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ORIGINAL ARTICLE

Ameliorative Potential of *Allium cepa* or Quercetin on the Histology, Immunohistochemistry and Oxidative Stress Biomarkers Parameters Against Potassium Bromate-induced Hepatotoxicity in Adult Male Rats

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Background: *Allium cepa* has been linked to a variety of health advantages, although little is known about its hepatoprotective properties. Using biochemical, immunohistochemical, and histological markers, the current study was conducted to examine the efficacy of quercetin-rich aqueous extract of *Allium cepa* (AEAC) against potassium bromate-induced hepatotoxicity in Wistar rats and the probable mechanism. There are a total of 40 male Wistar rats used in this study and were equally divided into eight (8) groups. Group A: Control. Group B: potassium bromate, $KBrO_3$ (dissolved in water), at 30 mg/kg. Group C: Quercetin as a standard (50 mg/kg). Groups D and E: AEAC, at 300 mg/kg and 150 mg/kg respectively. Groups F and G: $KBrO_3$ plus AEAC. Group H: $KBrO_3$ plus quercetin. On alternate days for 90 days, all administrations were done by intubation.

Results: $KBrO_3$ generated a significant difference in the levels of oxidative stress biomarkers and DNA fragmentation compared to the control (p 0.05). Histological and immunohistochemical examinations of liver samples revealed patterns that were comparable to those seen in enzyme biomarkers. In all cases, combining $KBrO_3$ with AEAC or pure quercetin attenuated the effects of $KBrO_3$.

Conclusion: Therefore, potassium bromate induced hepatocellular toxicity via oxidative stress while treatment with the AEAC ameliorated its effects.

Key words: Allium cepa, Hepatocellular toxicity, Histology, Immunohistochemistry, onion, rats

Potassium bromate ($KBrO_3$) is a by-product of ozonation water disinfection and may be found in both tap and bottled water (Liu and Mou, 2004). Furthermore, due to its oxidizing capabilities, $KBrO_3$ is commonly employed as a food ingredient in the bread-making process for wheat maturation and as a neutralizer in cold-wave hair treatments (Kurokawa *et al*., 1990). Previous research has shown that potassium bromate's harmful effects are caused by the production of reactive oxygen species (McDorman *et al*., 2005). Lipid peroxidation and genotoxicity are caused by reactive oxygen species (Jaloszynski *et al*., 2007). The increase of 8-hydroxyguanosine (8-OH dG) production from DNA deoxyguanosine residues by free radicals has been attributed to $KBrO_3$'s genotoxicity (Ahlborn *et al*., 2009). Furthermore, the International Agency for Research on Cancer has categorized $KBrO_3$ as a category 2B carcinogen (probably harmful to humans) (IARC, 2019). Several cases of human toxicity, including hearing and renal impairment, have been documented after incidental or intentional intake of a few grams of $KBrO_3$ (De Angelo *et al*., 2001)

The liver's high sensitivity to chemical injury has been linked to several characteristics, including its anatomical closeness to the blood supply and its involvement in xenobiotic biotransformation. As a result, preventing hepatocellular damage is extremely important. The mechanisms of cellular changes linked with $KBrO_3$ exposure in liver tissues are poorly understood. Toxic drug exposure impairs liver function, which is a direct result of alterations in the organ's histological features (Singh *et al*., 2001). Because ingestion of $KBrO_3$, like many harmful substances, might be unintentional, a suitable preventive/therapeutic agent for $KBrO_3$ therapy must be developed that is both inexpensive and widely available.

In several experimental models, medicinal herbs and plants are effective in treating oxidative stress-related disorders in various organs (Hussain *et al*., 2014).

The bulbs of red onions (Allium cepa) contain a variety of phytochemicals, the majority of which are hydrocarbons and their derivatives, such as cepaenes

and thiosulfinates (Dorsch and Wanger, 1999; Goldman *et al*., 1996).   There are additional pigments like anthocyanins, as well as flavonoids like kaempferol and quercetin (Dorant *et al*., 1994).

Quercetin (3, 3, 4, 5, 7- pentahydroxyflavone), is a flavonoid found in fruits, seeds, nuts, tea, and red wine (Hollman *et al*., 1999). Because of the discoveries that it inhibited numerous tumor-related processes such as oxidative stress, cellular proliferation, and metastasis, quercetin is gaining in popularity (Lugli *et al*., 2009).

