Effect of Selenium to High Doses of Nitrate and Nitrite in Immunoglobulin Production and Detoxifying Enzymes Activities.

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Abstract: The present work is conducted to study the effect of selenium on reducing the toxic effect of high doses of nitrate and nitrite. Selenium is added to high doses of nitrate and nitrite in rats diet as 100 mg/kg body weight. Potassium nitrate used at 0.6 and sodium nitrite used at 0.2% for two months of feeding. Nutritional, immunological parameters and detoxifying enzyme activities were estimated. The results indicated that present of nitrate and nitrite in rats diet affected a significant decrease in food intake, body weight gain and feed efficiency ratio when compared with control group. It can be observed that values of IgG and IgM production are significantly decreased as a result of adding nitrate and nitrite. Nitrate and nitrite lowered the activities of hepatic catalase and glutathione peroxidase, whereas it drastically increased Gamma glutamyl transeptidase activity in kidney. Addition of selenium to high doses of nitrate and nitrite affected increased food intake, body weight and food efficiency ratio. A significant increase of IgG and IgM were observed when compared with control group. Feeding of diet containing selenium at level 100mg/kg body weight has variable effects on the different antioxidant/bio-transformation enzymes. It can be concluded that presence of selenium in the diet that containing nitrate and nitrite salts is very important. Present study recommends the use of nitrate and nitrite in food products especially meat production must be monitored.

Key words: nitrate and nitrite salts – selenium- IgG- IgM- detoxifying enzymes.

INTRODUCTION

Nitrate and nitrite salts are used in food industry especially meat products to improve the color, taste and control undesirable gas and flavor production by anaerobic bacteria[1]. Nitrate and nitrite are added due to their protective effect against toxic bacteria as clostridium botulinium[2]. This bacteriocidal effect is referred to compound derived from it during food processing[1]. The antibody production and cell proliferation of B cells are significantly influenced by addition of nitrite[3]. IgG, IgA and IgM are decreased in these children while spontaneous blastogenesis of lymphocytes are increased[4]. High doses of nitrate and nitrite in food products decreased the food intake, body weight, vitamin A in liver and utilization of B-carotene[5]. NAS, [6] calculated the dose of nitrate per day which effect on the human health, this dose nearly was 317 mg. Pregnant women who living in region with high content of nitrate and nitrite may lead to effect on their fetus[7]. Chronic uses of nitrate at low level in food products may cause mental depression and headache[8]. Nitrites may increase methemoglobinemia, anemia, the risk of goiter and carcinogenic nitrosamines in human[9]. Meat products supply 98% of nitrites while the vegetable products supply 94-98% of nitrates especially potato and cabbage which contained around 32% and 24% respectively[10,11]. Selenium is an antioxidant which protects the body from aging and keeps tissues youthfully elastic[12]. Selenium has been found to be needed by body to from glutathione peroxidase and protects the body from oxidative damage by super oxides and hydrogen peroxide[13]. Epidemiological studies have reported an inverse association between selenium in blood and cancer occurrence and animal studies have reported the relation between selenium and the reduction of several tumor types[14]. Presence of selenium in food prevents nitrosation and rapidly reduces nitrite's so, it can prevent from endogenous nitroamines formation[15]. The present study was designed to evaluate the role of selenium on nutritional, immunological parameters and detoxifying enzyme activities in rats.

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MATERIAL AND METHODS

Materials: Potassium nitrate, sodium nitrite and sodium selenite were obtained from EL-Naser Pharmaceutical Chemical Company, Cairo, Egypt. Normal albino rats, their weight were between 95-100g, were obtained from International Research Center, Dokky, Cairo, Egypt.

Methods: Experimental animal design: A total of 30 male rats, weighing between 95-100 g were housed in cages under hygiene conditions in the biological laboratory of Research, Institute of Ophthalmology Medical Analysis Dep., Giza, Egypt. Rats were divided into 5 groups, each group contained 6 rats as following:

- Group (1) was fed on basal diet as a control group.
- Group (2) was fed on basal diet containing 6 g potassium nitrate / kg diet.
- Group (3) was fed on basal diet containing 6 g nitrate potassium/kg diet and 100 mg sodium selenite / kg body weight.
- Group (4) received basal diet containing 2g sodium nitrite /kg diet.
- Group (5) received basal diet containing 2g sodium nitrite /kg diet and 100 mg sodium selenite / kg body weight.

