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Effects of Gestational Exposure to Potassium Bromate as Food Additive on Reproductive and Immunological Parameters in Mouse Offspring

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Abstract

Potassium bromate ($KBrO_3$) is used as a food additive because of its high oxidizing characteristics, which have been linked to carcinogenic and mutagenic effects. The study looked into how $KBrO_3$ exposure during gestation affected the growth, reproductive performance, and immune cells of parental mice and their F_1 generation. Thirty Swiss Albino mice (*Mus musculus*) aged 27±3 days were collected from ICDDR'B in Dhaka, with 10 males and 20 females. When the mice reached 45 days of age, they were randomly separated into two groups, each with ten females and five males. Group A mice served as the untreated control, while group B mice were given $KBrO_3$ at 400 mg/liter in drinking water. Body weights were obtained at 2-week intervals. The results revealed that $KBrO_3$ reduced growth in both male and female parental mice. The $KBrO_3$-treated group showed considerably reduced amounts of estradiol, testosterone, and T_4 (P<0.01). Male mice treated with $KBrO_3$ showed significant (P<0.05) reductions in sperm count and motility. The $KBrO_3$-treated mice offspring weighed less and grew slowly. Neutrophil counts were somewhat higher in mice born in the $KBrO_3$-treated group. The F_1 offspring of mice in this group had decreased sperm count, motility, and testis weight (P<0.05). Testosterone and estradiol levels were lower in F_1 mice born from the $KBrO_3$ group than in F_1 mice from the control group. The findings of the study suggested that $KBrO_3$ may have multigenerational effects on growth and reproduction. However, more research on $KBrO_3$ is required to get clear understanding of its mechanism.

Keywords: $KBrO_3$, Reproductive Parameters, Testosterone, Gestational Exposure, Mice.

Introduction

Potassium bromate ($KBrO_3$) belongs to the bromide group and has potent oxidizing properties. It has been employed as a flour enhancer for more than fifty years. At the late dough stage, it encourages greater dough rise and results in a voluminous dough (Chauhan and Jain, 2016). Potassium bromate is utilized in the production of fish paste, cheese, and beer. It is also used in the pharmaceutical and cosmetic industries, as well as in cold-wave hair solutions (Kurokawa *et al.*, 1990). Additionally, when bromide-containing water is disinfected by ozonization, it is created as a secondary product.

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Long term administration of $KBrO_3$ was reported to have carcinogenic and mutagenic effects in liver and kidney of rats and mice (Mubarak *et al.*, 2020). It is suggested that oxidative stress may play a role in toxic and carcinogenic effects of bromate (Ahmad *et al.*, 2014). *In vitro* studies conducted using mammalian cell lines indicated that $KBrO_3$ caused the production of 8-hydroxydeoxyguanosine, a widely recognized indicator of oxidative DNA damage. This was achieved through the creation of bromine or its oxide radicals (Ballmaier and Epe, 2006).

The International Agency for Research on Cancer (IARC) has classified $KBrO_3$ as a B_2, meaning that it is a potential human carcinogen (Chauhan and Jain, 2016). As a result, numerous health organizations and agencies have implemented bans on its use (Achukwu *et al.* 2009). However, despite the fact that it is illegal in some countries, it is still permitted in others. Bakers illegally use high doses of $KBrO_3$ (Elsheikh *et al.*, 2016). Nevertheless, the FDA has established a safety limit of 20 parts per billion (ppb) or 0.02 milligrams per kilogram (mg/kg) for $KBrO_3$ in bread (Ekop *et al.*, 2008). However, it has been revealed that the amount of $KBrO_3$ left in some bread samples surpasses this limit by about 449 times. Therefore, The Joint Food and Agricultural Organization/World Health Organization expert committee on food additives has proposed an upper limit of 75 ppm of $KBrO_3$ for flour treatment, provided that baked goods made with this treated flour contain minimal $KBrO_3$ residues (WHO, 2007).

