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Original Research Article

The Histological Effect of Potassium Bromate on the Cerebellum of Adult Wistar Rats

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ABSTRACT

Objectives: The present study was aimed at assessing histological changes on the cerebellum following low- and high-dosage oral administration of potassium bromate to adult wistar rats.

Methods: Twenty healthy adult wistar rats weighing 180-200g were used for the study. They were fed with feed and water during an acclimatization period of two weeks before administration of potassium bromate began. They were divided into four groups: A, B C and D. Group A served as control while rats in groups B, C and D received 50, 100 and 150mg per kg body weight of potassium bromate respectively. Administration lasted for 21 days and body weight was recorded weekly. At the end of administration, they were sacrificed by chloroform inhalation method, and the cerebellum was harvested and processed for examination.

Results: The control group showed normal weight gain as well as normal physical activity. Group D showed unsteadiness in movement, difficulty in breathing, and two rats died after the 18th day of administration. Rats in group C experienced difficulty in breathing and slight unsteadiness in movement. Group B were mildly affected. Histopathological examination of groups C and D showed degenerative changes and haemorrhage in the cerebellum.

Conclusion: The present study showed that potassium bromate had harmful effects on the cerebellum in a dose-dependent manner, and its use should be discouraged.

Keywords: Potassium bromate, cerebellum, toxicity, lethal dose.

INTRODUCTION

Potassium bromate is a white crystal, granule or powder, which is colourless, odourless, and tasteless. It has no medicinal value but is added to flour as a maturing agent. [1] Potassium bromate is typically used by flour millers and bakers as a flour improver, strengthening the dough and allowing higher rising, [2] added to the fact that it is cheap and probably the most effective oxidizing agent. [3]

Bromate was first discovered to cause tumours in rats in 1982, and subsequent studies confirmed its damage to the kidney, liver, thyroid and other organs. [4-6] These led to its ban from use in food products in Canada, Nigeria, Brazil, Peru, Sri Lanka and China. [3] Parson and Chipman [7] in their study implicated

potassium bromate with inducing oxidative stress in tissues. According to Atkins, [8] potassium bromate could cause cough and sore throat when inhaled in humans, and abdominal pain, diarrhea, nausea, vomiting, kidney failure, hearing loss, bronchial and ocular problems, when ingested.

Although potassium bromate has been banned in Nigeria by the National Agency for Food, Drug Administration and Control (NAFDAC), Nigerians still consume its products, posing a great threat to food safety and public health. The only study reported on the brain was by Abuelgasim *et al*. [9] in Sudan, who amongst other things reported that potassium bromate caused vacuolation, neuronal degeneration, and haemorrhage. Owing to paucity of reported studies of potassium bromate toxicity to the cerebellum, the present study is concerned with the establishment of histological changes observed in the cerebellum of adult wistar rats on ingestion of potassium bromate.

MATERIALS AND METHODS

Twenty apparently healthy wistar rats of female sex weighing about 180g to 200g which were considered fit to withstand the experiment were used. They were housed in perspex cages and fed with animal feed and water for a period of two weeks during acclimatization with the new environment. Good ventilation system, favourable humidity and temperature condition of the room was ensured. The Guide for the Care and Use of Laboratory Animals was followed. [10]

Experimental Protocol

Potassium bromate ($KBrO_3$) manufactured by Windia Specialty Chemical PVT Ltd, Tamil Wadu, India was used. Twenty five grams (25g) was dissolved into 1000ml of distilled water. The rats were randomly divided into four groups A, B, C, and D each group containing five rats. Group A received distilled water, while groups B, C, and D were orally administered with $KBrO_3$ at concentration of 50, 100, 150mg/kg b.wt respectively. The weight of each rat per group was recorded according to group. This was done before and after potassium bromate administration to the wistar rats. The reported LD50 of $KBrO_3$ from a study by Kurokawa *et al* was 160-180mg/kg b.wt, and the adverse effect level was 63mg/kg b.wt. [11]

Dissection

The animals were sacrificed after anaesthesizing them with chloroform. The sagittal suture was traced with dissecting blade after the scalp had been reflected, in order to collect the cerebellum, and fixed in 10% formal saline.

Tissue Processing and Staining

After the sacrifice of the animals, tissue processing was done according to the standard procedures. The tissue was fixed in 10% formal saline. Other routine steps (dehydration, clearing, impregnation, embedding in paraffin wax, sectioning, staining with H&E and final mounting) followed. They were finally observed under the light photomicroscope and their photomicrographs were taken and analyzed.

Data Analysis

The mean and standard deviation for the body weight during administration was generated using the SPSS software, Version 16 (Chicago, Il, USA), and the paired t-test and one way ANOVA test were determined.

