The Relationship between Sodium Nitrate and Potassium Bromate Consumption and Hepatotoxicity in Rats

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ABSTRACT

The present study aims to discover the oxidative damage and changes in liver enzymes activities that may be occur of sodium nitrate, potassium bromate administration. Male rats were divided into four equal groups. Group 1 used as control, group 2, 3 and 4 treated with sodium nitrate (500 mg/L), potassium bromate (400 mg/L) and their mixture (500 mg sodium nitrate plus 400 mg potassium bromate), respectively. The activities antioxidant enzymes, thiobarbituric acid-reactive substances (TBARS), glutathione, liver and plasma enzymes were determined. The results showed that treatment with sodium nitrate and potassium bromate resulted in significant (p≤0.05) decrease in antioxidant enzymes activities and glutathione level, while, the level of TBARS significantly (p≤0.05) increased than control group. Also, treatment with sodium nitrate and potassium bromate caused significant (p≤0.05) increase in plasma lactate dehydrogenase (LDH), acid phosphatase (ACP), alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) while, all these enzymes were significantly (p≤0.05) decreased in liver. Treatment with sodium nitrate plus potassium bromate in the mixture group caused significant (p≤0.05) changes in these parameters compared to treated animals with either sodium nitrate or potassium bromate alone. In conclusion, the use of sodium nitrate and potassium bromate should be limited because they have negative effects on health, causing adverse changes in antioxidant enzymes activities and liver enzymes.

Key words: Synthetic food additive; Hepatotoxicity; Antioxidant enzymes; Liver enzymes; Lipid peroxidation.
1. INTRODUCTION

Nitrate is used as additives to improve food quality and inhibit microbial contamination (Cantwell and Elliott, 2017). According to WHO (2011), the major sources of nitrates are cured meat products, vegetables, fish and dairy products. Usually dairy products contain <3-27 mg nitrate/kg while, meat products might contain <2.7-945 mg nitrate/kg. Crops such as spinach green salad, beetroot and radish regularly contain nitrates at levels above 2500 mg/kg (Nujić and Habuda-Stanić, 2017). Sodium nitrate is commonly used in curing mixtures to inhibit microbial growth, develop characteristics flavors and develop the color of meat (Sindelar and Milkowski, 2012). The WHO estimates the mean daily dietary intake of nitrate from 43 to 141 mg (WHO, 2007).

Katan (2009) and Rangabhashiyam et al., (2014) indicated that high nitrate levels in drinking water can make threats to human health for the reason that in gastrointestinal tract, nitrates are reduced to nitrites, nitrates lead to methemoglobinemia, nitrites and nitrates have the probable to form carcinogenic compounds. Song et al. (2015) reported that processed food such as sausages and other processed meat are linked with gastric cancer risk due to its high contents of nitrates. High level of nitrate in meat products could inducemethemoglobinemia (reduced ability of the red blood cell to release oxygen to tissues). It might be responsible for the formation of carcinogenic nitrosamine in humans (Kapor, 2004 and Chan, 2011). Nitrates might increase lipid peroxidation which leads to harmful effects to different organs such as kidney and liver (Rocha et al., 2012).

Potassium bromate is an oxidizing agent that is commonly used; as a food additive; as a cake and bread improver (Kakehashi et al., 2013), in flour milling; as strong oxidizing agent; in cheese making; as an ingredient in fish-paste and beer malting (Kurokawaet al., 1990). Also, bromate is formed as a by-product of water disinfection by ozonation (Liu and Mou, 2004) and used in cosmetic products (Kakehashi et al., 2013). FDA (2007) permitted bromate salts for use as bread dough conditioners at a maximum concentration of 75 ppm. WHO
reported that potassium bromate has been used as an oxidizing reagent in the dyeing of textiles and in laboratories. Oloyede and Sunmonu (2009) mentioned that potassium bromate has been linked to several organs damage. Acute intoxication to potassium bromate in humans may results in neuropathological disorders, thrombocytopenia and renal failure, while chronic intoxications led to the increase of numerous nonrenal and renal tumors (Ajarem et al., 2016).