It has been reported that most bread sold in Bangladesh includes $KBrO_3$ (Mahmud *et al.*, 2021). Given the extensive usage of this compound, a large proportion of the population is at danger of being exposed to it, either as a food additive or as a result of occupational contact during the production process. In this setting, persons are vulnerable to different health concerns associated with the consumption of $KBrO_3$. Exposing prepubertal rats to $KBrO_3$ inhibits their growth, causes testicular underdevelopment, and impairs sperm production, which may signal future infertility or sterility (Elsheikh *et al.*, 2016). It is reported that genotoxic effects of $KBrO_3$ are linked to DNA and chromosomal destruction, mutations, base change chromosomal alterations, and changes in gene expression. These consequences eventually lead to the development of cancer. Elmahdy *et al.* (2015) discovered that exposure to $KBrO_3$ caused liver tissue damage and raised levels of blood enzymes. A separate experiment discovered that $KBrO_3$ caused harm to testicular cells, resulting in cell death. However, the presence of antioxidants mitigated the harmful effect (Nwonuma *et al.*, 2016). Additionally, it was reported that $KBrO_3$ had cytotoxic effects on the kidney cells of Fischer 344 rats (Dodd *et al.*, 2013). Stuti and D'souza (2013) investigated the negative effects of $KBrO_3$ on biochemical, hematological, and histological parameters of Swiss albino mice. Another study found that human erythrocytes exposed to $KBrO_3$ undergo cell lysis (Ahmad *et al.*, 2014). In this scenario, customers are susceptible to various health hazards linked to the ingestion of $KBrO_3$. Given the scarcity of studies on the effects of $KBrO_3$ on reproductive and immune function in humans and animals, the current study examined the effects of gestational $KBrO_3$ exposure on reproductive and immunological parameters in mouse offspring.

Materials and Methods

Experimental Design

The experiment was carried out at the Department of Physiology, Bangladesh Agricultural University, Mymensingh. Thirty Swiss Albino mice (*Mus musculus*), 10 males and 20 females, were collected from ICDDR'B, Mohakhali, Dhaka, at 27±3 days of age. When the mice reached 45 days of age, they were divided into two groups: Group A and Group B, each with an equal number of mice (five males and ten females). Group A, served as vehicle control, was provided with mice pellets and normal drinking water, while Group B was fed on the basal diets and provided potassium bromate at 400 mg/L with drinking water. Potassium bromate ($KBrO_3$, Sigma-Aldrich Co., Germany) was added to drinking water on a daily basis until the trial ended.

Body Weight

A digital balance was used to determine the body weight of each mouse. Body weight was measured on the first day of the trial and every 15 days after that.

Hematological Analyses

Blood analysis was performed using the protocol stated by Miah *et al.* (2023). Briefly, the mice were fasted overnight before collection of blood. After administering diethyl ether to each comatose mouse, blood was collected from the heart with a sterile syringe. Standard techniques were used to measure total leukocyte count (TLC), total erythrocyte count (TEC), and hemoglobin content (Hb conc).

Sperm Parameters

Upon completion of the experiment, all mice were euthanized under anesthesia and their testicles were excised to get the epididymis for sperm analysis. The cauda epididymis of mice was taken and coarsely chopped in a Petridish using sharp scissors. The torn epididymis was inserted in a test tube with 4 ml of phosphate buffered saline at 37°C. After a period of 5 to 10 minutes, the sperm cells were dispersed and quantified using a pipette in the Neubauer's chamber, as described by Sujan *et al.* (2021). Epididymal sperm motility was assessed by depositing a small amount of diluted sperm suspension (in PBS) on a warmed slide, covering it with a cover slip, and observing it under a low-magnification microscope. At least ten widely dispersed areas were examined to estimate the proportion of sperm cells capable of mobility. The percentage of sperm motility was quantified.

Hormonal Assay

Serum testosterone, estradiol, and serum thyroxine (T_4) levels were tested using the Radioimmunoassay Kit at the Institute of Nuclear Medicine and Allied Sciences (INMAS), Mymensingh Medical College, Bangladesh.

Statistical Analysis

The laboratory data was entered and stored in Microsoft Excel 2010, then imported into GraphPad Prism for analysis. A Student's t-test was used to compare the data and assess statistical significance. *P*-values <0.05 were considered significant.

Results and Discussion

Effects of Potassium Bromate ($KBrO_3$) on Live Body Weight in Parental Mice

The live body weight of both male and female mice of two groups at different time point is presented in Figure 1. Body weights of normal healthy control group (Group A) and $KBrO_3$-treated group (Group B) were recorded at 15-day intervals until the end. Initially both groups had the same body weight, but after 15, 30, 45, and 60 days, the body weight of $KBrO_3$-treated mice gradually decreased while the weight of control mice increased. The current finding showed that addition of $KBrO_3$ to drinking water hindered the growth in mice. The observed phenomenon can be attributed to the interaction between $KBrO_3$ and the iodine receptors, resulting in reduced iodine absorption by the thyroid gland. This, in turn, generates an iodine shortage, which leads to growth retardation (Fisher and Bull, 2006). The findings of this study are consistent with earlier research on the toxicity and carcinogenicity of $KBrO_3$. Specifically, both short and long-term oral administration of $KBrO_3$ reduces growth (DeAngelo *et al.*, 1998). In contrast, $KBrO_3$ was found to have no effect on the body weight of rats (Abuelgasim *et al.*, 2008). This discrepancy could be explained by differences in the ages of the experimental animals and the amount of $KBrO_3$ administered.

