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## Cataractogenic Potential of Bromate-Mediated Oxidative Stress in Rabbits

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The effects of bromate-induced oxidative stress on some cataractogenic indices were studied in the lens, cornea and retina of New Zealand White rabbits given 60 mg  $\text{kBrO}_3/\text{kg}$  body wt/day by gavage for 28 days using gravimetric, enzymatic, colorimetric and spectrophotometric procedures. The bromate treatment led to significant decreases in the activities of  $\text{Na}^+\text{-K}^+\text{-ATPase}$  in the lens and cornea and significant increases in corneal catalase and lenticular superoxide dismutase, SOD activities ( $p < 0.05$ ). However the retinal enzymes were unaffected. Significant reductions in the antioxidant vitamins A and C were accompanied by significant elevation of malondialdehyde, MDA in all the tissues investigated ( $p < 0.05$ ). These results are strongly indicative of bromate-induced oxidative stress. The elevation of SOD and catalase activities appear to be adaptive responses to cope with this challenge. The implication of these findings for cataractogenesis and the likelihood of existence of tissue-specific reactive species in bromate-induced oxidative stress in ocular tissues are discussed.

**Key words:** Antioxidant status, potassium bromate, vision

## INTRODUCTION

Potassium bromate,  $\text{KBrO}_3$  is an oxidizing agent employed widely as a maturing agent in flour and a dough conditioner in the bakery industry<sup>[1,2]</sup>. Bromate is a constituent of cold-wave hair formulations and may be present as a contaminant in drinking water due to the conversion of bromides to bromates during ozonation of raw water<sup>[3]</sup>. Several toxic effects of  $\text{KBrO}_3$ , mainly carcinogenic and mutagenic effects have been reported in experimental animals<sup>[4-8]</sup>. Lethal oral doses of bromate in humans have been estimated as 154-385  $\text{mg kg}^{-1}$  body wt, while serious poisoning results at doses of 46-92  $\text{mg kg}^{-1}$  body wt<sup>[9]</sup>. Oral doses of 185-385  $\text{mg kg}^{-1}$  body wt result in irreversible toxic effects like renal failure and deafness in humans, while lower doses are associated with vomiting, diarrhoea, nausea and abdominal pain<sup>[9]</sup>. Due to the harmful effects of  $\text{KBrO}_3$ , the Joint Expert Committee on Food Additives, JECFA has recommended that when bromate is used in food processing no residues should be left<sup>[10]</sup>. However, we recently reported toxic bromate residues in Nigerian bread<sup>[11]</sup>, which shows that humans are still being exposed to this toxin notwithstanding several existing legislations outlawing its use.

Despite the plethora of evidence on toxicity of  $\text{KBrO}_3$  in human and animal tissues, there is a dearth of information on its effect on ocular function. It is known that bromate induces oxidative stress in tissues<sup>[2,3,7]</sup>. Indeed oxidative DNA damage appears to be the basis of bromate-induced kidney carcinogenesis<sup>[7,12,13]</sup>. Evidence abound suggesting a direct causal relationship between oxidative stress and cataractogenesis<sup>[14-16]</sup>. This study was therefore aimed at ascertaining the effect of  $\text{KBrO}_3$  toxicity on some rabbit ocular tissues and their potential for cataractogenesis in this animal model.

## MATERIALS AND METHODS

**Animals and treatment:** Three month old New Zealand White rabbits [initial mean weight =1.40 kg ] were obtained from a breeder in Benin City. The animals were housed singly in clean, disinfected metal hutches and acclimatized on guinea growers mash (product of Bendel Feed and Flour Mills, BFFM Ltd, Ewu, Nigeria) for 2 weeks prior to the experiment. They were subsequently weighed and randomly assigned to 2 groups (5 per group). Members of one groups (test) were given 60  $\text{mg KBrO}_3$  /kg body wt /day for 28 days. The bromate was dissolved in physiological saline and administered by gavage. Members of the other group (control) received saline (by the same route) in place of bromate. Both groups were fed *ad libitum* on mash and

clean drinking water was liberally provided. Fresh feed was provided daily, while stale remnants were weighed and discarded. The rabbits in the test group were weighed weekly to allow for adjustments in bromate. At the end of 28 days, all animals were weighed and sacrificed painlessly by decapitation under chloroform anesthesia. The eye balls were rapidly excised and the lens, cornea and retina were quickly dissected out. Each tissue was ground thoroughly in a hand mortar with some grains of acid-washed sand and 5.0 ml of ice-cold physiological saline for 10 min. The resultant tissue homogenates were kept at 4°C and used for subsequent assays. All homogenates were analyzed within a few hours of preparation.

