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EFFECTS OF POTASSIUM BROMATE ON THE KIDNEY AND HAEMATOLOGICAL PARAMETERS OF SWISS ALBINO MICE

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ABSTRACT
The present study aimed to investigate the possible effects of potassium bromate toxicity on histological, haematological and biochemical parameters in Swiss albino mice. Mice were orally administered with potassium bromate at the rate of 150 mg/kg body weight daily in a single dose for 30, 60 and 120 days. The chemical significantly reduced the RBC count ($p < 0.01$) Hb% ($p < 0.01$) and platelet count ($p < 0.01$), while it increased significantly the urea ($p < 0.01$) and creatinine level ($p < 0.01$) and decreased total protein and Albumin ($p < 0.01$) Histopathological examination showed degenerative changes of tubular cells, cytoplasma vacuolation, cellular infiltration, tubular dilation with eosinophilic debris and clear cell cytoplasm were observed. These findings suggest that $KBrO_3$ affects the physiological and biochemical activities of Swiss albino mice.

INTRODUCTION

Potassium bromate ($KBrO_3$) is used as a food additive. It is white crystalline granules having no medicinal value, but is added to flour as a maturing agent, to dough, to fish paste, as a conditioner and also to beer or cheese (Chipman *et al*., 1988). It is cheap and probably the most efficient oxidizing agent. The bromate voluminizes the centre of bread and increases the size of bread artificially, producing bread with a pure comb structure (deMan, *et al.,* 1990) Potassium bromate ($KBrO_3$; mol. wt. 167.01) is highly soluble in water. It decomposes at about 370ºC and has a melting point of 350ºC; it reacts vigorously as a strong oxidizing agent with organic materials (Kurokawa *et al*., 1990). It has an adverse effect on health. It degrades Vit A_1, B_1, B_2, E and niacin, which are the main vitamins available in the bread (FAO/WHO, 1992; PCHRD.com, 2000).

Positive results were obtained for the mutagenicty of potassium bromate in *Salmonella typhimurium* strain TA 100 using the Ames test and for chromosomal aberrations in cultured Chinese hamster fibroblast cells (Ishidate *et al*., 1984). In *vivo* study of the acute cytogenetic effect of $KBrO_3$ on rat bone marrow cells (Fujie *et al*., 1988) and in the mouse micronucleus test (Nakajima *et al*., 1989, Hayashi *et al*., 1989) liver and kidney tissue may degrade bromate to bromide by a process involving glutathione (Tanaka *et al*., 1984) although only a small amount appears to be reduced in this way (Kutom *et al*., 1990) Potassium bromate administered to groups of F344 rats in water at concentration of 0, 150, 300, 600, 5000 or 10000 mg/L for 13 weeks has inhibited body weight gain in males and significant increases in serum parameter in both sexes at 600 mg/L (Kurokawa *et al*., 1990). Carcinogenic and mutagenic effect of potassium bromate have been reported in experimental animals (Kurokawa *et al*., 1987), studies on rats and mice confirmed tumour of the kidney, thyroid and other organs (CSPI 1999). Oxidative damage appears to be the basis of bromate-induced carcinogenesis (Chipman *et al*., 2006). Potassium bromate exposed to male rats in the drinking water has induced an accumulation of U-globulin, a male rat specific urinary protein, in the kidney (Umemura *et al*., 1993).Abdominal pain, kidney failure, hearing loss, peripheral neuropathy, hypotension anaemia and also causes cancer in the experimental animal and in humans (CSPI, 1999; Watson, 2000). In acute human intoxication by accidental ingestion or attempted suicide, degeneration of kidney tubules and liver paranchymal cells and acute myocarditis were observed (Norris 1965; Paul 1966; Stewat 1969). Decreased leucocyte count has also been reported due to consumption of bromate (Hoffbrand *et al*., 2004).Male Wister rats exposed to 0.04% potassium bromate in drinking water (approximately 30mg/kg/bw per day for upto 15 months, showed increased blood urea nitrogen (BUN) and marked structural abnormalities of the cortical tubules (Nakano *et al*., 1989).The kidney is an obligatory excretory route for most drugs,so renal insufficiency results in drug accumulation and increased concentration in the tubular fluid.The most frequently encountered nephrotoxins results in acute renal failures (Price Syliva *et al*. 2003). The present study was undertaken to determine if sub of chronic and chronic toxicity of potassium bromate can affect the haematological parameters and renal tissues of Swiss albino mice.