Effects of $KBrO_3$ on Selected Hematology in F_0 Mice

Table 1 depicts the results of an analysis of total leukocyte count (TLC), total erythrocyte count (TEC), and hemoglobin content (Hb conc) in two separate mouse groups. There was no significant growth in TLC. The $KBrO_3$-treated group had lower hemoglobin concentrations and total red blood cell counts, but the differences were not statistically significant ($P>0.05$).

The current findings are similar with those of Achukwu *et al.* (2009), but differ from those of Shehab and Ghadbhan (2021). They discovered that administering $KBrO_3$ alone to the animals resulted in a drop in red blood cell (RBC) count, white blood cell (WBC) count, Hb conc, and packed cell volume (PCV) compared to the control group. However, the levels are still significantly lower than those of the control group. Anemia is demonstrated by a transient drop in Hb conc and PCV levels in rats given a dose of 200 mg/kg BW $KBrO_3$ during the third week. According to Sai *et al.* (1992), $KBrO_3$ is capable of inducing methemoglobinemia and cyanosis. This is triggered by the oxidation of ferrous ions to ferric ions by reactive oxygen species (ROS) generated by $KBrO_3$.

Effects of $KBrO_3$ on Reproductive Parameters

Testosterone, estradiol and T_4 concentrations in male and female F_0 mice treated with $KBrO_3$ were measured and presented in Figure 2. In the control group, values were 1.31±0.79 ng/mL, 12.85 pg/mL, and 45.38±5.17 nmol/L. In the $KBrO_3$-treated group, values were 0.70±0.30 ng/mL, 7.85 pg/mL, and 26.06±3.02 nmol/L, respectively. Significant differences (P<0.05) were seen in testosterone, estradiol, and T_4 levels in the $KBrO_3$-treated group. The findings of this study are very similar to those of Zang *et al.* (2016) and Munir *et al.* (2017). The drop in testosterone concentration in blood plasma could be attributed to lower enzyme and protein expression, as well as decreased plasma LH levels.

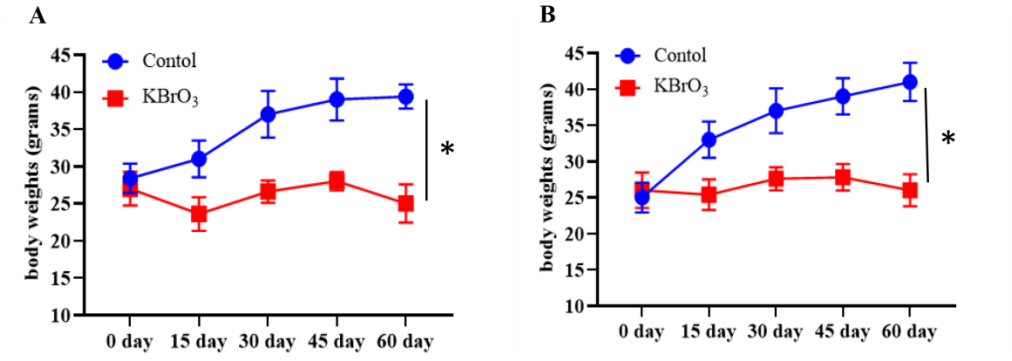

Figure 1. Effect of $KBrO_3$ on body weight (grams) in mice.

A = male parental mice and B = female parental mice. Values are expressed as Mean±SD; *values differ significantly at P<0.05.

Table 1. Effects of $KBrO_3$ treatment on hematological parameters in F_0 male mice

Group	TEC (x 10^6/uL)	Hb (g%)	TLC (x10^3/uL)
Control (Group A)	8.98±0.29	8.93±0.41	8.36±0.42
$KBrO_3$-treated (Group B)	8.13±0.21^{ns}	7.66±0.70^{ns}	9.12±0.64^{ns}

Values are expressed as mean±SD; ns = not significant.

