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Histological and Immunohistochemical Assessment of the Cerebral Cortex Following Potassium Bromate Intoxication in Wistar Rats

Agnes A. Nwakanma[1], Moses B. Ekong[2], Chukwuebuka O. Elemuo[1], Mokutima A. Eluwa[3], Igwe J. Okafor[1], Ann C. Idaguko[4]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria
[2]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo Nigeria
[3]Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria
[4]Department of Anatomy, Faculty of Basic Medical Sciences, Madonna University, Elele, Nigeria

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ABSTRACT

Potassium bromate ($KBrO_3$) is classified as mutagenic and carcinogenic although used in food and cosmetics industries, and also found in drinking water as a by-product of disinfection. Industrial pollution of drinking water and its ability to cross the blood-brain barrier lead to the investigation of its effect on the cerebral cortex. Twenty adult Wistar rats with average weight of 200 g were divided into four groups designated as 'A' for the control group, and 'B, C and D' for the test groups, with each group having five rats. All animals had free access to feed and water. The control group did not receive any treatment, while the test groups received 0.1 mg/kg, 0.2 mg/kg, and 0.3 mg/kg of $KBrO_3$ respectively, for 28 (twenty-eight) days through orogastric tube. On the 29th day, all animals were anaesthetized using ketamine hydrochloride (100 mg/kg, i.p.), and the brains were perfused transcardially with 0.9 % buffered saline followed by 10 % buffered formalin. The brains were removed and further preserved in 10% buffered formalin. The cerebral cortex was dissected, processed for paraffin sectioning and stained for histological study using Haematoxylin and Eosin technique and astrocytes immunolabelling with glial fibrillary acidic protein (GFAP). Results revealed that $KBrO_3$ caused histological alterations and degeneration of neurons and astrocytes in the $KBrO_3$ test groups especially the high dosage groups. These effects were dose dependent, and hence the degeneration of cells may lead to the functional impairment of the cerebral cortex.

Keywords: *Cerebral cortex, histology, potassium bromate, Glial fibrillary acidic protein, Wistar rat*

INTRODUCTION

Potassium bromate ($KBrO_3$) is a nephrotoxic and carcinogenic substance although used in food and cosmetics industry, and also found in drinking water as a by-product of disinfection (Kurokawa et al. 1990). $KBrO_3$ is a colourless, odourless and tasteless white crystal/powder initially used as a food additive and was a commonly used flour enhancing agent in Nigeria (Emeje et al. 2010). Its use in baking was to ensure an increase loaf volume and texture of the bread rise in the oven (Nakamura et al. 2006) probably due to its oxidizing properties.

The maximum concentration of $KBrO_3$ allowed in bread by the United States Food and Drug Agency (FDA) was 0.02 mg/kg (WHO 1992). However, $KBrO_3$ degrades essential vitamins in bread (Ekop et al. 2008), and has been classified by the International Agency for Research on Cancer (IARC) as a possible human carcinogen based on sufficient evidence that $KBrO_3$ is carcinogenic and mutagenic in experimental

Correspondence: Agnes A. Nwakanma, M.Sc., Department of Anatomy, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, P.M.B 02, Uli, Nigeria. Email: akudoekeoma@yahoo.com; +2348037738053

animals (IARC 1986). $KBrO_3$ has also been classified as a genotoxic carcinogen based on positive results in the Ames (Ishidate et al. 1984), chromosome aberration (Ishidate et al. 1984) and micronucleus tests (Hayashi et al. 1988). It is reported to be carcinogenic in the kidney, thyroid, and mesothelium in rats, while being carcinogen in the kidney in mice (DeAngelo et al. 1998; Wolf et al. 1998). Furthermore, Umemura et al. (2006) also reported *in vivo* mutagenic effects of $KBrO_3$ in the kidneys of rats.

The lethal dose (LD50) of $KBrO_3$ in the Wistar rat is higher in the female than in the male, and is reported to be 160-180 mg/kg body weight (Kawachi et al. 1980). Oinuma et al. (1974) reported increase in the levels of cholesterol and phospholipids in the brain and kidney of mice after a single intragastric administration of $KBrO_3$. It is reported to alter cholesterol and vitamin E levels (Keser et al. 2011), cause oxidative damage and histological changes in the kidney (Le Page et al. 1995; Wahba and Ibrahim 2013) and degenerative changes in the liver and the brain (Akanji et al. 2008; Oyewo et al. 2013; Wahba and Ibrahim 2013). Though there are some reports on the effect of $KBrO_3$ on the brain, this is however limited probably due to the ban on its use.

