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RESEARCH

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Effects of potassium bromate on *Rattus norvegicus* brain antioxidant markers, acetylcholinesterase activity, and DNA fragmentation: investigation of therapeutic effect of *Allium cepa*

Solomon Tsekohol Agu[1,2*], Akintola Ahmed Alabi[1], Deborah Oluwaseun Meshioye[1], Michael Adedapo Gbadegesin[1] and Oyeronke Adunni Odunola[1]

Abstract

Background *Allium cepa* is well-known for its antioxidant capabilities and contains potent antioxidant quercetin (3, 30, 4, 5, 7-pentahydroxyflavone). We investigated the therapeutic effects of aqueous extract of *Allium cepa* (AEAC) that is quercetin-rich against potassium bromate (KBrO3)-induced oxidative damage in the brains of male Wistar rats using biochemical, immunohistochemical, and histological markers. For 90 days, 40 male Wistar rats were administered KBrO3, KBrO3 + AEAC, and/or quercetin on alternate days, or AEAC and quercetin alone.

Results KBrO3 significantly ($p > 0.05$) suppressed and diminished antioxidant enzymes and acetylcholinesterase activities with notable decreased total protein levels. Additionally, oxidative stress biomarkers (MDA and NO), as well as DNA fragmentation, all increased significantly ($p > 0.05$). The immunohistochemical expression of P53, caspase 3, and COX2 protein also increased significantly in the cerebral cortex of the KBrO3-treated groups, but BCL-2 protein expression decreased significantly. Histological examination of brain tissues revealed patterns that corresponded to the enzyme markers. The effects of KBrO3 were all attenuated by the administration of AEAC and quercetin.

Conclusions This research demonstrates the therapeutic effects of *Allium cepa* on KBrO3-induced oxidative stress, and biochemical perturbation in the brain of *Rattus norvegicus*. Even though the exact mechanism of action of *Allium cepa* at the molecular level cannot be completely deduced from the results presented above, it could possibly be due to a combination of its antioxidant, anti-inflammatory, and apoptotic mechanisms. Further studies are required to examine the molecular pathways responsible for these aforementioned therapeutic effects.

Keywords Potassium bromate, *Allium cepa*, Oxidative stress, Cerebral cortex, *Rattus norvegicus*

*Correspondence:
Solomon Tsekohol Agu
agu.solomon@uam.edu.ng
[1] Cancer Research and Molecular Biology Laboratory, Department of Biochemistry, University of Ibadan, Ibadan, Oyo State, Nigeria
[2] Department of Veterinary Physiology and Biochemistry, Federal University of Agriculture, Makurdi, Benue State, Nigeria

Background

The inherent toxicity of food additives has been a major subject of interest in recent years. According to a growing body of research, a deluge of xenobiotics released during metabolism might produce free radicals, resulting in oxidative stress, a critical process that changes the internal redox equilibrium (Ben et al., 2014, 2015). The brain has peculiar features of the capillary endothelial cells surrounding the cerebral blood vessels that provide some protection to the brain by inhibiting the entry of various types of circulating molecules. However, a group of fairly lipophilic xenobiotics could easily diffuse through the endothelial cells of the brain capillaries and infiltrate the neuronal cells (Banks, 2016). And the brain, like other tissues in the body, is highly vulnerable to toxic compound-induced injuries because neurons in the brain perform specialized activities such as neurotransmission and other functions but it has limited regenerative capacity, and a weak endogenous antioxidant defense, making it more vulnerable to an imbalance in redox homeostasis. (Savaskan et al., 2007). As a consequence, such compounds exhibit cellular toxicity as parent compounds or active metabolites with high negative impart and irreparable disruption of neuronal function. (Stevens & Baker, 2009).

For the past 90 years, potassium bromate (KBrO3) has been used in food (Oloyede & Sunmonu, 2009). KBrO3 is most commonly used as a flour improver and maturation agent by flour millers and bakers (Vadlamani & Seib, 1999). In experimental animals, KBrO3 metabolism in vivo is linked to the production of free radicals, which causes oxidative stress, genomic damage, and cellular apoptotic death (Chipman et al., 1998). KBrO3 has been demonstrated in human and experimental animal investigations to cause numerous organ damage (Ahmad et al., 2015; Farombi et al., 2002; Kujawska et al., 2013) It's also been proven that $KBrO_3$ is irritating and harmful to tissues, particularly those in the central nervous system (CNS) and kidneys (Robert & William, 1996).

Some plant products are used in traditional medicine to treat a variety of disorders because they contain natural antioxidants, whereas other plants contain bioactive ingredients such as phenolics and polyphonic compounds that have anti-inflammatory and anti-oxidant properties (Zhou et al., 2016). Antioxidants have been shown in studies to protect the body from free radical damage (Pietta, 2000).