MATERIALS AND METHODS

Male albino mice with average body weight of 25-30 grams were selected for the experimental study. The animals were housed at controlled environmental conditions 22 ± 2ºC relative humidity 50 ± 10% and 12h dark-light cycle. Animals were housed and allowed free access to food and water. All experimental procedures were conducted as per the guide lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

For subchronic and chronic toxicity studies, selected pathogen free mice were sorted and potassium bromate was administered at 150 mg/kg body weight orally per day for 30, 60 and 120 days by gavage method.

Haematological Assessment

After proper anesthetizing of the animals, blood was collected through cardiac puncture for analyzing haematological parameters and serum was extracted for biochemical assessment enumeration of total RBC; haemoglobin content was determined by the method described by Schalm *et al.,* 1975. Kidney function test urea was determined by diacetylmonoxine (DAM) method using urea kit from Span Diagnostics Ltd. (Wybenga, 1971) and creatinine by alkaline picrate method. Experimental data were expressed as mean ± SE. Anova test was performed to find whether or not scores of two groups in different durations differ significantly. Difference was considered significant, if $P<0.01$, $P<0.05$.

Histopathological Studies

Mice were sacrificed from each group for histological study. The selected organ was dissected and fixed in 10% neutral formalin fixative and tissues were processed and stained with Haematoxylin – Eosin (H and E) and examined under a light microscope.

RESULTS

Oral $KBrO_3$ – induced changes in the haematological parameters

Table (1) shows that the haematological parameters (RBC, Hb and Platelet) decreased significantly. There was 9.48%, 14.55%, 15.6% loss in RBC, while 9.55%, 14.06%, 21.10% loss in Hb content and 19.90%, 22.22%, 31.06% loss in platelet count at the end of 30 days, 60 days and 120 days respectively.

Oral $KBrO_3$ – induced changes in the biochemical parameters

Table (2) shows that the serum Urea Creatinine levels were significantly elevated ($P<0.01$) in the treated animals. The increase of urea was 31.83%, 45.48%, 53.05% and serum creatinine increase was 51%, 64.35%, 74.75%, while serum total protein and Albumin decreased significantly ($P<0.01$). The decrease of total protein was 13.24%, 16.81%, 18.52% and decrease in Albumin was 22.30%, 28.38%, 33.75% at the end of 30 days, 60 days and 120 days respectively.

DISCUSSION

The results of the present investigation suggests that subchronic and chronic dose of potassium bromate cause alterations in haematology, biochemical parameters and histopathological changes.

Results of the present investigation revealed decreased erythrocyte (RBCs) count and hemoglobin (Hb). This change induced by potassium bromate may be due to the prevention of red blood cell synthesis via inhibition of erythropoiesis in the bone marrow. Erythropoietin stimulates the bone marrow to make an adequate number of red blood cells, assuring oxygen delivery to all tissues with marked damage to the renal tissues; the production of EPO decreases. So, the bone marrow produces fewer red blood cells and anemia develops. Anemia is caused due to drug and toxic chemical injestion which are stored in tissues (Swaminathan, 2004). The lower Hb content may be due to the lesser RBC concentration in the blood. Lower Hb content is due to the insufficient supply of iron for haemoglobin synthesis (Chakravarty *et al.*, 2005).