This study arose due to the differing concentrations of $KBrO_3$ in contaminated drinking water humans are exposed to. As the water concentrations of $KBrO_3$ cannot be standardized because of differences in the environment, this has really motivated the need for this study on the effect of a high bioavailability of $KBrO_3$ in the brain with emphasis on the cerebral cortex.

MATERIALS AND METHODS

20 female Wistar rats with average body weight 200 g were used for the study. The animals were handled according to the guidelines for animal care of the United State's National Institute of Health, and the research was approved by the Institutional Ethics Committee. The rats were divided into four groups (A, B, C, and D), of five rats each. Normal rat chow and water was provided *ad libitum* throughout the experimental period.

25 g of $KBrO_3$ (Windia Speciality Chemical LTD, India) was obtained from a reputable shop in Onitsha, Nigeria. This was dissolved in 1 litre of distilled water, and the appropriate dose for each group was calculated. The control group did not receive any treatment, while the test groups were given oral doses of 0.1 mg/kg, 0.2 mg/kg, and 0.3 mg/kg of $KBrO_3$ solution, respectively for 28 days via orogastric tube.

At the end of the experiment (day 29), all the animals were anaesthetized with ketamine hydrochloride (100 mg/kg, i.p.), and was secured into a perfusion tray. The rib cage was cut-open to expose the heart. The rat was quickly perfused transcardially with 0.9 % buffered saline for about four minutes and 10 % buffered formalin for three minutes. The whole brains were then removed and fixed in 10% buffered formalin. The cerebral cortex was dissected, processed for paraffin wax embedding and routinely stained for histological study using haematoxylin and eosin technique and glial fibrillary acidic protein immunohistochemical reaction for astrocytes.

RESULTS

Histological study

The control section of the cerebral cortex showed the developing cortical layers, namely; the marginal layer (I), cortical layer (II), and subcortical layers (III). There was scanty cellular population in the marginal layer, while the cortical and subcortical layers contained pyramidal and granular-shaped cells (Fig. 1A). In animals that received 0.1 mg/kg of $KBrO_3$, the section of the cerebral cortex showed some hypertrophied cells with some cells having karyorrhectic appearance compared to the control group (Fig. 1B). In animals that received 0.2 mg/kg of $KBrO_3$, the section of the cerebral cortex showed hypertrophied cells, with some cells having karyorrhectic appearance compared to the control group (Fig. 1C). In animals that received 0.3 mg/kg of $KBrO_3$, the section of the cerebral cortex showed cells with pyknotic appearance compared to the control group (Fig. 1D).

Astrocytes

The cerebral cortex of group A (control) animals showed immuno expression of GFAP within the cell body and processes of the astrocytes (Fig. 2A). The cerebral cortex of the 0.1 mg/kg of $KBrO_3$ test group showed no difference in the GFAP expression compared to the control (Fig. 2B). The cerebral cortical section of 0.2 mg/kg of $KBrO_3$ test group showed decreased expression of GFAP in the cell bodies and processes compared to the control group (Fig. 2C), while the section of the cerebral cortex of 0.3 mg/kg of $KBrO_3$ test group showed marked decreased expression of GFAP in the cell bodies and processes compared to the control group (Fig. 2D).

DISCUSSION

This study investigated the histological and immunohistochemical effects of $KBrO_3$ on the cerebral cortex of Wistar rats. The results of this study on the sections of the cerebral cortex showed hypertrophy of cells and karyorrhectic features in some, in the animals administered 0.1 mg/kg of $KBrO_3$. There was hypertrophy of cells and karyorrhectic features in some cells in the cerebral cortex of animals administered 0.2 mg/kg of $KBrO_3$, while the cerebral cortex of animals administered 0.3 mg/kg of $KBrO_3$ showed atrophied and pyknotic cells compared to the control group.

Hypertrophy and hyperplasia of cells are usual physiological mechanisms by which cells adjust to changes in their environment, however, this does not rule out the adjustment due to adverse conditions (Huether and McCance 2008). In this study, hypertrophy could have resulted from the adverse effect of $KBrO_3$ as it is reported to cause chromosome aberration (Ishidate et al, 1984), oxidative damage and histological changes in other body organs and the brain (Le Page et al, 1995; Wahba and Ibrahim 2013; Akanji et al. 2008; Oyewo et al. 2013). Pyknosis and karyorrhexis were also observed, and these are cellular degenerative changes resulting from either necrosis or apoptosis (Kumar et al. 2005; Kroemer et al. 2009), which may have been induced by $KBrO_3$ administration. Cortical cells undergoing these degenerative features are known to- result in loss or modification of functions (Bredesen et al. 2006). These degenerative changes may eventually lead to the death of the entire brain area.

