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IMPAIRMENT OF OVARIAN BIOCHEMICAL CONTENTS AND ENZYMES ACTIVITIES DURING POTASSIUM BROMATE ($KBRO_3^-$) INTOXICATION IN ALBINO MICE *MUS MUSCULUS*

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Keywords:

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ABSTRACT: Potassium bromate ($KBrO_3^-$) is a chemical compound commonly used as a oxidizing agent that has been used as a food additive for baking of white bread, bun, pav and pizza bread. Potassium bromate ($KBrO_3^-$) is mainly known to generate free radicals, which cause oxidative damage to essential cellular macro molecules, leading to cause nephrotoxicity in experimental animals. In this part of experiment biochemical profile *i.e.* alkaline phosphatase, acid phosphatase and protein content along with ovarian histopathological changes were observed. The animals were treated with $KBrO_3^-$, 0.7 mg/g b.wt. in 0.125 ml distilled water orally through canulla upto 15 and 30 days. It has been observed significantly lowered ALP, protein and ACP level elevated. However ovarian histophathological changes were not observed after 15 days $KBrO_3^-$ treatment. Along with the ovary treated with potassium bromate upto 30 days show degenerative cells in the thecal layer and less amount of cytoplasmic was noticed. Our result conclude that in a very low amount, potassium bromate if is available in the body may pose transitory reproductive disruption and are dependent upon the dose and durations employed.

INTRODUCTION: Potassium bromate ($KBrO_3^-$) is a chemical compound commonly used as a oxidizing agent that has been used as a food additive for baking of white bread, bun, pav and pizza bread. Apart from this, it is also used in cheese production, beer making and is commonly added to fish paste products [1]. Beside this it is used in pharmaceutical and cosmetic industries and is a constituent of cold wave hair solutions [2]. In addition to it, $KBrO_3^-$ is used in the treatment of flour, treating barley in beer making and it has been used for the improvement of the quality of fish-paste products in Japan [3].

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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 nephrotoxic in both man and experimental animals [4]. Breathing potassium bromate can irritate the nose, throat and lungs causing coughing, wheezing, headache, irritability, impaired thinking and personality changes.

Many reports have documented that $KBrO_3^-$ can induce multiple organ toxicity in humans and experimental animals [5, 6, 7]. Potassium bromated has deleterious effect on CNS of mice [8]. Potassium bromate causes oxidative damage to essential cellular macro molecules, leading to marked nephrotoxicity and cancer in experimental animals [9]. Bromate was first found to cause tumors in rats in 1982. Furthermore it induces renal cell tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid [10].

Subsequent studies on rats and mice confirmed that it causes tumors of the kidney, thyroid, and other organs. However, the impact of $KBrO_3^-$ on female reproductive system especially ovary are less documented. So, in present investigation we tried to evaluate the impact of potassium bromate on ovary of female mice, *Mus musculus* by observing histopathological and biochemical changes.

MATERIAL AND METHODS:

Experimental Animals: The present experiment was performed on mature female mice, *Mus musculus* weighing 25 ± 5 gm. All animals were acclimatized to laboratory conditions *i.e.* at 22 ± 3ºC temperature and light and dark photoperiod (14L: 10D h) in the Animal House of Laboratory of Endocrinology, Bioscience Department, Barkatullah University, Bhopal (M.P.). Hygienic conditions were maintained with rice husk bedding in separate polypropylene cages. Animals were provided with standard feed and water *ad libitum.* Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No.1885/GO/S/16/CPCSEA/IAEC/ B.U./12.

Chemical: Potassium Bromate (Brand name: Bromic acid, potassium salt) were obtained from Sigma - Aldrich 02108 company through V.K. Trader M.P. Nagar, Zone-2, Bhopal, (M.P.) for this experimental study.

Preparation of Dose: A dose of 0.7 mg / 30 - 35 g b.wt / 0.125 ml distilled water was prepared.

Experimental Design: The present experiment was performed on mature female healthy Swiss albino mice (female mice, 30 - 35 gm weight) *Mus musculus* were divided into three groups of 5 each. Dose of Potassium Bromate was finalized after observing various literatures and confirmed through experimental investigation, whereas LD_{50} of Potassium Bromate in female mice is 280 mg [11].

Group 1: The animals of this group were served as control, received balanced diet, water *ad libitum* for 15 and 30 days.