The onion is a well-known traditional nutraceutical and medicinal plant that is cultivated and used all over the world. It is typically consumed for its purported nutritional and medicinal properties. Allium is extremely therapeutic, and it is one of the vegetables used to reduce the risk of gastric cancer. (Zhou et al., 2011), cancer of the bladder (Malaveille et al., 1996), brain (Hu et al., 1999), breast (Challier et al., 1998), lungs (Khanduja et al., 1999), ovaries (Shen et al., 1999), and stomach (Dorant et al., 1994) and vascular disease (Da Silva 1998). It has been proven to have antidiabetic effects (Sheela et al., 1995) and it is helpful in the management of cataracts as it contains flavonols, flavones, and isoflavones, its inclusion in foods is associated with lowering oxidative stress (Juurlink et al., 1998). Kumari and Augusti (2007) found that sulfur compounds generated from onions, such as S-methyl cysteine sulfoxide and allyl propyl disulfide, had hypolipidemic properties. There are few studies on the impact of *Allium cepa* on brain regions such as the cerebral cortex of the frontal lobe, which is involved in sophisticated cognitive behavior planning, personality expression, decision making, and social behavior moderation (Yang and Raine, 2009). *Allium cepa's* broad culinary use is expected to have a positive impact on brain tissue (Muonagolu and Ekong, 2016). The aim of this study was to evaluate the therapeutic effects of AEAC and/or quercetin on KBrO3-induced toxicity in the brains of male Wistar rats.

Methods

Chemicals

Potassium bromate used was purchased from Sigma-Aldrich, St. Louis, MO. All the other chemicals used were of the highest purity grade.

Plant materials

Red Onion cultivated in Ibadan, Nigeria, was procured from Bodija, Ibadan vegetable market, and was authenticated and identified as *Allium cepa* by a Botanist (Adepoju Kolawole) at the Herbarium facility Life Sciences, Botany Department, University of Ibadan, Nigeria. The plant was compared with an existing specimen previously kept at the herbarium (voucher number: 01676).

Extraction of AEAC

The *Allium cepa* extract was produced with minor changes, as previously described by Nwaehujor et al. (2014). After washing in clean running tap water, the onion bulbs were rinsed in distilled water. The bulbs' outer scaly leaves were carefully removed by hand, and the fleshy portion of the onion was rewashed with distilled water. 10 g of the onion bulb was sliced into small pieces and mashed in 500 ml of water using a blending machine. The mixture was filtered using a muslin cloth, then Whatman no. 1 filter paper. The filtrate was dried at 45 °C to produce crude quercetin, which was then kept in a sterile container at 4 °C until needed.

Experimental animals and treatment

Forty male Wistar rats weighing between 100 and 120 g were obtained from the animal house-Faculty of Basic Medical Sciences, University of Ibadan. They were housed in clean polypropylene cages and kept in a room maintained at 24–28 °C, with controlled cycles of 12 h light and 12 h dark, and relative air humidity of 40–60% in the Department of Biochemistry, University of Ibadan. They were allowed to acclimatize for 14 days prior to the treatment and were fed standard commercial pellets (Vita Feeds, Ibadan) and water ad libitum. The experimental protocol was carried out in accordance with the guidelines on the care and wellbeing of research animals (N.I.H 1985) as approved by the university institutional animal ethical committee of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, with reference number UI/FVM/22/17.

Forty male Wistar rats were randomly divided into eight (8) groups of five (5) rats each and treated accordingly as follows.

Group A: Rats were administered distilled water only.

Group B: Rats were given KBrO3 (dissolved in distilled water) at 30 mg/kg body weight.

Group C: Rats were administered quercetin (50 mg/kg) as a standard.

Group D: Rats were given AEAC at a dose of 300 mg/kg.

Group E: Rats were given AEAC at a dose of 150 mg/kg body weight.

Group F: Rats were given 30 mg/kg KBrO3 plus 300 mg/kg of AEAC.

Group G: Rats were given 30 mg/kg KBrO3 plus 150 mg/kg of AEAC.

Group H: Rats were given 30 mg/kg KBrO3 plus 50 mg/kg of quercetin.

The doses of AEAC and KBrO3 were adopted from previous studies by Ozougwu et al., 2010 and Achukwu et al. (2009) respectively, and all treatments were administered via gavage once per day on alternate days for 90 days. During this treatment period, the rats were observed for signs of toxicity and death. Their body weights were recorded weekly using a digital weighing balance.

Samples collection

After the completion of the treatments, the rats were sacrificed by cervical dislocation, and blood for each animal was collected into a plain sample bottle for biochemical assays. The clotted blood sample was centrifuged at 3000 rpm for 5 min to obtain serum and stored at 4 °C until used for analysis.

The skull of each experimental rat was carefully excised with dissecting scissors and forceps and the brain was isolated, rinsed in 1.15% KCl solution, blotted with filter paper, and weighed. The brain was divided into two parts: one sectioned for histological examination stored in 10% formalin solution and the other section homogenized.

Histological preparation

The fixed tissues were dehydrated in ascending series of ethanol, cleared in two changes of xylene, infiltrated in three changes of molten paraffin wax (melting point 58–60 ℃), and embedded in molten paraffin. Sections of 4 microns thickness were cut by using a rotary microtome and stained with Ehrlich's hematoxylin and counterstained with eosin (Lillie & Fulmer, 1976).

Homogenization of brain samples

The remaining portions of the brain were homogenized in an ice-cold homogenizing buffer (0.1 M phosphate buffer, pH 7.4) with a Teflon homogenizer. To obtain the post mitochondrial fractions, the homogenates were centrifuged at 10,000 rpm for 15 min in a cold centrifuge at 4 °C. This fraction and serum were used to assess oxidative stress makers, DNA fragmentation, and acetylcholinesterase activity.

