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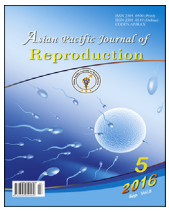

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Effects of potassium bromate on male rat growth and testicular histology

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ABSTRACT

Objective: To investigate the effects of supplementation of potassium bromate ($KBrO_3$) to drinking water on the growth rate, pubertal weight, testis & epididymal weights and testicular histology of growing male rats.

Methods: Thirty male Wizard rat [age = 21 d, mean BW = (40.0 ± 5.3) g] were used. The rats were grouped into 5 treatment groups each group consist of 6 rats. T_1 was offered drinking water supplemented with 100 mg, T_2 200 mg, T_3 300 mg, T_4 400 mg/L $KBrO_3$ for the duration of the experiment, while the control group was offered $KBrO_3$ free water. The BW weights were taken weekly. Eight weeks after treatment the rats were sacrificed, testes & epididymae were excised and weighted. The testes were fixed and histopathological sections of 5–6 μm were made, stained with H & E and examined under light microscope.

Results: The results showed that the growth rate, pubertal weight, testes & epididymal weights and testicular histology of growing male rats were significantly ($P < 0.001$) affected with $KBrO_3$ supplementation to drinking water. The growth rate of the control group and T_1 (100 mg/L $KBrO_3$) fit well a sigmoid pattern growth curve and no differences in their growth rates were recorded, while the sigmoid pattern of the growth curves of treatment T_2, T_3 and T_4 was disrupted. No difference ($P > 0.05$) in BW between the control and T_1 was recorded. However, it is clear that T_2, T_3 and T_4 significantly ($P < 0.05$) reduced the BW at puberty. Furthermore treatment groups recorded significantly ($P < 0.001$) low testicular and epididymal weights compared to the control. Supplementation of $KBrO_3$ to drinking water caused serious changes in testicular tissue of the rats. The treatment rats' testes had distorted or even collapsed seminiferous tubules and narrow interstitial spaces. Upon magnification the seminiferous tubules appeared very narrow, mostly devoid of spermatogenesis and with no sperms.

Conclusion: Exposure of prepubertal rats to $KBrO_3$ retards their growth, causes testicular hypoplasia and impairs spermatogenesis, which is a predictor of infertility or even sterility in the future.

1. Introduction

Infertility in men is steadily on the rise in many countries of the world [1,2]. Louis *et al*. has reported that the incidence of infertility among men in USA has reached 12% [3]. Many studies attributed the decline in male fertility to endocrine disrupting chemicals (EDCs) exposure especially the chlorinated compounds that are accused of inducing low sperm quality in men [2,4,5]. The EDCs are exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding and elimination of natural blood hormones that are present in the body and are responsible for homeostasis, reproduction and developmental process [6]. The EDCs are also thought to act on nuclear hormone receptors such as androgen, estrogen, progesterone, thyroid, retinoid as well as

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on nuclear & non-nuclear and neurotransmitter receptors. Bromides are a member of the halide family a group of elements that includes fluorine, chlorine and iodine. The bromides are known of their binding to the same receptors that are used to capture iodine and interfere with thyroid hormones function resulting in a low thyroid state [6]. Thus bromides can be classified as a member of the EDCs. Potassium bromate ($KBrO_3$), a member of the bromides group, is used in the maturation process of flour because of its oxidizing properties and has been used as a dough conditioner in the bread-making process for over 50 years. The WHO, Joint FAO/WHO Expert Committee on Food Additives [7] has temporarily recommended a maximum level of 75 ppm of $KBrO_3$ for treating flour, provided that baking products prepared from such treated flour contain negligible residues of $KBrO_3$ [7]. In Japan, the level has been set at 30 ppm under the same conditions as for WHO. In Canada $KBrO_3$ is permitted as food additive, but it was delisted in 1994, however, it was reported as an impurity in packaging food papers [8]. Also $KBrO_3$ has been introduced as an oxidizing agent, a primary standard, and a brominating agent in analytical chemistry. $KBrO_3$ is also used in barley, cosmetics and water purification industries. In many animal experimental investigations $KBrO_3$ has been classified as a possible carcinogenic substance [9–12]. Unfortunately in the under developing countries $KBrO_3$ is used injudiciously as bread conditioner to maximize the bakeries profits. In Sudan, although supplementation of $KBrO_3$ to bread is prohibited, the bakers illegally use unspecified quantities of $KBrO_3$ as bread conditioner. This disaster is not limited to humans because in the periods of drought and scarcity of pasture the farm animals have to consume the dried surplus bread. Thus unknown amounts of $KBrO_3$ reach the consumers through consumption of bread and/or animal products. Under this situation the consumers in Sudan are vulnerable to many of the health risks associated with consumption of $KBrO_3$. Due to the sparse researches on the effects of $KBrO_3$ on the growth and development of reproductive system, it is the objective of the current study to investigate the effects of $KBrO_3$ on the growth rate, pubertal weight, testes & epididymal weights and testicular histology of growing male rats kept on $KBrO_3$ during prepubertal period.