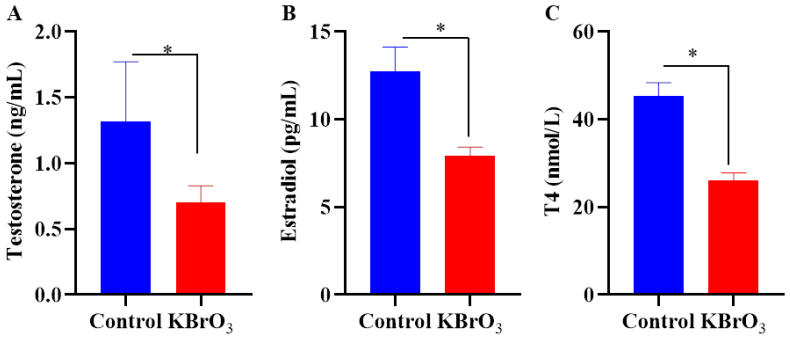

Figure 2. Effects of $KBrO_3$ on testosterone (A), estradiol (B) and T_4 (C) in mice.

Values are expressed as Mean±SD. *indicates significant differences (P<0.05) between control and $KBrO_3$-treated group.

In this work, mice exposed to $KBrO_3$ had slower growth (figure 1), and their testicular weight was similarly reduced. This is probably due to an interaction between $KBrO_3$ and iodine receptors, which lowers iodine absorption by the thyroid gland. This causes an iodine deficiency, which leads to

stunted growth (Elsheikh *et al.*, 2016). The cause of testosterone hormone deficiency could be due to the effect of $KBrO_3$ on the main enzymes that affect metabolism and steroid release in the testis. The $KBrO_3$-treated mice exhibited significant decreases in sperm count, proportion of head and tail abnormalities, and sperm motility (P<0.05) as shown in Table 2. The weights of the testes and epididymis were dramatically decreased. The findings are consistent with prior investigations on the toxicity and carcinogenicity of $KBrO_3$ (Kurokawa *et al.*, 1986; DeAngelo *et al.*, 1998). They showed that both short-term and long-term oral treatment of $KBrO_3$ reduces growth.

Effects of $KBrO_3$ on live body weight in F_1 Offspring

The live body weight of F_1 offspring mice of two groups at different time point is presented in figure 3. Body weights of normal healthy controls (Group A) and $KBrO_3$-treated group (Group B) were recorded at 15-day intervals for up to 120 days. $KBrO_3$-treated offspring mice were born with decreased body weights, which remained lower until the end of the trials. The body weights of $KBrO_3$-treated mice slowly increased, but the weights of control mice rapidly increased. The current study discovered that adding $KBrO_3$ to drinking water produced stunted growth in offspring mice.

Effects of $KBrO_3$ on Immune Cells in F_1 Mice

Similar to the parental mice, the effect of $KBrO_3$ on TLC and differential leukocyte count was modest; nevertheless, neutrophil levels were marginally raised in mice born from the $KBrO_3$ treated group, while total WBC increased non-significantly (Table 3).

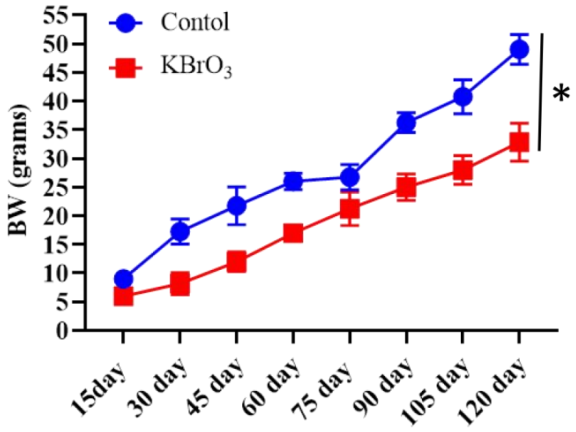

Figure 3. Effect of $KBrO_3$ on body weight (grams) in F_1 offspring mice.

Values are expressed as Mean±SD. *values differ significantly at P<0.05.

Table 2. Effect of $KBrO_3$ treatment on sperm count and motility in F_0 male mice

Groups	Sperm Concentration (million/mL)	Sperm Motility (%)	Testis Weight (mg)
Control (Group A)	21.37^{a}±1.10	48.25^{a}±6.23	108.5^{a}±4.93
$KBrO_3$-Treated (Group B)	17.92^{b}±0.94	30.75^{b}±4.74	92.75^{b}±5.31

Values are expressed as mean±SD; ^{ab}values with different superscript letter differed significantly (P<0.05).

