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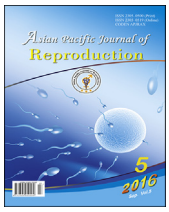

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Effects of potassium bromate on male rat growth and testicular histology

Adil Salim Elsheikh[1*], Thoyba Faroug Fadul[2,3], Eiman Mohamed-Elkheir Aboagla[3], Ahmed Abdel Rahim Gameel[4]

[1]*Department of Applied Medical Sciences, Community College, Najran, Saudi Arabia*

[2]*Sudan Academy of Sciences, Khartoum, Sudan*

[3]*Animal Production Center, Ministry of Animal Resources, Kuku, Sudan*

[4]*Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum, Sudan*

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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

*Corresponding author: Adil Salim Elsheikh, Department of Applied Medical Sciences, Community College, Najran, Saudi Arabia.
E-mail: adilelgarrai@yahoo.com
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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 $KBrO_3$

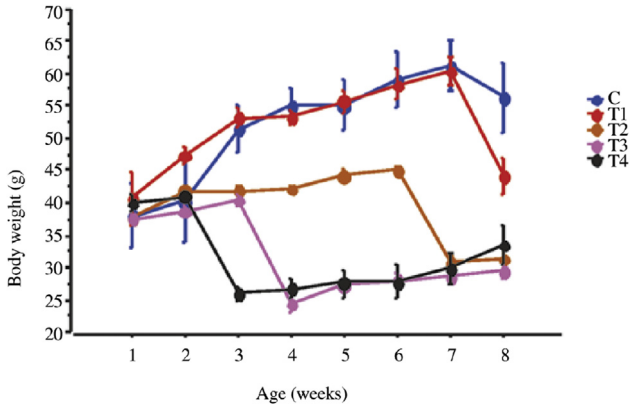

Figure 1. The effect of $KBrO_3$ on the growth rate of male Wizard rats. C: Control group, T_1: 100 μg/L $KBrO_3$, T_2: 200 μg/L $KBrO_3$, T_3: 300 μg/L $KBrO_3$, T_4: 400 μg/L $KBrO_3$.

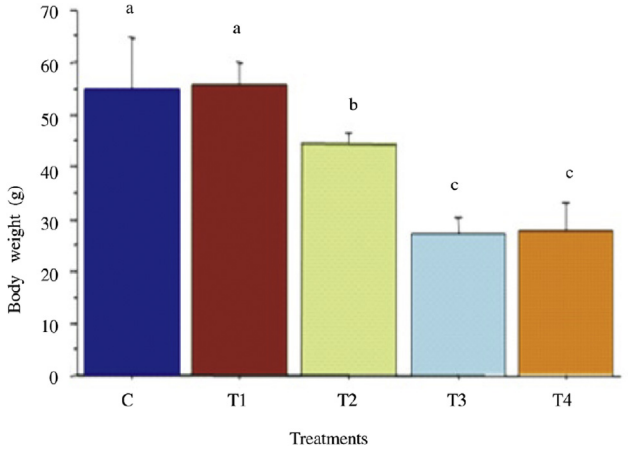

Figure 2. Effect of $KBrO_3$ on weight at puberty of male wizard rats. C: Control group, T_1: 100 μg/L $KBrO_3$, T_2: 200 μg/L $KBrO_3$, T_3: 300 μg/L $KBrO_3$, T_4: 400 μg/L $KBrO_3$. $^{a, b, c}$ $P < 0.001$.

concentrations significantly reduced ($P < 0.001$) the BW at puberty. The mean BW of the rats in control group, T_1, T_2, T_3 and T_4 were (55.17 ± 3.72), (55.75 ± 1.77), (44.27 ± 0.67), (27.48 ± 1.13) and (27.75 ± 2.18) g, respectively. No difference ($P > 0.05$) in BW between the control and T_1 was recorded. However, it is clear that the higher concentrations 200, 300, 400 mg/L $KBrO_3$ significantly ($P < 0.05$) reduced the BW at puberty.