The red onion bulb has also been proven to be beneficial in the treatment of a variety of human and animal ailments, including cancer and diabetes, as well as being a natural antioxidant, antibacterial, and anti-allergic resource (El-Meleigy *et al*., 2010)

The red onion has been shown to have antibacterial, antiparasitic, and antimycotic properties in several in vitro investigations (Rose *et al*., 2005). The goal of this study was to see how a quercetin-rich aqueous extract of Allium cepa (AEAC) affected potassium bromate hepatotoxicity in male Wistar rats

MATERIAL AND 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

Allium cepa (red onion bulb) was procured from Bodija, Ibadan vegetable market, and authenticated at the Department of Botany, University of Ibadan, Nigeria. A specimen of the plant was then deposited in the herbarium of the Department of Botany bearing voucher specimen identification number UIH 22631.

Extraction of quercetin-rich constituents of *Allium cepa*

This extraction method was a modification of Nwaehujor *et al.* (2014). The onion bulb was washed with clean tap water followed by distilled water. The outer scaly leaves of the bulbs were manually removed and the fleshy part of the onion was rewashed with distilled water. Exactly 10 g of the onion bulb was cut into small parts and crushed in 500 ml of water using a

blending machine. The mixture was filtered through a muslin cloth and then Whatman no. 1 filter paper. The filtrate was evaporated at 45 ^{0}C to dry to produce crude quercetin and the dried residue was kept in a sterile bottle at 4^{0}C until required for use.

Experimental animals and treatment

Male Wistar rats weighing 100-120 g were obtained f rom the animal facilities of the Faculty of Basic Medical Sciences.

In the Department of Biochemistry, University of Ibad an, they were housed in clean polypropylene cages and kept in a room maintained at 24-28^{0}C with controlled cycles of 12 hours light and 12 hours dark and relative air humidity of 40-60%.

They were permitted to acclimatize for seven (7) day s before the initiation of the treatment and provided regul ar commercial pellets (Vita Feeds, Ibadan) and water ad libitum.

The experimental protocol was carried out following the National Institute of Health's guidelines for the care and welfare of research animals (NIH, 1985), as approved by the university institutional animal ethical committee of the Federal University of Agriculture, Makurdi, Nigeria, with reference number UI/FVM/17/22.

The experiment was carried out on forty male Wistar rats that were randomly separated into eight (8) groups of five (5) rats each and were treated differently depending on their group.

- Group A: Rats were administered distilled water only

- Group B: Rats were given KBrO$_3$ (dissolved in distilled water) at 30 mg/kg body weight

- Group C: Rats were administered quercetin at a dose of 50 mg/kg

- 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 administered 30 mg/kg KBrO$_3$ + 300 mg/kg of AEAC

- Group G: Rats were given 30 mg/kg of KBrO$_3$ + 150 mg/kg of AEAC

- Group H: Rats were given 30 mg/kg KBrO$_3$ + 50

mg/kg of quercetin.

For 90 days, all treatments were given by gavage once a day on alternate days. The rats were monitored for indicators of toxicity and mortality throughout this time. A computerized weighing scale was used to record their body weights every week.

Termination of the experiment and harvest of tissues

The rats were sacrificed by cervical dislocation once the treatments were completed, and blood was taken in a plain sample bottle for biochemical assays. The serum was obtained by centrifuging the clotted blood sample at 3000 rpm for 5 minutes and storing it at 4 0C until it was utilized for analysis. Each experimental rat's liver was also thoroughly removed with dissecting scissors and forceps, cleaned in a 1.15 percent KCl solution, blotted with filter paper, and weighed. The liver was sectioned for histological analysis, and the remaining piece was homogenized using a Teflon homogenizer in an ice-cold homogenizing solution (0.1M phosphate buffer, pH 7.4).

To extract the post mitochondrial fractions, the homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C in a cool centrifuge. The liver biomarkers, oxidative stress, and antioxidant activity were all determined using this fraction and serum.

Biochemical analyses

A spectrophotometer was used to examine the biochemical parameters: the reduced glutathione (GSH) level was evaluated using the liver homogenate according to the technique of Jollow *et al* (1974). According to the approach described by Wright *et al*., lipid peroxidation was measured by measuring the quantities of Malondialdehyde formed as a result of lipid peroxidation (1981). According to Mohandas *et al*., the activity of the enzyme glutathione peroxidase (GPx) was determined (1985). Catalase (CAT) activity was determined according to the method of Claiborne (1985). The level of SOD activity was determined by the method of Misra and Fridovich (1972). Glutathione S-transferase (GST) was determined by the method of Habig *et al*. (1974)*.* The level of H$_2$O$_2$ was assessed by H$_2$O$_2$-mediated horseradish peroxidase-dependent oxidation of phenol red by the method of Pick and

Keisari (1981). Nitrite assay was done using Griess reagent with some modifications of the method of Green *et al*. (1982).

Determination of ɣGT was done using commercial kits (Rekon diagnostics. Pvt. Ltd).