Table 3. Effect of $KBrO_3$ on TLC and DLC in F_1 offspring male mice

Group	TLC (10^3/µL)	DLC (%)			
		Neutrophils	Lymphocytes	Monocytes	Eosinophils
Control (Group A)	9.48±0.26	26.66±2.00	60.00±2.04	7.60±0.47	6.00±1.41
$KBrO_3$-treated (Group B)	$8.72±0.42^{ns}$	28.66±2.49	54.33±2.49	$9.0±0.81^{ns}$	$8.43±1.24^{ns}$

Values are expressed as mean±SD; ns = non-significant.

Table 4. Effect of $KBrO_3$ on sperm count, motility and testis weight in F_1 offspring male mice

Groups	Sperm Count (million/mL)	Sperm Motility (%)	Testis Weight (mg)
Control (Group A)	18.85^{a}±2.13	50.25^{a}±4.32	110.3^{a}±5.24
$KBrO_3$-treated (Group B)	16.15^{b}±1.35	40.75^{b}±5.48	100.80^{b}±4.82

Note: Values are expressed as Mean±SD; ^{ab}values with different superscripts differ significantly (P<0.05).

Effects of $KBrO_3$ on Reproductive Parameters in F_1 Mice

Effect of $KBrO_3$ on sperm count, sperm motility and testis weight in F_1 offspring male mice is depicted in the Table 4. The $KBrO_3$-treated F_1 offspring mice had significantly lower sperm count, motility, and testis weight (P<0.05) compared to normal control mice. The results could be attributed to the negative effects that were passed down vertically from parents to children. Furthermore, the reduced size of the testis and sperm features may have an effect on the metabolism and steroid release in the testis of offspring mice (Zang *et al.*, 2016; Munir *et al.*, 2017).

Effects of $KBrO_3$ on Testosterone, and Estradiol in F_1 Offspring Mice

Testosterone and estradiol concentrations in male and female F_1 mice were also measured and presented in table 5. Testosterone and estradiol levels were reduced in $KBrO_3$-treated F_1 mice than in the control group. The mean values for these parameters were considerably different (P<0.05). This decrease in hormone levels could be due to the effect of $KBrO_3$ on the testis of offspring mice.

Table 5. Effects of $KBrO_3$ on testosterone and estradiol in F_1 offspring mice

Groups	Testosterone (ng/mL)	Estradiol
$KBrO_3$-treated (Group B)	1.20±0.78	7.67±1.10
Control (Group A)	0.75*±0.30	1.99*±0.32

Values are expressed as mean±SD; *values differ significantly (P<0.05).

Conclusions

Potassium bromate ($KBrO_3$) is classified as a bromide compound. It is well known for its potent oxidizing properties. For nearly half a century, it has been used to improve the quality of flour. A recent study discovered that 67% of bread samples contained an excess of $KBrO_3$. Given the substance's extensive use, a large proportion of the population is at danger, either as a food additive or as a result of occupational exposure during the production process. The study looked at how $KBrO_3$ exposure during gestation affected growth rate, reproductive performance, and immune cells in parental mice and their offspring.

The results demonstrated that administering $KBrO_3$ via drinking water lowered growth rates in both male and female parental mice. Potassium bromate treatment had no meaningful effect on hematological markers. Total leukocyte count increased modestly. Male mice treated with $KBrO_3$ showed significant reductions in testosterone and T_4 levels (P<0.01), while female parental animals showed reductions in estradiol levels. Mice treated with $KBrO_3$ showed a significant (P<0.05) drop in sperm count and motility. The offspring mice treated with $KBrO_3$ had a lower body weight. The number of neutrophils in mice born from the $KBrO_3$-treated group increased somewhat, but leukocyte counts did not differ significantly. The F_1 offspring mice treated with $KBrO_3$ showed reduced sperm count, motility, and testis weight (P<0.05). The level of testosterone and estradiol in male and female F_1 mice treated with $KBrO_3$ were lower than those in the control group.

The current findings indicated that $KBrO_3$ may have long-term effects on development and reproduction across generations. As a result, it is recommended that we seek alternatives to $KBrO_3$ or impose a prohibition or ban on it in our country. Further

research on $KBrO_3$ is required to make a definitive conclusion and understand its exact process.

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Declaration

The authors declare that this research findings reported in this article do not have any conflicting interest.

Authors' Contributions

MAM conceived the idea, designed and supervised the experiments, reviewed and edited the manuscript; PPM, MMR and MHR performed the sample collection, laboratory experiment, analyze the data and drafted the first version of the manuscript; AM and JAB acted as the resource person as well as reviewed and edited the manuscripts. The completed manuscript has been read and approved by all the authors.

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