Toxicological Properties of Potassium Bromate

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Abstract

Potassium bromate (KBrO₃), used in both the food and cosmetics industry, and a drinking water disinfection by-product, is a nephrotoxic chemical and rodent carcinogen. As KBrO₃ is primarily an oxidizing compound, reactive oxygen and other species generated from bromate have been held responsible for the genotoxic, carcinogenic and toxic effects. Bromate induces primary DNA oxidative damage, mutations, and DNA-strand breakage, structural chromosomal aberration types of chromatic breaks and exchanges. KBrO₃ induces micronuclei in different cells in vivo. Bromate is a clastogenic agent. Bromate administered in the drinking water was tumorigenic in the rat kidney, thyroid, and mesothelium and was a renal carcinogen in the male mouse. The incidence of mesotheliomas on the tunica vaginalis testis in rats was a dose-dependent manner. It was shown that KBrO₃ is an effective promoter of kidney neoplasia induced by N-ethyl-N-hydroxyethylnitrosamine. There are a lot naturally occurring compounds which may be used as effective chemopreventive agents against KBrO₃-mediated renal and other organ oxidative stress, toxicity and tumor promotion response.

Keywords: Potassium bromate; Oxidative damages; Genotoxicity; Carcinogenicity; Chemoprevention

Introduction

Potassium bromate (KBrO₃; CAS No. 7758-01-2) is not a naturally occurring compound, but is synthesized by passing bromine vapour through a solution of potassium hydroxide when heated. This compound exists as white crystals, crystalline powder or granules. It is highly soluble in water (75 g/L at 25°C), slightly soluble in ethanol; it is very stable in water solution at room temperature. KBrO₃ decomposes at temperature above 370°C, with the emission of oxygen and toxic fumes [1].

KBrO₃ is a strong oxidizing agent that has been used in flour milling, as an ingredient in fish-paste in Japan, in cheese making, in beer malting, and as a component of cold hair-wave liquids, and a oxidizing compound [1].

Moreover, bromate is formed as a by-product of water disinfection by ozonation and is frequently detected in top and bottled water. In fact bromate is one of the most prevalent disinfection by-product of surface water [2].

Ozone has been introduced for water disinfection because it is more efficient than chlorine for killing microbes and leads to much lower levels of carcinogenic trihalomethanes (THMs). Ozone leads to formation of hydrobromous acid in water with high bromine content and forms brominated organic by-products and bromate [3]. Occupational exposure to KBrO₃ occurs mainly in production plants during packaging processes.

Toxicity in humans

In Western countries most poison cases with KBrO₃ were by accidental ingestion, mainly among children. In Japan, KBrO₃ was more often ingested for tentative suicide by young women, especially hairdressers [1]. The lethal dose of this chemical in man has been estimated at 5-500 mg/kg of body weight (b.w.) [4,5]. In reported cases nine out of twenty four adults died 3-5 days after ingestion [5].

In the acute phase of poisoning, vomiting and diarrhea with abdominal pain were the main symptoms. Subsequent manifestations included oliguria, anuria, deafness, thrombocytopenia, hypotension, vertigo, and depression of the central nervous system. Acute renal failure was associated with hemolytic uremic syndrome [4]. In both children and adults, oliguria and death from renal failure were seen [4].

In biopsy of the kidney atrophy, necrosis, degeneration, and regeneration of the proximal tubular epithelium have often been observed. In the later stages, sclerosis of the glomeruli and interstitial fibrosis have been reported. Cardiotoxicity and hepatotoxicity have also been observed [5].

Partial hearing loss and complete deafness have also been reported [4,6]. Severe irreversible sensorineural hearing loss within 4-16 h of bromate ingestion was recorded in almost all of the cases. Moreover, strong irritating action of KBrO₃ on gastric mucosa was noted [6,7]. The signs and symptoms of chronic intoxication of humans by KBrO₃ have not been described.

Toxicity in experimental animals

The values of median lethal doses (LD₅₀) per os of KBrO₃ in rats, mice, and hamsters (Table 1) were comprised the range of 280 to 495 mg/kg of body weight [1]. Other data indicate that the oral LD₅₀ value of this compound in rats, determined by OECD Guideline 401 method, was estimated at level of 157 mg/kg b.w. Potassium bromate has been classified as a compound belonging to the category “Toxic” [8].