Immunohistochemical investigations

For immunohistochemical investigations, 4 microns paraffin sections were stained.

Immunohistochemically for visualizing cysteine-aspartic protease (caspase-3), BCL-2, COX_2, and p53 using the suitable antibodies in each staining time (Schneider et al., 2012). The intensity of PCNA and caspase-3 expression in brain sections were quantified by using NIH image j software.

Biochemical analyses

Glutathione S-transferase (GST) was determined by the method of Habig et al. (1974). The glutathione peroxidase (GPX) activity was measured using the Mohandas et al. (1984) method. Catalase (CAT) activity was assayed by the decomposition of hydrogen peroxide according to the method of Claiborne (1984). Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972). The cerebral cortex malondialdehyde (MDA) concentrations and index of lipid peroxidation, were spectrophotometrically according to the method of Draper and Hadley (1990). Nitrite assay was done using Griess reagent with some modifications of the method of Green et al. (1982). AChE activity in the cerebral cortex homogenates was measured by the method of Lombardi et al., (1999). The total protein levels were measured by an enzymatic colorimetric kit (Wako Chemicals USA, Inc.).

Assay of DNA Fragmentation by diphenylamine (DPA) method

The percentage of DNA fragmentation of the brain homogenate was determined by the method of Gibb et al. (1997).

Statistical analysis

All data are expressed as mean ± SEM. The results were statistically evaluated by using Graph Pad Prism ver. 8.01 for Windows. Significant differences between the experimental groups were assessed by the one-way ANOVA (analysis of variance) test followed by the Dunnett multiple comparison test. *p-values* less than 0.05 were considered to be significant.

Results

Physical monitoring

Both the control and AEAC and quercetin-treated rats appeared to have normal activity and normal adequate food and water intake. KBrO3-treated rats exhibited general weakness and loss of appetite. On the other hand, KBrO3 plus AEAC and or quercetin-treated rats did show a diminution in abnormality.

Enhancement of the cellular antioxidant defense by AEAC and quercetin in the brain tissue of rats administered KBrO3

There were no statistical differences in the levels of enzymic and non-enzymic antioxidant defense components between the control and AEAC and quercetin-treated groups, respectively. However, treatment of rats with KBr alone caused a significant ($p < 0.05$) changes in the activities of major detoxifying enzymes like the level of cellular glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in the brain tissues when compared with the control (group 1) given distilled water only. However, co-administration of rats with KBrO3 and AEAC (at 300 mg/kg or 150 mg/kg) or quercetin at 50 mg/kg (Group F, G, and H respectively) restored the activities of the enzymic and non-enzymic antioxidant defense components to levels significantly different from the group treated with KBrO3 (Group B) only and in fact similar to or not significant ($p > 0.05$) different from the negative control (Group A). It was observed that the groups of rats given AEAC alone (Groups D and E) or quercetin alone (Group C) have a significant ($p < 0.05$) increase in the level of GST and activities of Catalase, SOD, and GPx compared with the control, Group A (Fig. 1). This, therefore, confirmed

