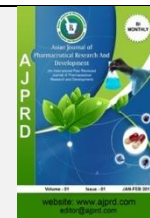


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Research Article

Ameliorative Effect of *Allium Cepa* L. (Red Onion) Extract against Potassium Bromate Induced Intestinal Injury In Rats

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ABSTRACT

Potassium bromate is an oxidizing agent and one of the cheapest dough improvers in the baking and food industry. This agent is classified as carcinogenic in rats and nephrotoxic in both man and experimental animals when given orally. The red onion, *Allium cepa*, is used in the daily diet for taste. Compounds in onions also have various medicinal properties, such as being anticancer, antifungal, antioxidant, anti-ulcer, and anti-inflammatory. We therefore sought to investigate whether aqueous extract of red onion would be protective against injury to the intestine.

Materials and Methods: 36 Wistar male rats were divided into 6 batches. Over a 4-weeks experimental period, group I rats served as a control and group II received 100 mg/kg b.w of KBrO₃ on the 24th and 27th days. Group III received red onion juice at 1ml/100 g bw every day and 100 mg/kg bw of KBrO₃ on the 24th and 27th days. Group IV received 50 mg/kg bw of KBrO₃ twice per week, group V received red onion juice daily at 1ml/100 g bw and 50 mg/kg bw of KBrO₃ twice per week, and group VI received 30 mg/kg KBrO₃ every day. The body and four organs weights, including the kidney, testis, lung and liver of all experimental rats were measured. The histopathological investigation was performed for the intestine tissues of all groups.

Results: KBrO₃-treated small intestines exhibited destruction in the villi, decreased number of goblet cells, crypt loss and cell infiltration in the epithelial lining. Treatment with extract of red onion appeared to significantly ameliorate the toxic effects of KBrO₃.

Conclusion: Our findings suggest that red onion ameliorates the extent of intestinal injury and appears to act as an antioxidant. This study supports that red onion has beneficial properties, although no direct clinical conclusions can be drawn from these data.

Key words: Potassium bromate, histopathological, red onion, intestine, Wistar rats, protective

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INTRODUCTION

A food additive is a substance or mixture of substances present in a food as a result of any aspect of production, processing, storage, or packaging¹. The use of these additives is a well practice but is not without controversy.

Potassium bromate (KBrO₃) is a chemical additive mixed in flour to improve the action of gluten and encourages the fermentation process leading to increase the volume of the bread and hold its shape². Bromate can be also found as a disinfection byproduct of the ozonation of drinking water³. The bromide in the source water is oxidized to bromate by the ozone. Furthermore, besides its use in the baking industry, potassium bromate is also widely used in

cosmetic, especially in hair lotions, in treating barley in beer making, and in fish paste and cheese production⁴. Despite the benefits of this chemical, toxicological studies have shown that bromates cause kidney, thyroid and gastrointestinal cancer in experimental animals, and the International Agency for Research on Cancer (IARC) classifies bromates as a group 2B carcinogen (a possible human carcinogen) with renal tumour risks at high doses (IARC 1986). As a result of extensive investigations into the potential risks associated with KBrO₃, this food additive is banned in many countries such as the United Kingdom, France, Nigeria, and Canada^{5,6}. Several antioxidants (AOs) have shown protection against bromate-induced toxicity. These antioxidants could reduce or prevent free radical formation by disrupting the oxidation chain reaction and

neutralize free radicals, resulting to delay or the inhibition of cellular damage⁷⁻¹⁰.

Onion (*Allium cepa* L.) is one of the most widely consumed and cultivated vegetable crops in the world¹¹. Traditionally, onions have been used as an herbal remedy for a wide range of disorders due to their association with various pharmacological effects¹². Therefore, the purpose of this study is to investigate the possible protective effects of red onion extract against KBrO₃ toxicity on intestine of Wistar rats.

