

Hepatotoxic Effects of Potassium Bromate on Adult Wistar Rats

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Abstract

Objective: We aimed to demonstrate the histopathologic effects of potassium bromate (KBrO₃) on the liver cells of rats following oral administration. **Method:** Twenty young Wistar rats of weights 196-215g were divided into four groups. The control group A was orally administered with 1ml of distilled water daily; the experimental groups B, C and D were orally administered with 50, 100, and 200 mg/kg body weight/day dosages of KBrO₃ for 35 days. Both the control and experimental groups were sacrificed using the chloroform inhalation method at the end of study period. **Results:** Rats which received 200 mg kg⁻¹ b.wt. of KBrO₃ died within the 20th day of administration. The body weights were significantly increased ($P < 0.05$) in the experimental groups from the 3rd to the 5th week of study while the relative weight of their liver were not affected compared to the control group. Histopathological examination of the experimental groups indicated; little sinusoidal dilatation in rats treated with 50 mg kg⁻¹ b.wt. of KBrO₃; hepatic vacuolation, large sinusoidal dilatation, degenerative changes and cellular congestion in rats, which received 100, 200 mg kg⁻¹ b.wt. of KBrO₃ compared with the control group, which maintained normal kidney tissues. These histological alterations appeared marked in rats administered with 200 mg kg⁻¹ b.wt. of KBrO₃. **Conclusion:** The present study indicated dose-dependent, histopathologic effects on the liver cells of rats administered with KBrO₃. Our findings therefore suggest that chronic KBrO₃ consumption may put the liver at some risk of adverse histopathological conditions.

Keywords: Liver, histopathology, potassium bromate, hepatotoxicity.

1. Introduction

Potassium bromate (KBrO₃) is a food additive, which exists as a white crystals or powder. It is used primarily as a maturing agent for flour and as a dough conditioner [1]. It is also generated as a by-product of ozonization of surface water in treated drinking water [2]. Several studies have reported the nephro and neuro-toxicity of KBrO₃ in man and its carcinogenicity in animals following exposure [3-6], thus demonstrating the danger, which potassium bromate poses to health if consumed in food or water. Other studies have also shown that it possesses the potential of inducing deafness, redness and pains of the eye and skin [7, 8]. In Nigeria, KBrO₃ has been declared unsafe and banned from the list of food additives by the National Agency for Food, Drug Administration and Control (NAFDAC). However, despite the ban and the awareness created by NAFDAC on the danger of using potassium bromate as flour enhancer, many bakers and water-treatment plant owners still use the substance. This poses a great threat to food safety and public health especially to most Nigerians who use bakery products and commercially prepared (ozonised) water, who may be ignorant of the adverse effects of KBrO₃ on body tissues.

Limited information exists concerning the cellular changes associated with toxicity of KBrO₃ in liver tissues. The impairment of organ function is a direct consequence of alterations in the histological structures of the organ, which may be dependent on the level of exposure to toxic substances [9]. These facts call for more studies to demonstrate the hepatotoxic effects of KBrO₃. We therefore aimed to demonstrate the histopathologic changes in the liver tissues of rats following exposure to different doses of KBrO₃.

2. Materials and Methods

2.1 Animals

Twenty apparently healthy Wistar albino rats of both sexes weighing 196 - 215 g were used. They were housed

in the animal house of the Department of Human Anatomy, Anambra State University, Uli, under standard conditions ($29 \pm 2^\circ\text{C}$ temperature, 40-55% humidity, good ventilation) and had free access to water and diet (normal rat chow). They were acclimatized for two weeks before the start of the experiment.