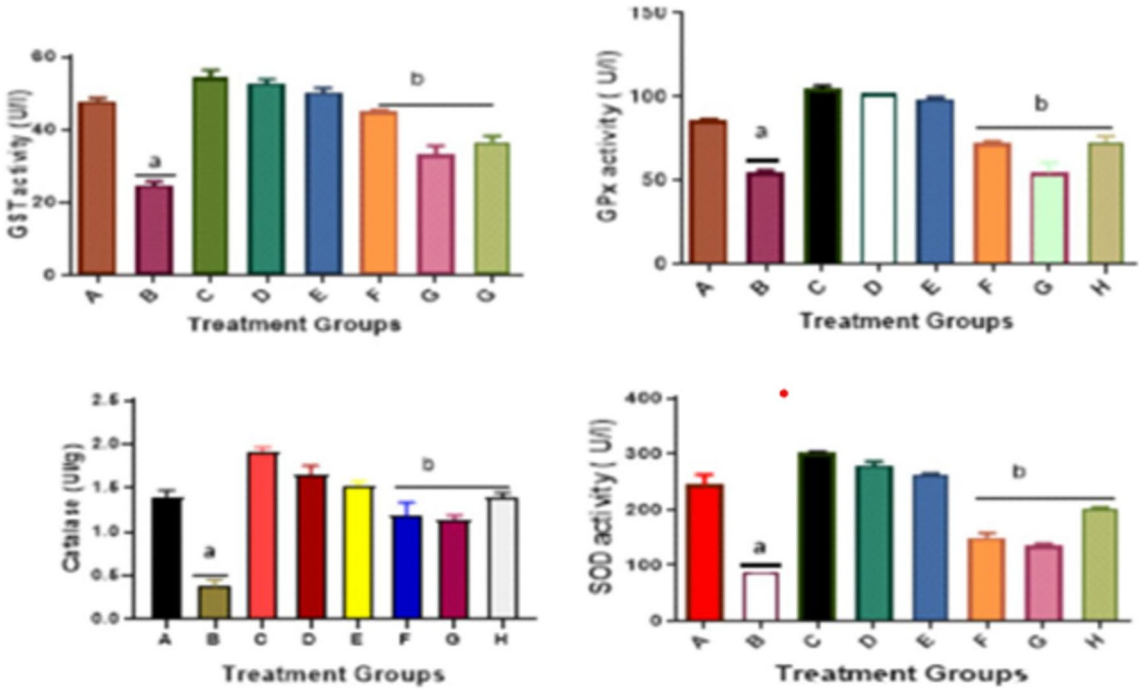

Fig. 1 Activities of different cellular antioxidant defense (GST, GPx, Catalase, and Superoxide dismutase) in the brain tissue of male Wistar rats in different experimental groups. Values are mean ± SD (n = 5), a = significantly different compared with the negative control group A ($p < 0.050$). b = significantly different compared with the positive control group B treated with KBrO3 only ($p < 0.05$). Group A received distilled water, B: 30 mg/kg KBrO3, C: 50 mg/kg Quercetin, D: 300 mg/kg AEAC, E: 150 mg/kg AEAC, F: KBrO3 + 300 mg/kg AEAC, G: KBrO3 + 150 mg/kg AEAC, H: KBrO3 + Quercetin

the tissue antioxidant system enhancement effects of the extracts.

Abrogation of the KBrO3-induced inflammatory biomarkers (Malondialdehyde and Nitric Oxide) by AEAC

Administration of KBrO3 (group B) caused a significant ($p < 0.05$) increase in Malondialdehyde (MDA) and Nitric oxide (NO) levels when compared with the control group (group A) that were given distilled water as shown in Fig. 5. On the other hand, when rats were treated with KBrO3, there was a significant ($p < 0.05$) decrease in the levels of MDA and NO in the groups given KBrO3 and AEAC or quercetin (Group F/G/H versus Group B) compared with the group given KBrO3 alone as observed in Fig. 2.

AEAC abated KBrO3-induced DNA fragmentation in the brain of rats

There was significant ($p < 0.05$) DNA fragmentation in the brain tissues of rats treated with KBrO3 (Group B) when compared with those given distilled water only (Group A) (as shown in Fig. 3). Quercetin and AEAC abrogated the effect of KBrO3 in Groups F, G and H treated with KBrO3 and AEAC or quercetin (Fig. 3).

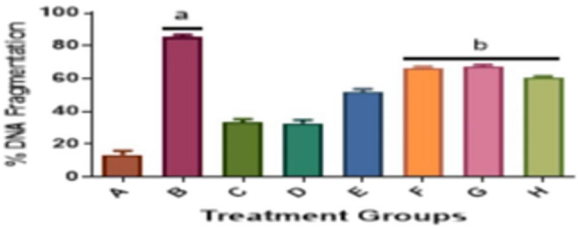

Fig. 3 AEAC and Quercetin abrogated KBrO3-induced DNA fragmentation in the brain of male Wistar rats. Values are mean ± SD (n = 5), a = significantly different compared with the negative control group A ($p < 0.050$). b = significantly different compared with the positive control group B treated with KBrO3 only ($p < 0.05$). Group A received distilled water, B: 30 mg/kg KBrO3, C: 50 mg/kg Quercetin, D: 300 mg/kg AEAC, E: 150 mg/kg AEAC, F: KBrO3 + 300 mg/kg AEAC, G: KBrO3 + 150 mg/kg AEAC, H: KBrO3 + Quercetin

AEAC and quercetin improved the levels of total protein and acetylcholine in the brain tissues of rats administered KBrO3

Administration of KBrO3 alone (group B) caused a significant decrease ($p < 0.05$) in acetylcholine concentration and total protein levels in the brain samples when compared with the control group (group A) that were given distilled water as shown in Fig. 4. There was a significant ($p < 0.05$) increase in the levels of acetylcholine in the groups administered KBrO3 and AEAC or quercetin compared with the group given KBrO3 alone (Group F/G/H versus Group B).

Administration of AEAC improved cerebral cortex histoarchitecture in KBrO3 treated rat

Histopathological finding in the cerebral cortex in KBrO3 treated group (Fig. 5B) is characterized by chromatolysis and severe necrosis of the neuronal cell relative to the control group and the groups administered AEAC alone in graded doses and quercetin alone that showed normal neuronal architecture (Fig C, D, and E) respectively . The degree of neuronal damage induced by KBrO3 treatment was ameliorated in the groups co-administered KBrO3 and the graded doses of AEAC and/or quercetin.

Effects of KBrO3 and/or aqueous extract of *Allium cepa* (AEAC) on the expression of apoptotic-related proteins in the cerebral cortex of rats

(Figure 6).