Preparation of diet: The basal diet consisted of protein 13%, fat 7%, vitamin mixture (1%), salt mixture (4%), fiber (cellulose) (5%), and the remaining was starch choline chloride (0.2%) according to Reeves et al.,[16]; Hegested,[17] and[18].

Nutritional parameters: Body weight and food intake were measured once a week of the experimental period (2 months). Feed efficiency ratio was calculated according to Hsu et al.[19]. At the end of the experiment, rats were anesthetized and scarified. Blood samples were collected from portal veins. Organs such as liver and kidney were rapidly excised and part of the organs was stored frozen in liquid nitrogen container for assay of glutathione (GSH) and anti-oxidant/detoxifying enzymes.

Analytical methods: Blood samples were centrifuged and serum was separated to estimate some biochemical parameters. Detection of IgG and IgM by direct ELISA, according to Engvall and Perlman.[20] Liver glutathione content was determined by the method of Weiss et al.[23]. Hepatic glutathione-S-transferase (E.C.2.5.1.18, GST) activity was determined by the procedure of Habig et al.[24]. g-glutamyl transpep-tidase (E.C.3.3.2, GGT) in kidney was estimated by the method of Meister et al.[25].

Histopathological study: On the last day of the experiment, rats were anesthetized then liver and kidney were removed and kept in 10% formaldehyde. Dehydration and clearing of the tissues were formed automatically. The prepared 5-micron thickness sections were stained with hematoxilin and eosin (H/E) and Gomeri aldehyde-fuchsin (GAF) according to Gomeri.[26]

Statistical analysis: Data obtained from this study was analyzed statistically for standard error and Significant differences between treatment means were determined using Duncan’s multiple range tests[27].

RESULTS AND DISCUSSIONS

Nutritional parameters: Effects of potassium nitrate and sodium nitrite with and without sodium selenite on nutritional parameters were studied. The parameters included food intake, body weight gain and feed efficiency ratio.

From data in table (1), it could be observed both of the nitrate and nitrite caused high decrease of food intake, body weight gain and feed efficiency ratio, compared with control group. Addition of sodium selenite to nitrate and nitrite in the diet lead to increase food intake, body weight gain and feed efficiency ratio of rats.

The highest values of food intake and body weight gain is observed in control group followed by groups 3 and 5 which fed on the basal diet containing 6 g nitrate/kg diet and 100 mg selenite sodium / kg body weight and the group fed on basal diet containing 2g/kg diet and 100 mg selenite sodium/ kg body weight. The lowest value of food intake and body weight are noticed in group which fed on basal diet containing 2g nitrite /kg diet. Feed efficiency ratio is changed and improved as a result of selenite sodium addition.

Biochemical parameters: Immunoglobulins production: Effect of adding selenite sodium at the level of 100 mg/kg bodyweight on the IgG and IgM production was studied. IgG and IgM of control group were higher than that of all the tested groups. The values were 213±1.03 and 106±1.15 mg/l for IgG and IgM respectively. Adding selenite sodium to rats diet which feed on nitrite and nitrate compounds lead to significant increase values of
immunoglobulins production more than rats received selenite sodium free diets containing nitrite and nitrate compounds. The decreasing of IgG and IgM were increased with diet which containing nitrite and nitrate. The lowest IgG and IgM values were observed in group of diet containing 2% nitrite. The relative values were increased by adding selenite sodium but still lower than control group (100%).

**Glutathione and Bio-transformation Enzymes in Liver and Kidney:** Glutathione (GSH) level in control group was the highest recorded value in groups 2 and 4 which fed on basal diet containing nitrate and nitrite (Table 3). In groups 3 and 5, the values of the glutathione were increased with adding selenite sodium when compared with the groups of selenite sodium-free diet.