Group 2: The animal of this group received balanced diet, water *ad libitum* and treated daily with Potassium Bromate (0.7 mg / 30 - 35 g b.wt / 0.125 ml /day) orally *via* cannula upto 15 days.

Group 3: The animals of this group received balanced diet, water *ad libitum* and treated daily with Potassium Bromate (0.7 mg / 30 - 35 g b.wt / 0.125 ml / day) orally via cannula upto 30 days.

The initial body weights of all mice were taken out on day first *i.e.* 0 days and then 16th and 31st day of the experiment with the help of laboratory weighing balance and the value were expressed in gram/animals. All animals of each group were sacrificed by cervical dislocations on 16th & 31st day of experiments. The ovaries were taken out, washed in normal saline (0.9%) NaCl, dried by filter paper then weighed and kept one side of ovary in Bouin's fixative for histological studies and remaining ovaries were used for biochemical estimations.

Assay of Tissue Homogenate for Biochemical Studies: A known amount of ovarian tissue was homogenized in chilled 0.25M sucrose solution using homogenizer and centrifuged at 25000 rpm for 15 min. The supernatant was used for acid phosphatase (ACP) and alkaline phosphatase (ALP) enzyme activities. The ACP and ALP were estimated by adopting the methodology of Bergmeyer (1963). However, the total protein estimation was done by using Folin-phenol method [12] was used. The ovarian tissue was homogenized in 20% trichloroacetic acid (TCA) and centrifuged at 15000 rpm for 20 min. The supernatant was discarded. The residue was centrifuged with definite volume of 0.1 N NaOH and the supernatant was used for protein estimation. These parameters were measured spectrophoto-metrically at different nano-meters (nm) respected to their optimized parameters.

Statistical Analysis: Results of the experiments were expressed as mean and standard error of mean of different groups. The differences between the mean values were evaluated by student t test. The values for $p<0.005$ were considered significant and $p<0.05$ were considered more significant.

Histopathological Study of Ovary: After different intervals *i.e.* 15 and 30 days the ovary were dissected out quickly, washed thoroughly in 0.87% physiological saline (NaCl), removed excess tissue, blotted dry and fixed in aqueous Bouin's fixative (5µm thick paraffin sections were cut and stained

with Ehrlich's Haematoxylin and Eosin (Ehrlich, 1886) for normal histology to observe the histopathological changes. These sections were observed under microscope at magnifications of 100X and 400X than subjected to microphotography using microphotography unit.

RESULT: Potassium bromate treated animals showed significant biochemical changes in their ovaries in comparision to control animals. When potassium bromated (0.7 mg / 30 - 35 g b.wt / 0.125 ml / day) administered to the animal orally for 15 days and 30 days decreased their body weight and protein content choronologically in comparison to control group (**Table 1** and **2** and **Fig. 1** and **2**).

TABLE 1: BODY WEIGHT (gm) OF CONTROL AND POTASSIUM BROMATE TREATED FEMALE MICE (*MUS MUSCULUS*) AFTER 15 AND 30 DAYS

Groups	Control	Treated group (15 days)	Treated group (30 days)
Initial weight	28.66 ± 1.6	28.96 ± 1.2	28.10 ± 1.7
Final weight	28.78 ± 1.7	24.30 ± 0.87*	26.33 ± 0.44**

It has been also seen that the levels of ovarian ACP significantly increased after 15 and 30 days of potassium bromate exposed animals in comparision to control group (**Table 3** and **Fig. 3**) While, the ovarian ALP levels were significantly decrease after 15 and 30 days of potassium bromate exposed animals in respect to control (**Table 4** and **Fig. 4**).

