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Toxicologic Interaction of Potassium Bromate and *Allium cepa*, *Allium sativum* or Sodium Selenite in Wistar Rats

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Abstract: Although diets containing mixtures of 600 mg kg^{-1} of $KBrO_3$ plus 2% *Allium sativum*, 600 mg kg^{-1} $KBrO_3$ plus 2% *Allium cepa* and 600 mg kg^{-1} of $KBrO_3$ plus 1 mg kg^{-1} sodium selenite did not adversely affect the growth of Wistar rats, a significant decrease in body weight gain, hepatonephropathy, desquamation of the intestinal epithelium into the lumen and lymphocytic accumulation in vital organs were observed in the rats fed a diet consisting of 600 mg kg^{-1} of $KBrO_3$ singly for 4 weeks. These changes associated with macrocytic normochromic anemia were correlated with alterations in serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities and concentrations of cholesterol, urea and other serum constituents. However, none of the rats died during the 4-week period.

Key words: *Allium cepa*, *Allium sativum*, selenite, antihepatonephrotoxic activity

INTRODUCTION

The toxicity to rats of potassium bromate given in drinking water at 600 mg L^{-1} or higher for seven weeks is characterized by significant inhibition of body weight gain and hepatonephropathy (Kurokawa *et al*., 1990). Renal cell tumors were induced in female B6C3F1 mice and male F344 rats by potassium bromate in concentrations of 250 or 500 mg L^{-1} in drinking water for 78 or 104 weeks (Kurokawa *et al*., 1986).

Allium cepa, Onion (Liliaceae) contains organic sulphur compounds (Fenwick and Hanley, 1985) and is used in African traditional medicine as antiseptic, antispasmodic, antihelmenthic, carminative, expectorant and diuretic and for the treatment of diabetes mellitus and skin afflictions (Abdel Gadir *et al*., 2006).

Allium sativum, Garlic (Liliaceae)contains allicin, a hypotensive diallyl disulphide oxide and is used in local traditional medicine as bacteriostatic and antidiabetic agents (Bakhiet and Adam, 1995).

Selenium is one of antioxidants that help limit the oxidation of LDL cholesterol and thereby help to prevent coronary heart disease (Ozer *et al*., 1995). Daily selenium supplements played a role in immune system, reduced the occurrence of prostatic, colonrectal, pulmonary and other cancers and protected against aflatoxicosis (Dafalla, 1985; Corvilain *et al*., 1993; Combs *et al*., 1997).

The open literature contains no studies on the interaction of potassium bromate and *A. cepa*, *A. sativum* or sodium selenite in rodents, birds and livestock. The choice of inclusion in the diets of 2% *A. cepa*, 2% *A. sativum* and 1ppm selenite is based on a previous study in Wistar rats (Abdel Gadir *et al*., 2006).The present study was, therefore, undertaken to investigate the combined effect of low dietary inclusions of these substances and potassium bromate on the growth, pathological, biochemical and hematological parameters of Wistar rats.

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MATERIALS AND METHODS

Study Design

Sixty four 2-month-old male Wistar rats were housed within the premises of the Medicinal and Aromatic plants Research Institute, National Center for Research, Khartoum under light/dark cycle with feed and water provided *ad libitum*.

The rats were randomly allotted to 8 groups, each of 8 rats. Group 1 was fed the normal diet and served as control. Groups 2 and 3 received diets containing 600 mg kg^{-1} of potassium bromate (BDH, England) and 2% (w/w) of garlic, respectively. Groups 4 and 5 received diets containing 2% (w/w) Onion and 1ppm sodium selenite, respectively. Groups 6, 7 and 8 received diets that had mixture of 600 mg kg^{-1} of potassium bromate plus 2% (w/w) garlic, 600 mg kg^{-1} of potassium bromate plus 2% (w/w) Onion and 600 mg kg^{-1} of potassium bromate plus 1 ppm sodium selenite. All rats were fed their designated experimental diets for 4 weeks.

Average body weight and body weight gain were estimated weekly for each group. After 4 weeks of treatments, rats were killed under diethyl ether anesthesia. Blood samples were collected from each of the killed rats for serum chemistry and hematology.

Blood Analyses

Serum samples were analysed for the activities of aspartate Aminotransferase (AST), alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) and for concentrations of total protein, albumin, globulin, cholesterol and urea.

Hemoglobin (Hb) concentration, Red Blood Cell (RBC) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and White Blood Cell (WBC) counts were determined by standard methods (Schalm *et al*., 1975).