Laba (2003) confirmed that potassium bromate has harmful effects on bread nutrition value by lowering various vitamins such as B2, A1, E, B1 and niacin. Starek and Starek-Świechowicz (2016) reported that the major toxic symptoms and signs after treatment with potassium bromate were hypothermia, lacrimation, tachypnoea, ataxic gait, diarrhea and suppression of movement. Therefore, the present study aims to evaluate the effects of sodium nitrate, potassium bromate and their mixture on the activities of antioxidant enzymes, the levels of TBARS and glutathione and liver enzymes activities in rats.

2. MATERIALS AND METHODS

2.1. Animals

Forty male Sprague dawley rats weight 150-200 g were housed in the animal house at Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt.

2.2. Chemicals

Sodium nitrate and potassium bromate were obtained from Sigma Company Brand, Egypt. Aqueous solutions of both compounds were prepared freshly every day. Chemical kits were obtained from Biosystems S.A. (Spain), Diamond (Germany).

2.3. Diet

Animals were divided into four equal groups (10 rats each). Group 1 used as control. Group 2, 3 and 4 treated with sodium nitrate at 500mg/L according to Eskiocaket et al., (2005) and Anwar and Mohamed (2015), potassium bromate at 400mg/L according to Deangelo et al. (1998) and Dodd et al. (2013) and their mixture, respectively. Animals administrated sodium nitrate and potassium bromate and their mixture in drinking-water daily for eight weeks.
2.4. Blood samples collection and tissue preparation

At the end of the experimental period (56 days), animals were anesthetized with ether and sacrificed. The blood samples were collected in heparinized tubes. The heparinized blood samples were placed immediately on ice. Plasma samples were obtained by centrifugation at 4000 rpm for 10 min, and were stored at -20°C until used for analyses. Stored plasma samples were analyzed by kits for the activities of plasma aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH).

Liver was removed at the end of the experiment, washed using chilled saline solution. Tissue was minced and homogenized. The homogenate was centrifuged at 10,000 × g for 20 min at 4°C. The resultant supernatant of the organ was used for determination of different enzyme activities in liver.

2.5. Oxidative stress markers
Liver glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione S-transferase (GST), catalase (CAT), glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) were determined.

2.6. Statistical analysis
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Quantitative data were described using mean, standard deviation. Significance of the obtained results was judged at the 5% level.

The used test was:
F-test (ANOVA)
For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (LSD) for pairwise comparisons.

3. RESULTS AND DISCUSSION
Antioxidant enzymes activities in liver of rats treated with sodium nitrate, potassium bromate and their mixture are tabulated in Table (1). Data indicated that treatment with either sodium nitrate or potassium
bromate resulted in significant (p≤0.05) decrease in antioxidant enzyme activities compared to control group. Interestingly, rat's liver treated with mixture of sodium nitrate plus potassium bromate resulted in significant (p≤0.05) decrease in the activities of antioxidant enzymes when compared to sodium nitrate, potassium bromate and control. The table confirmed that the values of total antioxidant capacity in sodium nitrate, potassium bromate and their mixture group were found to be lower than control. Thiobarbituric acid reactive substances (TBARS) level and glutathione (GSH) level of rat's liver treated with sodium nitrate, potassium bromate and their mixture are also shown in Table (1). As depicted in the table, the level of TBARS in sodium nitrate, potassium bromate and their mixture group are significantly (p≤0.05) higher than control group while, the level of glutathione was significantly (p≤0.05) lower than control group.

Oxidative stress attributed to imbalance between antioxidant defense system and formation of free radicals resulted in liver injury, which disturbs cellular functions lead to different pathological conditions (Sabiu et al., 2014). Manassaram et al. (2007) mentioned that nitrate has ability to generate free radicals which induce oxidative stress causing toxicity. The obtained results of TBARS is supported with earlier finding by Microalwski (1994) who reported that there was significant increase in TBARS might be due to the harmful effects of free radicals which were started by the peroxidation of poly unsaturated fatty acids of cholesterol, phospholipids and lipoprotein in cell membrane. Eskiocak et al. (2005) concluded that increase thyroid gland weights, decreased iodine uptake, changed serum thyroid hormone levels and histopathological changes in female Wistar rats that received sodium nitrate for 30 weeks at range between 50 and 500 mg/L tap water. Alyet al. (2010) found that decreased the activity of enzymatic markers of spermatogenesis, sperm motility and Sperm count of male Swiss albino rats exposed orally to sodium nitrate in drinking-water at range between 50 and 200 mg/kg bw per day compared to control group. Ward et al. (2005) indicated that intake of nitrate resulted in increased risks for
neural tube defects. *Croen et al. (2001)* mentioned that nitrate levels above 45 mg/L led to increased risk of anencephaly.