Randox Laboratories Limited kits (Code: TP245 370343 C8 WJ 2 x 500ml) were used for determining the total protein level. Randox Laboratories Limited kits (Cat NO.:AS101 2 x 100ml) were used to determine AST level. Randox Laboratories Limited kits (AL 100 1 x 100ml) were used to determine ALT level.

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

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).

Immunohistochemical investigations of COX-2, Bcl-2, Caspase-3, and p53 expression level

For immunohistochemical investigations, 4 microns paraffin sections were stained immunohistochemically for visualizing cysteine-aspartic protease (caspase-3), BCL-2, P53, and COX-2 positive cells, using the suitable antibody in each staining time (Hsu *et al*., 1981). The intensity of caspase-3, P53, BCL-3, and COX-2 expression in liver sections was quantified by using NIH image j software.

Statistical analysis

All results are expressed as mean ± standard deviation (Mean ± SD). The data were analyzed by one-way analysis of variance (ANOVA) with Graph pad prism version 8.01.

RESULTS

AEAC and Quercetin ameliorated potassium bromate (KBrO₃)-induced hepatocellular toxicity in Wistar rats

Administration of KBrO₃ only at a dose of 30 mg/kg body weight caused a significant ($p < 0.05$) increase in the activity of alanine amino transaminase (group B) as compared with the control given distilled water only (group A), as in Figure 1A. On the other hand, treatments of rats with KBrO₃ and AEAC at the dose of 300 or 150 mg/kg (Groups F and G respectively) led to a significant reduction in the KBrO₃ -induced elevated enzyme activity when group B is compared with group F or G. The effect of the AEAC on KBrO₃ toxicity as measured by ALT activity is comparable with that of quercetin (group H, Figure 1A). The activities of other enzymes markers of liver toxicity, aspartate amino transaminase (Figure 1B) and γ-glutamyl transferase (Figure 1C) follow patterns similar to that of alanine amino transaminase activity in the control and experimental animals. In all cases, the higher dose of AEAC (300 mg/kg) seemed to be more effective in ameliorating the KBrO₃ -induced activities of the enzymes (Figures 1 A-C). Histological analysis of the liver of the experimental rats corroborated the observation made with the enzyme indices of hepatotoxicity (Figure 2)

Enhancement of the cellular antioxidant defense by AEAC and quercetin in the liver tissue of rats treated with KBrO₃

Administration of KBrO₃ alone to rats caused significant (p<0.05) changes in the activities of major detoxifying enzymes like superoxide dismutase (SOD), catalase, glutathione S-transferase (GST), and glutathione peroxidase (GPx) and the level of cellular reduced glutathione (GSH) in the hepatic tissues when compared with the control given distilled water only (Figure 3). However, treatment of rats with KBrO₃ and AEAC (at 300mg/kg or 150 mg/kg) or quercetin at 50 mg/kg (Group F, G, and H respectively) caused a restoration of 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 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 GSH and activities of GST and GPx compared with the control,

Group A (Figure 3). This, therefore, confirmed the tissue antioxidant system enhancement effects of the extracts.

Mitigation of the KBrO₃-induced inflammatory biomarkers (Malondialdehyde and Nitric Oxide) by AEAC and/or Quercetin

Administration of KBrO₃ (group B) caused a significant ($p<0.05$) increase in Malondialdehyde (MDA) levels when compared with the control group (group A), given distilled water as shown in Figure 4. The level of MDA was significantly ($p<0.05$) lower in the groups given KBrO₃ and AEAC or quercetin compared with the group given KBrO₃ alone (Group F/G/H versus Group B). Assessment of the effects of KBrO₃ on hepatic Nitric Oxide (NO) levels in the treated rats revealed trends similar to the observations made with MDA levels above (Figure 4).

Changes in the KBrO₃-induced Liver injury in rats

Treatment of rats with 30 mg/kg body weight KBrO₃ alone resulted in severe portal congestion, with moderate periportal vacuolar degeneration due to disruption of hepatic architecture, however, rats in groups administered distilled, quercetin, and AEAC alone showed normal hepatocellular architecture. In the groups co-administered KBrO₃ + AEAC at the graded doses, there was a significant reduction in the degree of the hepatic architecture disruption showing minimal portal congestion and mild periportal cellular infiltration.

Variation of KBrO₃-induced DNA fragmentation in the liver cells of rats

There was significant ($p<0.05$) DNA fragmentation in the liver homogenate of rats treated with KBrO₃ (Group B) alone when compared with those given distilled water only (Group A) (as shown in Figure 5). Quercetin and AEAC abrogated the effect of KBrO₃ in Groups F, G and H treated with KBrO₃ and AEAC or quercetin (Figure 5).