**Table 1:** Effects of nitrite and nitrate salts with and without selenite sodium on food intake, weight gain and feed efficiency (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Food intake</th>
<th>Body weight gain</th>
<th>Feed efficiency ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (1)</td>
<td></td>
<td>13.14</td>
<td>47.90±0.98</td>
<td>0.093</td>
</tr>
<tr>
<td>Basal diet + 6 g nitrate / kg diet group (2)</td>
<td></td>
<td>9.35</td>
<td>**29.54±1.42</td>
<td>0.083</td>
</tr>
<tr>
<td>Basal diet + 6 g nitrate / kg diet + 100 mg Selenite sodium / Kg b w group (3)</td>
<td></td>
<td>11.62</td>
<td>40.24±0.56</td>
<td>0.089</td>
</tr>
<tr>
<td>Basal diet + 2 g nitrite / kg diet group (4)</td>
<td></td>
<td>7.47</td>
<td>**21.14±0.89</td>
<td>0.081</td>
</tr>
<tr>
<td>Basal diet + 2 g nitrite / kg diet + 100 mg Selenite sodium / Kg b w group (5)</td>
<td></td>
<td>10.89</td>
<td>32.75±1.06</td>
<td>0.086</td>
</tr>
</tbody>
</table>

**Table 2:** Effects of nitrite and nitrate salts with and without selenite sodium on Immunoglobulins production (Mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>IgG mg/l</th>
<th>Relative value (%)</th>
<th>IgM mg/l</th>
<th>Relative value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (1)</td>
<td></td>
<td>213±1.03</td>
<td>100</td>
<td>106±1.15</td>
<td>100</td>
</tr>
<tr>
<td>Basal diet + 6 g nitrate / kg diet group (2)</td>
<td></td>
<td>**151±2.19</td>
<td>71.08</td>
<td>**73.4±0.08</td>
<td>69.24</td>
</tr>
<tr>
<td>Basal diet + 6 g nitrate / kg diet + 100 mg Selenite sodium / Kg b w group (3)</td>
<td></td>
<td>198±1.55</td>
<td>92.96</td>
<td>89.25±1.64</td>
<td>84.20</td>
</tr>
<tr>
<td>Basal diet + 2 g nitrite / kg diet group (4)</td>
<td></td>
<td>**142±2.01</td>
<td>66.70</td>
<td>**60.01±2.16</td>
<td>56.61</td>
</tr>
<tr>
<td>Basal diet + 2 g nitrite / kg diet + 100 mg Selenite sodium / Kg b w group (5)</td>
<td></td>
<td>*188±1.61</td>
<td>88.26</td>
<td>86.53±1.90</td>
<td>81.63</td>
</tr>
</tbody>
</table>

* high significant p<0.001 ** very significant p<0.001

**Table 3:** Level of glutathione and antioxidant and detoxifying enzymes in liver and kidney of rats fed control or nitrate and nitrite salts diets with or without treatment of selenite sodium

<table>
<thead>
<tr>
<th>Diets</th>
<th>GSHP</th>
<th>Catalase x10⁴</th>
<th>GSSH</th>
<th>GSSGR+</th>
<th>GST++</th>
<th>GGT³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (1)</td>
<td>2.62±0.23</td>
<td>37.14±0.19</td>
<td>0.38±0.14</td>
<td>0.53±1.13</td>
<td>24.21±1.02</td>
<td>0.58±1.63</td>
</tr>
<tr>
<td>Basal diet + 6 g nitrate / kg diet group (2)</td>
<td>2.41±0.26</td>
<td>**20.68±2.05</td>
<td>*0.29±0.43</td>
<td>0.49±1.82</td>
<td>21.79±3.71</td>
<td>**2.02±0.38</td>
</tr>
<tr>
<td>Basal diet + 6 g nitrate / kg diet + 100 mg Selenite sodium / Kg b w group (3)</td>
<td>2.54±0.85</td>
<td>34.07±3.11</td>
<td>0.34±1.03</td>
<td>0.54±2.08</td>
<td>25.38±2.72</td>
<td>**0.67±0.54</td>
</tr>
</tbody>
</table>

**Catalase activity in liver of rats fed on control diet with nitrite was drastically reduced to less than half of the value obtained of control animals followed by group fed on nitrate. Values of Catalase activity in liver for groups fed on basal diet containing 6 g nitrate/kg diet and 100 mg selenite sodium / kg body weight and the group fed on basal diet containing 2g/kg diet and 100 mg selenite sodium / kg body weight were higher than rat groups fed on selenite sodium-free diet.**