Materials and Methods

Animals

Albino Wistar rats (12–13 weeks old, weighing 150–200g each) were obtained from the Animal House of the National Medical Research Centre in the city of Al-Zawia, Libya. 30 male Wistar rats were randomly allocated to 5 groups of 6, where each group was subjected to one of the treatments as prescribed later. They were housed in standard clear plastic cages and maintained under standard animal housing conditions. They were allowed to acclimatize for 2 weeks before the experiments were conducted, and were given free access to standard laboratory food and water. All animals were grown for the four-week period of the experiment.

Preparation of *Allium cepa* Extract (AcE)

Fresh onion, *Allium cepa*, was obtained from the local market in Al-Zawia. The bulbs were rinsed thoroughly with water and cut into small pieces and prepared as described in earlier study¹³.

Chemicals

Potassium bromate was purchased in powder form from the Sigma-Aldrich Company. Stock of potassium bromate (KBrO₃) solution was prepared by dissolving 25 g of KBrO₃ in 1000 ml distilled water, followed by storage at 4 °C¹⁴. Bromate working solutions were prepared by diluting the stock solution to the required concentrations as needed.

Experimental animals grouping

The animals were divided into 5 equal groups, each contains 6 male rats:

1) **Control Group (G1)**: Rats were fed with a normal diet and drinking water only for four weeks. 2) **Treated Group 2 (G2)**: Rats were given KBrO₃ by oral gavage (100 mg/kg bw) at days 24 and 27 of the experiment. 3) (AcE + KBrO₃)

Group3 (G3): Rats were treated with 1 mL/100 g bw/day of AcE extract via gavage for 28 days, and were given KBrO₃ (100 mg/kg b w) at days 24 and 27 of the experiment. 4) (KBrO₃) **Group 4 (G4)** rats were given KBrO₃ by oral gavage in doses of 50 mg/kg bw twice per week. 5) (KBrO₃ + AcE) **Group 5 (G5)** rats were treated with (1 mL/100 g bw/day) of AcE extract via gavage for 28 days, and were given KBrO₃ (50 mg/kg bw) twice a week. 6) (KBrO₃) **Group6 (G6)** rats were given KBrO₃ by oral gavage in doses of 30 mg/kg bw/day) for 4 weeks.

Body and Organ Weights

The body weights of the rats were recorded throughout the experiment. Change in body weight was calculated by subtracting the final body weight from the initial body weight. Additionally, the liver, kidneys, testes and lungs were also weighed.

Preparation of tissue samples

Portions of the duodenum were immediately fixed in 10% neutral buffered formalin for histological study. Tissues were dehydrated through a series of ethanol solutions, cleared in xylene, embedded in paraffin and routinely processed for histological analysis¹⁵. Sections of 5 µm thickness were cut using a rotary microtome and stained with haematoxylin-eosin.

Statistical analysis

Data were subjected to one-way ANOVA using GraphPad Prism. The data are presented as mean ± SEM. The cut-off value for statistical significance was $p < 0.05$.

RESULTS

Effects of KBrO₃ and/AcE on Body and Some Organ Weights

In order to investigate the protective effects of red onion juice against KBrO₃-induced toxicity, the body weights of all experimental rats were measured. There were no significant changes in the body weight of rats that survived at the end of the experiment, (Table 1) neither were the relative weights of some selected target organs (Table 1). Four organs, including the kidney, testis, lung and liver showed slightly lower weights in the treated groups compared to these values in the control group, although these results were not statistically significant.

Table 1: Effect of red onion juice on body weight relative organs weights in KBrO₃ intoxicated experimental rats.