3.3. The effect of $KBrO_3$ on the testes weight

Figure 3 shows that the administration of potassium bromate reduced ($P < 0.001$) the testis weight of male rats. The mean testicular weight of control group, T_1, T_2, T_3 and T_4 were (0.73 ± 0.11), (0.49 ± 0.04), (0.24 ± 0.04), (0.24 ± 0.05) and (0.18 ± 0.03) g, respectively. No significant difference ($P > 0.05$) in the testicular weight among treatments was recorded.

3.4. The effect of $KBrO_3$ on epididymal weight

The weight of the epididymae of the male rats were significantly ($P < 0.001$) influenced by potassium bromate (Figure 4). The mean epididymal weight of control group, T_1, T_2, T_3 and T_4

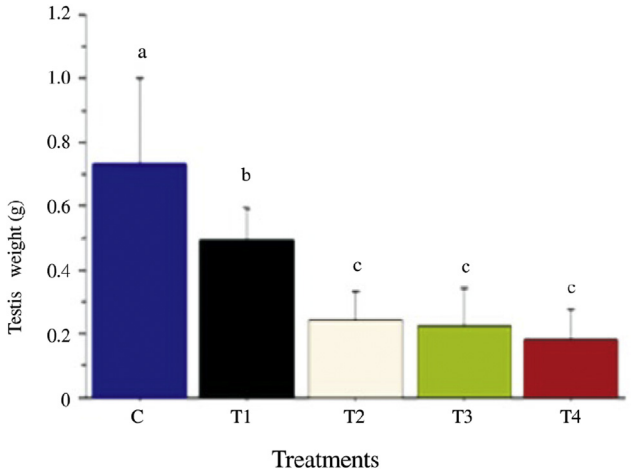

Figure 3. Effect of $KBrO_3$ on testicular weight of male rats. C: Control group, T_1: 100 μg/L $KBrO_3$, T_2: 200 μg/L $KBrO_3$, T_3: 300 μg/L $KBrO_3$, T_4: 400 μg/L $KBrO_3$. $^{a, b, c}$ $P < 0.001$.

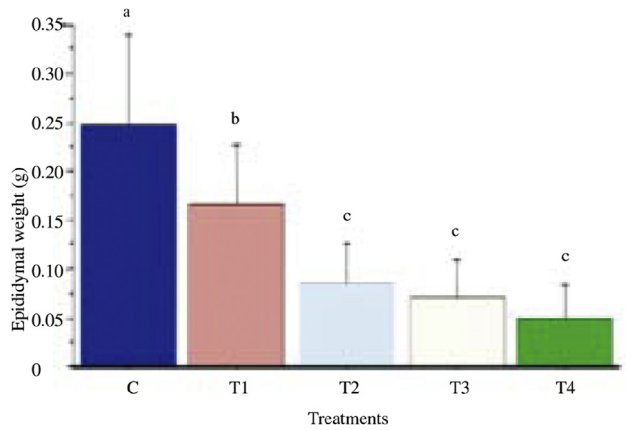

Figure 4. Effect of $KBrO_3$ on epididymal weight of male rats. C: Control group, T_1: 100 μg/L $KBrO_3$, T_2: 200 μg/L $KBrO_3$, T_3: 300 μg/L $KBrO_3$, T_4: 400 μg/L $KBrO_3$. $^{a, b, c}$ $P < 0.001$.

were (0.25 ± 0.04), (0.17 ± 0.03), (0.09 ± 0.02), (0.07 ± 0.02) and (0.05 ± 0.01) g, respectively. However, no differences ($P > 0.05$) between the weights of the epididymae of T_2, T_3 and T_4 were observed.

3.5. Effect of $KBrO_3$ on testicular histology

Figure 5 shows that $KBrO_3$ altered the tissues of the testis of treated rats compared to that of the control. Plate 1 shows a testis of a control rat; the ST are round, the interstitial spaces are wide enough, the testes appear very active, with may cellular layers, spermatids, sperm formation and the tubules have many layers of germinal epithelium. However in plate 2 (T_1) the ST of the testes appear elongate and there are few cellular layers and spermatids in ST. In plate 3 (T_2) the ST had different shapes and the interstitial spaces were almost absent, so the tubules appear compact with very few spermatids formation. In plate 4 (T_3) the STs are polyhedral and the interstitial spaces are narrow. Plate 5 (T_4) shows that the STs are almost collapsed with peripheral dark stain and the tunica albuginea is shrunken. Plate 6 (T_4) shows a magnified ST of plate 5, the tubule is devoid of spermatogenesis with basal vacuolated small cells and large cells central cells and the lumen contains no sperms.