2.2 Experimental Design

The animals were divided randomly into four groups A, B, C, D, each containing five rats. The rats were also separated into males and females in each cage. Group A was left as the control group, which were orally administered with 1ml of distilled water daily for 35 days. The other groups B, C, D served as the experimental groups and were orally administered with potassium bromate (manufactured by Windia Speciality Chemicals LTD, Tamil Nadu, India) for 35 days respectively. The concentration of KBrO_3 used was 25 g per liter of distilled water; the average bromated water consumption was 0.6 ml/day (equivalent to 15 mg KBrO_3 /day) and the average body weight was 0.2 kg. The corresponding daily dosages of KBrO_3 were then calculated and measured as 50, 100 and 200 mg/kg b.w./day and administered to groups B, C, and D respectively. Kurokawa et al. reported that the oral LD50 of KBrO_3 for both male and female Wistar rats were approximately 160-180 mg/kg body weight [4]. They also identified 63 mg KBrO_3 /kg-day as an adverse effect level. The present experiment was designed to be dose-dependent with the different doses of KBrO_3 administered daily to the experimental groups for a period of 5 weeks. Both the control and experimental groups were sacrificed (using the chloroform inhalation method) after 35 days from the onset of administration of KBrO_3 .

2.3 Preparation and Staining of Tissues and Histological Examination

As soon as the animals were sacrificed, they were quickly dissected, the liver removed, and the slices immediately fixed in a fixative (10% buffered formalin), transferred into specimen bottles, and kept frozen for 48 hours before being used. The liver tissues were embedded in paraffin wax, sectioned at 5 μm and stained by hematoxylin and eosin [10]. The photomicrographs were observed using Nikon research microscope (Novex, Holland). The micrograph pictures were taken with digital camera (DCM 510.5M Pixels, CMOS chip) connected to the microscope.

2.4 Data Analysis

Data was expressed as mean \pm standard deviation. Comparative analysis was done using analysis of variance (ANOVA). Post-hoc comparative analysis was performed using Bonferroni comparison test. Statistical significance was set at $P < 0.05$. All statistics were done using SPSS for windows (version 16.0).

3. Results

All rats, which received 200 mg/kg b.w. potassium bromate, died within twenty days. Significant differences were observed in the body weights of rats administered with potassium bromate compared with the controls in the 3rd, 4th, and 5th weeks of study respectively (Table 1).

Table 1. Mean body weight of rats orally administered with potassium bromate according to the study periods.

STUDY PERIODS	GROUP A	GROUP B	GROUP C	GROUP D	F-STAT	P
1 st Week	200.6 \pm 3.28	201.8 \pm 4.60	204.4 \pm 4.56	203.6 \pm 6.50	0.62	0.61
2 nd week	205.6 \pm 6.65	210.0 \pm 7.90	209.0 \pm 7.41	222.0 \pm 38.34	0.63	0.60
3 rd week	222.2 \pm 10.68	218.4 \pm 7.09	201.6 \pm 11.73*	-	5.96	0.02
4 th Week	252.0 \pm 4.47	219.6 \pm 10.16*	190.0 \pm 10.0*	-	64.59	0.000
5 th Week	260.0 \pm 10.0	220.0 \pm 9.35*	188.0 \pm 10.95*	-	63.48	0.000

Data are mean \pm standard deviation. * Significantly ($P < 0.05$, $P < 0.001$) different from group

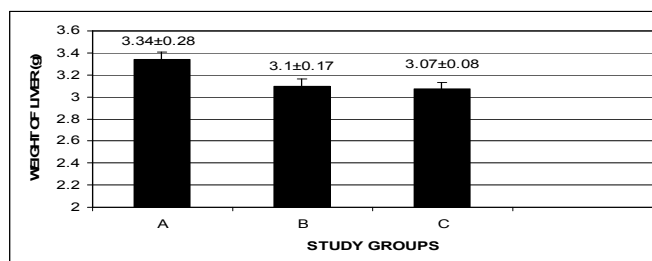


Figure 1. Mean weight of liver in rats orally administered with potassium bromate at the end of study period.

