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Biochemical and Histopathological Changes in Potassium Bromate- fed Rats

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Abstract: ***The present study aimed to clarify the toxic effect of potassium bromate in Wister albino rats. Eighteen 60- days-old, rats were allotted at random to three groups. The first group served as control, and the other groups received potassium bromate dietary at doses of 300 and 600mg /kg body weight for 8 weeks. The results revealed that there was no death through the period of the study of 8 weeks. There was no significant change (P<0.05) in the body weights and organs weights of all groups during the first 30 days but there was significant decrease (P<0.05) in body weights and significant increase in kidney, liver and brain weights for the group treated with 600 mg/kg of potassium bromate. There was also a significant increase (P<0.05) in the kidney weight for the group treated with 300 mg/kg body weight of potassium bromate compared with the control group. Significant increases of urea, creatinine and albumins levels beside significant decreases in Package Cell Volume (PCV), Hemoglobin(HB) and Red blood cell(RBC) were evident in groups treated with 300 and 600 mg/kg body weight of potassium bromate. Significant increases (P<0.05) ocuured in Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the group treated with 600 mg/kg body weight of potassium bromate. Histopathological examination of the group of rats treated with 600 mg/kg body weight of potassium bromate showed congestion in the liver beside marked demylenation change in brain (brain edema). In the study, no renal tumor, no changes in the heart, spleen or stomach were observed probably because of the short duration (8 weeks) of feeding.***

Keywords: Potassium Bromate, Biochemical and Histopathological Changes

1. Introduction

Potassium bromate ($KBrO_3$) is an oxidizing agent that has been used as a food additive; it has long been used to increase the volume of bread with a fine crumb structure (ABA and AIB, 2008). Potassium bromate is colorless and odorless crystals, which can be a granular or powder form and have negligible vapor pressure, soluble in water and dissociate in water to the metal and bromate ions (Merck, 1983; CDC, 2003; Health Canada, 1999). It has a melting point of 350 °C (approx.) and a decomposition temperature of 370 °C (Lewis, 1991; NTP, 1991).

The problems of potassium bromate started with ozonation of drinking water to form promate as major by-product (WHO, 1993). When research was done to confirm the safety of ozonated water, it was found that potassium bromate caused renal cancer in rats when they drank water with potassium bromate. Following this discoveries, many countries, Health organization and agencies started banning the use of potassium bromate (NAFDAC, 2003). Some of the countries in which potassium bromate has been banned include United Kingdom in 1990 and Canada in 1994. Other countries where potassium bromate has been removed from the list of permitted food additives are Belgium, Greece, Norway, Denmark, Spain, Portugal, Japan and Switzerland (NAFDAC 2003). WHO also banned the use of potassium bromate in 1993.

Despite the awareness created by NAFDAC on the danger of using potassium bromate as flour enhancer, many bakers still use the restricted substance (NAFDAC 2003).

Several researches have been carried out in different parts of the world to prove that potassium bromate is dangerous to health if consumed in food or water. It has been shown to be nephro-toxic in both man and experimental animals (Uchida et al 2006).

2. Materials and Methods

Experimental animal

Eighteen 60-day-old, clinically health male Wistar rats were allotted at random to three groups each of 6 rats. Group 1 was fed untreated diet and served as control. Potassium bromate (BDH, England) was thoroughly mixed with the normal diet and fed to rats at 300mg/kg (Group 2) and 600mg/kg (Group 3) for 60 days.

Three rats from each group were slaughtered in 30 days and another three rats from each group were slaughtered in 60 days. Specimens of the liver, intestines, kidneys, spleen, stomach, bone marrow and heart were immediately fixed in 10 % neutral buffered formalin and processed for histopathology.

Blood samples were collected at slaughter for hematology and serum analysis.