Glutathione peroxidase (GSHP) activity liver was significantly reduced (P<0.001) in groups were fed on
the basal diet with nitrite and nitrate. While, it was increased in control, nitrite and nitrate containing diet groups supplemented with selenite sodium. The activity of glutathione reductase (GSSGR) was increased in rats fed selenite sodium containing diet, compared with those of diet with nitrite and nitrate. Glutathione-S-transferase (GST) activity was found to increase in selenite sodium diet group (3) when compared to the control.

Gamma glutamyl transpeptidase (GGT) activity in kidney was found to be elevated in nitrite and nitrate treatment. Thus, nitrite and nitrate treatment to control rats is associated with increasing GGT level nearly four folds; whereas nitrite and nitrate treatment to selenite sodium diet group increased the values only one or two fold indicating effect of selenite sodium absorption.

**Fig. 1:** Histological examination of liver tissues of rats fed on control or nitrate and nitrite compounds diets with or without treatment of selenite sodium.
Histopathological study: No change in normal histological pattern of liver (group 1) and kidney (group 1) tissues were noted changing under both of nitrate and nitrite (groups 2 and 4). Appearances of intercellular spaces and increase in the number of cytoplasmic vacuoles and eosinophilic granules were noted in the liver cells of nitrate and nitrite treated rats. The nuclei showed diffused chormatin bodies under the experimental conditions. After supplementation of selenite sodium at the level 100 mg per kg body weight (groups 3 and 5), the intercellular spaces disappeared and the cytoplasmic vacuoles and eosinophilic granules became less abundant, but the nuclei still showed diffused chromatin bodies. In case of kidney tissues under nitrate and nitrite toxic (groups 2 and 4), there was an increase in the space between glomerulus and the capsule in the nephrons. Occasional hemorrhages in the kidney tissues were also noted under these conditions. There were an increase in the eosinophilic granules. Supplementation with selenite sodium (groups 3 and 5) to the toxic groups of rats could reverse these defects significantly.
Discussions: Selenium is a trace element distributed in the human body and especially concentrated in the renal cortex. It is an antioxidant which protects the body from premature aging and keep the tissues healthy\cite{12,13}. Nitrate and nitrite ions are both highly soluble in water. These ions are considered as a part of the diet and also produced from nitric oxide. When taken with food, they are readily absorbed from the small intestine\cite{28}. Nitrate salt is partially converted to nitrite by oral bacteria and by stomach acids, helping to reduce gastrointestinal tract infection\cite{14}. Nitrate is in high concentration in drinking water and meat products which induced methemoglobinemia in infinite. It has been implicated in the formation of methemoglobin and carcinogenic nitrosamine in humans \cite{29}.

Food intake of rats in groups 2 and 4 was decreased when compared with the control group, the decreasing in food intake is about 28.84% in nitrate group while it is 43.27% in nitrite group. Adding selenite sodium to basal diet of groups 3 and 5 lead to increase the food intake by about 17.27% for nitrate and 21.64% for nitrite. Body weight gain in control group have the highest value (47.50±0.98) followed with group 3 (40.24±0.56) and then group with (5) 32.75±1.06. Increased rate in body weight gain for rats in these groups may be attributed to increase of food intake and improvement in feed ratio efficiency. Presence of nitrate and nitrite in the diet of rats results in a significant decrease in food intake, body weight gain and feed ratio efficiency. This decrease is due to deleterious effect of nitrate and nitrite formation of nitrosamine and inhibitory effect of nitrite on digestive enzyme. Our results were in agreement with the results obtained by Hassan\cite{2} and Majek et al.\cite{5} who concluded that increasing amount of nitrate and nitrite in the diet decrease the food intake, body weight gain and the utilization of beta carotene which leads to decrease of vitamin A level in liver (60 and 80%).