Table 1: Effects of $KBrO_3$ on the haematological parameters (RBC, Hb and Platelet count)

DaysParameters		30 days	60 days	120 days
RBC(10^6/mm^3)	Control	10.13 ± 0.28	10.31 ± 0.23	10.32 ± 0.26
	150 mg/kg	9.17 ± 0.18**	8.81 ± 0.17**	8.71 ± 0.21**
Hb(gm/100 mL)	Control	12.87 ± 0.25	12.87 ± 0.21	13.50 ± 0.03
	150 mg/kg	11.63 ± 0.18**	10.99 ± 0.23**	10.65 ± 0.18**
Platelet(10^3/mm^3)	Control	294.29 ± 14.61	295.71 ± 16.59	294.29 ± 18.75
	150 mg/kg	235.71 10.65**	230 ± 14.14	202.86 ± 16.57**

**P<0.01

Table 2:Effects of $KBrO_3$ on the biochemical parameters (Urea, Creatinine, Total Protein and Albumin)

Parameters		30 days	60 days	120 days
Urea(mg/100 mL)	Control	22.43 ± 0.8	23.57 ± 0.80	23.71 ± 0.60
	150 mg/kg	29.57 ± 0.8**	24.29 ± 1.50**	36.29 ± 1.30**
Creatinine (mg/100 mL)	Control	1 ± 0.05	1.01 ± 0.05	1.03 ± 0.05
	150 mg/kg	1.51 ± 0.07**	1.66 ± 0.04**	1.80 ± 0.05**
Total Protein(gm/dL)	Control	6.87 ± 0.15	6.90 ± 0.15	6.91 ± 0.15
	150 mg/kg	5.76 ± 0.15	5.74 ± 0.13**	5.63 ± 0.10**
Albumin(gm/dL)	Control	3.90 ± 0.07	3.91 ± 0.01	3.94 ± 0.09
	150 mg/kg	3.03 ± 0.09**	2.80 ± 0.08	2.61 ± 0.12**

**P<0.01

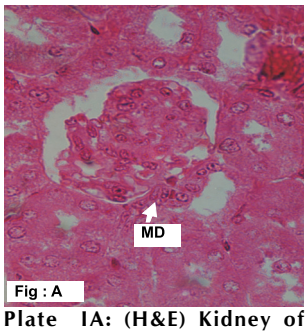


Plate IA: (H&E) Kidney of control mice showing renal cortex with well defined glomerulus (G), Bowman's capsule (BC) and macula densa (MD). X1000

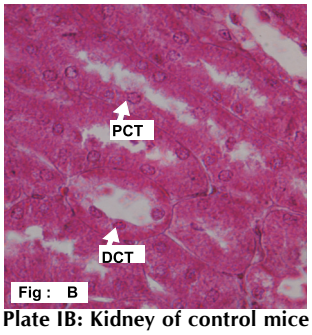


Plate IB: Kidney of control mice showing normal architecture of proximal convoluted tubule (PCT) and distal convoluted tubule (DCT). X1000

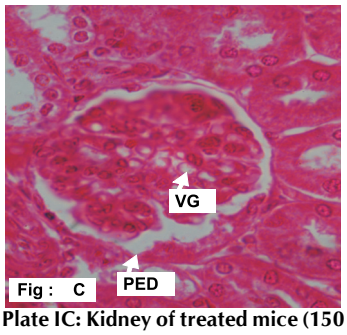


Plate IC: Kidney of treated mice (150 mg/kg bw/day $KBrO_3$ administered orally for 30 days) showing parietal epithelial cells damaged (PED) and vacuolization of glomerulus (VG). X1000

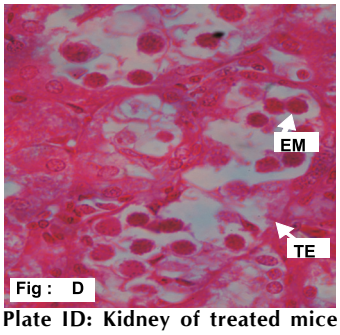


Plate ID: Kidney of treated mice (150 mg/kg bw/day $KBrO_3$ administered orally for 30 days) showing atrophic tubules with thinned epithelium (TE), eosinophilic material (EM) and degenerated cytoplasmic materials in the tubules. X1000