Major toxic signs and symptoms after a single intragastric administration of KBrO₃ were tachypnoea, hypotermia, diarrhea,
lacrimation, piloerection, suppression of locomotor movement, ataxic gait, and animals lying in a prone position. At autopsy the major findings were strong hyperemia of the glandular stomach mucosa and congestion of the lung. Microscopically, epithelial dilatation and desquamation of the distal convoluted tubules were observed in rats 1 h after KBrO₃ administration. Necrosis and degenerative changes of the proximal tubular epithelium were noted after 3 h and regenerative alterations of the tubular epithelium occurred within 48 h. In mice and hamsters, however, these histopathological changes were later observed and to a lesser intensity [1]. A nephrotoxic single dose of KBrO₃ (100 mg/kg b.w.) administered to rats resulted in a decline in the specific activities of leucine aminopeptidase (LAP), γ-glutamyl transferase (γ-GT), maltase, and alkaline phosphatase (AP), enzymes of brush border membrane (BBM), and also induced oxidative stress in kidney. The enzymes of carbohydrate metabolism, i.e., lactate dehydrogenase, malate dehydrogenase, glucose-6-phosphatase dehydrogenase, glucose-6-phosphatase, and fructose-1,6-bisphosphatase were also altered and suggest a shift in energy metabolism from the aerobic to anaerobic mode. Maximum changes in all the parameters were 48 h after administration of KBrO₃, after which recovery took place [9].

Table 1: LD₅₀ (mg/kg b.w.) values in rat, mouse, and hamster after p.o. administration [1,8]. *Numbers in parentheses are 95% confidence limits. Observation time amounted 7 days.

<table>
<thead>
<tr>
<th>Species/Strain</th>
<th>Male (mg/kg b.w.)</th>
<th>Female (mg/kg b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat F344</td>
<td>400 (348-460)*</td>
<td>495 (446-549)</td>
</tr>
<tr>
<td>Rat Wistar</td>
<td>157</td>
<td>157</td>
</tr>
<tr>
<td>Mouse B6C3F1</td>
<td>280 (250-314)</td>
<td>355 (311-405)</td>
</tr>
<tr>
<td>Mouse MS/Ae</td>
<td>471</td>
<td>-</td>
</tr>
<tr>
<td>Mouse CD-1</td>
<td>289</td>
<td>-</td>
</tr>
<tr>
<td>Hamster Syrian golden</td>
<td>388 (318-473)</td>
<td>460 (400-529)</td>
</tr>
</tbody>
</table>

In the same experiment similar effects were observed in the intestinal mucosa [10]. KBrO₃ administered to rats resulted in increased lipid peroxidation, protein oxidation, hydrogen peroxide levels and decreased the reduced glutathione (GSH) content indicating the induction of oxidative stress in blood. Methemoglobin levels, methemoglobin reductase activity, and nitric oxide concentrations were significantly increased while the total anti-oxidant power and vitamin C concentration were decreased [11].

The chronic effects of KBrO₃ administered via drinking water or by feeding animals with bread treated with the compound were observed. In male Fischer 344 (F344/N) rats exposed to KBrO₃ in drinking water at dose levels of 0, 1.5, 7.9, 16.9, and 37.5 mg/kg-day for up to 100 weeks were found non-neoplastic alterations of treatment-related increases in hyperplasia of the transitional cells of the renal papilla and pelvis, which termed urothelial hyperplasia. This effect at and above 7.9 mg/kg-day KBrO₃ was reported, with the no-observed-adverse-effect level (NOAEL) of 1.5 mg/kg-day. In rats administered only with 37.5 mg/kg-day weights significant increases in the kidney and thyroid weights and elevated relative liver, kidney, thyroid, and spleen weights were seen [12,13].

Male F344 rats were treated with KBrO₃ in drinking water at dose of 0, 0.4, 1.6, 8.1, 16.2, and 32.4 mg/kg-day for 2 or 13 weeks. Increases in kidney weights were observed in rats of at the highest dose (32.4 mg/kg-day) group following 2- and 13-weeks exposure. In renal tubules of rats exposed to 16.2 and 32.4 mg/kg-day of KBrO₃ for 2 weeks and in rats of the 32.4 mg/kg-day group at 13 weeks hyaline droplets were seen. A no observed effect level (NOEL) of 8.1 mg/kg-day was selected on the base of the absence of microscopic alterations in the kidney [14].

Eighteen rats, three dogs, and three monkeys were fed a diet containing 84% flour treated with KBrO₃ at a level of 75 mg/kg for a period of 4, 12 and 8 weeks respectively. No adverse effects were found in any of the species. Twelve rats and two dogs were fed with bread made from flour containing 200 mg/kg KBrO₃ for 16 days, and three dogs given diets containing flour treated with 70 mg/kg KBrO₃ for 6 weeks, and also four dogs were given bread made from flour containing 200 mg/kg KBrO₃ for 17 months showed no adverse effects associated with the intake diets [15].

Mutagenicity and genotoxicity

No data were available on the subject of mutagenicity and genotoxicity in humans. Potassium bromate was found to be weakly mutagenic in Salmonella typhimurium TA100 at a concentration of 3 mg/plate after metabolic activation. However, the compound proved not mutagenic active in S. typhimurium TA98, TA1535, TA1537, TA1538, Escherichia coli WP2try- and E. coli WP2try-his- with or without metabolic activation [16,17]. KBrO₃ was also mutagenic in S. typhimurium TA102 and TA104, strains which are sensitive to chemicals that generate reactive oxygen species (ROS) and active oxygen radicals [18].