Methodology

a)Hematological parameters

The parameters measured were Hemoglobine (Hb), Packege Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), differential WBC counts and erythrocyte series, Mean cell volume (MCV), Mean cell hemoglobin (MCH) and Mean cell hemoglobin concentration (MCHC). These measurments were performed using an automatic Hematology Analyzer (Sysmex kx-21, Japan).

b) Serobiochemical parameters

The parameters measured were aspartate aminotrantransferas (AST), alanine aminotrantransferas (ALT), alkaline phosphatase (ALP) total protein, albumin, cholesterol and urea. **T**hese parameters activity was measured by a Hitachi 902 analyzer using commercial kits (Biosystem Chemicals, Barcelona, Spain). Globulin

concentration was obtained by subtracting albumin concentration from that of total protein and T-bilirubin and D- bilirubin.

a) Pathological methods
b) Statistical analysis

All obtained data were statistically analyzed by using SPSS program, 2002. Mean ± standard deviation was occurred. Also, P values (<0.05).

3. Results and Discussion

Hematological changes

The effects of various doses of potassium bromate on hematological constituents were indicated in Tables (1 and 2). Results indicated that WBC values were significantly lower in Groups 2 & 3 through the 60 days of feeding. However, PCV values were higher in group2 significantly lower in Group 3 through the 60 days. The RBC, Hb and PCV were significantly lower in Group 3 after 60 days. Potassium bromate was found to have effects on hemopoiesis at two doses of (300 and 600 mg-1 b.wt) in all periods of feeding, resulting in anaemia. Chioman et al (1988) reported the induction of methaemo- globinaemia in rats when potassium bromate was used. They also claimed that oxidation of ferrous ion to ferric by reactive species generated from potassium bromate ingestion. The study also revealed that there was no statistically significant difference in the test and control samples for the MCHC and haematocrit values. In the report of a two and half years, boy who swallowed a neutralizing solution containing potassium bromate, the only haematological finding was a change in hemoglobin concentration from 11.4g/dl to 10.7g/dl in a period of two months (Thompson and Westfall 1949).

Table 1: Hematological changes in rats fed with various levels of dietary Potassium Bromate for30 days

Parameters	Treatments		
	Group1 (Control)	Group 2 300mg/kg ($KBrO_3$	Group 3 600mg/kg ($KBrO_3$)
Hb (g/dl)	13.76 ± 0.54	13.20 ± 0.25	12.33 ± 0.84
RBC (× 10^6 mm)	9.30 ± 0.84	8.40 ± 0.09	7.05 ± 0.43
PCV (%)	62.70 ± 0.59	68.47 ± 4.07*	48.23 ± 3.53*
MCV (m^3)	73.67 ± 2.90	74.50 ± 0.29	68.40 ± 1.30
MCH (pg)	14.90 ± 0.90	17.50 ± 0.26	16.67 ± 0.12
MCHC (%)	18.90 ± 0.58	25.60 ± 0.15	21.27 ±1.08
WBC (× 10^3 mm)	7.76 ± 0.82	4.16 ±1.20*	2.10 ± 0.12*
LY	56.73 ±1.99	42.47 ± 0.43*	35.30 ±1.12*
MO	5.63 ±1.88	3.17 ± 0.03	6.33 ±1.34
GR	37.63 ± 3.02	54.37 ± 0.43*	58.37 ± 2.37*

Figures are Means ± SD
* indicates significance at $p \leq 0.05$

Table 2: Haematological changes in rats fed with various levels of dietary Potassium Bromate for60 days

Parameters	Treatments		
	Group 1 (Control)	Group 2 300mg/kg ($KBrO_3$	Group 3 600mg/kg ($KBrO_3$)
Hb (g/dl)	14.80 ± 0.57	13.53 ± 0.47	12.63 ± 0.23*
RBC (× 10^6 mm)	10.53 ± 0.67	9.91 ± 0.28	8.98 ± 0.55*
PCV (%)	60.47 ± 3.79	64.90 ± 2.18	34.90 ± 0.95*
MCV (m^3)	57.33 ± 0.33	69.50 ± 0.29	54.67 ± 1.45
MCH (pg)	14.10 ± 0.38	15.23 ± 0.57	15.47 ± 0.32
MCHC (%)	24.57 ± 0.61	20.10 ± 0.12	27.400 ± 0.06
WBC (× 10^3 mm)	11.77 ± 0.32	8.53 ± 0.06	7.17 ± 0.15*
LY	39.17 ± 11.17	54.43 ± 3.67*	69.80 ± 6.62
MO	24.10 ± 3.71	13.93 ± 1.24*	13.50 ± 4.54*
GR	36.73 ± 13.31	41.63 ± 4.58	16.70 ± 10.32*

