Effect of Potassium Bromate on Liver and Blood Constituents of Wistar Albino Rats

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Abstract: Twenty four Wistar albino rats were divided into 4 groups and treated orally with potassium bromate at doses of 0, 50, 100 and 200 mg kg\(^{-1}\) body weight (b.wt.) for 21 days. Rats received 200 mg kg\(^{-1}\) b.wt. died within 18 days. A significant reduction in Hb, PCV and MCHC values were observed in animals received 200 mg kg\(^{-1}\) b.wt. in the second week while no changes occurred in the groups treated with 50 and 100 mg kg\(^{-1}\) b.wt. The activity of alanine transaminase (ALT) was significantly increased in rats received 100 and 200 mg kg\(^{-1}\) b.wt. of potassium bromate from the first week, while total protein and albumin were significantly decreased from the first week in animals treated with 200 mg kg\(^{-1}\) b.wt. and the second week at the dose 100 mg kg\(^{-1}\) b.wt. Histologically liver degeneration and haemorrhage was evident in the groups treated with 100 and 200 mg kg\(^{-1}\) b.wt. The dose of 50 mg kg\(^{-1}\) b.wt. did not cause any changes compared to the control.

Key words: Potassium bromate, blood constituent, liver

INTRODUCTION

Food additives are substances added directly and intentionally to food, usually in small quantities for improvement of specific purpose. However, non-intentional additives may be an integral part of food. Substances should be considered as additives if it has no ill effect and its margin of safety is adequate.

Potassium bromate has been evaluated for acceptable level of treatment for flour to be consumed by man (FAO/WHO, 1964). It is also used in treating barley in beer making and for improvement of the quality of fish-paste products in Japan.

Potassium bromate is generated as a contaminant in drinking water due to conversion of bromide found naturally in water to bromate by ozone which is used as disinfectant (Ueno et al., 2000).

Several researches on safety evaluation of potassium bromate were carried out. It was found to be a genotoxic and carcinogenic (Kurokawa et al., 1990; Sai et al., 1992). Hence was ruled unsafe and banned from the list of food additives.

In Sudan, however, food adulteration and discriminate addition of food additives continue to be a problem despite the precaution undertaken. The present study was designed to evaluate the toxic effect of potassium bromate on the liver and haemopoiesis.

MATERIALS AND METHODS

This study was conducted on April 2005.

Potassium Bromate

Potassium bromate in a form of powder was supplied by Sudanese Consumer Protection Association at Khartoum, Sudan.

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Animals

Twenty four Wistar albino rats of both sex weighing 60-70 g were supplied by Medicinal and Aromatic Plant Research Institute, at Khartoum. They were kept under standard conditions and had free access to water and standard diet. The animals were left for a week, as an adaptation period.

Experimental Design

The animals were randomly divided into four groups, 6 rats each. The first group was left as control and the others were administered orally with potassium bromate using nasogastraic tube daily for 21 days at concentration of 50, 100 and 200 mg kg\(^{-1}\) b.w.t.

Blood samples were collected by puncturing retro-orbital plexus with heparinized capillary tube into dry clean tube containing EDTA (ethylenediaminetetraacetic acid) as anticoagulant for haematology and into dry clean tubes for serology. The blood was allowed to clot at room temperature for 30 min then centrifuged at 3000 rpm for 5 min Sera were separated and stored at -20°C until analyzed. Haematological indices were measured according to Schalm (1980). RBCs and WBCs were counted using Neubaur haemacymeter. PCV was measured using hematocrit method. Hb determination was based on the conversion of Hb to cyanomet hemoglobin. Mean corpuscular haemoglobin (MCH) concentration and mean corpuscular volume (MCV) were calculated.

Sera were analyzed for total protein concentration by Biurat method described by Reinhold (1953). Albumin was measured using Bromo Cresole Green method as described by Spencer and Prince (1997). Globulin was obtained by subtracting albumin from total protein concentration and albumin globulin ratio was calculated.

The enzyme ALT (alanne transaminase) was determined using the method described by Reitman and Frankel (1957).

Animals were sacrificed after 21 days. Slices of livers were fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 μm and stained by Hematoxyline and Eosin using Drury and Wallington (1980) method.

Statistical Analysis

Data were analyzed statistically by student t-test according to Mendenhall (1971).

RESULTS

Control rats and those received 50 mg kg\(^{-1}\) b.w.t. potassium bromate showed no clinical signs throughout the experimental period, where depression and difficulty in breathing were observed in rats received 100 and 200 mg kg\(^{-1}\) b.w.t. of potassium bromate. All rats received 200 mg kg\(^{-1}\) b.w.t. died within 18 days.