Changes in the expression of apoptotic-related proteins in the rats

Cox-2, p53, and caspase-3 genes were expressed at very low levels in the hepatic tissues of the control rats given distilled water only (Figure 6 panel A). However, in the group of rats treated with KBrO₃ only (Group B), the corresponding tissue sections showed intense staining for cox-2, p53, and caspase-3 expressions (Figure 6 panel B). Treatment with AEAC or quercetin ameliorated the KBrO₃-induced expression of cox-2 and caspase-3 and p53 genes in the liver cells in the groups treated with KBrO₃ and AEAC or quercetin (Groups F, G, and H) compared with those treated with KBrO₃ only (Group B). On the other hand, BCL-2 protein was mildly expressed in group A and also in the KBrO₃-treated, group B. There was a significant increase in expression of the BCL-2 protein in the groups treated with KBrO₃ and AEAC or quercetin.
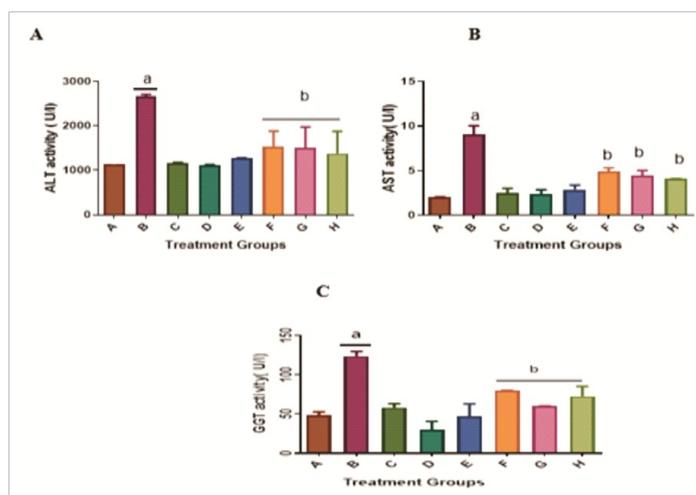