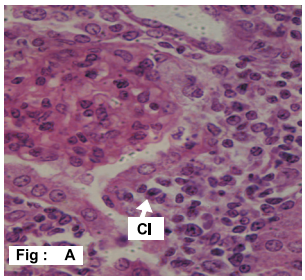
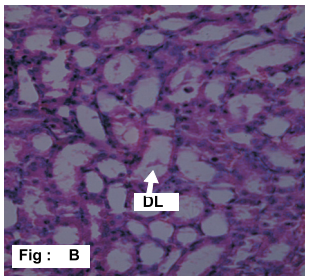


Plate IIA: (H&E) Kidney of treated mice (150 mg/kg bw/day $KBrO_3$ administered for 60 days) showing extensive cellular infiltrate (Cl) necrosis of parietal epithelium, flattening of tubular cells and cluster of nuclei observed in glomerulus. X1000



IIB: $KBrO_3$ treated (150 mg/kg bw/day administered for 60 days). Showing dilatation of lumen (DL) of the tubules, flattening of the epithelial cells with loss of brush border and degeneration of nuclei. X400

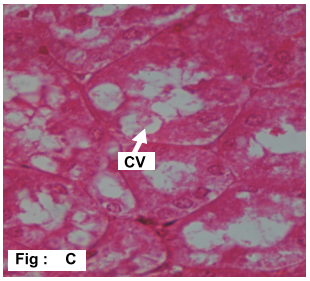
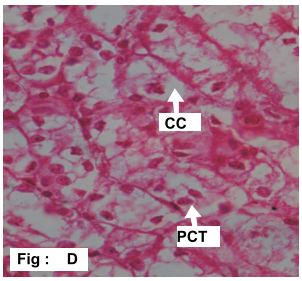


Plate IIC: $KBrO_3$ treated (150 mg/kg bw/day administered for 120 days), showing that epithelium of the tubules are ragged. In the tubular cells cytoplasmic vacuolation (CV), loss of brush border and nuclear degeneration were observed. X1000



IID: $KBrO_3$ treated (150 mg/kg bw/day administered for 120 days), showing complete cytoplasmic degeneration, eccentric nuclei and clear cells (CC) cytoplasm observed in the kidney tubules. X1000

The decrease in the platelet count is due to DNA strand breakage in these cells induced by the oxidative stress caused by potassium bromate. The elevation in serum urea and creatinine indicates its adverse effect on kidney function. This is in agreement with previous study by Khan *et al*. (2003). The decrease in total protein and albumin may be due to drop in protein synthesis which is attributed to liver damage, similar findings were observed by Khan *et al*. (2012). Our results also revealed changes in the epithelium of Bowman's capsule. Other changes observed were pycnotic nuclei and vacuolization of glomerular cells. Proximal convoluted tubule and distal tubules of nephron showed marked changes, such as, ragged tubular cells, flattened and detached epithelium and necrosis of individual tubular epithelial cells. Dilated tubules, eosinophilic debris were observed in the tubules; similar findings were recorded by Onodera *et al*. (1986). Dilatation of tubules with loss of brush border were seen Ravindra *et al*. (2010) observed that cisplatin toxicity caused marked dilation of proximal convoluted tubules with cellular debris in the tubular lumer. The clear cells appeared with no cytoplasmic staining and eccentric nucleus were seen in the tubules. Presence of inflammatory cellular infiltrates composed of lymphocytes and monocytes are seen. This result is consistent with the studies of Tahashi (1984). Cytoplasmic vacuolation observed was due to damage to subcellular organelles. Histopathological damage to the different subdivisions of the kidney is mainly due to the presence of free radicals, generated because of the oxidative stress induced by potassium bromate. The pars recta of the proximal convoluted tubule is the segment most sensitive to oxidative stress and hence most affected. These degenerative changes in the proximal convoluted tubules reinforce the views of Koechel *et al*. (1984) and Damjanov (1996) who found that many chemicals have a direct nephrotoxic action and exerted their effects principally on the proximal convoluted tubule.

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