**Enzyme and metabolite assays:** Catalase was assayed spectrophotometrically by estimating the rate of decomposition of hydrogen peroxide,  $\text{H}_2\text{O}_2$ <sup>[17]</sup>. SOD was assayed by monitoring the inhibitory effect of the enzyme on the auto-oxidation of epinephrine<sup>[18]</sup>.

The assay of  $\text{Na}^+\text{-K}^+\text{-ATPase}$  was carried out colorimetrically by estimating the amount of inorganic phosphate,  $\text{P}_i$  liberated upon incubation of the tissue homogenates with disodium ATP<sup>[19]</sup>. The  $\text{P}_i$  was determined colorimetrically using ammonium molybdate<sup>[20]</sup>. MDA was estimated colorimetrically based on its reaction with thiobarbiturate to yield a pink-coloured complex that absorbs strongly at 532 nm<sup>[21]</sup>.

Ascorbic acid was assayed in a colorimetric reaction using 2,4-dinitrophenyl hydrazine, 2,4-DNP<sup>[22]</sup>. In this assay, ascorbate reacts with 2,4-DNP to give a red osazone which absorbs strongly at 540 nm.

Vitamin A assay involved the precipitation of proteins with absolute ethanol followed by extraction of tocopherols and carotenes with petroleum ether (60-80°C) and estimation of the carotenes by their absorbance at 459 nm<sup>[23]</sup>.

**Statistics:** The Means $\pm$ SEM of the values of the various parameters for the test and control groups were analyzed for statistically significant differences using students t-test.

## RESULTS

Feed intake and weight gain were significantly lower ( $p<0.05$ ) in the bromate-treated rabbits when compared to controls (Table 1). Catalase activity was significantly increased ( $p<0.05$ ) in the cornea of the rabbits given  $\text{KBrO}_3$ , while the enzyme was virtually unaffected in the lens and retina (Fig. 1). The bromate treatment led to significant increase ( $p<0.05$ ) in lenticular SOD activity,

Table 1: Feed intake and weight changes for rabbits in the test and control groups

	Control	Test	Statistics
Mean feed intake (g/rabbits/day)	58±4 <sup>a</sup>	45±3 <sup>b</sup>	p<0.05
Mean weight gain (g/rabbit)	333±14 <sup>a</sup>	113±11 <sup>b</sup>	p<0.05
Feed efficiency (Weight gain /g feed)	5.7	2.5	

Values are Mean±SEM (n=5). For each parameter, those having different superscript across differ significantly (p<0.05)

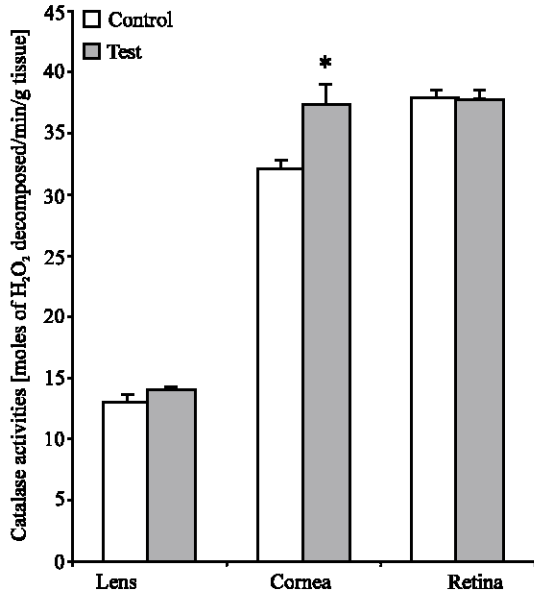


Fig. 1: Catalase activities in the lens, cornea and retina of KBrO<sub>3</sub>-treated rabbits and controls (\*p<0.05 compared to control, n=5 per group)

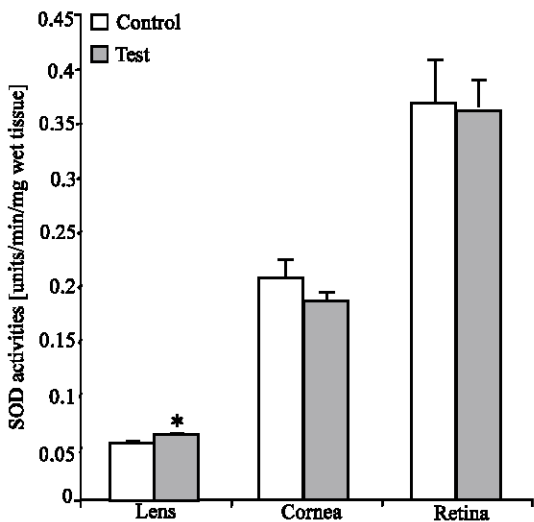


Fig. 2: SOD activities in the lens, cornea and retina of KBrO<sub>3</sub>-treated rabbits and control (\*p<0.05 compared to control, n=5 per group)