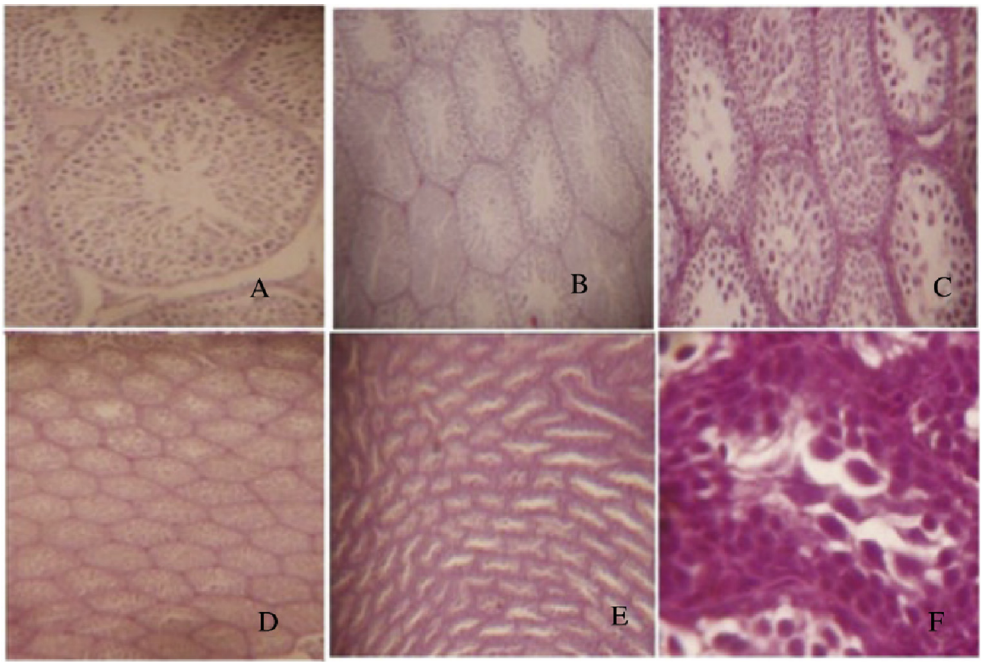

Figure 5. Histopathological picture of the testes of rat treated with $KBro_3$.
Plate 1. control with normal histological picture. Plate 2,3,4 and 5: testicular tubular structure of rats treated with potassium bromate. Notice the abnormalities (less spermatogonia layers, arrangement of tubules, narrow lumen and degeneration of interstitial tissue). Plate 6 shows the structure of a ST of rat in T_4: notice the abnormalities (collapsed STs, no spermatogenesis, vacuolated cells, two layers of spermatogonia of different shape and size).

4. Discussion

The results of this study showed that $KBrO_3$ supplementation to drinking water retarded the growth in growing rats and consequently reduced the pubertal, testicular and epididymal weights. Furthermore exposure to $KBrO_3$ alters the histology of the rat testis and impaired spermatogenesis.

The growth of rats exposed to $KBrO_3$ in this study was retarded and their testes weights were reduced. This was probably due to the binding of $KBrO_3$ to the iodine receptors, minimizing iodine uptake by thyroid gland and causing iodine deficiency which lead to growth retardation [17]. Consequently the testis and the epididymal weights were reduced. The finding of this study is in agreement with toxicity and carcinogenicity studies on $KBrO_3$ where short and long term oral administration of $KBrO_3$ was proved to reduce the growth [10,18–20]. Contrarily $KBrO_3$ was reported to have no effects on rat's body weight [21–23]. This discrepancy may be due to the differences in experimental rats age used and the dose of $KBrO_3$ administered.