Potassium bromate as a strong oxidant induces primary DNA oxidative damage. KBrO₃ increases 8-hydroxydeoxyguanosine (8-OHdG) DNA adduct levels, a representative marker of oxidative DNA modification, in vivo and in vitro [19-21]. Under cell-free conditions, no modification was induced by KBrO₃ alone, but evident DNA damage was seen in the presence of reduced glutathione (GSH). In L1210 mouse leukemia cells and LLC-PK1 porcine kidney cells, KBrO₃ led to a DNA damage profile similar to that observed after treatment of cell-free DNA with bromate and GSH [22]. Speit et al. [23], using the Comet assay in V79 Chinese hamster cells treated with KBrO₃, found that this chemical significantly increased levels of 8-OHdG. They observed four Hprt point mutations and three of them were G:C to T:A transversions which usually arise after replication of 8-OHdG. The induction of DNA breakage was confirmed in the other studies [24,25]. Oxygen radicals can damage bases in DNA to result in double-strand breakage. KBrO₃ induced DNA-strand breakage in V79 cells, human leukocytes and rat kidney epithelial cells [23,26].

Double-strand breaks occurring spontaneously or caused by DNA-demaging agents are repaired in mammalian cells through recombinational repair or end rejoining. Recombinational repair involves filling DNA gaps with homologous sequences existing on homologous chromosomes or sister chromatids, whereas end rejoining joins the ends of the two broken strands in a nonhomologous fashion [27]. Both of these repair mechanisms could lead to loss of heterozygosity (LOH) produced by deletion, recombination or other chromosomal alteration. LOH is considered to be involved with the initiation and promotion of cancer because it is an important mechanism in the inactivation of autosomal genes. Mutations in tumor suppressor genes are generally recessive. A mutation in several alleles of the tumor suppressor gene disturbs growth control of cell and is a crucial in the development of cancer [28].
Potassium bromate is a strong, dose-related mutagen in the Thymidine kinase gene of L5178Y/Tk+/- 3.72C mouse lymphoma cells. This compound induced very high mutant frequencies, reaching about 1400 × 10^-6 at the highest concentration (3 mM) [28]. This is in contrast to the relatively weak mutagenic activity demonstrated in the Hprt mutation assay and the microbial mutation assays. The mutant frequencies induced by KBrO3 in the Hprt gene of V79 Chinese hamster cells reached 4, 7, 30 and 72 × 10^-6 at the concentration 0, 5, 10 and 20 mM, respectively [23]. Thus, the mutant frequency caused by KBrO3 in the Tk gene of mouse lymphoma cells is over 200 times higher than that in the Hprt gene of V79 cells at comparable concentrations. The large difference in these results are probable related to the potent clastogenicity of bromate. Chemicals acting mainly by a clastogenic mechanism induce detectable mutagenicity in the Tk gene but are weakly mutagenic or nonmutagenic in the Hprt gene and in microbial assays [29].

Structural chromosomal aberrations in Chinese hamster lung cells treated with KBrO3 in vitro at doses of 0.0625-0.250 mg/mL, without metabolic activation, and in bone marrow cells of male Long-Evans rats after administration this chemical at doses of 250.5 mg/kg i.p. or 344.0 mg/kg p.o. were observed [16, 17, 30]. The main aberration types observed were chromatid breaks and chromatid exchanges. The clastogenic activity of KBrO3 was considered to be relatively strong [1].

Potassium bromate induced of micronuclei in polychromatic erythrocytes in male ddY mice in a dose-dependent manner when it was administered at doses higher than 25 and 100 mg/kg b.w. by i.p. and p.o. routes, respectively, was found [31]. Similar effects were seen in peripheral blood reticulocytes of CD-1 and ddY mice, and F344 rats [32-34], and also in the cells of glandular part of stomach and liver of rats treated with KBrO3 p.o. at dose of 40-80 mg/kg b.w. for 4, 14, and 28 days [35]. In other study conducted on male mice Ms/Ae and CD-1, administration of KBrO3 at doses of 18.8-150 mg/kg b.w. i.p. or 37.5-300 mg/kg b.w. p.o., respectively, led to an increase in a dose-dependent manner in micronuclei in bone marrow polychromatic erythrocytes. In mice CD-1 observed effect was more pronounced after chemical administration i.p. than p.o. [36].

It has been suggested that oxidative DNA damage can cause mutations that contribute to the activation of oncogenes and/or the inactivation of suppressor genes, thereby leading to tumorigenesis [37].

