997). However, elevated activities of alanine aminotransferase and aspartate aminotransferase are a common sign of impaired liver function. Alkaline phosphatase is present on cell surfaces in most human tissues, especially those of the intestine, liver, bones, spleen 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.

However, the activities of these enzymes were reduced significantly after treatment of the rats by olive oil or long recovery times, suggesting that olive oil may suppress sodium-benzoate-induced hepatic carcino genesis. This phenomenon may be occurring due to modulating the antioxidant defence status of the animals in response to antioxidant action of their contents of flavinoids and polyphenolic compounds (Papudopoulos & Boakon 1991; Baldioli et al. 1996). In conclusion, olive oil and long recovery time may suppress some but not all of the endogenous toxic injuries of rat organs induced by sodium benzoate administration.

References


مستخلص الدراسة

يهدف هذا البحث إلى دراسة قياسات الدم وبعض الدراسات البيوكيميائية في بعض فئات الفئران وذلك نتيجة إعطائهم بنزوات الصوديوم ودراسة تأثير زيت الزيتون مع أو بدون منهج وقت للاستشفاء على هذه القياسات المختلفة. أجري البحث على أثناين وأربعون من ذكور فئران بيضاء بالغة الذي يترواح وزنها من 180-200 جرام. تم تقسيم الفئران إلى ثمان مجموعات عشوائية متساوية في العدد واعتبرت المجموعة الأولى ضابطة وأعطيت 2 مل من الماء المشرب عن طريق الفم باستخدام كمية محددة. أما المجموعة الثانية أعطيت جرعة قدرها 50 ملجم/كيلو. وبنزوات الصوديوم، وأعطيت المجموعة الثالثة نفس المقدار من البنزوات إضافة إلى 4 مل. وبناءً على الماء المشرب. أما المجموعة الرابعة أعطيت نفس الجرعة من بنزوات الصوديوم لمدة 26 يومًا، بينما أعطيت المجموعة الخامسة نفس جرعة الزيت وزيت الزيتون لفترة 26 يومًا. وتم معالجة المجموعة الأخيرة (مجموعة الاستشفاء) بنفس طريقة المجموعة الخامسة ولكنها تركت أربعة أسابيع قبل إجراء القياسات المذكورة.

في نهاية التجربة أخذت عينات من كل مجموعة لفحص القياسات الدموية وبعض القياسات البيوكيميائية وأستخدم برنامج SPSS لتحليل النتائج إحصائيًا.

أظهرت دراسة القياسات الدموية أن أدى بنزوات الصوديوم إلى زيادة واضحة في عدد كرات الدم البيضاء والخلايا المفيدة. بينما أدى ذلك إلى نقص معدل الهيموكرتيد والهيموجلوبين وعدد كرات الدم الحمراء والصفائح الدموية. أدى إعطاء جرعة زيت الزيتون وتوفر إعطاء البنزوات لفترة أربع أسابيع (مجموعة الاستشفاء) إلى زيادة في عدد كرات الدم البيضاء والخلايا المفيدة وكذلك لوحظ انخفاض كاثر القياسات السالفة الذكر. وكذلك في معدل زيادة الجلوكوز مع إعطاء زيت الزيتون وتوقف إعطاء البنزوات.

من ناحية أخرى، أعطاء البنزوات زاد من معدل الكرياتين وأليوريا وحمض البوليك والأليروتات الكليمة والحلزول والكلوريدول والأنزيمات الخاصة بالفيبرين بشكل واضح في جميع الحالات ولكن انخفضت هذه الزيادة بعدها تتوقف إعطاء البنزوات. تستخلص هذه الدراسة أن توقف إعطاء بنزوات الصوديوم مع الاستمرار في إعطاء الزيت أدى إلى تقليل من سمية البنزوات لدى فئران التجربة.

الكلمات المفتاحية: ذكور الفئران البيضاء، بنزوات الصوديوم، القياسات الدموية، البيوكيميائية، زيت الزيتون، مدة الاستشفاء.