Figure 1: Effect of AEAC on serum alanine, serum aspartate Aminotransferase, and γ-glutamyl transferase activities in male Wistar rats treated with KBrO₃. Values are means + SD (n=5). a= significantly different compared with negative control, group A, and b= significantly different compared with positive control group B, treated with KBrO₃ only ($p<0.05$). Groups 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 and H: KBrO₃ + Quercetin.
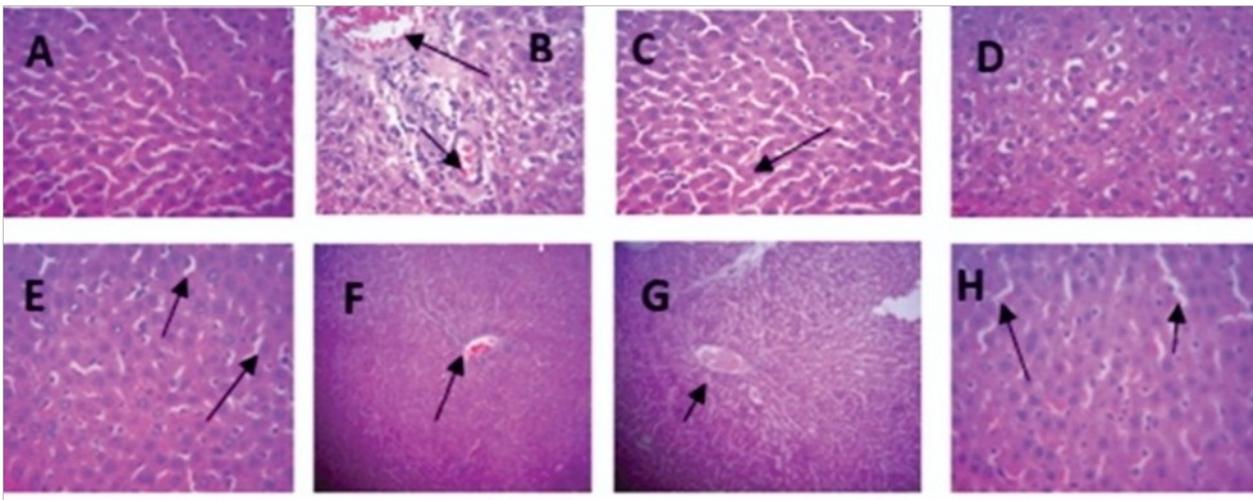
Figure 2: Selected photomicrographs of liver tissues sections (×100) in male Wistar rats treated with $KBrO_3$ and/or AEAC: There are no lesions in the liver of the control group. (A) but severe portal congestion, with moderate periportal cellular infiltration in the group, treated with only (B). There is a mild to moderate periportal vacuolar degeneration of hepatocytes in the group treated with Quercetin only (C) while treatment with AEAC at 300 and 150 mg/kg produced no visible lesion (D and E). Treatment with $KBrO_3$ and AEAC at 300 mg/kg (F) shows mild diffuse vacuolar degeneration whereas mild diffuse sinusoidal congestion was observed in the livers of rats that received $KBrO_3$ and AEAC at 150 mg/kg (G). There is mild central venous and sinusoidal congestion in the group treated with $KBrO_3$ and Quercetin (H).
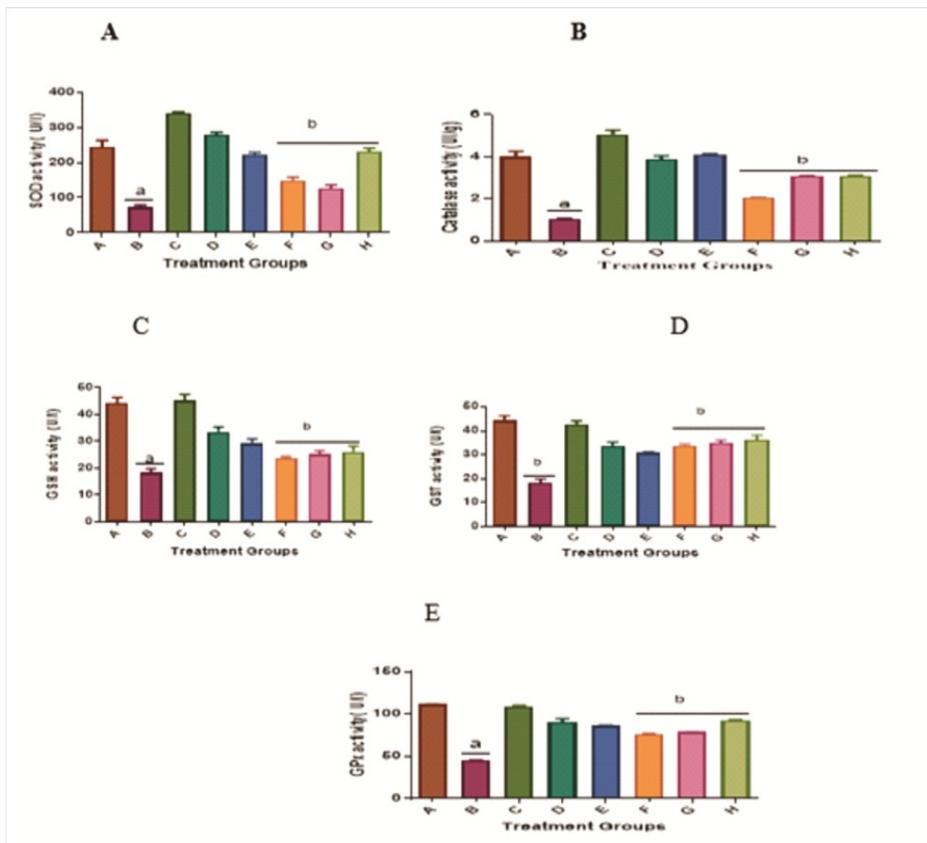