Pathologic Examinations

Post-mortem findings were recorded for all rats and specimens of liver, kidneys, heart, spleen and intestines were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 6 μm and stained with hematoxylin and eosin (H and E) for histopathologic examination.

Statistical Analysis

The significance of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

RESULTS

Effect on Growth

The rats fed a diet containing 600 mg kg^{-1} of $KBrO_3$ (Group 2) had the lowest ($p<0.05$) growth while the rats on diets containing mixture of 600 mg kg^{-1} of $KBrO_3$ plus 2% *A. sativum* (Group 6), 600 mg kg^{-1} of $KBrO_3$ plus 2% *A. cepa* (Group 7) and 600 mg kg^{-1} of $KBrO_3$ plus 1ppm sodium selenite (Group 8) as well as the control (Group 1) had the highest ($p<0.05$) growth (Table 1).

Pathological Changes

Four weeks after beginning the test feeding, no significant changes were observed in the tissues of the rats in Groups 3, 4 and 5 compared with the control rats (Group 1). On the other hand, renal tubular cell degeneration and necrosis (Fig. 1), hepatocellular fatty vacuolation and focal necrosis with lymphocytic accumulation between the cardiac muscle fibers, focal desquamation of the intestinal

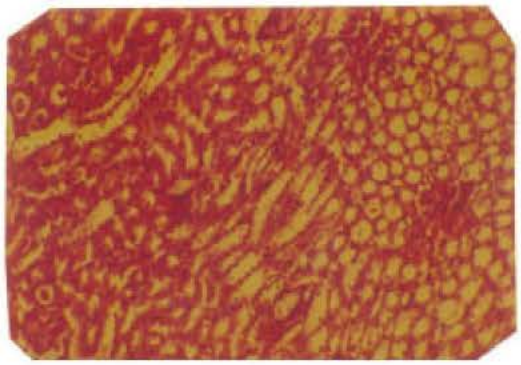

Fig. 1: Renal tubular cell necrosis and congestion in a rat fed a diet containing 600 mg kg^{-1} of $KbrO_3$ for 4 weeks. H and E x 100

Table 1: Growth changes in rats fed potassium bromate alone or combined with *A. sativum, A. cepa* or sodium selenite for 4 weeks

Groups	Body weight (g)	Body weight gain (g)
1	107.6±4.1^{a}	42±2^{a}
2	81.3±2.9^{b}	20±1.2^{c}
3	98.7±3.6^{a}	34.6±2.3^{b}
4	98.7±3.6^{a}	34.6±2.3^{b}
5	98.7±3.6^{a}	34.6±2.3^{b}
6	104±3.7^{a}	44.2±1.4^{a}
7	99.4±3.7^{a}	40.3±1.7^{a}
8	100.3±3.7^{a}	40.3±1.5^{a}

Values are means±SE, Means within columns with no common letter(s) are significantly different ($p<0.05$)

Table 2: Serobiochemical changes in rats fed potassium bromate alone or combined with *A.sativum, A. cepa* or selenite for 4 weeks

	Groups							
Parameters	1	2	3	4	5	6	7	8
AST (IU)	10±1.5^{b}	25±2.1^{a}	21±1.6^{a}	21±2.2^{a}	21±2.2^{a}	15±1.9^{ab}	11±1.5^{b}	21±1.6^{a}
ALT (IU)	5±0.4^{c}	18±1.9^{ab}	20±1.9^{ab}	20±1.9^{ab}	19±1.8^{ab}	27±2^{a}	12±1.2^{b}	17±1.4^{ab}
ALP (IU)	393.8±6^{a}	277±3.3^{ab}	191.6±4.9^{bc}	46.5±2.4^{c}	173.2±4.3^{bc}	230±4.1^{b}	230±3.1^{b}	200.4±4.9^{bc}
Total protein ($g\ dL^{-1}$)	9.3±0.7^{a}	9.3±0.8^{a}	8.8±0.6^{a}	8.2±1.2^{a}	8.04±1.2^{a}	7.4±0.8^{b}	7.4±0.8^{b}	9.2±0.9^{a}
Albumin ($g\ dL^{-1}$)	3.4±0.2^{a}	3.4±0.3^{a}	2.9±0.6^{ab}	3.4±0.8^{a}	3.01±0.8^{a}	3.3±0.3^{a}	3.3±0.2^{a}	2.6±0.4^{b}
Globulin ($g\ dL^{-1}$)	5.9±0.4^{ab}	5.9±0.3^{ab}	5.9±0.5^{ab}	4.8±0.9^{ab}	5.03±0.9^{ab}	4.1±0.5^{b}	4.1±0.2^{b}	6.6±0.5^{a}
Cholesterol ($mg\ dL^{-1}$)	78.4±3.1^{b}	107.5±4.5^{ab}	75.4±1.9^{b}	114.7±3.2^{ab}	89.98±3.2^{b}	149.5±5.1^{a}	149.5±5.1^{a}	82.9±3.9^{b}
Urea ($mg\ dL^{-1}$)	8.5±1.9^{b}	11.7±1.1^{b}	4.9±0.9^{c}	10.2±1.9^{b}	21.1±1.3^{ab}	47.9±2.1^{a}	47.9±2^{a}	27.4±2^{ab}