### Carcinogenicity

No data were available on the subject of carcinogenicity in humans. Potassium bromate is a rodent carcinogen. It is possibly carcinogenic to humans (Group 2B) [1, 38].

Male and female F344 rats developed renal cell tumors and thyroid follicular tumors, and the male rats had also an increased incidence of abdominal mesotheliomas [12, 13, 39, 40]. DeAngelo et al. [12] demonstrated that KBrO3 is carcinogenic in the rat kidney, thyroid, and mesothelium and that it is a renal carcinogen in the male B6C3F1 mouse. KBrO3 was carcinogenic in rats at water concentrations as low as 20 mg/L and dose of 1.5 mg/kg-day [12].

F344 rats of both sexes were given KBrO3 in drinking water at concentrations of 250 and 500 mg/L for 110 weeks. Daily intakes of this compound amounted 12.5 and 27.7 mg/kg-day in males and 12.5 and 25.5 mg/kg-day in females in the high- and low-dose groups, respectively. All dosed groups of rats had statistically significant high incidences of renal cell tumors (RCT), dysplastic foci (DF), which are preneoplastic lesions for RCT, and also adenocarcinomas, and adenomas. Other tumors found in the kidney were two transitional cell papillomas, two transitional cell carcinomas, and one angiosarcoma in treated rats and one liposarcoma in a control rat [40]. The renal cell tumors in rats are morphologically similar to human renal cell cancer of the clear-cell type [13]. In male rats given 250 or 500 mg/L KBrO3 also occurred mesotheliomas of the peritoneum at a significantly higher incidence than in the controls. On the other hand, no mesotheliomas were observed in treated and control female rats [40] (Table 2).

<table>
<thead>
<tr>
<th>Group (mg/L)</th>
<th>KBrO3</th>
<th>Number of rats</th>
<th>Mean induction time ± SD, week</th>
<th>Number of rats (%) bearing DF</th>
<th>RCT</th>
<th>Adeno-carcinomas</th>
<th>Adenomas</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>52</td>
<td>111.0 ± 0.0</td>
<td>6(11)</td>
<td>3(6)</td>
<td>3(6)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>53</td>
<td>103.7 ± 9.1</td>
<td>32(60)**</td>
<td>32(60)**</td>
<td>24(45)**</td>
<td>10(19)*</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>53</td>
<td>88.9 ± 18.9</td>
<td>40(77)**</td>
<td>46(88)**</td>
<td>44(85)**</td>
<td>5(10)*</td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>47</td>
<td>-</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>50</td>
<td>107.6 ± 5.8</td>
<td>13(25)*</td>
<td>28(56)**</td>
<td>21(40)**</td>
<td>8(15)*</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>49</td>
<td>107.9 ± 5.6</td>
<td>9(173)*</td>
<td>39(80)**</td>
<td>36(69)**</td>
<td>9(17)*</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Incidences of renal cell tumors (RCT), dysplastic foci, and other neoplastic changes in F344 rats given KBrO3 in drinking water [40].

*Males and females which survived longer than 14 and 85 weeks, when the earliest RCTs were found, *p ≤ 0.01 when compared with controls, **p ≤ 0.001 when compared with controls.

In the other study F344 male rats were treated with 0, 15, 30, 60, 125, 250 or 500 mg/L KBrO3 in drinking water for 104 weeks. The mean survival time of the animals treated with 500 mg/L (82.8 weeks) was significantly shorter than that of controls (103.1 weeks). In all exposed groups the rats had RCT, but there were statistically significant increases only at doses of ≥125 mg/L. The incidences of
renal adenomas and RTC were significantly elevated in rats receiving concentrations of 125, 250, and 500 mg/L.

Also, significant dose-related increases in the incidences of DF were observed in all groups exposed to concentrations over 30 mg/L. Folicular adenomas and adenocarcinomas of the thyroid were seen in the groups treated with 60, 250, and 500 mg/L. In the rats of the 500 mg/L dose group the combined incidences of benign and malignant follicular alterations were significantly increased. Mesotheliomas of the peritoneum were significantly elevated in animals receiving 500 mg/L (Table 3) [41].

<table>
<thead>
<tr>
<th>Group (mg/L)</th>
<th>KBrO₃</th>
<th>Number of rats (%) bearing/Effective number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Renal adenocarcinomas</td>
</tr>
<tr>
<td>0</td>
<td>0/19</td>
<td>0/19</td>
</tr>
<tr>
<td>15</td>
<td>0/19</td>
<td>0/19</td>
</tr>
<tr>
<td>30</td>
<td>0/20</td>
<td>0/20</td>
</tr>
<tr>
<td>60</td>
<td>0/24</td>
<td>1/24(4)</td>
</tr>
<tr>
<td>125</td>
<td>0/2</td>
<td>5/24(21)*</td>
</tr>
<tr>
<td>250</td>
<td>0/20</td>
<td>5/20(25)*</td>
</tr>
</tbody>
</table>

Table 3: Incidences of renal tumors, thyroid tumors and mesotheliomas in male F344 rats in the dose-response study [41]. *p ≤ 0.05 when compared with control, **p ≤ 0.001 when compared with control.

