Saudi Journal of Biological Sciences xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Deleterious effects of potassium bromate administration on renal and hepatic tissues of Swiss mice

Naif G. Altoom [a], Jamaan Ajarem [a], Ahmed A. Allam [a,b], Saleh N. Maodaa [a], Mostafa A. Abdel-Maksoud [a,*]

[a] King Saud University, College of Science, Department of Zoology, Riyadh 11451, Saudi Arabia
[b] Beni-suef University, Faculty of Science, Department of Zoology, Beni-Suef, Egypt

ARTICLE INFO

Article history:
Received 19 January 2016
Revised 29 January 2017
Accepted 30 January 2017
Available online xxxx

Keywords:
Platelets
Lipid profile
LDH
Creatinine
Reduced glutathione

ABSTRACT

Potassium bromate ($KBrO_3$) is widely used as a food additive and is a major water disinfection by-product. The present study reports the side effects of $KBrO_3$ administration in Swiss mice. Animals were randomly divided into three groups: control, low dose $KBrO_3$ (100 mg/kg/day) and high dose $KBrO_3$ (200 mg/kg/day) groups. Administration of $KBrO_3$ led to decreased white blood corpuscles (WBCs), red blood corpuscles (RBCs) and platelets count in the animals of both the high and the low dose groups. Altered lipid profile represented as low density lipoprotein (LDL), high density lipoprotein (HDL) and cholesterol levels were observed in plasma samples of both $KBrO_3$ treated groups of mice. Also, an increased plasma level of LDH was detected in both $KBrO_3$ treated groups. Histological investigations showed impaired renal and hepatic histology that was concomitant with increased plasma Creatinine level in both of $KBrO_3$-treated groups. Nevertheless, decreased glutathione (GSH) level in both renal and hepatic tissue of mice after $KBrO_3$ intake was detected. These results show that $KBrO_3$ has serious damaging effects and therefore, its use should be avoided.

1. Introduction

Potassium bromate ($KBrO_3$) is a well-known flour improver that acts as a maturing agent (Vadlamani and Seib, 1999). It has been in use as a food additive for the past 90 years (Oloyede and Sunmonu, 2009). It acts principally in the late dough stage giving strength and elasticity to the dough during the baking process while also promoting the rise of bread. $KBrO_3$ is also used in beer making, cheese production and is commonly added to fish paste products (Ahmad and Mahmood, 2014). Additionally, it is used in pharmaceutical and cosmotic industries and is a constituent of cold wave hair solutions (Oloyede and Sunmonu, 2009). Moreover, $KBrO_3$ can appear as a byproduct in an ozonization of water containing bromide. As a result of $KBrO_3$ biotransformation, free radicals generation can cause oxidative damage to essential cellular macromolecules, leading to marked nephrotoxicity and cancer in experimental animals (Chipman et al., 1998). International Agency for Research on Cancer (IARC) has classified $KBrO_3$ as a possible human carcinogen (group 2B) and its application in food processing was restricted. Indeed, many previous reports has documented that $KBrO_3$ can induce multiple organ toxicity in humans and experimental animals (Farombi et al., 2002; Kujawska et al., 2013; Ahmad et al., 2015) and that kidney is considered to be the primary target organ of these dangerous compound (Kurokawa et al., 1990; Ahmad et al., 2013). $KBrO_3$ is extremely irritating and injurious to tissues especially those of the central nervous system (CNS) and kidneys. The pathological findings included renal tissue damage and haemolysis (Robert and William, 1996). Carcinogenic and mutagenic effects of $KBrO_3$ have been also reported in experimental animals (Kurokawa et al., 1987). Several cases of accidental poisoning in children resulting from ingestion of bromate solution and sugar contaminated with bromate were reported as the source of an outbreak of mild poisoning in New Zealand (Paul, 1966). Consequently, $KBrO_3$ has been banned in several countries including the United Kingdom in 1990, Nigeria in 1993 and Canada in 1994 (Oloyede and Sunmonu, 2009). Toxicological studies have convincingly shown that $KBrO_3$ affects

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

* Corresponding author at: Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.
E-mail addresses: nalotaiby1@hotmail.com (N.G. Altoom), jajarem@ksu.edu.sa (J. Ajarem), aallam@ksu.edu.sa (A.A. Allam), maodaa_28@yahoo.com (S.N. Maodaa), harrany@gmail.com (M.A. Abdel-Maksoud).

http://dx.doi.org/10.1016/j.sjbs.2017.01.060
1319-562X/

Please cite this article in press as: Altoom, N.G., et al. Deleterious effects of potassium bromate administration on renal and hepatic tissues of Swiss mice. Saudi Journal of Biological Sciences (2017), http://dx.doi.org/10.1016/j.sjbs.2017.01.060

the nutritional quality of bread as the main vitamins available in bread are degraded (Sai et al., 1992). It is known that $KBrO_3$ induces oxidative stress in tissues (Sai et al., 1991; Watanabe et al., 1992; Parsons and Chipman, 1992, 2000) that could be the basis of bromate-induced carcinogenesis (Chipman et al., 2006). The present study attempts to assess the effects of oral administration of $KBrO_3$ on the lipid profile in plasma, oxidative stress, hepatic and renal histomorphology of Swiss mice using two different doses of $KBrO_3$ to compare their effects.