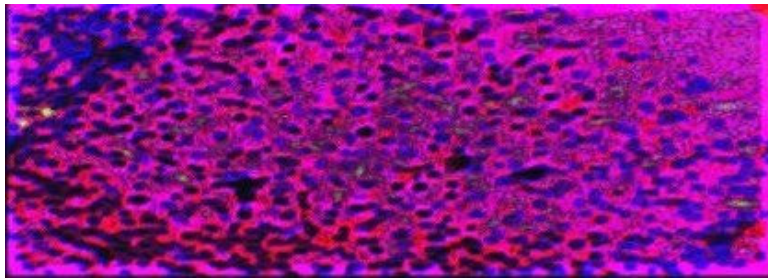


Figure 2. Photomicrograph of Hepatocyte of group A rats administered with distilled water showing normal histology (H&E x 100).

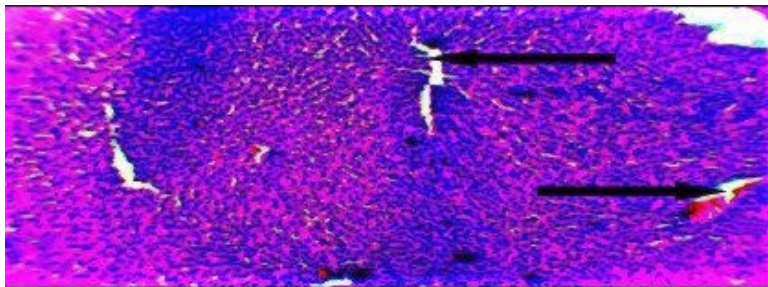


Figure 3. Photomicrograph of liver cell of group B rat administered with 50 mg/kg b.w./day KBrO₃ showing little sinusoidal spaces (H&E x 100).

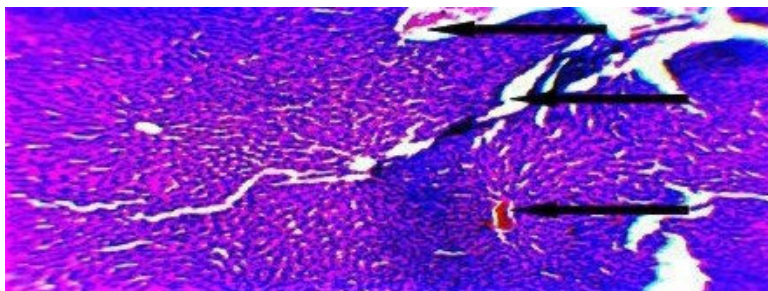


Figure 4. Photomicrograph of hepatocyte sinusoidal blood vessel of group C rat administered with 100 mg/kg showing fenestrated endothelial cells and acute sinusoidal spaces (H&E x 100).

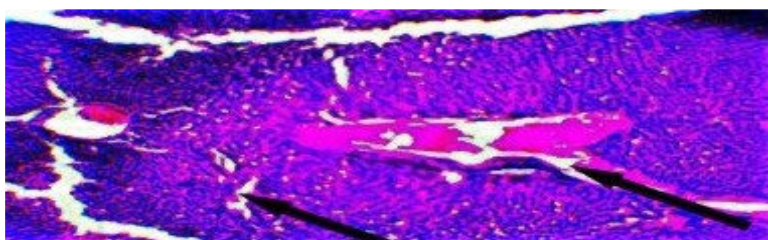


Figure 5. Photomicrograph of hepatocyte of group C rat administered with 100 mg/kg b.w./day KBrO₃ showing degenerative changes and wide hepatic sinusoidal spaces or dilation (H&E x 100).

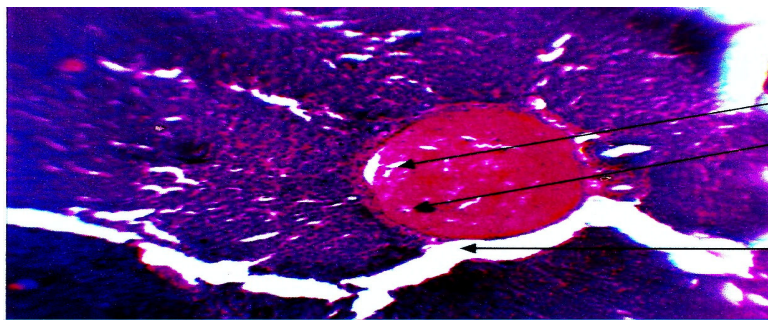


Figure 6. Photomicrograph of liver cell of group D rat administered with 200 mg/kg b.w./day KBrO₃ showing congestive hepatopathy demonstrating perisinusoidal fibrosis and centrilobular (zone III) sinusoidal dilatation (H&E x 100).