The carcinogenicity of KBrO₃ was studied also in male B6C3F1 mice and male F344/N rats, which were treated with 0, 9.1, 42.4 and 77.8 mg/kg-day, and 0, 1.5, 7.9, 16.9, and 37.5 mg/kg-day, respectively. KBrO₃ was administered in the drinking water for up to 100 weeks. This study showed that KBrO₃ is carcinogenic in the rat kidney, thyroid, and mesothelium and is a renal carcinogen in the male mouse. There was a treatment, but not a dose-related increase in the incidence of mouse renal tumors after 100 weeks of KBrO₃ intake in the drinking water. A dose-dependent increased incidence of mesotheliomas on the tunica vaginalis testis was seen in exposed groups (Table 4). In many affected rats, mesothelioma was also present on the serosal surface of some or all of the abdominal viscera and the mesentery. In all rats with mesotheliomas on the abdominal viscera, there were also mesotheliomas of the vaginal tunic. Rats treated with KBrO₃ at dose of 37.5 mg/kg-day for up to 100 weeks had an increase in renal adenoma and carcinoma incidences. Most of the tumors occurred in the cortex, with a few extending into the outer medulla. Rats exposed to KBrO₃ at dose of ≥16.9 mg/kg-day had an increased incidence of thyroid follicular proliferative changes, including hyperplasia, adenoma, and carcinoma [12].

<table>
<thead>
<tr>
<th>Dose (mg/kg-day)</th>
<th>Number examined rats</th>
<th>Number (%) of affected rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47</td>
<td>0(0)</td>
</tr>
<tr>
<td>1.5</td>
<td>49</td>
<td>4(8.2)</td>
</tr>
<tr>
<td>7.9</td>
<td>49</td>
<td>5(10.2)*</td>
</tr>
<tr>
<td>16.9</td>
<td>47</td>
<td>10(21.3)**</td>
</tr>
<tr>
<td>37.5</td>
<td>43</td>
<td>27(62.8)***</td>
</tr>
</tbody>
</table>

Table 4: Incidence of mesotheliomas on the tunica vaginalis testis in KBrO₃-treated male F344 rats [12], *p ≤ 0.05 when compared with control, **p ≤ 0.002 when compared with control, ***p ≤ 0.002 (significant trend).

Although the carcinogenicity of KBrO₃ in rats was definitely established by several experimenters, it was thought necessary to test the promoting properties of this chemical. In the experiments N-ethyl-N-hydroxyethylnitrosamine (EHEN) was used as initiator, because this carcinogen is known as a potent initiator useful for assessing the promotion potential of exogenous compounds on kidney and liver neoplasia. In male F344 rats pre-treated with EHEN at concentrations of 500 and 1000 mg/L, drinking water for 2 weeks and then exposed to 500 mg/L KBrO₃ orally for the following 24 weeks were significantly increased incidences of both DF/cm² and RCT/cm² in kidney as compared to animals treated with EHEN alone. Therefore these findings clearly demonstrated enhancing activity of bromate on kidney lesion development [1]. In the other study the mean numbers of DF/cm² were found to be significantly increased in a dose-related manner in rats exposed to >30 mg/L KBrO₃. It was shown that threshold of the promoting effect of KBrO₃ in drinking water exists at range concentrations of 15-30 mg/L. The promoting effect of potassium bromide (KBr) was also tested since KBrO₃ is thermically easily degraded to KBr during the baking process. No promoting effect was observed with KBr [42].

Bromate is thought to induce its toxic and carcinogenic responses through oxidative damage resulting from increased levels of lipid peroxide [43]. Bromate forms oxygen radicals, which are known to damage DNA, as evidenced by increased 8-OHdG levels in response to oxidative stress [21,44]. Bromate must undergo cellular metabolism to cause DNA damage because DNA damage did not occur directly in mixture of DNA and bromate in vitro [1]. The mechanism of bromate-induced carcinogenesis may include lipid peroxidation, which generates oxygen radicals that cause DNA damage [1]. The apparent species differences in the induction of renal cell tumors are correlated with the different levels of lipid peroxidation (LPO) [21]. LPO not only act as intermediates for free radical chain reactions, but also generate...
various reactive products, such as malondialdehyde and trans-4-hydroxy-2-nonenal, which directly form exocyclic DNA adducts [45,46]. A single exposure of male rats to KBrO₃ at high doses by i.g. intubation or by single i.p. injection causes an increase of thiobarbituric acid-reactive substances (TBARS) along with 8-OHdG formation [47,48]. However, exposure to carcinogenic doses in the drinking water failed to increase TBARS, in spite of the increase of 8-OHdG. These results suggest that DNA oxidation induced by KBrO₃ may occur independently of lipid peroxidation and more than 250 mg/L this compound in the drinking water can exert a carcinogenic effect by way of oxidative stress [48]. It has been reported that reduction of KBrO₃ by GSH and cysteine results in the generation of bromine oxides and short lived bromine radicals acting as reactive intermediates which can effectively oxidize guanine [49].