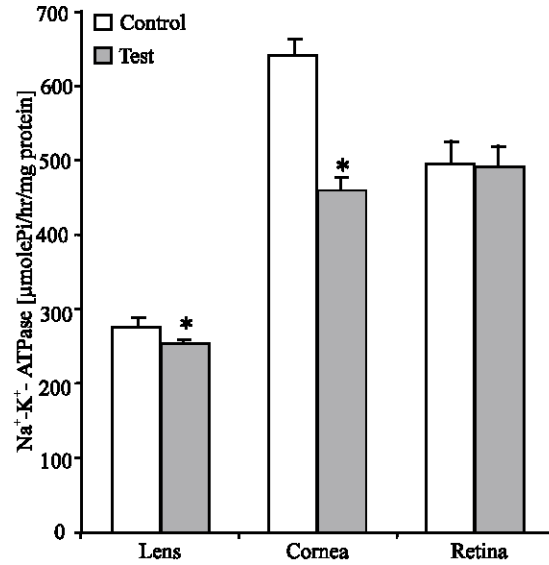


Fig. 3: Na<sup>+</sup>-K<sup>+</sup>-ATPase activities in the lens, cornea and retina of KBrO<sub>3</sub>-treated rabbits and controls (\*p<0.05 compared to control, n=5 per group)

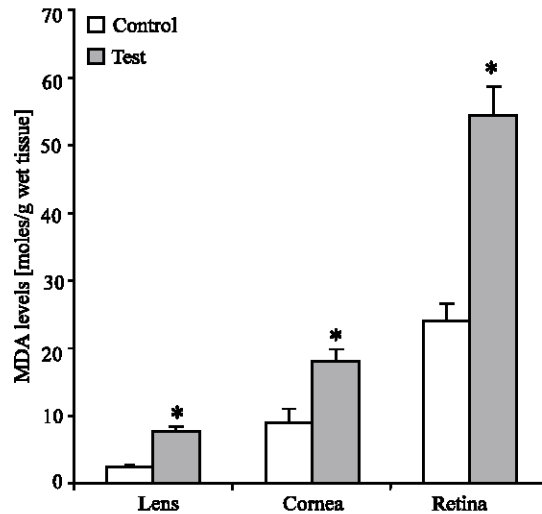


Fig. 4: MDA levels in the lens, cornea and retina of KBrO<sub>3</sub>-treated rabbits and controls, n=5 per group)

while the corneal and retinal enzymes were relatively unchanged (Fig. 2). Na<sup>+</sup>-K<sup>+</sup>-ATPase activity was significantly decreased in the lens and cornea (Fig. 3), while significant elevation of MDA, accompanied by significant decreases in the levels of vitamins A and C were obtained in all the tissues studied (Fig. 4, 5 and 6, respectively).

## DISCUSSION

Most of the available data on acute toxicity studies on bromate refer to rats, mice and hamsters<sup>[6,24,25]</sup>. The LD<sub>50</sub>

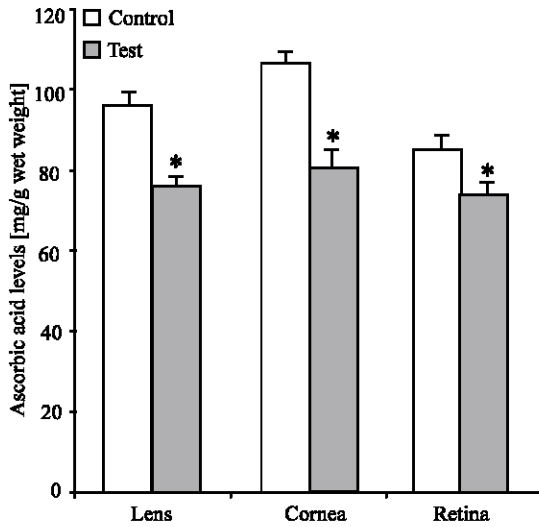


Fig. 5: Ascorbic acid levels in the lens, cornea and retina of  $\text{KBrO}_3$ -treated rabbits and controls (\* $p < 0.05$  compared to control,  $n = 5$  per group)

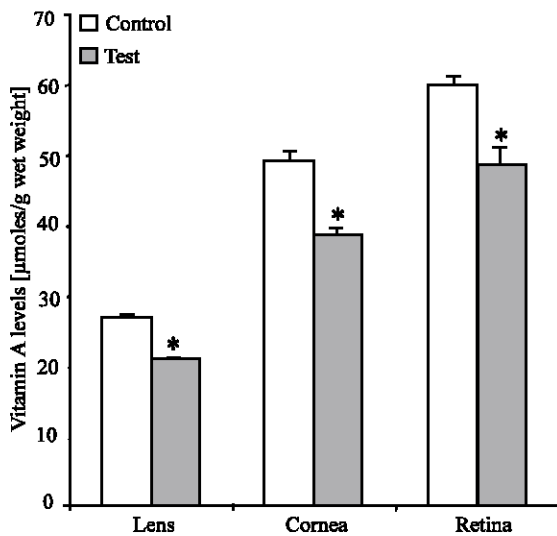


Fig. 6: Vitamin A levels in the lens, cornea and retina of  $\text{KBrO}_3$ -treated rabbits and controls (\* $p < 0.05$  compared to control,  $n = 5$  per group)