TABLE 2: PROTEIN ESTIMATION (mg/gm) AFTER DIFFERENT INTERVALS. *i.e.* 15 AND 30 DAYS TREATMENT OF POTASSIUM BROMATE AND CONTROL OVARIES OF FEMALE MICE *MUS MUSCULUS*

Group	Duration in days	
	15 Days	30 Days
Control	3600.07 ± 221.895	3709 ± 115.025
Treated	2501.08 ± 150.99*	1500.01 ± 91.23**

TABLE 3: ACID PHOSPHATASE (ACP IU/gm) LEVEL IN OVARY OF FEMALE MICE *MUS MUSCULUS* AFTER THE EXPOSURE OF POTASSIUM BROMATE AFTER 15 AND 30 DAYS

Groups	Duration in days	
	15 days	30 days
Control	0.51 ± 0.01	0.54 ± 0.01
Treated	0.73 ± 0.02*	0.86 ± 0.02**

TABLE 4: ALKALINE PHOSPHATASE (ALP IU/gm) LEVEL IN OVARY OF FEMALE MICE *MUS MUSCULUS* AFTER THE EXPOSURE OF POTASSIUM BROMATE AFTER 15 AND 30 DAYS

Groups	Duration in days	
	15 days	30 days
Control	0.81 ± 0.02	0.84 ± 0.03
Treated	0.67 ± 0.01*	0.52 ± 0.03**

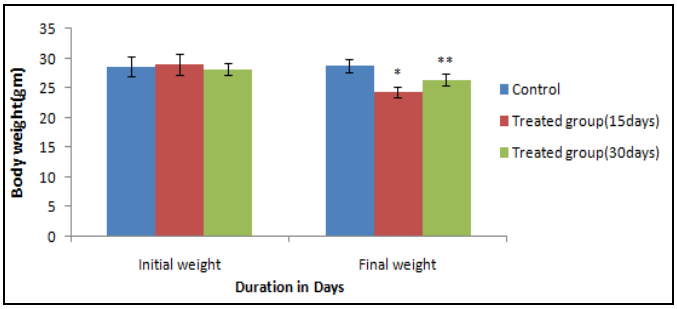

FIG. 1: BODY WEIGHT (gm) OF CONTROL AND POTASSIUM BROMATE TREATED FEMALE MICE (*MUS MUSCULUS*) AFTER 15 AND 30 DAYS

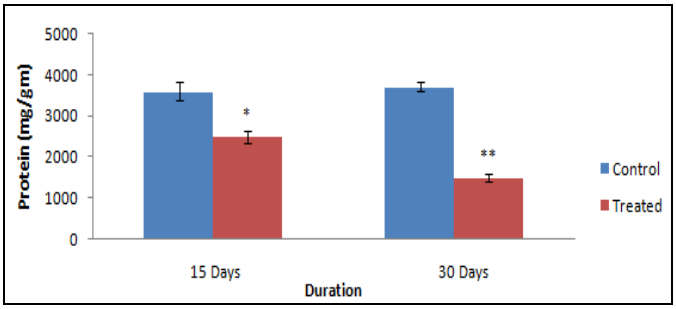

FIG. 2: PROTEIN ESTIMATION (mg/gm) AFTER DIFFE RENT INTERVALS *i.e.* 15 AND 30 DAYS TREATMENT OF POTASSIUM BROMATE AND CONTROL OVARIES OF FEMALE MICE *MUS MUSCULUS*

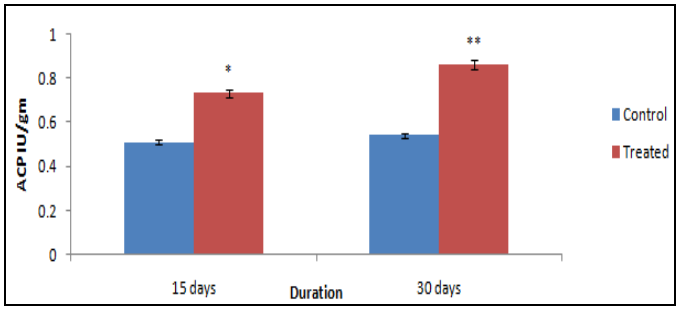

FIG. 3: ACID PHOSPHATASE (ACP, IU/gm) LEVEL IN OVARY OF FEMALE MICE *MUS MUSCULUS* AFTER THE EXPOSURE OF POTASSIUM BROMATE AFTER 15 AND 30 DAYS