Figure 3: Enhancement of the cellular antioxidant defense by AEAC in the liver tissue of rats treated with $KBrO_3$. Values are means + SD (n=5). a= significantly different compared with negative control, group A, and b= significantly different compared with positive control group B, treated with $KBrO_3$ only (p<0.05). Groups A received distilled water, B: 30 mg/kg $KBrO_3$, C: 50 mg/kg Quercetin, D: 300 mg/kg AEAC, E: 150 mg/kg AEAC, F: $KBrO_3$ + 300 mg/kg AEAC, G: $KBrO_3$ + 150 mg/kg AEAC and H: $KBrO_3$ + Quercetin.
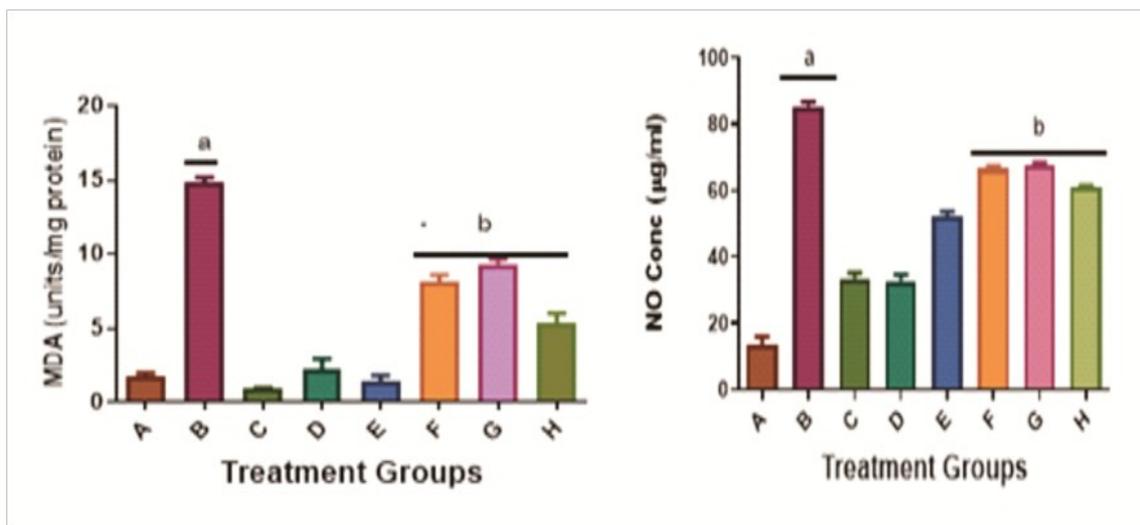
Figure 4: Mitigation of the KBrO₃-induced inflammatory biomarkers (Malondialdehyde and Nitric Oxide) by AEAC levels in the liver of male Wistar rats. Values are means + SD (n=5). a= significantly different compared with negative control, group A (p<0.05). b= significantly different compared with positive control group B, treated with KBrO₃ only (p<0.05). Groups 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 and H: KBrO₃ + Quercetin.
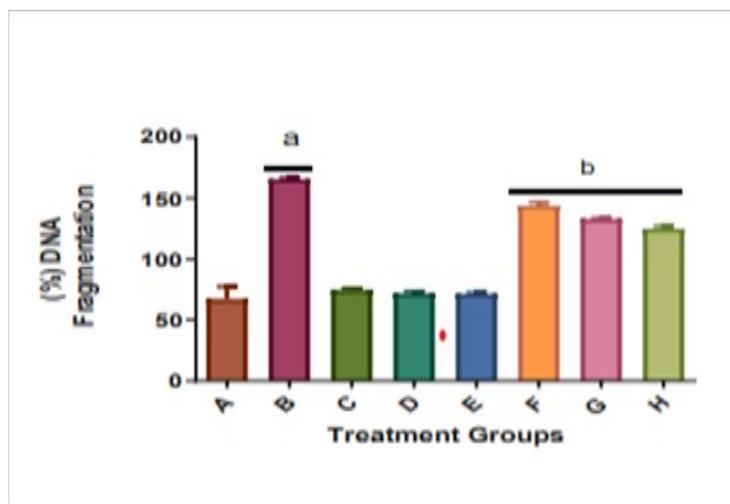

Figure 5: Effect of AEAC on the proportion of DNA fragmentation in the liver of male Wistar rats treated with potassium bromate. Values are means + SD (n=5). a= significantly different compared with negative control, group A, and b= significantly different compared with the positive control group B, treated with KBrO₃ only (p<0.05). Groups 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 and H: KBrO₃ + Quercetin.
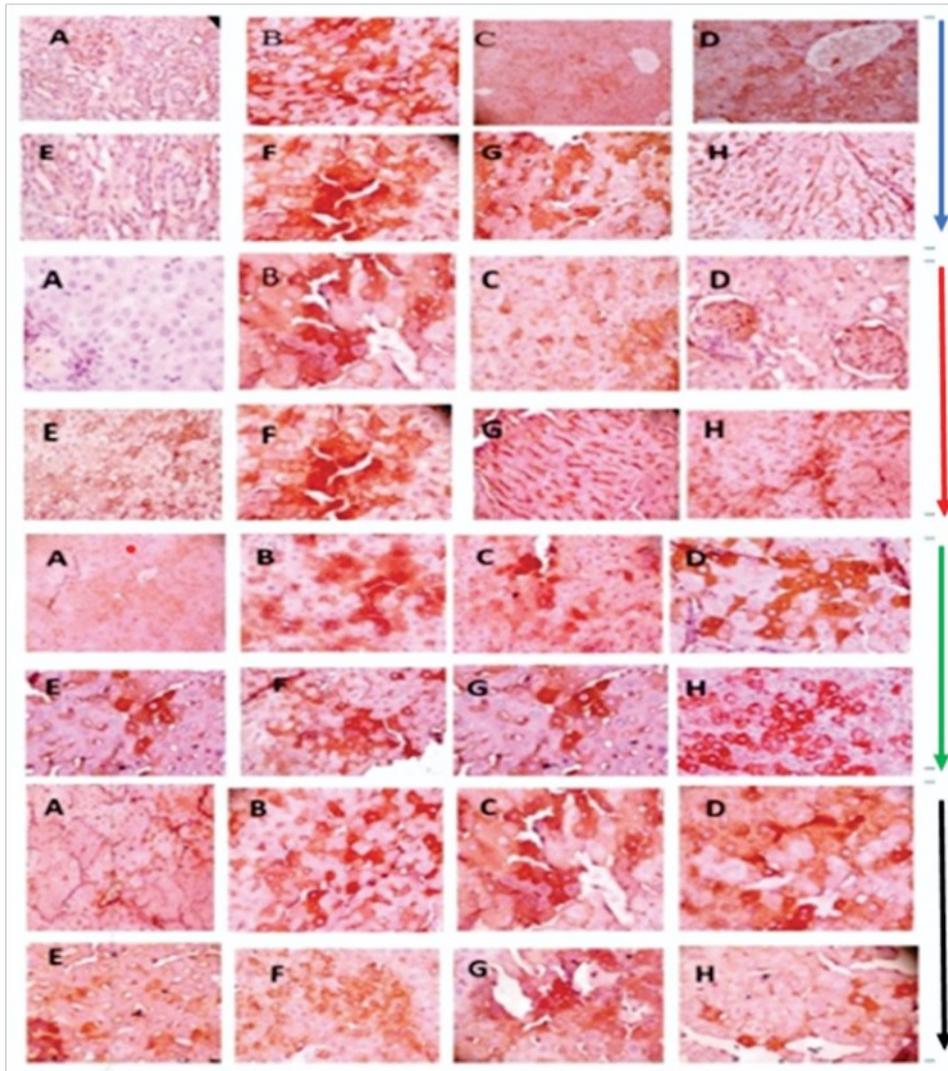
Figure 4: Representative photomicrographs of liver tissues comparing the expression levels of COX-2 (Blue arrow), P53 (Red arrow), Caspase-3 (Green arrow), and BCL-2 (Black arrow) proteins in male Wistar rats treated with potassium bromate and/or AEAC. There is a negligible or mild expression of Cox-2, P53, Caspase-3, and BCL-2 in the negative control group (A), quercetin treated group (C) and AEAC treated groups (D and E); severe expression in $KBrO_3$ treated group (B); There is a moderate expression in the group treated with $KBrO_3$ and AEAC or quercetin (F, G, and H).