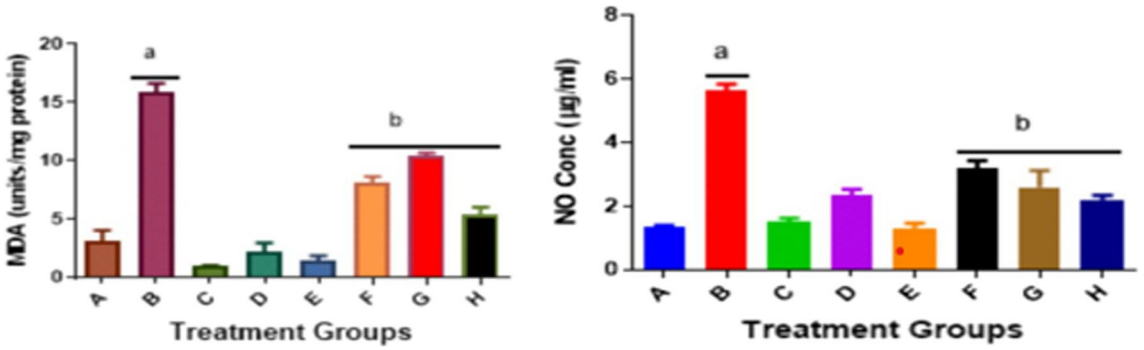

Fig. 2 AEAC and quercetin ameliorated KBrO3 mediated inflammatory biomarkers (Malondialdehyde and Nitric oxide) in male Wistar rats. Values are mean ± SD (n = 5), a = significantly different compared with the negative control group A ($p < 0.050$). b = significantly different compared with the positive control group B treated with KBrO3 only ($p < 0.05$). Group A received distilled water, B: 30 mg/kg KBrO3, C: 50 mg/kg Quercetin, D: 300 mg/kg AEAC, E: 150 mg/kg AEAC, F: KBrO3 + 300 mg/kg AEAC, G: KBrO3 + 150 mg/kg AEAC, H: KBrO3 + Quercetin

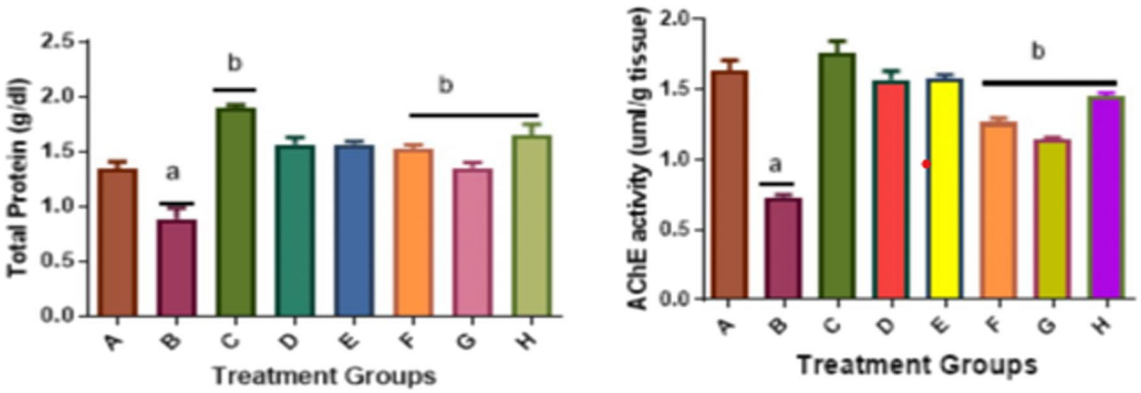

Fig. 4 Levels of total protein and acetylcholinesterase activity in the brain of male Wistar rats of different experimental groups. Values are mean ± SD (n = 5), a = significantly different compared with the negative control group A ($p < 0.050$). b = significantly different compared with the positive control group B treated with KBrO3 only ($p < 0.05$). Group A received distilled water, B: 30 mg/kg KBrO3, C: 50 mg/kg Quercetin, D: 300 mg/kg AEAC, E: 150 mg/kg AEAC, F: KBrO3 + 300 mg/kg AEAC, G: KBrO3 + 150 mg/kg AEAC, H: KBrO3 + Quercetin

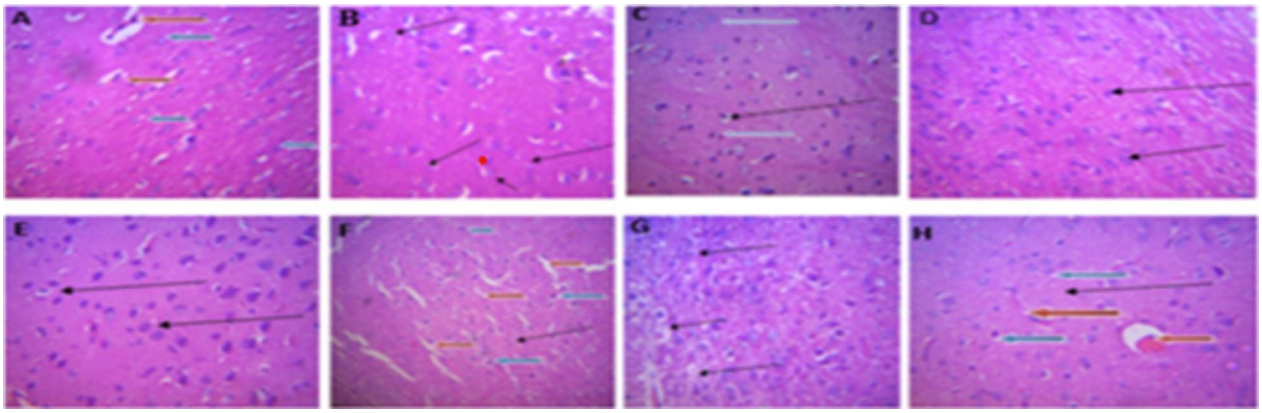

Fig. 5 Photomicrographs obtained from hematoxylin- and eosin-stained cerebral cortex sections of the rat treated with KBrO3 and AEAC and or quercetin. Group A: control showed normal neuronal architecture, group B was administered KBrO3 only and shows chromatolysis and severe necrosis of the neuronal cells, groups C, D, and E groups administered AEAC alone in graded doses and quercetin alone showed normal neuronal architecture. The groups F, G, and H co-administered KBrO3 and AEAC/ or quercetin shows some normal cellular architecture, with modest degeneration of cells in the cerebral cortex.