At the end of study period, the relative weights of liver in the rats administered with 50 mg and 100 mg/kg b.w./day potassium bromate were not significantly affected compared to the control (Figure 1). The control group A, administered with distilled water, maintained their normal histology at the end of the study (Figure 2). The hepatocytes of group B rats administered with 50 mg/kg b.w./day KBrO₃ indicated small sinusoidal spaces (Figure 3). The sinusoidal blood vessels of group C rats administered with 100 mg/kg b.w./day KBrO₃ indicated fenestrated endothelial cells. There were also the presence of wide hepatic sinusoidal dilatation and degenerative changes (Figures 4 & 5). In group D rats administered with 200 mg/kg b.w./day KBrO₃ there were evidences of congestive hepatopathy such as perisinusoidal fibrosis and centrilobular (zone III) sinusoidal dilatation (Table 6).

4. Discussion

In the present study, there were significant reductions in body weights of rats administered with KBrO₃ compared with the controls. This was more evident in the 4th and 5th weeks of study where rats administered with 50 mg and 100 mg/kg b.w./day KBrO₃ had significantly lower body weights compared with the controls. This agrees with a previous study [11], which reported a significant reduction in bodyweight of rabbits administered with potassium bromate. In contrast, other studies [12, 13, 14] have reported absence of KBrO₃ effect on body weights of rats. In addition, no significant difference was observed in the liver weight of rats administered with KBrO₃ compared with the controls in the present study. This finding agrees with a previous study by Farombi et al [13], but in contrast with Watanabe et al and Abuelgasim et al [12, 13], which reported relative liver weight increase in rats administered with 100 mg kg⁻¹ b.wt. of potassium bromate.

Histopathologic findings indicated normal liver tissues in the control group, administered with distilled water. In contrast, hepatic sinusoidal dilatations were observed in groups B, C and D. The dilatation appeared small in group B but became wider with increase in KBrO₃ dosage in groups C and D. Vacuolation and sinusoidal dilatation of liver cells have been previously associated with reduction of antioxidant enzymes and enhancement of xanthine oxidase and lipid peroxidase by KBrO₃ [15, 16]. Degenerative changes and fenestration of endothelial cells observed in group C rats administered with 100 mg kg⁻¹ b.wt. of potassium bromate, may be an indication of the destruction of the capillary endothelium of the liver by the chemical substance. This may result in reduction in total protein and albumin synthesis and increase in alanine transaminase (ALT), which are consequent with hepatic cell damage and injured cell membrane permeability [15]. The total protein and albumin has been shown to decrease significantly, while the activity of ALT increased in rats administered with 100 and 200 mg kg⁻¹ b.wt. of potassium bromate [15]. The incidence of congestion of cells indicated by the liver following administration of 200 mg kg⁻¹ b.wt. of KBrO₃, may be due to damage to the hepatocytes by the chemical compound. This may be an indication of cirrhosis, which usually disrupts the normal flow of blood through the liver [17].

5. Conclusion

In conclusion, the present study indicated that a long-term exposure to small and high dosages of KBrO₃ caused alterations in the histology of the liver of Wistar rats. Some of the histopathologic effects on the liver cells were either mild or absent in rats administered with 50 mg kg⁻¹ b.wt., but marked in those exposed to high-dose KBrO₃, thus indicating dose-dependent effect. These alterations may account for the various hepatotoxic effects associated with exposure to KBrO₃.

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