RESULTS

Table 1: Weights of the control and test groups, and analysis of variance (ANOVA)

GROUP	WEEK 1	WEEK 2	WEEK 3
A (Control)	234 (5.48)	244 (5.47)	256 (5.48)
B (50mg/kg)	240 (7.07)	248 (4.47)	254 (5.48)
C (100mg/kg)	248 (4.47)	250 (0.00)	246 (5.48)
D (150mg/kg)	258 (4.47)	260 (0.00)	250 (0.00)
P-Value	P<0.001	P<0.001	0.02

Data are means and standard deviation. Analysis of variance (ANOVA) showed significant difference ($p<0.05$) in

body weights between the groups for the three weeks of administration of potassium bromate. The result showed that the effect of potassium bromate on weight was dosage-dependent.

Table 2: Initial and final weight of the control and test groups, and paired t-test.

GROUP	INITIAL	FINAL	P-Value
A (Control)	234 (5.48)	256 (5.48)	P<0.001
B (50mg/kg)	240 (7.07)	254 (5.48)	0.03
C (100mg/kg)	248 (4.47)	246 (5.48)	0.37
D (150mg/kg)	258 (4.47)	250 (0.00)	0.02

Data are means and standard deviation. Paired sample t-test was used to compare initial and final weight for the four groups. The control group and group B showed significant increase ($p<0.05$) in weight, group C had an insignificant decrease ($p>0.05$) while group D had a significant decrease ($p<0.05$) in weight. The result showed that potassium bromate had duration-dependent effects on weight.

Physical Observation

The control group showed normal weight gain as well as normal physical activity throughout the duration of the experiment, however, the treated group showed less active behaviour. Group B were mildly or not affected compared to the control, and showed weight gain. Difficulty in breathing and slight unsteadiness in movement occurred in group C, as well as weight reduction by the third week. Rats in group D showed unsteadiness in movement, difficulty in breathing, and two rats died after the 18th day of administration. There was also significantly reduced weight after the third week. Histopathological examination of groups C and D showed degenerative changes and haemorrhage in the cerebellum.

Histopathological Studies

The photomicrographs of relevant stained sections of the cerebellum were subsequently taken with the aid of a light microscope at magnification of X100.

- Group A (Control): Showed no significant histopathological change (**Error! Reference source not found.**)
- Group B (50mg/kg): Showed evidence of mild degeneration of Purkinje cells (**Error! Reference source not found.**).
- C (100mg/kg): There was degeneration of Purkinje cells and haemorrhage (**Error! Reference source not found.**).
- D (150mg/kg): There was evidence of degeneration of Purkinje cell layer, inflammation of the granular layer, neuronal degeneration and haemorrhage (**Error! Reference source not found.**).

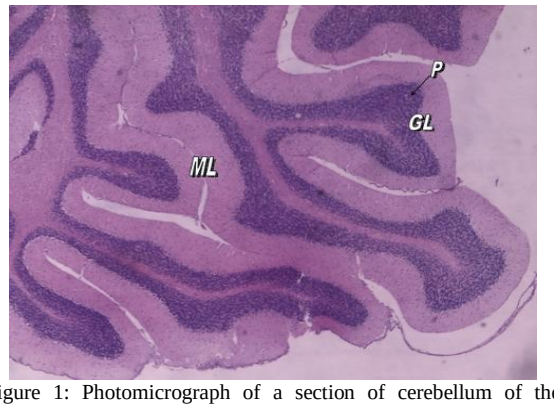

Figure 1: Photomicrograph of a section of cerebellum of the control group showing normal histological features. *Purkinje cell layer (p); granular layer (GL); molecular layer (ML)*. [H & E; X100]

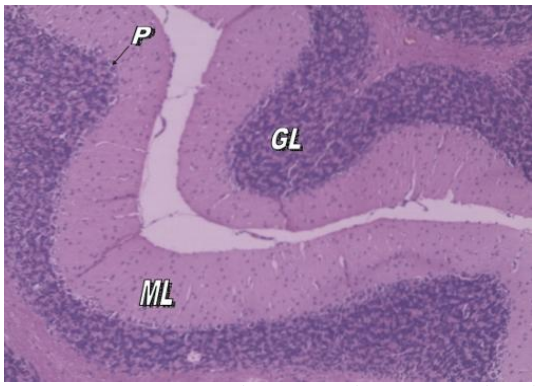

Figure 2: Photomicrograph of a section of cerebellum of rat fed with 50mg/kg potassium bromate, showing evidence of mild or absence of degeneration of Purkinje cells. *Arrow indicates sites of mild degeneration*. [H & E; X100]