Discussion

According to an expanding wealth of data, the toxicity of varied xenobiotics, including potassium bromate, is associated with increased production of reactive oxygen species (ROS), resulting in oxidative stress in cells (Khan et al., 2001); a detrimental condition that is accompanied by damage to a variety of cellular macromolecules (Halliwell & Gutteridge, 2007a, 2007b). The derivatives of superoxide anions are the most abundant ROS generated and are particularly highly reactive and damaging as hydroxyl radicals.

Onion (*Allium cepa* L.) is a known quercetin-rich vegetable (Azuma et al, 1999) and quercetin glycosides (mainly quercetin 4′-glucoside and quercetin 3, 4′-diglucoside) has high antioxidant potentials (Price et al., 1997; Tsushida & Suzuki, 1996). Onion intake was found to protect the DNA against oxidative damage, lowering of peroxidized lipids in the circulation and urine, and hypoglycemic and hypocholesterolemic effects (Babu & Srinivasan, 1999).

In the present study, oral administration of KBrO3 alone to rats resulted in a significant decrease in the activities of antioxidant enzymes such as GPx, SOD, and catalase in brain tissue (Fig. 1). This finding is consistent with a previous study by Watannabe et al. (2004) who discovered that KBrO3 can inhibit the activities of important antioxidant enzymes. Farombi et al., 2002 also highlighted the unswerving inhibitory effect of KBrO3 on the endogenous physiological defense system. And since the brain has high metabolic activity and low antioxidant