Figures are Means ± SD
* indicates significance at $p \leq 0.05$

Serobiochemical changes

Tables (3 and 4) show the effects of various doses of Potassium bromate on seriobiochemial constituents in rats. The activities of serum AST and ALP were significantly higher in Group 2 and Group3. ALT was higher with no significant in Group2 and Group 3 within 60 days. The urea, creatinine and albumin levels significantly increased in Group3 and showed insignificant increase in Group2 after 30 days compared with the control group. However, cholesterol levels increased in Groups 2 and 3 after 30 days. Measurement of enzymic activities of AST and ALT is of clinical and toxicological importance, as changes in their activities are indicative of liver damage by toxicants or in diseased conditions (Singh et al., 2001).

The observed decrease in the activities of liver followed by concomitant increase in serum AST and ALT activities suggested that there may be a leakage of these enzymes from the liver to the serum (Hanley et al., 1986). Abdel-Tawwab et al. (2001) in separate studies described the reduction in transaminases activity to liver necrosis caused by the toxicants and possible damage to the hepatocytes. The reduction in the activities of ALT and AST in the liver may be due to the interference with protein metabolism in the cells or inhibition of the enzyme (Karmen et al., 1995). The significant increase ($p < 0.05$) in ALP activity observed in the serum of rats fed bromate-containing diet compared with the control may be attributable to the loss of membrane components due to a possible reaction between potassium bromate and the membranes of liver and kidney cells, causing leakage of the enzyme into the serum. This observation was supported by Fleischer and Schwartz (1971) who reported that any damage done to the cell membrane may lead to leakage of ALP, which is a marker enzyme in the plasma membrane into extracellular fluid. Increased activities of serum enzymes have been reported in case of tissue damage (Hanley et al., 1986). The increase in serum levels of urea and creatinine in this study are indication of renal toxicity. This is in agreement with previous study by Khan et al. (2003) who stated that 125 mg/kg b.wt. of potassium bromate given intraperitoneally to rats resulted in marked elevation of BUN and creatinine. Similar findings were also reported by Watanabe et al. (2004). El-Sokkary (2000) reported that potassium bromate caused degeneration

and necrotic change. Mack (1988) reported signs of renal failure and deafness in human at an oral dose of 185-385mg/kg b.wt. of potassium bromate beside gastrointestinal disturbances.

Table 3: Serobiochemical changes in rats fed with various levels of dietary Potassium Bromate for30 days

Parameter	*Treatment groups*		
	Group *1* *(Control)*	Group *2* *300mg/kg* *($KBrO_3$*	Group *3* *600mg/kg* *($KBrO_3$)*
AST (iu)	140.33 ± 0.89	165.00 ± 2.89*	167.50 ± 3.75*
ALT (iu)	30.00 ± 3.46	36.00 ± 3.06	39.33 ± 1.45
ALP (iu)	264.00 ± 2.37	300.00 ± 5.77*	304.50 ± 7.79*
Total protein (g/dl)	6.13 ± 0.22	6.60 ± 0.26	64.33 ± 0.29
Albumin (g/dl)	3.70 ± 0 .58	3.87 ± 0.09	3.90 ± 0.06
Globulin (g/dl)	2.43± 0.22	2.73± 0.18	2.53 ± 0.23
Cholesterol (mg/dl)	71.00 ± 2 .89	62.33 ± 3.92	65.33 ± 2.19
D.Bill	0.02 ± 0.01	0.03 ± 0.01	0.02 ±0.01
T.Bill	0.04 ± 0. 01	0.05 ± 0.00	0.03 ± 0.00
Urea (mg/dl)	42.33 ± 2.19	40.33 ± 5.84	40.00 ± 4.51
Creatin	0.80 ± 0 .06	0.60 ± 0.15	0.83 ± 0.03