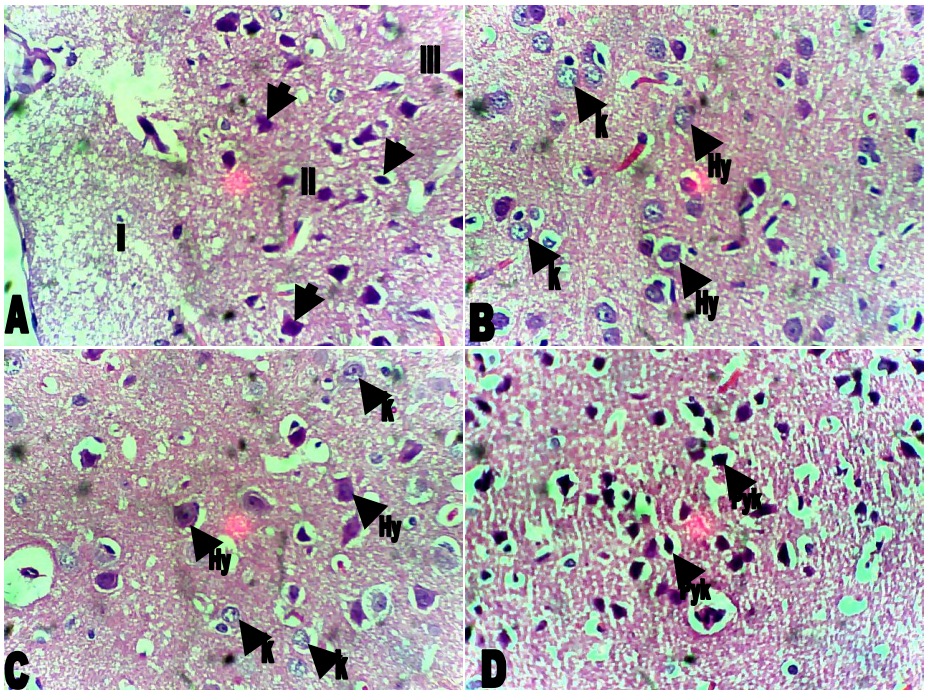

Fig. 1: Representative photomicrographs of sections of the cerebral cortex of Wistar rats. A. The control group showing layers of the cerebral cortex: I= marginal layer, II= cortical layer, III= subcortical layer. Within these layers were different cell types (arrows). B. 0.1 mg/kg KBrO3 group showing some hypertrophied cells (Hy) with some having karyorrhectic appearance (k). C. 0.2 mg/kg $KBrO_3$ group showing hypertrophied cells (Hy), with some cells having karyorrhectic appearance (arrows). D. 0.3 mg/kg $KBrO_3$ group showing showed cells with pyknotic (Pyk) appearance. H & E, ×400.