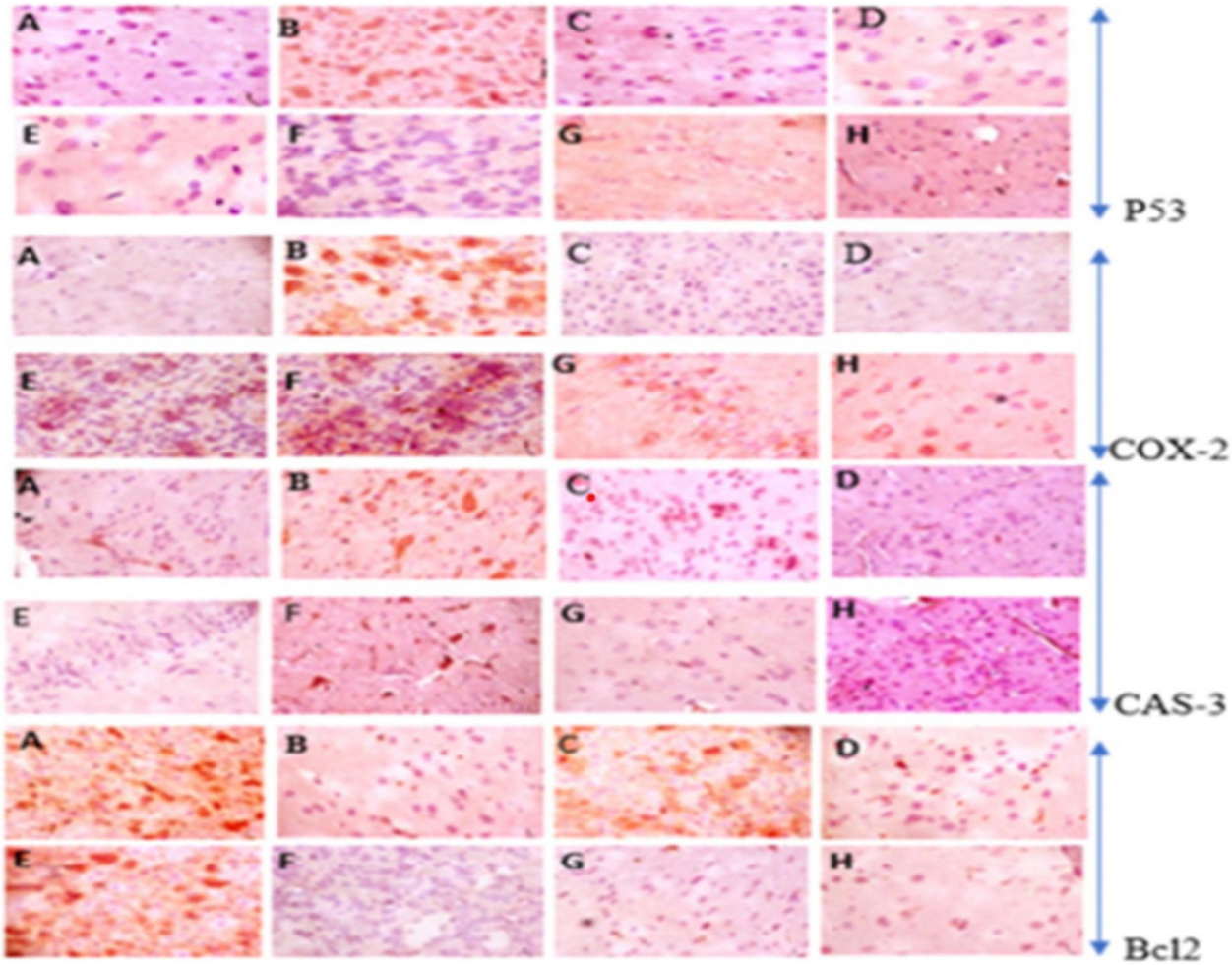

Fig. 6 Shows slides of cerebral cortex comparing the expression levels of COX-2, P53, Caspase-3, and Bcl-2 proteins in male Wistar rats treated with KBrO3 (**B**) plus AEAC (**D** and **E**) or quercetin (**C**). The group adminsistered KBrO3 (**B**) only shows more expression (browner staining) of COX-2, p53, Caspase-3, and Bcl-2 proteins relative to the control group (**A**). The control group and those administered the extract (**D** and **E**) and quercetin (**C**) only showed insignificant expression (lesser brown staining) of the proteins. There is little or no expression of these proteins in the group treated with KBrO3 and AEAC or quercetin (**F**, **G**, and **H**).

capacity and this makes the brain most vulnerable to oxidative stress (Cobley et al., 2018). The above observation from this study delineates that KBrO3 elicited harmful effects in the internal milieu of the brain of the treated rats. Conversely, AEAC administration offers an antioxidant first line of defense against ROS in the groups co-administered KBrO3 with AEAC and or quercetin. The rat in groups (C, D, and E) that received AEAC in graded doses and quercetin alone (Fig. 1) showed high activity of the antioxidant enzyme when compared to the rats in group A and this report has buttressed the fact that *Allium cepa* and quercetin are known to protect cells from exogenous insults by activating the endogenous defense system, which involves catalase, superoxide dismutase, and glutathione (Alam et al., 2014; Bas et al., 2014). The notably increased level of antioxidant enzyme and nonenzymatic component in the ACEAC treated groups, gives credence to the antioxidant properties of *Allium cepa.*

One index of membrane damage and alteration in the function and structure of cellular membranes is the formation of free malondialdehyde (MDA) (Herrero et al., 2001). Moreover, an elevated level of NO^{-} a reactive free radical is known to induce nitrosative stress that subsequently damages cellular lipids, and nucleic acids (Moldogazieva et al, 2018; Taysi et al, 2019). $ONOO^{-}$ is the initiator of induction of lipid peroxidation which disrupts the cell membranes and lipoproteins. The $ONOO^{-}$ and MDA collectively act as cytotoxic as well as mutagenic (Barrera, 2012). The results of this study revealed that KBrO3 administration alone increased MDA and NO concentrations in treated rats' brain

tissues (Fig. 2), implying that nitrosative and oxidative stress was involved in the toxicity. Because of its high oxygen consumption, high level of redox metal ions, lesser antioxidant defense mechanism, and high level of polyunsaturated fatty acids, the brain is more susceptible to lipid peroxidation (Magalingam et al., 2018). Furthermore, it has been established that extreme generations of NO in the brain can collaborate with super oxygenated constituents to yield ONOO- in nerve cells, and this phenomenon is extremely toxic to the membrane lipids, DNA, and white matter of the brains, resulting in oxidative stress after the antioxidant defense mechanisms are exhausted or overwhelmed (Alexander et al., 2015). Gratifyingly, concurrent treatment with KBrO3 and AEAC or quercetin in the groups E and F extirpated KBrO3 induced higher levels of MDA and NO (Fig. 2). This marked reduction in MPO activity and NO level in the brain tissues of rats following the administered AEAC, therefore, ascribes the antioxidative impact of AEAC to alleviating KBrO3-mediated harmful effect in treated rats. Jakaria et al. (2009) asserted the anti-neuroinflammatory activities of *A. cepa* extract in down-regulating the mRNA NO, thereby attenuating NO release, which is consistent with our findings, and similarly, Hwang et al., 2009 also reported the antioxidant activity and lipid peroxidation inhibiting properties of *A. cepa* in the brain tissue.

Khan and Sultan005) confirmed that KBrO3 can cause oxidative DNA damage in rats, culminating in DNA fragmentation, which endorses the finding of our research. The level of percentage DNA fragmentation in the brain tissues of rats administered KBrO3 only increased significantly ($p < 0.05$) when compared to the control (Fig. 3). The groups F, G and, H co-administered KBrO3 with AEAC and quercetin, there was a significant reduction in the levels of the percentage DNA fragmentation. The groups treated with graded doses of AEAC and quercetin showed no DNA fragmentation (Fig. 3). A study has shown that a significant increase in the percentage of DNA fragmentation could be due to the DNA strand breakage triggered by KBrO3 -induced oxidative stress (Parsons & Chipman, 2000). These mitigating effects of AEAC show that this AEAC contains bioactive compounds that play an important role in DNA repair by annulling the deleterious effect of the KBrO3 on brain tissues.