Generally, there were no significant differences between the control and those given 50 and 100 mg kg\(^{-1}\) b.w.t. potassium bromate during the experimental period. However, animals received 200 mg kg\(^{-1}\) b.w.t. showed a significant (p<0.05) reduction in Hb concentration PCV and MCHC on the second week (Table 1).

Total protein level was reduced significantly (p<0.05) in the second week while albumin reduction occurred in the first and second week in animals received 200 mg kg\(^{-1}\) b.w.t. potassium bromate; relevant to this reduction in albumin, the albumin-globulin ratio was decreased. On the other hand total protein and albumin levels were not affected in the first week in the rats treated with 100 mg kg\(^{-1}\) b.w.t. but in the second and third week significant (p<0.05) reduction was evident (Table 2). The rats given 50 mg kg\(^{-1}\) b.w.t. exhibited no significant change in total protein and albumin levels.

The ALT activity was significantly increased throughout the experimental period at doses of 100 an 200 mg kg\(^{-1}\) b.w.t. while at the dose 50 mg kg\(^{-1}\) b.w.t. the activity of ALT was unchanged.
Table 1: The haematological values of rats treated orally with various levels of potassium bromate

<table>
<thead>
<tr>
<th>Duration (week)</th>
<th>Doses (mg kg⁻¹ b.wt.)</th>
<th>RBCs ×10⁶/mm³</th>
<th>WBCs ×10⁶/mm³</th>
<th>Hb (g day⁻¹)</th>
<th>PCV (%)</th>
<th>MCHC (%)</th>
<th>MCV (μL)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>4.9±0.25</td>
<td>6.3±0.85</td>
<td>13.0±0.72</td>
<td>33.7±0.96</td>
<td>38.6±0.80</td>
<td>69.0±0.3</td>
</tr>
<tr>
<td>50</td>
<td>4.9±0.69*</td>
<td>6.9±1.10*</td>
<td>12.9±0.33*</td>
<td>33.8±1.60*</td>
<td>37.8±0.25*</td>
<td>68.0±0.6*</td>
<td>69.0±0.3</td>
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<tr>
<td>100</td>
<td>4.8±0.60**</td>
<td>6.6±1.00**</td>
<td>11.9±0.67**</td>
<td>31.5±2.70**</td>
<td>37.7±0.56**</td>
<td>66.0±1.8**</td>
<td>69.0±0.3</td>
</tr>
<tr>
<td>200</td>
<td>5.3±0.58*</td>
<td>7.2±1.40*</td>
<td>11.5±1.00*</td>
<td>34.0±1.80*</td>
<td>33.8±0.19*</td>
<td>64.0±1.8*</td>
<td>69.0±0.3</td>
</tr>
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<td>2</td>
<td>0</td>
<td>5.1±0.28</td>
<td>6.2±0.28</td>
<td>13.4±0.70</td>
<td>35.8±0.96</td>
<td>37.0±0.65</td>
<td>70.0±1.1</td>
</tr>
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<td>50</td>
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<td>6.6±0.44**</td>
<td>13.2±0.66**</td>
<td>36.2±1.20**</td>
<td>36.0±0.30**</td>
<td>74.0±1.2**</td>
<td>69.0±0.3</td>
</tr>
<tr>
<td>100</td>
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<td>7.0±0.99**</td>
<td>13.3±0.24**</td>
<td>35.6±1.80**</td>
<td>38.7±1.00**</td>
<td>74.0±0.5**</td>
<td>69.0±0.3</td>
</tr>
<tr>
<td>200</td>
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<td>5.6±0.51**</td>
<td>10.4±0.50**</td>
<td>29.3±0.2*</td>
<td>35.4±2.5*</td>
<td>68.0±1.2*</td>
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<td>3</td>
<td>0</td>
<td>5.3±0.82</td>
<td>6.7±0.36</td>
<td>13.3±0.53</td>
<td>34.3±1.50</td>
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<td>6.2±0.47**</td>
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<td>32.8±1.50**</td>
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<td>69.0±0.3</td>
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<tr>
<td>100</td>
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<td>6.2±0.54**</td>
<td>11.3±1.40**</td>
<td>30.3±2.50**</td>
<td>37.2±1.50*</td>
<td>70.0±3.1*</td>
<td>69.0±0.3</td>
</tr>
</tbody>
</table>

Mean values±SD; NS = Not significant; * = p<0.05

Table 2: The level of some blood parameters of rats treated orally with various levels of potassium bromate