**Table (1):** liver TBARS and GSH level, the activities of SOD, GPx, CAT and GST and total antioxidant capacity of male rats treated with sodium nitrate, potassium bromate and their mixture.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Sodium nitrate</th>
<th>Potassium bromate</th>
<th>Sodium nitrate + potassium bromate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (n mol/g tissue)</td>
<td>86.1±1.52</td>
<td>120.0±1.14</td>
<td>119.6±2.91</td>
<td>167.0±0.55</td>
</tr>
<tr>
<td>GSH (µmol/g tissue)</td>
<td>6.4±0.11</td>
<td>3.8±0.06</td>
<td>3.9±0.06</td>
<td>2.3±0.03</td>
</tr>
<tr>
<td>SOD (U/g tissue)</td>
<td>39.8±0.37</td>
<td>31.6±0.51</td>
<td>31.6±0.51</td>
<td>25.4±0.63</td>
</tr>
<tr>
<td>CAT (U/g tissue)</td>
<td>138.5±3.62</td>
<td>118.7±4.64</td>
<td>110.4±3.87</td>
<td>95.4±1.91</td>
</tr>
<tr>
<td>GST (µmol/h/g tissue)</td>
<td>2.1±0.06</td>
<td>1.9±0.07</td>
<td>1.9±0.09</td>
<td>1.2±0.06</td>
</tr>
<tr>
<td>GPX (U/g tissue)</td>
<td>11.7±0.32</td>
<td>8.4±0.55</td>
<td>9.6±0.68</td>
<td>7.2±0.37</td>
</tr>
<tr>
<td>TAC (µmol/h/g tissue)</td>
<td>6.1±0.01</td>
<td>5.6±0.02</td>
<td>5.7±0.04</td>
<td>4.8±0.05</td>
</tr>
</tbody>
</table>

The values represent mean ±SE. Means in the same row with superscript by different letters are significantly different, (p≤0.05).

TBARS= thiobarbituric acid-reactive substances, GSH = Reduced glutathione, GPX= Glutathione peroxidase, GST= Glutathione S-transferase, CAT= Catalase, SOD= Superoxide dismutase, TAC= Total antioxidant capacity.

*Khan and Sultana (2005)* reported that treated with potassium bromate led to reduction of renal glutathione at 30% and dropping the activities of glutathione-S-transferase at 42% with compared to control, reduction of renal glutathione attributed to rapid oxidation reaction between oxidants generated by potassium bromate and the glutathione. The obtained results of superoxide dismutase activity and glutathione level are consistent with the previous report by *Bayomyet et al. (2016)* who reported that the decreased levels of both SOD and GSH are suggestive of excessive free radicals formation attributed to lipid peroxidation resulted in tissue damage. The obtained result of TBARS of rats treated...
with potassium bromate is consistent with the previous report by Bayomy et al. (2016) who found that oxidative damage induced by potassium bromate which significantly increased TBARS and decreased the levels of GSH and SOD activity in rat's liver. Ajarem et al. (2016) indicated that significant reduction in the level of glutathione of mice's brain as compared with control group due to treated with potassium bromate at two doses (100, 200 mg/kg).

Plasma and liver lactate dehydrogenase (LDH), acid phosphatase (ACP), alkaline phosphatase (ALP), alanine transaminase (AIT) and aspartate transaminase (AST) of rats treated with sodium nitrate, potassium bromate and their mixture are tabulated in Table (2) and (3). It was observed that treatment with sodium nitrate significantly (p≤0.05) increased plasma LDH, ACP, ALP, AIT and AST activities and significant (p≤0.05) decreased liver LDH, ACP, ALP, AIT and AST activities compared to control group. On the same path, treatment with potassium bromate resulted in significant (p≤0.05) increased plasma enzymes activities and significant (p≤0.05) decreased liver enzymes activities compared to control. In addition, treatment with sodium nitrate plus potassium bromate in combination group led to significant (p≤0.05) impact on plasma and liver enzymes activities. It observed that the increase in the activities of plasma LDH, ACP, ALP, AIT and AST in the sodium nitrate, potassium bromate and their mixture treated rats may be an indication of liver dysfunction.