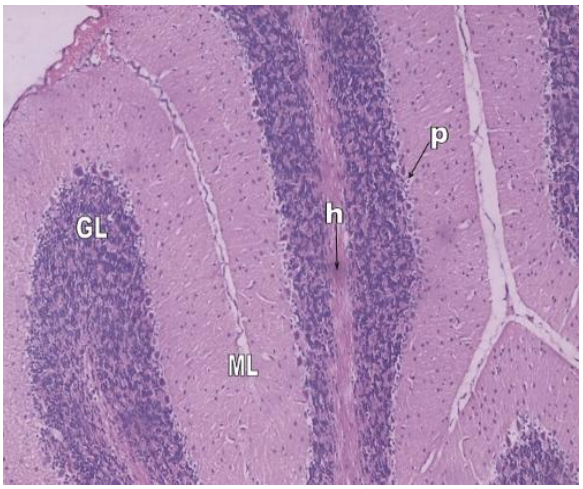

Figure 3 represents a photomicrograph of a section of cerebellum of rat fed with 100mg/kg potassium bromate, showing evidence of degeneration of Purkinje cells and haemorrhage *(h). Arrows indicate site of mild degeneration and haemorrhage (h). (GL=granular layer; ML=molecular layer)* [H & E; X100]

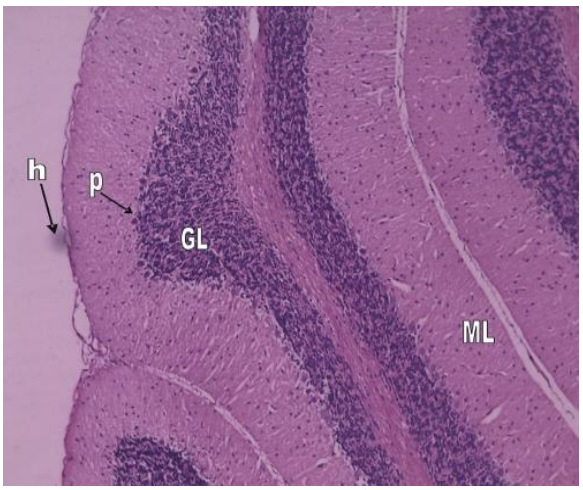

Figure 4 represents a photomicrograph of a section of cerebellum of rat fed with 150mg/kg potassium bromate showing degeneration of Purkinje cell layer (p), inflammation of the granular layer (GL), neuronal degeneration and haemorrhage (h). *Arrows indicates site of degeneration and haemorrhage (h). (ML=Molecular layer).* [H & E; X100]

DISCUSSION

Over time and with increasing dosage, the present study showed that potassium bromate ingestion caused a reduction in body weight. This was clearly seen in high-dosage groups C and D between the second and third week of $KBrO_3$ administration. This is in line with Okalie and Ikewuchi [12] who reported a significant reduction in body weight of rabbits fed with $KBrO_3$, and also Dimkpa et al, [5] who stated that there was significant weight reduction in wistar rats, especially towards the last two weeks of administration. In contrast, Farombi *et al.* [13] and Watanabe *et al*. [14] reported absence of potassium bromate effect on the body weight.

In the present study, death occurred within eighteen days when potassium bromate was administered to rats orally at a dose of 150 mg/kg b.wt. The death could be probably due to multiple problems such as renal failure, liver damage as well as cerebellar damage, and not cerebellar damage in isolation. This result is similar to that reported by Kurokawa *et al*. [11] who pointed out that the oral LD50 of potassium bromate in rats range between 160-180 mg/kg b.wt, and also agrees with studies by Abuelgasim *et al.* [9] where the rats died on the 18th day after a dosage of 200 mg/kg b.wt. However, rats dosed with 100 mg/kg b.wt of potassium bromate exhibited signs of poisoning by difficulty in breathing and slight uncoordination in movement. Histopathological examination in the present study showed evidence that potassium bromate had harmful effects ranging from neuronal degeneration to haemorrhage on the brain. In concurrence, Abuelgasim *et al*. [9] reported such effects as vacuolations, neuronal degeneration and haemorrhage on the brain, though not making specific description to the cerebellum. The result implied that potassium bromate was capable of crossing the blood-brain barrier and exerting negative influence on the cerebellum.

CONCLUSION

The present study indicated that a long term exposure, and high doses of potassium bromate ($KBrO_3$) caused alteration in the histology of the cerebellum of wistar rat. Some of the histopathological

effect on the cerebellar cells were either mild or absent in administration of 50mg/kg b.wt, but there was a marked increase in those exposed to high dose of potassium bromate 100 and 150mg/kg b.wt thus indicating its dose dependent effect**.** Surveillance by the National Agency for Food, Drug Administration and Control (NAFDAC) on confectionaries and punishment to perpetrators should ensure potassium bromate is not used illegally, especially in Nigeria.

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