2. Materials and methods

2.1. Experimental animals housing and management

Twenty one day old male Wizar rats ($n = 30$) of mean BW of (40.0 ± 5.3) g were employed in this study. The rats were kept in rooms at 20–26 °C and a humidity of 40%–70% [13]. The lighting was set as described by Wong and Pace [14]. The rats were kept on a locally made pelleted ration (minced meat 1/2 kg, wheat flour 1/4 kg, salt 50 g and water). Fresh alfalfa was also provided daily. The body weight of the rats was monitored weekly using a digital balance and the weight was recorded in grams.

2.2. Supplementation of potassium bromate ($KBrO_3$) to drinking water

The rats were identified and grouped into 5 treatments groups each group consist of 6 rats. T_1 was offered drinking water supplemented with 100 mg, T_2 200 mg, T_3 300 mg, T_4 400 mg/L $KBrO_3$, while the control group was offered $KBrO_3$ free water, for the duration of the experiment (5 weeks).

2.3. Histopathological samples

The rats were anesthetized with chloroform and sacrificed with cervical dislocation [15]. The testes and epididymidae were dissected and weighed. The testes were fixed and histopathological sections were made as described by Johannsen [16]. Briefly the tissues were dehydrated with alcohol and cleared with chloroform or xylene. After cleaning they were immersed in melted paraffin wax and quickly cooled to fill the intracellular spaces. Then section of 5–6 μm were made with rotary microtome, transferred to a warm water bath containing little amount of gelatin powder and left floating until mounted onto the slides. The slides were then incubation for 30 min at 50 °C to dry. The wax was removed and the slides were stained with H & E and examined under light microscope.

2.4. Experimental designs

This study is a one factorial design study to investigate the effects of supplementation of $KBrO_3$ to drinking water on the growth rate, pubertal weight, testes & epididymal weights and testicular histology of growing male rats. Thirty male Wizard rat [age = 21 d, mean BW = (40.0 ± 5.3) g] were employed in this experiment to test the effects of $KBrO_3$ on the above mentioned parameters. $KBrO_3$ was supplemented to drinking water as described above. The BW of the rats was taken weekly and the BW at puberty was recorded 5 weeks after the treatment (the rats were 52 d the assumed age of puberty). The rats were sacrificed on week 8 of the treatment and their testes and epididymidae were weighted. Histopathological sections were prepared as above and examined under light microscope at 400× to investigate the changes that might happen in the STs and the process of spermatogenesis.

2.5. Statistical analysis

Data were subjected to ANOVA and differences at $P < 0.05$ were considered significant.

3. Results

3.1. The effect of $KBrO_3$ on growth

As shown in Figure 1, $KBrO_3$ treatment significantly ($P < 0.001$) influenced the growth rate of the growing rats. The growth of the control group and the rats in treatment 1 fit well a sigmoid pattern growth curve and no differences ($P > 0.05$) in their growth rate were recorded. The growth of rats in the remaining groups was significantly ($P < 0.001$) reduced by $KBrO_3$ and the sigmoid pattern was disrupted.

3.2. The effects of $KBrO_3$ on the weight at puberty

The puberty in male rat is around 52 d, since the rats were used after 3 week after birth, they will reach puberty on week 5 of the treatment. From Figure 2 it is clear that high $KBrO_3$