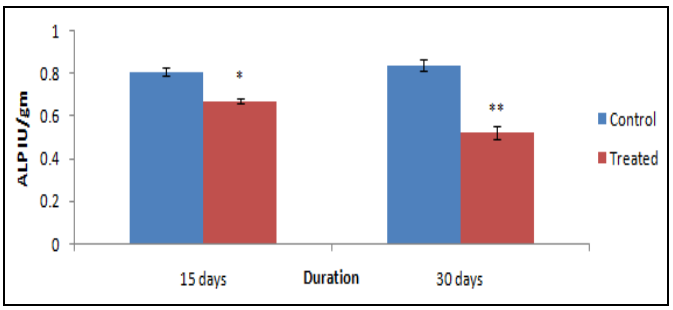

FIG. 4: ALKALINE PHOSPHATASE (ALP, IU/gm) LEVEL IN OVARY OF FEMALE MICE *MUS MUSCULUS* AFTER THE EXPOSURE OF POTASSIUM BROMATE AFTER 15 AND 30 DAYS

Values are mean ± SEM of 5 animals. *Significant different ($p \leq 0.005$) from control *vs.* experimental by student's t'test. **More Significant different ($p \leq 0.05$) from control *vs.* experimental by student's t'test

Histopathological Observations of Ovary: Apart from this, the ovarian histoarchitecture structure showed well developed oocyte, granulose cells and stromal cells **Fig. 5**. However, the ovary of treated animals with potassium bromate upto 15 days does not showed much demarkable changes in their histoarchitecture structure **Fig. 6**. But the animals exposed with potassium bromated upto 30 days showed marked degenerative changes in the thecal layer and less amount of cytoplasmic material were noticed in the stromal cells **Fig. 7**.

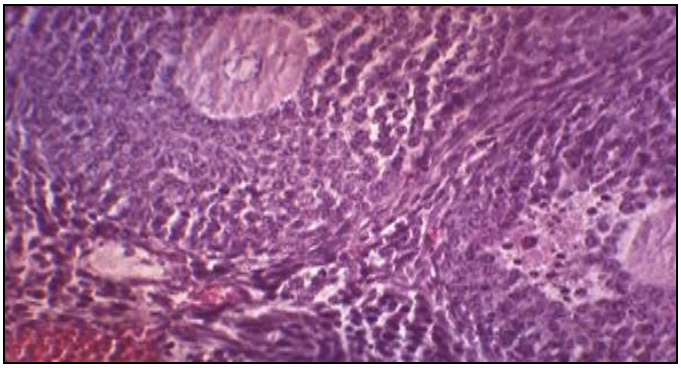

FIG. 5: TS OF CONTROL OVARY SHOWING THE NORMAL HISTOARCHITECTURE STRUCTURES. THE SECTION SHOWING WELL DEVELOPED OOCYTE WITH GRANULOSA CELL, WELL DEVELOPED THECAL LAYER AND THE STROMAL CELL. THE GRANULOSA CELL AND THECAL CELLS ARE HAVING WELL NUCLEATED WITH CYTOPLASMIC MATERIALS

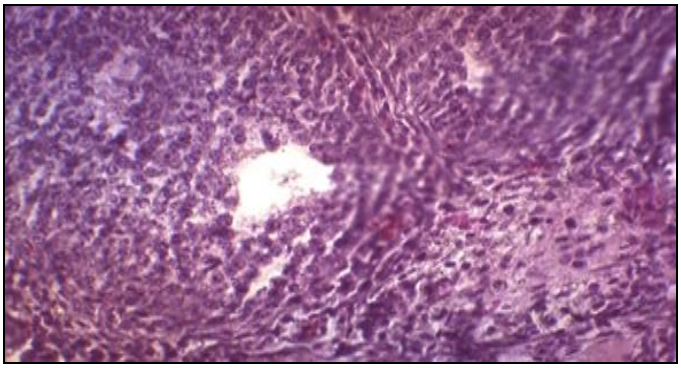

FIG. 6: THE OVARY TREATED WITH POTASSIUM BROMATE UPTO 15 DAYS SHOWING NO SUCH DEMARKABLE CHANGES IN GRANULOSE CELLS AND STROMAL CELLS. HOWEVER, SLIGHT DEGENE-RATIVE CHANGES WILL NOTICE IN THE CELL LAYER OF THE MATURE FOLLICLES