The obtained results of liver enzymes in rats treated with potassium bromate are consistent with the earlier finding by Sabiu et al. (2014) who concluded that there was hepatic injury as indicated to cellular release of intracellular enzymes and loss of liver cell membrane stabilization. Also, Khan et al. (2012) found increase in AST and ALT in rats administered with potassium bromate at the range between 20 to 200 mg/kg. Galaly and Mahmoud (2012) concluded that noticeable changes in the liver architecture like alterations of sinusoidal lumen and inflammatory cells infiltration in rats attributed to sodium nitrate exposure. The obtained results of liver enzymes activities are consistent with previous report by Aliet al. (2018) who confirmed that the plasma
activities of LDH, AST and ALT were significant (p≤0.05) increased by treatment with potassium bromate 135 mg/kg and cause renal dysfunctions. *Iweka et al. (2016)* suggested that the increase in AST and ALT attributed to cell death or inflammation resulted in high permeability of hepatocytes. *Dimka et al. (2013)* confirmed that treatment with potassium bromate at 100 and 200mg/kg led to increase the level of liver enzymes such as AST and ALT in rats. *Subramoniam and Pushpagada (1999)* reported that the integrity of liver cells is affected by toxic compounds that increase the level of AST and ALT.

*Akanjiet al. (2008)* concluded that there was a significant increase (P<0.05) in alkaline phosphatase activity in the serum after 10mg/kg body weight of potassium bromate administration. *Yakubu et al. (2003)* concluded that the increase in serum alkaline phosphatase activity of rats' treatment with potassium bromate attributed to damage plasma membrane, which resulted in the compromise of its integrity. *Yakubu et al. (2002)* reported that the reduction in alkaline phosphatase tissues activities subsequent to the treatment with potassium bromate might attributed to a reduction in concentration or total absence of specific phospholipids required by this membrane bound enzyme.

Table (2): Plasma LDH, ACP, ALP, AIT and AST of male rats treated with sodium nitrate (NaNo3), potassium bromated (KBrO3) and their mixture.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Potassium bromate</th>
<th>Sodium nitrate + potassium bromate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/ml)</td>
<td>67.7±0.68</td>
<td>86.9±0.43</td>
<td>85.9±0.37</td>
<td>121.8±0.39</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>47.9±0.44</td>
<td>116.1±0.69</td>
<td>117.1±0.76</td>
<td>185.2±0.50</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>141.7±7.05</td>
<td>234.1±8.85</td>
<td>229.5±12.46</td>
<td>287.4±12.17</td>
</tr>
<tr>
<td>ACP (U/L)</td>
<td>44.7±1.37</td>
<td>54.4±0.31</td>
<td>53.6±0.89</td>
<td>73.9±0.59</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1010.5±18.08</td>
<td>1238.4±21.77</td>
<td>1275.8±24.59</td>
<td>1595.8±11.72</td>
</tr>
</tbody>
</table>

The values represent mean ±SE. Means in the same row with superscript by different letters are significant different, (p≤0.05).

AST= aspartate transaminase, AIT= alanine transaminase, ALP= alkaline phosphatase, ACP= acid phosphatase, LDH= lactate dehydrogenase
Table (3): Liver LDH, ACP, ALP, AIT and AST of male rats treated with sodium nitrate, potassium bromate and their mixture.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Sodium nitrate</th>
<th>Potassium bromate</th>
<th>Sodium nitrate + potassium bromate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/g wet tissue)</td>
<td>219.4±0.23</td>
<td>181.7±0.24</td>
<td>181.8±0.39</td>
<td>125.9±0.23</td>
</tr>
<tr>
<td>ALT (U/ g wet tissue)</td>
<td>405.4±1.00</td>
<td>309.2±4.22</td>
<td>305±2.20</td>
<td>208.5±4.58</td>
</tr>
<tr>
<td>ALP (IU/ g wet tissue)</td>
<td>201.4±0.51</td>
<td>137.3±0.58</td>
<td>129±0.58</td>
<td>81.2±0.59</td>
</tr>
<tr>
<td>ACP (U/ g wet tissue)</td>
<td>83.2±2.06</td>
<td>65.2±0.68</td>
<td>59.7±0.23</td>
<td>52.5±0.29</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>931.1±14.52</td>
<td>606.9±3.11</td>
<td>491.5±2.17</td>
<td>391.2±16.80</td>
</tr>
</tbody>
</table>