Parameters Group	Body (mean ± SE)	Kidney (mean ± SE)	Testis	Liver (mean±SE)	Lung (mean±SE)
Control	222.4±7.34	0.72±0.02	1.10±0.15	7.01±0.25	1.24±0.09
KBrO ₃ (100 mg/kg bw)	206.6±17.01	0.73±0.03	1.08±0.14	7.24±0.23	1.46±0.21
AcE + KBrO ₃ (100 mg/kg bw)	191.4±9.19	0.80±0.07	1.16±0.11	6.45±0.43	1.34±0.15
KBrO ₃ (50 mg/kg bw)	184.4±12.69	0.70±0.03	1.11±0.11	6.8±0.52	1.26±0.04
AcE + KBrO ₃ (50 mg/kg bw)	213.6±4.82	0.74±0.05	1.32±0.07	6.82±0.23	1.46±0.11
KBrO ₃ (30 mg/kg bw)	214.2±12.06	0.74±0.4	0.96±0.15	6.48±0.40	1.44±0.10

A one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test was used for statistical analysis. The values shown are means $\pm$ SE.

Effects of Treatment on Histology of the Intestine Tissues

Histological evaluation of the tissue samples of control rats demonstrated a normal architecture of intestine (Figure 1. A) with the appearance of a prominent mucus layer. KBrO₃-induced considerable changes were observed in the small intestine. Microscopic examination of the intestinal tissue from rats treated with 100 mg/kg bromate revealed the loss of some superficial epithelium, necrosis of the upper villi and decreased number of goblet cells. Also, cellular debris was observed in the lumen of the crypt (Figure 1. B). When rats treated with bromate group (50

mg/kg), there were indicated damaged microvillar structures on the apical surface of crypt epithelium. The decreased number of goblet cells was easily recognized in these areas (Figure 1. D). Furthermore, decreases in villus height and crypt depth were marked in 30 mg/kg/day bromate group. Shortening of villus or fusion of villi, desquamation of surface epithelium was observed. Decreased number of goblet cells, crypt loss and cell infiltration in the epithelial lining were also detected (Figure 1. F). Microscopic examination of the intestinal tissue from rats treated with AcE + KBrO₃ revealed negligible lesions of surface epithelium and the histological appearance of these groups (Figure 1 C&E).