A remarkable decrease in pubertal body weight was observed in the current study. Similarly many investigators reported that EDCs reduce the pubertal body weight [24–26]. Factors that are known to modify the thyroid functions are also accused of limiting the biologically active iodine [27]. Sufficient iodine in tissues protects ATP (the biological units of energy) and helps cells to produce a healthy 36 ATPs through the Krebs cycle, which has profound implications on function of all cells. Also in the current study it is probable that the $KBrO_3$ disrupted the antioxidant activity of iodine and its carbon-bonded products and iodolactones and consequently disturbed the thyroid gland functions [28] as a results the growth rate and epididymal & testicular weights were reduced. The low epididymal weights are due to growth retardation as well as azoospermia resulting from testicular hypoplasia. The histopathological sections of the testes revealed severe hypoplasia of testicular structure and impairment of spermatogenesis process. These changes are due to a probable iodine deficiency induced by $KBrO_3$ supplementation to water. Iodine helps to eliminate the oxidative stress, a known factor that induces germ cell apoptosis, because it neutralizes the hydroxyl ions [29]. Furthermore, it was reported that neonatal persistent and transient hypothyroidism cause prevalence of oxidative stress that is characterized by elevated lipid peroxide levels, protein carbonyl contents with decreased antioxidant enzyme levels. Since the testis is very rich in polyunsaturated fatty acids and has poor antioxidant defense system; the iodine deficiency induced by $KBrO_3$ in the current study made the testes of the rats in this experiment vulnerable to oxidative stresses [29–31]. The iodine deficiency induced by $KBrO_3$ perhaps hampered the biosynthesis of the thyroid hormones that are critical for coordination of physiology within and between cells and tissues and consequently affected the development and function of the gonads [32].

In conclusion, prepubertal exposure of male rats to $KBrO_3$ retards growth, reduces testicular weight, alters testicular histology and impairs spermatogenesis. Thus it affects the male future fertility.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Hauser R, Skakkebaek NE, Hass U, Toppari J, Juul A, Andersson AM, et al. Male reproductive disorders, diseases, and costs of exposure to endocrine-disrupting chemicals in the European Union. *J Clin Endocrinol Metab* 2015; **100**: 1267-1277.