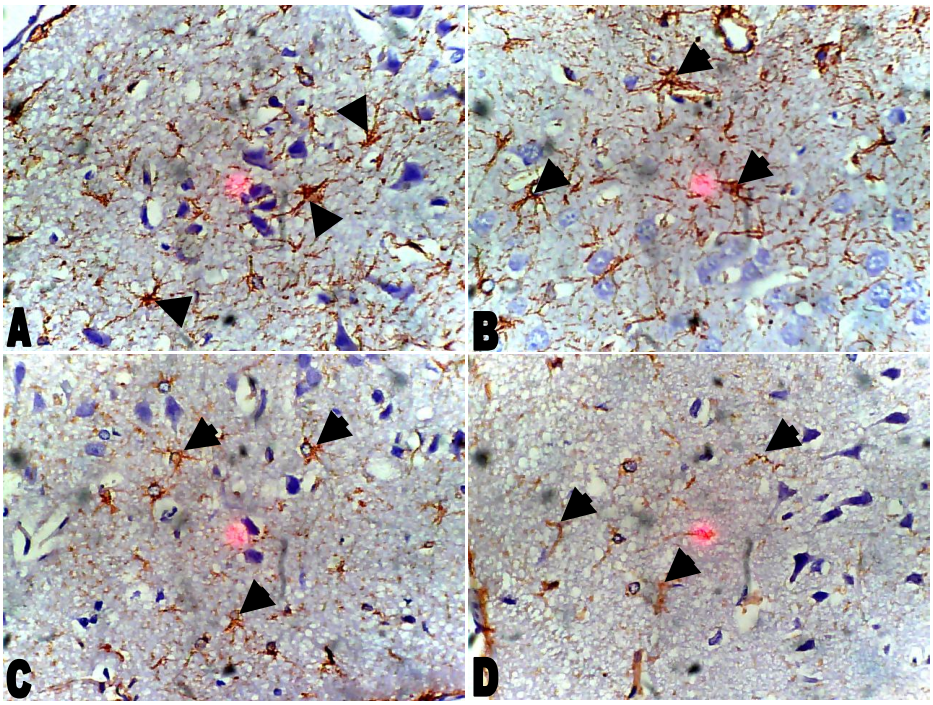

Fig. 2: Representative photomicrographs of sections of the cerebral cortex of Wistar rats. A. The control group showing expression of GFAP within the cell body and processes of astrocytes (arrow heads). B. 0.1 mg/kg $KBrO_3$ group showing no difference in the GFAP expression (arrows) compared with the control group. C. 0.2 mg/kg $KBrO_3$ group showing decreased expression of GFAP in the cell bodies and processes (arrows) compared with the control group. D. 0.3 mg/kg $KBrO_3$ group showing marked decreased expression of GFAP in the cell bodies and processes (arrows) compared with the control group. GFAP, ×400.

The cerebral cortex from animals that received 0.1 mg/kg of $KBrO_3$ showed no difference in the GFAP expression compared to the control. This may be that the 0.1 mg/kg of $KBrO_3$ may not have been sufficient to alter astrocytic protein expression. There was less expression of GFAP in animals that received 0.2 mg/kg of $KBrO_3$ and 0.3 mg/kg of $KBrO_3$ compared with the control group. Less expression of glial fibrillary acidic protein (GFAP) in the 0.2 mg/kg of $KBrO_3$ and 0.3 mg/kg of $KBrO_3$, test groups is indicative of inhibitory or destruc-

tive action which these concentrations of $KBrO_3$ may have had on the astrocytic protein expression. It has been reported that GFAP expression decreases in Down's syndrome, schizophrenia, bipolar disorder and depression, in response to acute infection or neurodegeneration (Johnston-Wilson et al. 2000), in Wernicke's encephalopathy (Cullen and Halliday 1994), in chronic infections involving HIV-1 (Levi et al. 1993), varicella zoster (Kennedy et al. 1994) and pseudorabies (Rinaman et al. 1993). As neurodegeneration was reported in the present study, this may be a reason for the decreased GFAP expression.

GFAP is an intermediate filament expressed in the central nervous system in astrocytes (Jacque et al. 1978; Venkatesh et al. 2013). The low GFAP expression may be detrimental to the structure and functions of the astrocyte, as it is involved in many important CNS processes, including cell communication and the functioning of the blood brain barrier, metabolism and in adjusting the filament network present in the cell (Kimelberg et al. 1993; Figley and Stroman 2011).

On the other hand, GFAP expression usually increases when the tissue is exposed to adverse conditions, often as a result of reactive astrocytes or astrogliosis. In astrogliosis, the astrocyte demonstrate stellate morphology, increased GFAP, immunoreactivity, increased number of mitochondria, as well as elevated enzymatic and non enzymatic antioxidant toxic activities (Eng et al. 2000; Gabyryel and Tizeciak 2001). However, this was not the case in the present study. The ability of astrocytes to protect neurons against toxic action of free radicals depends on their specific energy metabolism, high glutathione level and increased antioxidant enzyme activity (Gabyryel and Tizeciak 2001), which may have been altered negatively with $KBrO_3$ administration.

Chemically induced neurodegeneration is usually characterized by different patterns of neuronal cell death, gliosis, swollen or destroyed axons or destruction of the myelin sheath (Cavenagh 1984). However, $KBrO_3$ effect on the cerebral cortex presented different degenerative features that may affect the normal structural and functional activities of this brain area, which may eventually lead to a total breakdown of the whole body.

CONCLUSIONS

The results of this study showed that the tested high doses of $KBrO_3$ resulted in adverse cellular adaptations and loss of astrocytic proteins especially at the higher dosages. This may result in the functional impairment of the cerebral cortex.

Conflict of Interest

None declared.

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