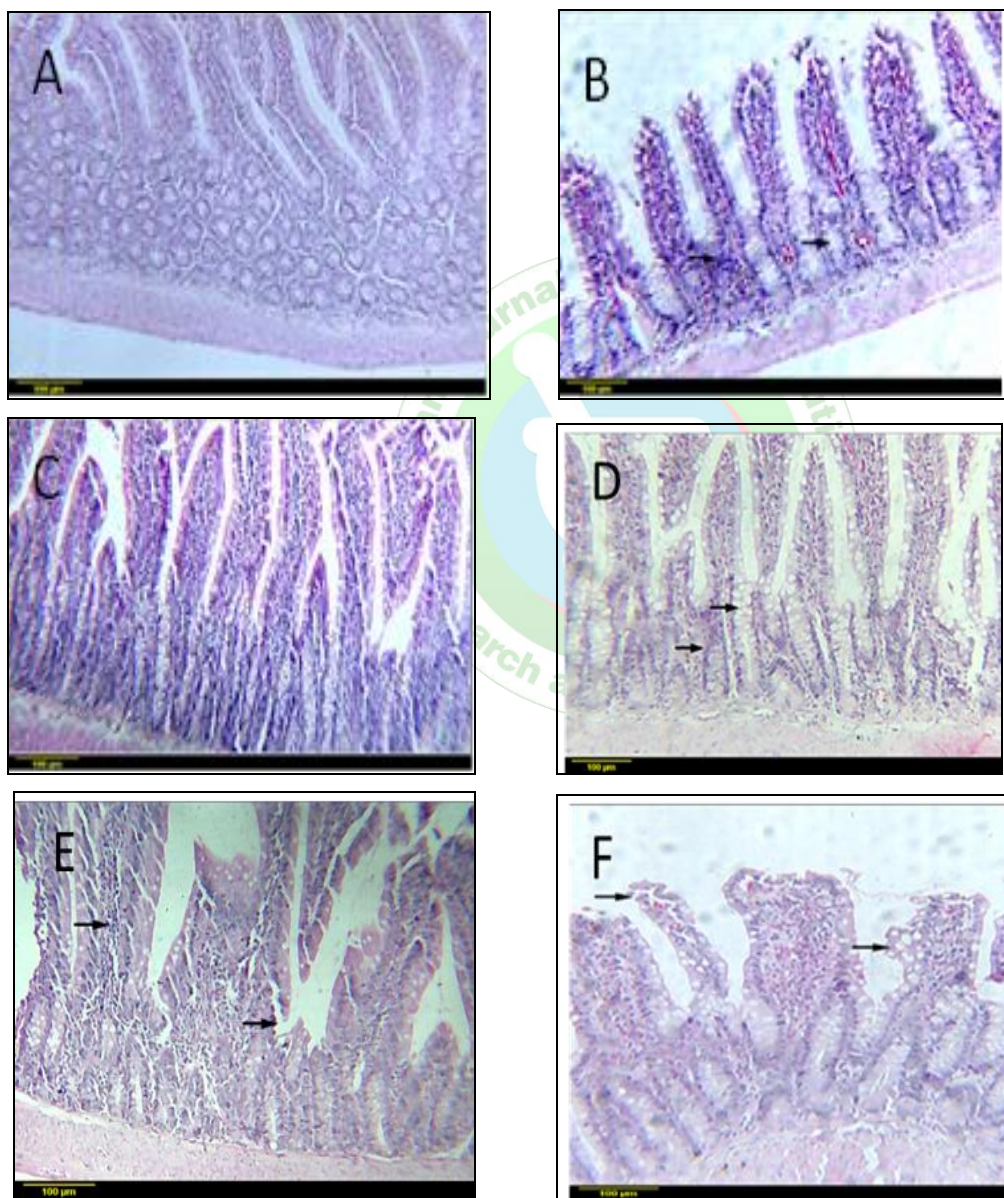


Figure 1: Micrograph of the small intestine, harvested from control and treated groups. (A) Normal architecture of duodenum from control rats **(B)** loss of some superficial epithelium (arrowhead), decreased number of goblet cells and cellular debris (arrow) was observed in the lumen of the crypt in rats treated with KBrO₃ (100 mg/kg bw). **(C)** Rats received AcE + KBrO₃ (100 mg/kg bw) shows less damaged tissue. **(D)** damaged microvillar structures on the apical surface of crypt epithelium (arrows) and decreased number of goblet cells are seen in rats receiving KBrO₃ (50 mg/kg bw). **(E)** treated group section by AcE + KBrO₃ (50 mg/kg bw) shows negligible lesions of surface epithelium. **(F)** Shortening of villus or fusion of villi, desquamation of surface epithelium (arrowheads), and cell infiltration (Asticks) in the epithelial lining treated are seen in rats treated by KBrO₃ (30 mg/kg bw). (sections stained with H&E, x100).

DISCUSSION

Histopathological Observations of Intestine Tissue

Antioxidant characteristics of red onion have been previously reported in different organs, such as the liver^{16, 17}, kidney^{18, 19} and testis²⁰. Therefore the aim of this study is to explore the possible protective effect of red onion against the injuries to rats caused by KBrO_3 in the intestine through histological study. This study shows that the histological features of the intestinal mucosa of rats treated with 50 or 100 mg/kg bw of KBrO_3 were characterized by epithelial desquamation, loss of the crypt, and inflammatory infiltration along with decreases in goblet cells. Similar results presented by Ahmad *et al.*⁴ were that the administration of 100 mg/kg bw of KBrO_3 induced DNA degradation in the intestinal tissue of treated rats and led to extensive intestinal damage such as mucosal cell damage, especially to the membrane. Also the lumen became full of debris and the intestinal villi lost their form²¹ conducted an extensive study of the effects of KBrO_3 on rats, and concluded that it affects intestinal morphology and induces adenomas in the small intestine in experimental animals.