DISCUSSION

According to research, chemical toxicity is connected to an increase in reactive oxygen species (ROS) production, which causes oxidative stress in cells (Khan *et al*., 2001). Superoxide anions and their derivatives (hydroxyl radical), the most common ROS produced in living cells are extremely reactive, causing peroxidation of cell membrane lipids resulting in membrane damage and cellular content leakage. Lipid peroxidation and associated membrane damage have been linked to the etiology of a variety of diseases, including hepatocellular carcinoma.

In the current study, $KBrO_3$ administration caused a significant ($p > 0.05$) increase in the transaminases (ALT and AST) and γ-glutamyl transferase (GT) activities when compared to the control group. These findings are consistent with previous research that found an increase in rat alanine and aspartate amino transaminases in the rats treated with $KBrO_3$ (Khan *et al*., 2012; Dimkpa *et al*., 2013). The alterations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities are biochemical indicators of hepatocellular injuries (Atawodi *et al*., 2014). When compared to the group

given just KBrO₃, treatment of KBrO₃ with AEAC at doses of 300 mg/kg or 150 mg/kg resulted in lower ALT, AST, and GT activity, suggesting that the extract can counteract the toxic effects of KBrO₃ on the liver.

The liver tissue photomicrographs are commonly used to evaluate the extent of tissue damage, by the microscopic study of their structural architectures. Thus, histopathological findings in tissue sections of animals are commonly used to validate the biochemical findings of a study (Al-Attar *et al*., 2015). Whereas there were no apparent abnormalities in the liver architecture of rats in the groups that were administered distilled water and pure quercetin alone, the group treated with KBrO₃ alone had portal congestion and mild periportal vacuolar degeneration due to disturbance of hepatic architecture. The harmful effects of KBrO₃ that are present here are comparable to those reported by other researchers (Dimkpa *et al*., 2013; Abuelgasin *et al*., 2008; Akanji *et al*., 2008). A combined treatment of AEAC at doses of 300 mg/kg or 150 mg/kg, as well as quercetin with KBrO₃, showed a diminution in the hepatic architectural disturbance, with low portal congestion and modest periportal cellular infiltration. This indicates that the AEAC has an abrogative effect against KBrO₃-mediated hepatotoxicity in rats. Previous studies have shown that plant extracts could prevent pathological changes in liver tissues of animals and every so often may reverse pathological changes already induced by toxicants to animal tissues (Adejor *et al*., 2003; Alqasoumi, 2014).

Different exogenous anxieties, including KBrO₃, can cause oxidative pressure. Past exploratory proof has exhibited that KBrO₃ incites oxidative pressure (Delker *et al*., 2006).