The participation of oxidative stress among possible causative factors in KBrO₃-induced carcinogenesis is of importance from the perspective of human health protection.

In two-stage model carcinogenesis using EHEN as an initiator, KBrO₃ enhanced renal carcinogenesis in both male and female rats [42,50]. Also, short-term exposure to KBrO₃ in males led to significantly an increase in bromodeoxyuridylidine-labeling indices (BrdU) in proximal convoluted tubules (PCTs) in the same dose-dependent manner as evidenced in the promotion assay [51]. Moreover, this compound induced renal ornithine decarboxylase (ODC) activity and led to thymidine incorporation in renal DNA with concomitant renal damage. Both indices are the characteristic changes of proliferative response [52]. As a possible mode of action, it was proposed involvement of α2u-globulin accumulation in KBrO₃-induced cell proliferation in males [48]. The fact that BrdU in PCT of females exposed to KBrO₃ were also increased, albeit at higher doses than in males, implies the existence of other causative factors. On the ground of the results of other study it was concluded that oxidative stress generated by KBrO₃ might take part in induction of cell proliferation in PCT of female rats, leading to tumor promoting potential. In males, however, α2u-globulin accumulation independent of oxidative stress plays a major role in cell proliferation [53].

In genetic study, the gene expression in kidneys from male F344 rats, chronically exposed to KBrO₃ at a non-carcinogenic (20 mg/L) and carcinogenic concentration (400 mg/L), was investigated. At high dose it was found alterations of gene transcripts involved in oxidative stress, lipid metabolism, kidney function/ion transport, and cellular function. The data obtained indicate that carcinogenic dose of KBrO₃ showed marked gene expression differences from the non-carcinogenic dose. Comparison of kidney development gene expression showed that the adenoma patterns are more characteristic of embryonic than adult kidneys. Moreover, the high dose kidney gene expression resemble an adenoma-like expression pattern [54].

The study on a gene expression profiles in the TK6 human lymphoblastoid cells treated with KBrO₃ in vitro revealed up-regulation of some genes involved in oxidative stress, apoptosis, and DNA repair [55]. The gene expression pattern of immortalized rat peritoneal mesothelial cells after 4 or 12 h exposure to KBrO₃ in vitro indicated oxidative stress, mitotic arrest, and apoptosis in treated cells. Increases occurred in oxidative stress responsive genes (among other heme oxygenase-1, quinone reductase, HPSP70, growth arrest and DNA damage protein 45 and 153, and p21); transcriptional regulators c-jun, c-fos, jun B, c-myc, and 1kB; protein repair components; DNA repair components; lipid peroxide excision enzyme phospholipase A₂; and apoptogenic components tumor necrosis factor α, inducible nitric oxide synthase and fas-ligand. Decreases occurred in bcl-2 (antiapoptotic) bax α, bad, and bok (proapoptotic) and cell cycle control elements (cyclines). Cyclin G and p14/cyclin-dependent kinase inhibitor 4b (which inhibit entry into cell cycle) were increased. Numerous signal transduction, cell membrane transport, membrane-associated receptor, and fatty acid biosynthesis and repair components were altered. As a model for KBrO₃-induced carcinogenicity in rat mesothelium has been proposed: KBrO₃ generates a redox signal that activates p53 gene and lead to transcriptional activation of oxidative stress and repair genes, deregulation of growth control, and defective DNA repair and than results in carcinogenic process [56].

puerperal fever, boils, cough, rheumatism and inflammation. The aerial part of the plant contains biological active components, such as carvone, d-limonene, cymene, and a carbonyl compound, nigellone isolated from the volatile oil fraction [61].

Female Wistar rats were pretreated with Nigella sativa extract by gavage once daily for 5 days and then received a single i.p. injection of KBrO₃ at a dose of 125 mg/kg b.w. The effect of prophylactic treatment of rats with Nigella sativa extract resulted in a significant decrease in renal micromosal lipid peroxidation, γ-GT, H₂O₂ and xanthine oxidase activity as compared with animals given KBrO₃ alone. There was significant recovery of renal glutathione level and glutathione metabolizing enzymes and antioxidant enzymes. There was also reversal in the enhancement of renal ODC activity and DNA synthesis. These data suggest that Nigella sativa is a potent chemopreventive agent and may suppress renal oxidative stress, toxicity and tumor promotion response to KBrO₃ in rats [61].