of orally-administered  $\text{KBrO}_3$  in mice is  $223\text{--}363 \text{ mg kg}^{-1}$  body wt<sup>[26]</sup>, while  $\text{LD}_{50}$  of  $280\text{--}495 \text{ mg kg}^{-1}$  body wt have been reported for rats<sup>[24]</sup>. Based on various tissue toxicity effects, the least observable adverse effect levels, LOAEL of bromate in rats are  $6.1\text{--}30 \text{ mg kg}^{-1}$  body weight/day<sup>[27-29]</sup>. However there is a dearth of information regarding bromate toxicity studies involving rabbits. Prior to the present study, preliminary exposure trials revealed that a dose of  $120 \text{ mg kg}^{-1}$  body weight/day was lethal to rabbits and resulted in mortalities within 5 days. On the

other hand, when this dose was halved to  $60 \text{ mg kg}^{-1}$  body wt/day, it was well tolerated without any obvious adverse effects. The observed significant decrease in weight gain for the  $\text{KBrO}_3$ -treated rabbits is probably a consequence of depressed feed intake due to the toxic effects of bromate. Several workers have reported similar bromate-induced decreases in body weight gain in rats<sup>[24,27,29,30]</sup>.

Increases in blood and tissue levels of thiobarbituric acid-reactive substances, mainly MDA, are very reliable indices of oxidative stress and lipid peroxidation<sup>[31]</sup>. Thus the profiles of MDA and antioxidant vitamins in the bromate-treated rabbits clearly indicate that bromate provokes oxidative stress in ocular tissues. Several reactive oxygen species, ROS are derivable from bromate viz., peroxy nitrile,  $\text{ONOO}^-$ , hydrogen peroxide,  $\text{H}_2\text{O}_2$ , superoxide anion,  $\text{O}_2^-$  and hydroxyl radical,  $\text{OH}^-$ <sup>[12]</sup>. The elevation of corneal catalase activity in the bromate-treated rabbits is most likely an adaptive response to cope with the challenges of raised  $\text{H}_2\text{O}_2$  levels in this tissue. Similarly, adaptive response may account for the elevation of SOD in the lens, which implies that in this tissue  $\text{O}_2^-$  rather than  $\text{H}_2\text{O}_2$  might be the principal agent for bromate-provoked oxidative damage. In contrast, retinal SOD and catalase were practically unaffected by  $\text{KBrO}_3$  not with standing the MDA and antioxidant vitamin profiles in this tissue. Thus it is likely that a ROS different from  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  may be implicated in bromate-induced oxidative stress in the retina. The involvement of tissue-specific ROS in bromate-mediated ocular damage is in agreement with the finding that SOD and catalase have no inhibitory effects on bromate-mediated renal damage<sup>[32]</sup>. Indeed studies have revealed that bromine oxides and bromine radicals, rather than  $\text{H}_2\text{O}_2$  are responsible for the oxidative stress and renal tumours induced in rat by  $\text{KBrO}_3$ <sup>[5,12,30,32]</sup>.

Cataracts is the final stage of a degenerative ocular disease resulting in opacification of the lens. Evidence abound linking ocular oxidative stress to cataractogenesis<sup>[14-16]</sup>. Thus cataracts may result if ocular antioxidant status is compromised<sup>[14,34]</sup>. Ocular oxidative stress leads to changes in electrolyte balance in the lens and cornea, as a consequence of depression of  $\text{Na}^+\text{K}^+$ -ATPase activity in these tissues.  $\text{Na}^+\text{K}^+$ -ATPase functions to maintain electrolyte balance in the lens and cornea<sup>[35]</sup>. The enzyme is inhibited in cataractous lens<sup>[36,37]</sup> and by cataractogenic factors such as free radicals<sup>[28,39]</sup>,  $\text{H}_2\text{O}_2$ <sup>[40,41]</sup> and changes in membrane lipid composition<sup>[42]</sup>. Consequently the pattern of  $\text{Na}^+\text{K}^+$ -ATPase and other antioxidant parameters in the lens and cornea of the  $\text{KBrO}_3$  toxified rabbits are strongly indicative of the cataractogenic potential of bromate. Thus of animal-to

man extrapolation is allowed, our results suggest that aside from its well established carcinogenic and mutagenic effects, bromate may be a strong predisposing factor in cataractogenesis.

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