Furthermore, when the rats were administered KBrO₃ alone, the levels of malondialdehyde (MDA) and nitric oxide (NO) were significantly ($p > 0.05$) increased compared to the control group. This finding was in consonance with the report of Raouia *et al*., (2021). MDA and NO levels were ameliorated by AEAC treatment in a dose-dependent manner. Additionally, as compared to control, KBrO₃ administration depleted cellular reduced glutathione (GSH) levels.

The CAT, SOD, GST, GSH, and GPX represent an armoury of antioxidants usually produce by the body to neutralize or 'mop up' free radicals that can harm the cells. They, therefore, defend the cells against oxidative stress. The ability of the body to produce these antioxidants is controlled by genetic makeup and influenced by exposure to environmental factors such as chemicals as well as dietary intakes (Halliwel, 1999).

KBrO₃ treatment caused a significant ($p > 0.05$) reduction in the activities of antioxidant enzymes: SOD, catalase, GST, and GPX in rats, whereas the administration of AEAC along with KBrO₃ significantly ($p > 0.05$) restored GSH and antioxidant enzyme activities (SOD, catalase, GST, and GPX) near normal in a dose-dependent manner, implying that the AEAC increased the production of cellular antioxidants. Consequently, the AEAC replaced the GSH levels and the antioxidant enzymes that were depleted during the neutralization of oxidative stress caused by KBrO₃. As a result, the extract may have acted as a hepatoprotective agent against KBrO₃ by restoring GSH levels, GST activity, and the glutathione redox cycle enzymes in hepatic tissues as shown. This is in keeping with Watanabe *et al*., prior's recommendation (2004).

The observation made with the AEAC may be linked to the presence of reputable antioxidants such as quercetin in the extract. Quercetin has been shown to possess potent antioxidant properties (Read, 1995; Orsolic *et al*., 2004). Moreover, the findings with the extract here, as a protective agents and antioxidants system restorer against KBrO₃ -induced toxicity and oxidative stress, are similar to the result obtained by administration of pure quercetin only.

In likewise manner, the administration of KBrO₃ caused a significant increase in the percentage of DNA fragmentation in the liver issues. The increased proportion of DNA fragmentation in the liver has been linked to oxidative DNA damages (Khan and Sultana, 2005). The findings that groups of animals treated with AEAC or quercetin along with KBrO₃ recorded a lower percentage of DNA fragmentation than those treated with KBrO₃ alone support the fact that the extract contains bioactive compounds which play a vital role in the inhibition of DNA fragmentation or DNA repair or both by abrogating the injurious effect of the KBrO₃ on the hepatocytes.

The expression of apoptotic-related proteins is modulated by toxicants that affect cellular redox status such as $KBrO_3$ (Corsby *et al*., 2000). Changes in the expression of apoptotic-associated proteins including caspase-3, BCL-2, and p53 might be linked to genome instability. The p53 gene, in particular, is a tumor suppressor gene that regulates the cell cycle, apoptosis, and DNA repair (Bowen *et al*., 2006).

In this work, we found that the group treated with $KBrO_3$ only had greater P53, Caspase-3, COX-2, and lower BCL-2 expression than the control group. However, co-administration of $KBrO_3$ with AEAC and/or quercetin suppressed the high production of P53 and Caspase-3 and raised the expression of the BCL-2 protein. It is now well known that apoptosis modulation is a part of several phases of oncogenesis, and that BCL-2, anti- and pro-apoptotic BCL-2 family members, as well as P53 and Caspase-3, may all play key roles. The impact of transcription factors controlling cellular proliferation is similar to the apoptotic/anti-apoptotic system. COX-2 inhibition appears to be a promising target for the prevention of a variety of proliferative disorders, including cancer (Konurek *et al*., 2005). COX-2 is controlled by the transcription factor NFkB, which creates a favorable environment for tumor growth. Following exposure to $KBrO_3$, the expression of COX-2 in liver tissues increased in the current investigation. Inhibition of $KBrO_3$-induced COX-2 expression in the hepatic tissues was noticed following treatment of $KBrO_3$ with graded dosages of AEAC or quercetin.

As a result of the preceding, it may be inferred that AEAC administration prevents $KBrO_3$-induced hepatocellular damage in rats. Hepatic architectural damage caused by $KBrO_3$ exposure was significantly reduced by the extract. In addition, in treated rats, the AEAC controls the production of transcription factors and proteins involved in the cell cycle and apoptosis pathways and increases antioxidant defenses against $KBrO_3$-induced distortion. The AEAC's capacity to limit the indicators of inflammation, oxidative stress, and apoptotic regulation in Wistar rats suggests it might be a promising option for chemoprevention of hepatocellular damage.

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CONFLICTS OF INTEREST

The author declare that they have no potential conflicts of interest.

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