Potassium bromate has been reported to induce tumours in the small intestine of the mouse after oral administration^{22, 23}. In addition, a more recent study by Aoki *et al.*²⁴ investigated tumour formation in the small intestine of *gpt* delta mice induced by the oral administration of KBrO_3 . They showed that the formation of the oxidative DNA base modification 8-oxo-deoxyguanosine (8-oxo-dG) was significantly increased at doses of 0.6 and 2 g/L in these mice. It is well known that the small intestine is the site at which the digestion and absorption of 90% of ions and molecules occurs. On the other hand, this may cause undesirable results; for example, when food contain drugs, toxic pollutants or harmful chemicals such as KBrO_3 ²⁵. Thus, seems likely that any alterations in metabolic pathways caused by toxicants could affect the function of the small intestine⁹. The intestinal brush border membrane (BBM) is one of the most important cellular membranes, because it is a major site of antioxidant action besides its role in nutritional absorption and digestion²⁶. The administration of KBrO_3 to rats has been found to induce oxidative stress (OS) and to lower the activity levels of several enzymes in the BBM and to cause extensive damage to the villi and intestinal gland cells, with the lumen being filled by debris⁹. The decrease in the activity of BBM enzymes may be linked to KBrO_3 -generated free radicals and ROSs which lead to leakage or loss of the enzymes after ROS-induced damage to the epithelial cells lining the intestine, and especially to the cell membrane²⁷. In addition, Ahmad *et al.*⁴ concluded that increased lipid peroxidation may affect the structure of the intestinal membrane and its function, leading to the decreased activity of these enzymes.

In this study, rat body weight decreased after KBrO_3 treatment compared with those in the control group, although the difference was not statistically significant. This finding is consistent with research carried out by Rezaq²⁸ which showed that the oxidative stress induced by KBrO_3 in rats caused significant decreases in body weight. The reduction in body weight could be attributed to the direct

toxic effect of KBrO_3 on the gastrointestinal tract, which perhaps results in the poor digestion of food or malabsorption of nutrients²⁸. The small intestine is exposed continuously to high levels of ROSs and requires digestive and absorptive functions to be tightly controlled by a series of antioxidant substances²⁹. In this study, the protective effect of AcE on KBrO_3 -induced intestinal damage could have been due to its effectiveness in inhibiting of KBrO_3 generated free radicals before they attacked their cellular target. The subsequent reduction in lipid peroxidation and oxidative modification of BBM enzymes might have contributed to the efficacy of the antioxidants in ameliorating the effects of KBrO_3 ²⁸. The present results are in accordance with those of Rezaq²⁸, who showed that the intake of sesame oil or jojoba oil may be useful in improving liver and kidney function and might protect against KBrO_3 -induced oxidative stress in rats by providing stronger antioxidant activity. Similarly, taurine alleviates KBrO_3 -induced tissue toxicity and oxidative damage by improving antioxidant defense, tissue integrity and energy metabolism⁴.

Finally, red onion exhibits important biological activity for health maintenance due to its huge content of antioxidant compounds, which have radical scavenging potential which can help to prevent or slow down the oxidation of free radicals before damage to cells and tissues can occur.

CONCLUSION

Potassium bromate (KBrO_3) causes toxicity in humans and experimental animals. It is a class IIB carcinogen, and its application in food processing is restricted in many countries. The present study has attempted to examine the effects of KBrO_3 -induced oxidative stress in small intestine. Histological observations of the small intestine (duodenum) from KBrO_3 -treated rats showed various types of intestinal damage. The lumen was full of debris and inflammatory cells, the intestinal villi had lost their contours, and goblet cells had decreased.

Red onion exhibits important biological activity for health maintenance due to its huge content of antioxidant compounds, which have radical scavenging potential which can help to prevent or slow down the oxidation of free radicals before damage to cells and tissues can occur. Red onion is also inexpensive and non-toxic and can be administered safely and significantly to people who are exposed to KBrO_3 and related compounds. We suggest that the red onion or its active compounds have a protective role to maintain accurate intestinal toxicity caused by potassium bromate.

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