Values are means±SE, Means within rows with no common letter(s) are significantly different ($p<0.05$); 1: Control; 2: $KBrO_3$ 600 mg kg^{-1}; 3: Garlic 2%; 4: Onion 2%; 5: Selenite 1 ppm; 6: $KBrO_3$+Garlic 600 mg kg^{-1} + 2%; 7: $KBrO_3$+Onion 600 mg kg^{-1}+2%; 8: $KBrO_3$+Selenite 600 mg kg^{-1}+1 ppm

epithelium into the lumen and congestion of the splenic red pulp were observed in the rats of Group 2. In the rats fed a diet containing 600 mg kg^{-1} $KBrO_3$ plus 2% *A. sativum* (Group 6), the epithelial cells of the proximal convoluted tubules showed mild degeneration or individual cell necrosis. The hepatocytes were normal but the kidney convoluted tubules were slightly affected in rats of group 7 as compared with those of group 2. In the rats fed mixture of 600 mg kg^{-1} $KBrO_3$ plus 1ppm selenite (Group 8), the lesions in vital organs were less marked. No significant changes were detected in the control rats (Group 1).

Table 3: Hematological changes in rats fed potassium bromate alone or combined with *A. sativum, A. cepa* or selenite for 4 weeks

	Groups							
Parameters	1	2	3	4	5	6	7	8
Hb (g dL^{-1})	9.2±0.9^{a}	10.5±1^{a}	9±0.8^{a}	12.6±0.9^{a}	12.6±0.9^{a}	10.3±9.1^{a}	12.3±1^{a}	11.16±1.1^{a}
RBC ($\times10^{6}$ mm)	5.4±0.5^{a}	4.24±0.2^{b}	5.2±0.3^{a}	3.6±0.7^{b}	3.9±0.8^{b}	3.96±0.4^{b}	3.4±0.1^{b}	4.25±0.3^{b}
PCV (%)	29±0.3^{ab}	32±0.3^{a}	27±0.6^{b}	24.6±1.3^{b}	38±1.2^{a}	31.6±0.6^{a}	37±0.2^{a}	33.5±0.3^{a}
MCV (m^{3})	53.7±2.1^{c}	75.5±2.5^{ab}	51.9±3.1^{c}	68.3±2.3^{b}	97.4±2.3^{a}	79.9±3.9^{ab}	108±4.2^{a}	78.8±3.4^{ab}
MCH (pg)	17.03±1.3^{b}	24.8±1.2^{ab}	17.3±1.3^{b}	35±2.3^{a}	32.3±1.9^{a}	26.6±1.3^{ab}	36±1.2^{a}	26.3±0.9^{ab}
MCHC (%)	31.7±1.7^{b}	32.8±1.2^{b}	33.3±1.4^{b}	51.2±3.3^{a}	33.2±1.7^{b}	33.3±1.3^{b}	33±1.2^{b}	33.3±1.4^{b}
WBC ($\times10^{3}$ mm)	6.15±1.2^{a}	7.3±1.3^{a}	4.5±0.9^{b}	5.9±0.7^{a}	4.7±0.6^{b}	6.4±0.9^{a}	7.3±1.3^{a}	6.2±1.2^{a}

Values are means±SE, Means within rows with no common letter(s) are significantly different ($p<0.05$) 1: Control; 2: $KBrO_3$ 600 mg kg^{-1}; 3: Garlic 2%; 4: Onion 2%; 5: Selenite 1 ppm; 6: $KBrO_3$+Garlic 600 mg kg^{-1}+2%; 7: $KBrO_3$+Onion 600 mg kg^{-1}+2%; 8: $KBrO_3$+Selenite 600 mg kg^{-1}+1 ppm