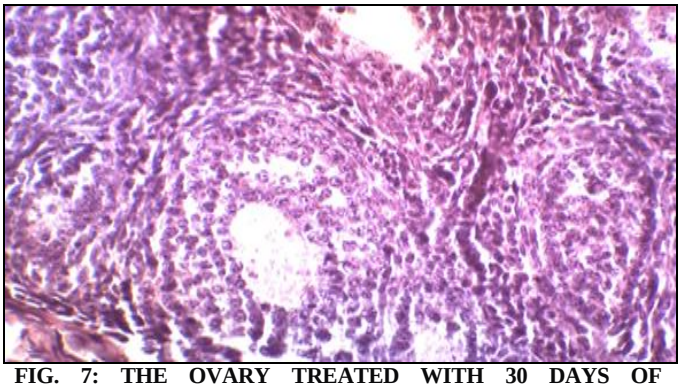

FIG. 7: THE OVARY TREATED WITH 30 DAYS OF POTASSIUM BROMATE IS SHOWING MARKED CHANGES CHARACTERIZED BY DEGENERATIVE CELLS WITH LESS AMOUNT OF STROMAL CELLS IN THECAL LAYER AS WELL AS CYTOPLASMIC LAYER

DISCUSSION: Potassium bromate is widely used as improving additive for bread making [7, 13]. However, several reports of bromated induced cancer in experimental animals are available [10, 14, 15, 16]. Toxicity studies in animals are commonly used to assess potential health risk in humans caused by intrinsic adverse effects of chemical compounds [11]. These adverse effects may manifest significant alterations in the levels of biomolecules, normal functioning and histo-morphology of the organs [17]. The current study was designed to investigate some of the histological and biochemical changes induced by $KBrO_3^-$ intake in female albino mice. In our study we have observed that oral intubation of $KBrO_3^-$ at the dose of 0.7 mg / 30 - 35 g b.wt / 0.125 ml distilled water, decreased significantly body weight of the animals after 15 and 30 days.

Dose dependent inhibition of body weight increase in both male and female F344 rats after oral administration of $KBrO_3^-$ [15]. The research during past two decades has identified, small intestine is one of the major target of ROS that are generated by orally ingested xenobiotics like $KBrO_3^-$ [1, 18, 19]. The process of digestion can be altered by drugs and toxicant [20]. The decrease with body weight may suggest that the small intestine is the major site of complete digestion and potassium bromate toxicity which may cause alteration in physiological and cellular functions of organism, which will be major cause of decreased body weight in treated animal as compared to control animal. It has been well reported that the measurement of activities of ‘marker’ enzyme in the body tissue can be used to access the toxicity of chemical compound on organ or tissues [21, 22].

Alkaline phosphatase (ALP), a 'marker' enzyme for the plasma membrane and endoplasmic reticulum [23, 24] is frequently used to assess the integrity of the plasma membrane [25]. In our study, ovarian ALP enzyme level significantly decrease in 15 days and 30 days in treated groups as compared to control group, such alteration in activity of the enzyme in the tissue would indicate damage to plasma membrane of cell [26].

The degradation of intracellular protein and other cytoplasmic components plays an important role in regulation of intracellular homeostasis, removal of waste and damaged cellular constituents and in response to environmental stresses [27, 28, 29]. Autophagy is also activated in response to certain environmental stressor, such as nutritional deprivation and oxidizing condition, however bromated cause nutritional deprivation by alteration in digestive system [1, 18, 19].

Acid phopshatase (ACP), a 'marker' enzyme for lysosomal membrane [30, 31]. ACP activity increase in lysosomal mediated cell damage, there is a evidence linking acid phosphatise activation with cellular degeneration and autophagy [32, 33, 34]. In our study ACP level significantly increases in a chronological fashion along the 15 days and 30 days treatment groups as compared to control group. In female phosphatase enzymes have been shown to regulate the growth, differentiation and atresia of ovarian follicles. It has been reported that phosphatase enzymes are constituents of follicular fluids of cows [35], pigs [36] and women [37].

In our study 30 days treated ovarian tissue showing marked changes characterized by degenerative cells with less amount of stromal cells in thecal layer as well as cytoplasmic layer, which support the previous study ovarian follicular atresia is a cellular degenerative process which is associated with increased lysosomal enzyme activity [30]. This may suggest that alterations in these enzymes may inhibit the ovarian function of *Mus musculus* which results histopathological changes in ovaries.

CONCLUSION: Our result conclude that in a very low amount, potassium bromate if is available in the body may pose transitory reproductive disruption and are dependent upon the dose and durations employed.

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CONFLICT OF INTEREST: Nil

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