**Tephrosia purpurea** is a polymorphic perennial herb occurring on the Indian subcontinent. It is used in the treatment of several diseases such as fever, chronic diarrhea, asthma, typanitis, heumatism and inflammation. It has hepatoprotective properties, and is used as a purifier of blood and is applied externally on leprosy and skin eruptions [62].

Prophylactic treatment of the rats with *T. purpurea* extract at doses of 5 mg/kg b.w. and 10 mg/kg b.w. prevented N-diethylnitrosamine-initiated and KBrO₃ promoted renal oxidative stress and toxicity. The depleted levels of glutathione, the inhibited activities of antioxidant enzymes, phase II metabolizing enzymes and the enhanced levels of serum creatinine and blood urea nitrogen (BUN) were recovered to a significant level. The susceptibility of renal microsomal membrane for lipid peroxidation and xanthine oxidase activity were significantly reduced. All the antioxidant enzymes were recovered dose-dependently. These data indicate that *T. purpurea* extract can be a potent chemopreventive agent against renal oxidative stress and carcinogenesis induced by N-diethylnitrosamine and bromate [63].

Similar effects were observed in the same experiment on rats pretreatment with soy isoflavones (the mixture of genistin 20 mg, daidzin 5.4 mg, and glycitin 4.6 mg, plus saponins and proteins found naturally in soy) by gavage once daily for 5 days at a dose of 5 mg/kg b.w. or 10 mg/kg b.w. and then administered with a single i.p. injection of KBrO₃ at a dose of 125 mg/kg b.w. Treatment of rats with soy isoflavones resulted in a significant decrease in xanthine oxidase activity as a pro-oxidative enzyme, lipid peroxidation, γ-GT, H₂O₂ generation, blood urea nitrogen, serum creatinine, renal ODC activity and thymidine [3H] incorporation into renal DNA as a tumor promotion markers. The significant recovery of renal glutathione content, anti-oxidant enzymes and phase-II metabolising enzymes were also observed. These results indicate that soy isoflavones act as a potent chemopreventive agent against KBrO₃-induced renal oxidative stress, organ toxicity and subsequent cell proliferation response in female Wistar rats [64].

In the other study it was demonstrated the modulatory effects of kolaviron (200 mg/kg b.w.), a bioflavonoid from Garcinia kola seeds on the antioxidant defense mechanisms, cellular redox status and oxidative stress in the kidney and liver of male Wistar rats pretreated or treated simultaneously with KBrO₃ i.g. in a single dose of 300 mg/kg b.w. Kalaviron administered three times a week for 4 weeks inhibited the decrease of the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) mediated by KBrO₃ in the kidney. Similarly, this bioflavonoid reduced the decrease in the activities of γ-GT and microsomal Ca²⁺ ATPase, and also increase in malondialdehyde (MDA) and lipid hydroperoxide formation induced by KBrO₃ in the kidney. Moreover, kolaviron attenuated the KBrO₃-decreased activities of membrane enzymes, i.e., glucose-6-phosphatase, 5'-nucleotidase and alkaline phosphatase. These data indicate the antioxidant effect of kolaviron, as a natural antioxidant. Kolaviron may therefore neutralize in the cellular redox status and depression of membrane enzyme activities caused by KBrO₃ in the kidney [65].

Also, extract from the red marine algae *Alsidium corallinum* exhibited cytoprotective effects against KBrO₃-induced erythrocyte oxidative damages in mice. *Alsidium corallinum* is widespread in the Mediterranean Sea, Madeira and the Canary Islands. One gram of algae contained 85.7 mg gallic acid (phenolic compound) and 63.4 mg quercetin (flavonoid) and richness of minerals (Ca, Mg, Na, K, Fe and small amounts of Zn, Ni, Cu, and Pb). Adult mice received KBrO₃ in drinking water (500 mg/L) and 7% of algae ethanolic extract via their diet for a period of 15 days. In comparison with animals which received KBrO₃ alone, the mice co-administered with bromate and algae extract showed a significant increase in erythrocyte (RBC), platelet, hemoglobin, and hematocrit values, and SOD and CAT activity, and also GSH and vitamin C levels in RBC. MDA and erythrocyte osmotic fragility values were reduced. In this study, the administration of *A. corallinum* extract had a potent protective effect against oxidative stress in RBC induced by KBrO₃. Moreover, have been shown the protective effects of algae extract against KBrO₃ genotoxicity [66].

Coumarin (1,2-benzopyrone) is a naturally occurring, heterocyclic organic compound. It is an aromatic constituent of many plants, among other *Dipertxy odorata, Asperula odorata, Cinnamonum cassia* and *Lavandum officinalis*. Coumarin is used as a fixative agent for odor in essential oils and perfumes. It is present in various soap, detergents, hair and cosmetic preparations.