2. Materials and methods

2.1. Animals

Forty five (45) Swiss Webster (SW) mice were obtained from animal house-College of pharmacy-king Saud University and maintained and monitored in a specific pathogen-free environment. All animal procedures were performed in accordance with the standards set forth in the Guidelines for the Care and Use of Experimental Animals issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Animal Ethics Committee at King Saud University. All animals were allowed to acclimatize in plastic cages inside a well-ventilated room for one week prior to the experiment. The animals were maintained under standard laboratory conditions (temperature of 23 °C, relative humidity of 60–70% and a 12-h light/dark cycle), fed a diet of standard commercial pellets and given water *ad libitum*.

2.2. $KBrO_3$ preparation and dosing schedule

Potassium bromate salt, a product of British drug home limited, Poole England was supplied in its white crystalline form by ASILA chemicals (Saudi Arabia). It was then dissolved in water to prepare the 100 mg/kg dose (0.5 gm/L) and the 200 mg/kg dose (1 gm/L). Animals were divided into 3 groups as follows: Group (I) control group (was given distilled water); Group (II) Low dose $KBrO_3$ group (was given 100 mg/kg); Group (III) High dose $KBrO_3$ group (was given 200 mg/kg). $KBro_3$ was orally administered daily through oral intubation at the two doses of 100 and 200 mg/kg/day for 42 days.

2.3. Sample collection

Blood was collected from the heart in heparinized tubes and plasma was obtained for biochemical investigations. Plasma was stored at −80 °C until use. Small pieces of liver and kidneys were removed, cut and put in sterile saline. The pieces were then fixed in 10% neutral buffered formalin and then embedded in paraffin.

2.4. Histological analysis of hepatic and renal tissues

The preparation of tissues for histological examination was done as described by Krause (2001); the photomicrographs were observed using the Leitz, DIALUX research microscope at x200. Pathological evaluation in H/E stained tissue sections was done by a pathologist blinded for the experimental regimen.

2.5. Cell blood count (CBC)

Whole blood samples were analyzed with an automatic Vet abc™ Animal Blood Counter (Horiba ABX, Montpellier, France) using the hematology kits specified for that instrument (Horiba ABX, France) according to the manufacturer's instructions.

2.6. Determination of creatinine level in plasma

Plasma samples were analyzed using commercial kits (bioMerieux, Marcy l'Etoile, France) for Creatinine according to the instructions of the manufacturer. Absorbance was measured with an Ultrospec 2000 U/V spectrophotometer (Amersham Pharmacia Biotech, Cambridge, England).

2.7. Lactate dehydrogenase (LDH)

Lactate dehydrogenase was determined using specified kits LiquiUV Test (Human, Germany) according to the manufacturer's instructions. Briefly, 20 µl of plasma was added to 1000 µl buffer solution (provided by in the kit) then incubated in cuvettes for 5 min at 30 °C. After that, 250 µl of the substrate was mixed with the solution and the absorbance was monitored after 1, 2 and 3 min. The colour development was detected at 340 nm in a spectrophotometer.

2.8. Lipid profile in plasma

LDL, HDL and total cholesterol levels were measured by an enzymatic colorimetric kit (Wako Chemicals USA, Inc.). Briefly, 10 µl of plasma were put into tubes and 1 ml of colour reagent solution was then added. 10 µl of standard solution (provided by in the kit), were put into tubes and 1 ml of colour reagent solution was then added. The solution was mixed well and incubated at 37 °C for 5 min. The colour development was detected at 500 nm in a spectrophotometer.

2.9. Glutathione (GSH) assay

Glutathione content was determined according to the procedure of Beutler et al. (1963) with some modification. Briefly, 0.20 ml of tissue supernatant was mixed with 1.5 ml precipitating solution containing 1.67% glacial metaphosphoric acid, 0.20% Na-EDTA and 30% NaCl. The mixture was allowed to stand for 5 min at room temperature and centrifuged 1000*g* for 5 min. One ml clear supernatant was mixed with 4 ml 0.30 M Na_2HPO_4 and 0.50 ml DTNB reagent (40 mg 5, 5′dithiobis-(2-nitrobenzoic acid dissolved in 1% sodium citrate). A blank was similarly prepared in which 0.20 ml water was used instead of the brain supernatant. The absorbance of the color was measured at 412 nm in a spectrophotometer.

Table 1
Effect of $KBrO_3$ treatment on the RBCs, WBCs and platelets count RBCs, WBCs and platelets count were measured in the three groups of mice, and the results are presented as the means ± SEM (n = 10), $^{*}P < 0.05$ for low dose $KBrO_3$ group vs. control; $^{\#}P < 0.05$ for high dose $KBrO_3$ group vs. control.

	Mean total leukocyte count ($\times 10^9$/L)	Mean total reticulocyte count ($\times 10^9$/L)	Mean platelet count ($\times 10^9$/L)
Control	11.3 ± 0.62	8.3352 ± 0.13200	875 ± 38
$KBrO_3$ (100 mg/dl)	10.0 ± 0.58*	7.2550 ± 0.3294*	423 ± 42*
$KBrO_3$ (200 mg/dl)	9.5 ± 0.93$^{\#}$	7.1375 ± 0.4019$^{\#}$	405 ± 63$^{\#}$

Please cite this article in press as: Altoom, N.G., et al. Deleterious effects of potassium bromate administration on renal and hepatic tissues of Swiss mice. Saudi Journal of Biological Sciences (2017), http://dx.doi.org/10.1016/j.sjbs.2017.01.060