The values represent mean ±SE. Means in the same row with superscript by different letters are significant different, (p<0.05).

AST= aspartate transaminase, AIT= alanine transaminase, ALP= alkaline phosphatase, ACP= acid phosphatase, LDH= lactate dehydrogenase

Akanji et al. (1993) suggested that loss of alkaline phosphatase activity from the tissues might be due to disruption of the ordered lipid-bilayer of the membrane structure attributed to the attendance of oxygen in the chemical compound, which oxidized the polyunsaturated fatty acids lead to leakage of ALP out of the cell to the extracellular fluid. The increase in ACP activities may be due to the raise in functional activity of the tissues (Yakubu et al., 2001). Akanji and Yakubu (2000) indicated that the significant loss of lactate dehydrogenase activity due to it is in close proximity to the plasma membrane. Also, Akanji et al. (1993) reported that small damage to the plasma membrane will easily resulted in release of lactate dehydrogenase from the cell interior to the extracellular environment.

4. CONCLUSIONS

In conclusions, the obtained results showed that sodium nitrate and potassium bromate and their mixture induced hepatotoxicity in rats. This was pronounced from the disturbances in the oxidative stress markers and changes in liver functions enzymes. It was clear that the mixture of sodium nitrate and potassium bromate caused harmful effects compared to either sodium nitrate or potassium bromate alone. Therefore, there is a need to explore natural preservatives for use to get rid of the harmful effects of sodium nitrate and potassium bromate.
5. REFERENCES


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العلاقة بين المتناول من نيترات الصوديوم وبرومات البوتاسيوم وتسمم الكبد في الفئران

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تهذ هذه الدراسة لاختبار أضرار الصوديوم والبوتاسيوم ونظام جنِارة البوتاسيوم مخلوطه معًا في الفئران. تم تقسيم ذكور الجرذان إلى أربع مجموعات متساوية. المجموعة الأولى استخدمت كمجموعة ضابطة، المجموعات الثانية والثالثة والرابعة تم معاملتهم بنترات الصوديوم (500 مللجم/لتر ماء) وبرومات البوتاسيوم (400 مللجم/لتر ماء) مشتقت من 50 ملليلترات الصوديوم مع 400 مللجم بروميات البوتاسيوم، على التوالي. تم قياس نشاط الإنزيماضاضاءة للأكسيدة ومستويات TBARS والجلوتاتيون في كبد الجرذان المعالجة بنترات الصوديوم وبرومات البوتاسيوم من خلال تأثيرات البوتاسيوم (p≤0.05) بشكل ملاحظي (p≤0.05) مقرونًا بالمجموعة الضابطة. كذلك أظهرت المعالمة بنترات الصوديوم وبرومات البوتاسيوم زيادة معنوية (p≤0.05) في نشاط الإنزيماضاضاءة للأكسيدة في كبد الفئران (LDH) ، إنتزيم الراكتيدريدوجينز (LDH) ، إنتزيم الفوسفاتيز الحمضي (ACP) ، إنتزيم الفوسفاتيز الحمضي (ALT) ، إنتزيم الفوسفاتيز الحمضي (AST) ، الأنزيم الناقل لحصب الألوان (AST) ، الأنزيم الناقل لحصب الألوان (ALT) ، الأنزيم الناقل لحصب الألوان (ALT) ، الأنزيم الناقل لحصب الألوان (ALT) ، الأنزيم الناقل لحصب الألوان (ALT).

كلمات المفاتيح: المواد المضافة الصناعية، السمية الكبدية؛ الإنزيماضاضاءة للأكسيدة؛ نيترات الصوديوم ؛ دهون المؤكسدة.

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