<table>
<thead>
<tr>
<th>Duration (week)</th>
<th>Doses (mg kg⁻¹ b.wt.)</th>
<th>Total protein (g dL⁻¹)</th>
<th>Albumin (g dL⁻¹)</th>
<th>Globulin (g dL⁻¹)</th>
<th>Alb/Glob ratio</th>
<th>ALT (U L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>6.6±0.53</td>
<td>3.8±0.67</td>
<td>2.9±0.67</td>
<td>1.30±0.90</td>
<td>23.7±5.30</td>
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<td>50</td>
<td>6.8±0.46</td>
<td>3.6±0.46</td>
<td>3.3±0.79</td>
<td>1.09±0.79</td>
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</tr>
<tr>
<td>100</td>
<td>6.2±0.40</td>
<td>3.1±0.48</td>
<td>3.0±0.80</td>
<td>1.03±0.70</td>
<td>32.2±3.70</td>
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</tr>
<tr>
<td>200</td>
<td>6.0±0.43</td>
<td>2.9±0.22</td>
<td>3.0±0.16</td>
<td>0.97±0.20</td>
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<tr>
<td>2</td>
<td>0</td>
<td>7.8±0.68</td>
<td>3.7±0.55</td>
<td>4.2±1.20</td>
<td>0.88±0.60</td>
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<tr>
<td>50</td>
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<td>3.1±0.65</td>
<td>3.9±0.59</td>
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<td>6.0±0.49</td>
<td>2.7±0.37</td>
<td>3.5±0.53</td>
<td>0.77±0.45</td>
<td>44.5±2.0</td>
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</tr>
<tr>
<td>200</td>
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<td>3.1±0.37</td>
<td>0.74±0.30</td>
<td>56.3±3.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>7.3±0.96</td>
<td>3.7±0.50</td>
<td>3.6±0.10</td>
<td>1.03±0.80</td>
<td>31.0±2.9</td>
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<tr>
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<td>6.3±0.45</td>
<td>3.0±0.14</td>
<td>3.3±0.40</td>
<td>0.91±0.30</td>
<td>37.4±4.0</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5.8±0.54</td>
<td>2.5±0.42</td>
<td>3.0±0.61</td>
<td>0.83±0.52</td>
<td>57.0±6.5</td>
<td></td>
</tr>
</tbody>
</table>

Mean values±SD; NS = Not significant; * = p<0.05

Fig. 1: Liver in the group treated with 200 mg kg⁻¹ potassium bromate. Vacuolisation and dilatation of sinusoids. H and E × 100

At postmortem the liver was dark in colour and friable in rats received 100 and 200 mg kg⁻¹ b.wt. Histologically the liver showed vacuolisation and dilatation of sinusoids (Fig. 1) beside congestion and haemorrhage.
DISCUSSION

In the present study, potassium bromate was found to have no effects on hemopoiesis at doses 50 and 100 mg kg\(^{-1}\) b.wt. but when the dose increased to 200 mg kg\(^{-1}\) b.wt. a reduction in Hh, PCV and MCHC were evident in the second week of the experiment, hence anaemia occurred. Chipman et al. (1998) reported the induction of methaemo-globinaemia in rats when potassium bromate was used. They also claimed oxidation of ferrous ion to ferric by reactive species generated from potassium bromate.

The reduction of total protein and albumin was dose dependant and occurred from the first week at the dose 200 mg kg\(^{-1}\) b.wt. and from the second week when the dose was lowered to 100 mg kg\(^{-1}\) b.wt. This change in total protein and albumin may be due to reduction in protein synthesis as the liver damage was illustrated by histopathology. Moreover the haemorrhage that occurred may play a role.

A remarkable increase in ALT suggests high permeability of hepatocytes due to liver damage which was supported histo-pathologically. ALT is consequent with hepatic cell damage and injured cell membrane permeability. This finding is in line with Kurokawa et al. (1990) who reported an increase in ALT in rats received 600 mg kg\(^{-1}\) potassium bromate in drinking water.

Khan et al. (2003) reported a reduction of antioxidant enzymes and enhancement of xanthine oxidase and lipid peroxidation when rats were treated with 125 mg kg\(^{-1}\) b.wt. potassium bromate administered intraperitoneally. El-Sokkary (2006) observed significant increase in malondialdehyde as an indicator of lipid peroxidation. These findings support the histological changes occurred in the liver such as vacuolation as lipid peroxidation play a role in liver injury. In contrast to our findings, Umemura et al. (1995) reported no pathological change in the liver.

In the present study the dose 50 mg kg\(^{-1}\) b.wt. potassium bromate caused no changes in hematology as well as pathological changes in the liver. The metabolism of potassium bromate is stable in the body and small amounts can be reduced to bromide by glutathione process in the liver (Kutom et al., 1990). However, portion of potassium bromate may be excreted in urine and this will lower its level in blood and tissues. Therefore, this may be the reason for absence of pathological changes at low doses.

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