Immunoglobulins are specific groups of glucoprotein and produced by one type of immune cells. In an important immune response in the body. This helps to prevent and control infections\cite{30}. Rats fed on diet containing nitrate and nitrite at high dose have had lower values of IgG and IgM when compared with the control group. It can be observed that there is a significant increase in IgG and IgM values in rats that received selenite sodium (groups 3 and 5) when compared with groups (2 and 4) which fed on the same diet without selenite sodium. These results are completely agreement with Kolb et al.\cite{15} who stated that antibody production of all B cell hybridoma is significantly suppressed by addition of nitrite. Also IgG and IgM were decreased at 42.3 and 59.6% in children who living in a region having a high content of nitrate in drinking water. Kolb et al.\cite{15} reported that nitrosamine formation can reliably be prevented by selenite sodium.

The purpose of this study was to examine the modulation of the detoxifying enzymes in rats fed on diet with nitrate and nitrite in high dose in rats fed on the same diet with addition of selenite sodium. It can be observed that, addition of nitrite and nitrate to the diet of rats resulted in a significant decrease in glutathione, Catalase activity in the liver, Glutathione peroxidase (GSHP) and increase in Gamma glutamyl transpeptidase (GGT) in the kidney of groups (2 and 4) when compared with control group. These changes may be attributed to the harmful effect of nitrate nitrosamine formation and the damage in liver cells. Presence of selenite sodium in food prevent nitrosamine and the decreasing of important enzymes in liver and kidney which prevent the man from cancer\cite{10}.

GSH represents an important defense mechanism in protecting cells against oxygen free radicals. Its presence in excess in vivo could scavenge the electrophilic moieties produced by xenobiotics by conjugation to less toxic products\cite{32}. The present study revealed that addition of nitrate and nitrite lead to decrease the hepatic GSH but adding selenite sodium to the same diet elevated the hepatic GSH. The increase of GSH by selenite sodium diet could be described as the result of modulation of several enzyme systems\cite{33}. The major responsibility for cellular protection against oxygen mediated toxicity rests on glutathione peroxidase/ reductase redox cycle enzymes, catalase and superoxide dismutase\cite{34}. There was a significant increase in the GSSGR activity on selenite sodium diet as a result of increasing in GSH on selenite sodium diet. The absence of decrease in hepatic GSH level on nitrate and nitrite treatment was also possible due to the reaction of the reactive intermediates of the carcinogen with GSH being at slow rates insufficient to deplete the pool of glutathione in the liver.

Enhancement of GST activity has been shown to increase the ability for detoxification of some carcinogens\cite{35}. Presence of an inhibitory effect on this enzyme as well as catalase by continuous feeding of selenite sodium diet after nitrate and nitrite treatment shows a protective effect of selenite sodium. Feeding of higher levels of selenite sodium or food rich in selenite sodium has been reported by some workers to result in enhanced GST activity in rats\cite{36}.

Gamma glutamyl transpeptidase in cells can be considered a marker enzyme of preneoplastic lesions. Who has recognized elevated GGT in liver cells (determined by employing a histochemical technique as GGT positive foci) as a valid in vivo test of preneoplasia in short-term animal experimentation in animals such as rat\cite{37}. In our study, nitrate and nitrite
treatment showed a more than two fold increase in GGT level in kidney indicating possible preneoplastic changes. The elevated level of GGT is attributed to the exposure of rats to the chemical carcinogen, nitrate and nitrite. On the other hand, feeding on diet containing the nitrate and nitrite with a high level of selenite sodium lead to a significant decline in GGT levels, which is attributed to the selenite sodium content of the diet. Thus, incorporation of selenite sodium with nitrate and nitrite in the diet elicited a beneficial effect. [35]. So, the administration of high dose selenium is effective in practically reversing some of the alterations produced by nitrate and nitrite either at the enzymatic and histological properties of the tested liver and kidney.

Conclusion: The present study, concluded that high dose intake of selenium 100 mg / kg bw result in decrease the toxic effect of nitrate and nitrite compounds on the nutritional parameters (BWG, FI and FER), Immunoglobulins production ( IgG and IgM) and Hepatic glutathione and bio-transformation enzymes in liver and kidney. So, it should be added high level of selenium 100 mg/kg body weight to food as meat products which had high content of nitrite and nitrate compounds.

REFERENCES