Figures are Means ± SD
* indicates significance at $p \leq 0.05$

Table 4: Serobiochemical changes in rats fed with various levels of dietary Potassium Bromate for 60 days

Parameter	*Treatment groups*		
	Group *1* *(Control)*	Group *2* *300mg/kg($KBrO_3$*	Group *3* *600mg/kg* *($KBrO_3$)*
AST (iu)	140.00 ±0.05	150 .5.0 ± 2.02*	159.50 ± 0.05 *
ALT (iu)	14.00 ±1.15	16.33 ± 1.33	17.00 ± 2.31
ALP (iu)	65.00 2.89	74.50 ± 2.60	80.00 ± 5.77*
Total protein (g/dl)	7.67±0.22	7.47 ± 0.15	7.20 ± 0.30
Albumin (g/dl)	3.47 ±0.13	3.83 ± 0.03	4.167 ± 0.09*
Globulin (g/dl)	4.20 ± 0.35	3.63± 0.123	3.50 ± 0.46
Cholesterol (mg/dl)	59.67 ± 2.03	61.50 ± 2.02	63.33 ± 1.76
D.Bill	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
T.Bill	0.03 ± 0.01	0.22 ± 0.19*	0.02 ± 0.00
Urea (mg/dl)	50.00 ± 0.58	61.00 ±1.15*	63.50 ± 4.33*
Creatin	0.87 ± 0.03	.90 ± 0.000	1.01 ± 0.03*

Figures are Means ± SD
* indicates significance at $p \leq 0.05$
Iu= international units

Histopathological changes
Histopathological findings revealed congestion in the liver (Fig1) beside marked demylenation change in brain (brain edema) of rats treated with 600 mg/kg for 30 days (Fig2). In this investigation, none of dietary KBro3 concentrations has caused renal tumor, probably because of the short duration (60 days) of feeding. There was no change of heart, spleen and stomach It has been shown that $KBro_3$ induced methemoglobinemia in mice due to the reduction of glutathione peroxide activity in the blood with an increase in superoxide and nitrous oxide (Watanabe et al., 2002). In this study, methemoglobinemia was not observed in KBro3 fed rats. There was evidence that potassium bromate had an effect on the brain. The lesion in the brain indicates that potassium bromate may cross the brain barrier and exert its effects on the endothelium permeability as well as brain tissue. This may indicate that it has a neurotoxic effect.

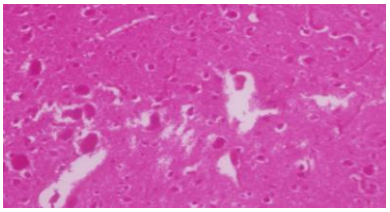

Figure 1: Brain of rats treated with 600 mg/kg Potassium Bromate. Note mild demylenation p40

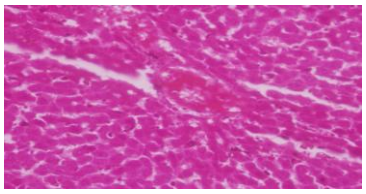

Figure 2: Liver of rats treated with 600 mg/kg Potassium Bromate. Note congestion p 40

P:power

4. Conclusion

It was clear that potassium bromate is a health risk. Effort to stop the use of potassium bromate in preparation of food should be intensified. NAFDAC should also focus its attention not only on bread, but on other bakery products in which bromated flour is used as a raw material. The physical properties of KBrO3 make it easy to be taken or administered as a poison to human, thus its use and handling should be highly regulated by the relevant authorities.

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