Serobiochemical Changes

In the rats on 600 mg kg^{-1} $KBrO_3$ (Group 2), 2% *A. sativum* (Group 3) 2% *A. cepa* (Group 4), 1 ppm selenite (Group 5) or combination 0f 600 mg kg^{-1} $KBrO_3$ plus 2% *A. sativum* (Group 6), 600 mg kg^{-1} $KBrO_3$ plus 2% *A. cepa* (Group 7) or 600 mg kg^{-1} $KBrO_3$ plus 1ppm selenite (Group 8), there were decreases in ALP activity and increases in AST and ALT activities. The concentration of cholesterol was higher ($p<0.05$) in groups 2, 4, 6 and 7 and lower ($p<0.05$) in groups 3, 5 and 8 than control. Hypoprotinemia was only observed in Groups 6 and 7 and urea concentration was found to increase in Groups 2, 4, 5, 6, 7 and 8 (Table 2).

Hematological Changes

The values of RBC was lower ($p<0.05$) in groups 2, 4, 5, 6 and 7 and those of PCV were lower ($p<0.05$) in Groups 3 and 4. The values of MCV increased in groups 2, 4, 5, 6, 7 and 8 but neither Hb nor MCHC values changed. The values of WBC only decreased in Groups 3 and 5 (Table 3).

DISCUSSION

There were no differences in mean body weight gains between the groups of rats for the 2-week pretrial period. This may be explained by the feeding of identical diets to each group and the useful randomized assignment for examination. The results of the present study indicate that feeding rats with *A. cepa* and *A. sativum* at 2% of the normal diet for 4 weeks is not toxic as evidenced by the absence of mortality, of clinical changes and of significant lesions in the vital organs. It seems, therefore, that the susceptibility of animals to feeding with plant materials is dependent, at least, on the type and concentration of the active constituents in the amount added to the diet as well as the rate of metabolic conversion in the liver to metabolites and consequent excretion (Abdel Gadir *et al*., 2006).

Despite the therapeutic uses of the two plants in traditional medicine their toxicity in rats or other animals has not been evaluated.

It is clear from the results of the present investigation that the liver and kidneys are the sensitive organs to the toxic action of $KBrO_3$.

The combination of dietary selenite at 1ppm and 600 mg kg^{-1} of $KBrO_3$ decreased the incidence and severity of damage to the vital organs and of congestive and hemorrhagic tendency produced by $KBrO_3$. Dafalla (1985) found that the combination of dietary selenite at 3 ppm and aflatoxin B_1 at 0.5 ppm increased the incidence and severity of hemorrhage on the thigh of chicks and that hemorrhage can persist on the thigh for 3 weeks after removal of the experimental diet. Similar findings were described in Hubbard chicks fed a combination of dietary aflatoxin B_1 and ochratoxin A (Huff *et al*., 1983).

Potassium bromate which is used to strengthen bread dough was found to cause cancer of the kidneys, thyroid gland and other organs of rats and mice and was consequently banned in many countries like the United Kingdom, Canada and California (Umemura *et al*., 1995). The absence of hypoproteinemia in rats fed a diet containing 600 mg kg^{-1} $KBrO_3$ could be due to hemoconcentration arising from slight fluid loss. Indeed, feeding $KBrO_3$ singly showed desquamation of the intestinal epithelium into the lumen. In this study, the incorporation of 600 mg kg^{-1} $KBrO_3$ in the diet causes anemia. The increase of MCV without effects on MCHC indicate macrocytic normochromic anemia. It has been found that ascorbic acid is technically feasible to replace 80 ppm of $KBrO_3$ for 20 ppm of ascorbic acid (Corrals *et al*., 1993) and Sai *et al*. (1992) found that glutathione plays an essential protective role against renal oxidative DNA damage and nephrotoxicity caused by $KBrO_3$ in rats. Khan *et al*. (2003) suggested that *Nigella sativa*, Black Cumin, is a strong chemoprotective agent and may suppress $KBrO_3$ -mediated renal oxidative stress, toxicity and tumor promotion response in rats.

The data of the present study suggest that the incorporation of 2% *A. cepa*, 2% *A. sativum* or 1 ppm selenite in the diet is chemoprotective and suppresses the development of hepatonephrotoxicity induced by $KBrO_3$ as evidenced by the reduction or absence of nephrotoxicity and hepatotoxicity, as well as improvement of growth, pathological, serobiochemical and hematological parameters. However, the increase in serum urea concentration may be attributed to delayed renal recovery.

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