Acetylcholine is a neurotransmitter that plays a number of vital roles in the brain. As a result, studying the brain level of this molecule following KBrO3 treatment is especially important. In comparison to the control group, there was a marked decrease in acetylcholinesterase activities in the KBrO3 treated group (Fig. 4), which is consistent with the findings of Hajer et al., 2017. Interestingly, there was a significant increase in acetylcholinesterase activities in the groups that received KBrO3 alongside AEAC and quercetin. The result of the brain tissue total protein level presented a similar pattern as that of acetylcholinesterase (Fig. 4).

Running simultaneously, cerebral cortex histopathology results confirmed the biochemical findings of AEAC's anti- KBrO3 cytotoxic effects. The rats in the groups that were only given distilled water, pure quercetin, and AEAC (Fig. 5A, C, D, and E) had no obvious abnormalities in their cerebral cortex architecture. When compared to the control group (Fig. 5 B), there were neuronal cells with chromatolysis and severe necrosis in the cerebral cortex of rats treated with KBrO3 alone. Following KBrO3 treatment, rats' brain tissue slices showed bleeding, neuronal degeneration, and vacuolation (Abuelgasim et al., 2008; Ajarem et al., 2016). Chromatolysis can be triggered by axotomy, ischemia, and toxicity to the cell leading to the disintegration of Nissl substances (Richard, 2000). We assumed that these abnormalities resulted from increased ROS production, which was confirmed by increased lipid peroxidation and decreased GSH. In contrast, treatment of KBrO3 in combination with AEAC and/or quercetin resulted in normal cellular architecture, with modest degeneration of cells in the cerebral cortex in group H treated with KBrO3 and a lower dosage of AEAC (Fig. 5F, G, and H). This finding indicates that the AEAC can reduce the toxicity caused by KBrO3. The neuroprotective activity of A. cepa extracts in transient cerebral ischemia was discovered by Shri and Singh in 2008 elucidated the neuroprotective effect of *A. cepa* extracts in transient cerebral ischemia. Prior to this, *allium cepa* was shown to inhibit frontal lobe degeneration and hippocampal cell death (Chun et al., 2003; Moriguchi et al., 1997).

Changes in the expression of apoptosis-related proteins (p53, Caspase 3, and Bcl-2) and inflammatory protein (COX 2) have been recently recognized as an important component of the neuronal response to stress (Li, et al., 1997). P53-protein has been shown to play a pivotal role in neuronal apoptosis. It functions as a site-specific transactivator of transcription and has been shown to activate the proapoptotic gene, caspase 3, and the observed increase in p53 expression is a natural defensive mechanism (Rotter, 1993). Bcl-2 belongs to the B cell leukemia-2 gene product (Bcl-2) family, with apoptosis being prevented by Bcl-2 (Kroemer, 1997). Caspase 3 is a cell death agonist and Bcl-2 has been shown to protect against various stimuli that induce apoptotic neuronal death (Clark et al., 1997). KBrO3 exposure resulted in modulation in the cellular redox state with an increase in p53 expression (Corsby et al., 2000). In the present study, KBrO3 treatment shows higher expression of p53,

Caspase-3, and COX 2, as seen in the slide with brow stains, and lower Bcl2 expression in the group treated with KBrO3 only when compared with the control (Fig. 6). Activation of caspase-3, p53, and COX2 had been linked to neuronal apoptosis and caspase 3 is considered a key stimulator of cell death. The activation of cell death signals occurs when neurons are injured by neurotoxins (Beer, 2000). Allium has been shown to decrease COX2 activity (Ali, 1995), and co-administration of KBrO3 with AEAC or quercetin reduced the high production of p53, caspase-3, and COX 2, as well as up-regulating the expression of Bcl2 protein (Fig. 6). The pattern of expression of these proteins after AEAC and Quercetin administration shows that AEAC is anti-apoptotic and anti-inflammatory, according to the results of this study.

Conclusions

Our findings show that oral treatment of KBrO3 directly influences the Wistar albino rat's cerebral cortex, DNA fragmentation, antioxidant status, proteins, and brain histomorphology and that *Allium cepa* is a viable chemoprevention candidate.

Abbreviations

Caspase-3	Cysteine-aspartic protease
COX-2	Cyclooxygenase-2
BCL-2	B-cell lymphoma 2
p53	Tumor suppressor gene
CAT	Catalase
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
GST	Glutathione-s-transferase
NO	Nitric oxide
MDA	Malondialdehyde
AEAC	Aqueous extract of *Allium cepa*
KBrO3	Potassium bromate

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Author contributions

AST, AAA, OMD, MAG, and AOA contributed to the design, AST, AAA, and OMD prepared materials and figures, MAG and AOA supervised the practical experiments, AST wrote and AAA, OMD, MAG, and AOA revised the manuscript. AAA, OMD, MAG, and AOA read and approved the final manuscript. AST, AAA, and OMD carried out the practical experiments, prepared data analysis, materials, and figures, and wrote the first draft of the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Experimental rats were cared for according to standard international (N.I.H, 1985) as approved by the university institutional animal ethical committee of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, with reference number UI/FVM/22/17.

Consent for publication

Not applicable.

Competing interest

The authors declare no competing interests.

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