[2] Hond ED, Tournaye H, Sutter PD, Ombelet W, Baeyens W, Covaci A, et al. Human exposure to endocrine disrupting chemicals and fertility: a case-control study in male subfertility patients. *Environ Int* 2015; **84**: 154-160.
[3] Louis JF, Thoma ME, Sorensen DN, McLain AC, King RB, Sundaram R, et al. The prevalence of couple infertility in the United States from a male perspective: evidence from a nationally representative sample. *Andrology* 2013; **1**: 741-748.
[4] Toft G. Persistent organochlorine pollutants and human reproductive health. *Dan Med J* 2014; **61**: B4967.
[5] Toft G, Jonsson BA, Lindh CH, Giwercman A, Spano M, Heederik D, et al. Exposure to perfluorinated compounds and human semen quality in Arctic and European populations. *Hum Reprod* 2014; **27**: 2532-2540.
[6] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev* 2009; **30**: 293-342.
[7] WHO. *Evaluation of certain food additives and contaminants*. Geneva: WHO Press; 2007.
[8] Environment Canada, Health Canada. Proposed risk management approach for bromic acid, potassium salt (potassium bromate). Chemical Abstracts Service Registry Number (CAS RN): 7758-01-2. [Online] Available from: https://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=D1613C58-1#top [Accessed on September, 2010].
[9] Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y. Toxicity and carcinogenicity of potassium bromate–a new renal carcinogen. *Environ Health Perspect* 1990; **87**: 309-335.
[10] DeAngelo AB, George MH, Kilburn SR, Moore TM, Wolf DC. Carcinogenicity of potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N rats. *Toxicol Pathol* 1998; **26**(5): 587-594.
[11] IARC. *Monographs on the evaluation of carcinogenic risks to humans, Vol. 71. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide*. Lyon: IARC Press; 1999, p. 1325-1533.
[12] Cunningham W, Warner CR. Br concentration as an indication of pre-baking bromination of bread products. *Food Addit Contam* 2000; **17**: 143-148.
[13] Clough G. Environmental effects on animals used in biomedical research. *Biol Rev* 1982; **57**: 487-523.
[14] Wong MA, Pace SM. Feeding pattern and gastrointestinal transit rate of rats under different room lighting schedules. *Lab Anim Sci* 1981; **31**: 251-258.
[15] Elsheikh AS, Takahashi Y, Hishinuma M, Kanagawa H. Effect of encapsulation on development of mouse pronuclear stage embryos in vitro. *Anim Reprod Sci* 1997; **48**: 317-324.
[16] Johannsen OA. *Histological technique: a guide for use in a laboratory course in histology*. Hoboken: J. Wiley & Sons; 1927.
[17] Fisher J, Bull RJ. Development of a rat dosimetry model for bromated. *Toxicology* 2006; **221**(2–3): 235-240.
[18] Kurokawa Y, Aoki S, Matsushima Y, Takamura N, Imazawa T, Hayashi Y. Dose-response studies on the carcinogenicity of potassium bromate in F344 rats after long-term oral administration. *J Natl Cancer Inst* 1986; **77**: 977-982.
[19] Nakano K, Okada S, Toyokuno S, Midorikawa O. Renal changes induced by chronic oral administration of potassium bromate or ferric nitrilotriacetate in Wistar rats. *Jpn Arch Intern Med* 1989; **36**(2): 41-48.
[20] Kurokawa Y, Hayashi Y, Maekawa A, Takahasi M, Kokubo T, Odashima S. Carcinogenicity of potassium bromate administered orally to F344 rats. *J Natl Cancer Inst* 1983; **71**: 965-972.
[21] Farombi EO, Alabi MC, Alkuru TO. Kolaviron modulates cellular redox status and impairment of membrane protein activities induced by potassium bromate in rats. *Pharmacol Res* 2002; **45**: 63-68.
[22] Watanabe S, Tajima Y, Yamaguchi T, Fukui T. Potassium bromate induced hyperuricemia stimulates acute kidney damage and oxidative stress. *J Health Sci* 2004; **50**: 647-653.
[23] Abuelgasim AL, Omer R, Elmahdi B. Serobiochemical: effects of potassium bromate on Wistar albino rats. *Am J Food Technol* 2008; **3**: 303-309.
[24] Reynolds RW, Bryson G. Effect of estradoil on the hypothalamic regulation of body weight in the rat. *Res Commun Chem Pathol Pharmacol* 1974; **7**: 715-724.
[25] Stoker TE, Goldman JM, Cooper RL. The dithiocarbamate fungicide thiram disrupts the hormonal control of ovulation in the female rat. *Reprod Toxicol* 1993; **7**: 211-218.
[26] Klinefelter GR, Strader LF, Suarez JD, Roberts NL, Goldman JM, Murr AS. Continuous exposure to dibromoacetic acid delays pubertal development and compromises sperm quality in the rat. *Toxicol Sci* 2004; **81**: 419-429.
[27] Beverly T, Towery MD. The physiology of iodine. *Bull World Health Org* 1953; **9**: 175-182.
[28] Smyth PPA. Role of iodine in antioxidant defence in thyroid and breast disease. *Biofactors* 2008; **19**: 121-130.
[29] Sahoo DK, Roy A, Bhanja S, Chainy GBN. Hypothyroidism impairs antioxidant defence system and testicular physiology during development and maturation. *Gen Comp Endocrinol* 2008; **156**: 63-70.
[30] Peltola V, Huhtaniemi I, Ahotupa M. Antioxidant enzyme activity in the maturing rat testis. *J Androl* 1992; **13**: 450-455.
[31] Chlubek D. Floride and oxidative stress (editorial). *Fluoride* 2003; **36**: 217-228.
[32] Crockford SJ. Evolutionary roots of iodine and thyroid hormones in cell-cell signaling. *Integr Comp Biol* 2009; **49**(2): 155-166.