The structure of coumarin consists of an aromatic ring fused to a condensate lactone ring. The ring-opened products of these lactones serve as nucleophiles to scavenge the reactive, ultimate, carcinogenic metabolites. The anti-oxidative activity of phenolic compounds such as coumarin depends on the reaction of the phenol towards the chain carrying peroxy radicals and on the stability of the phenoxyl radical formed in the reaction. Vicinal diol in phenolics chelate iron ions and transmitted electrons for the redox cycle of iron ions. The anti-oxidant activity of coumarin and its derivatives is based on the coumarin nucleus. Coumarin derivatives such as 7,8-dihydroxycoumarin and 7,8-diaxetoxy-4-methylocoumarin have strong anti-oxidant and radical-scavenging properties [67].

Female Wistar rats were pretreated with coumarin p.o. once daily for 5 days at a dose of 10 or 20 mg/kg b.w. and then received a single i.p. injection of KBrO₃ at a dose of 125 mg/kg b.w. Treatment of rats with coumarin resulted in a significant decrease in γ-GT and xanthine oxidase activity, lipid peroxidation, H₂O₂ generation, BUN, serum creatinine, renal ODC activity and DNA synthesis in comparison with animals treated with KBrO₃ alone. Renal glutathione level and antioxidant enzyme activities, i.e., CAT, GPx, and glucose-6-phosphate dehydrogenase were also recovered to significant level. These results indicate that coumarin exerts an effective chemopreventive action against KBrO₃-mediated renal oxidative stress, organ toxicity and tumor promotion response in female Wistar rats [68].

Taurine (2-aminoethane sulfonic acid) is the major intracellular-free β-amino acid and is one of the few known naturally occurring...
sulfonic acids. It is present in various foods like eggs, milk and is especially abundant in seafood and meat. Taurine has many essential biological roles such as conjugation of bile acids, osmoregulation, antioxidation, stabilization of membrane and modulation of calcium signaling pathway. This amino acid has been shown to be essential for the development and survival of mammalian cells, especially those of the cerebellum, retina, and kidney [69].

In the study on male Wistar rats treatment with taurine at dose of 100 mg/kg b.w. for 5 days, prior to administration of KBrO3 at single dose of 100 mg/kg b.w., resulted in significant attenuation in nephrotoxicity, renal lipid peroxidation and protein carbonyls, inhibition of renal BBM enzymes, and also DNA damage and DNA-protein cross-linking [70]. Similar effects were observed in the same experiment in rat intestine [71] and peripheral blood [72].

Catechol-type polyphenols are a natural or semi-synthetic products with chemoprotective activity. Oligonol is a novel biotechnology product that emanated from the oligomerization of polyphenols, typically proanthocyanidin from a variety of fruits, such as grapes, apples, and persimmons. Proanthocyanidins are naturally occurring polyphenols widely present in fruits, vegetables, barks, nuts, seeds and flowers. The extracts from the bark of pine (Pinus maritimus) contain flavan-3-ol derivatives and have antioxidant and neuroprotective properties [73]. Grape seed extracts contain monomeric flavanols, e.g. catechin and epicatechin, oligomeric proanthocyanidins and polymeric procyanidin [74]. In the comparative study on the chemoprotective activity of above-mentioned products the order of the antioxidant activity was (-)-epigallocatechin 3-O-gallate (EGCG) > catechin > oligonol > grape seeds extracts (GSE). Oligonol significantly restored blood levels of lipid peroxidation product to the level in untreated with KBrO3 rats and reduced the increased creatinine concentration in the blood. Catechin had the lowest effectiveness in normalization of the BUN and creatinine levels. Oligonol abilities to modulate KBrO3-induced lipid peroxidation and the levels of BUN and creatinine suggest its chemopreventive activity and possibility of application in mitigating toxicity effects due to long-term exposure to KBrO3 [75].

The compounds being an effective antioxidants, among other melatonin (N-acetyl-5-methoxytryptamine), indole-3-propionic acid, and propythiouracil, a thryostatic drug, exerted protective effect against lipid peroxidation in the thyroid induced by KBrO3 in vivo and in vitro [76].

Conclusion

This review presents data on some important findings concerning the toxicity and carcinogenicity of KBrO3, used in both the food and cosmetics industry and being a drinking water disinfection by-product. Bromate is a strong oxidizer, and its toxicological effects are mediated via the induction of oxidative stress. Exposure to KBrO3 leads to multiorgan lesions, mutagenicity, genotoxicity and carcinogenicity. These toxic effects observed in experimental animals were frequently occurred in a dose-related manner. The herbal medicines, derived from plant extracts, have been used to reduce free radical-induced tissue injury. In fact, herbal drugs and other antioxidants have protective effects against the toxic impact of KBrO3 and other environmental contaminants due to their efficiency, the low side effects incidence, and the low cost.

Conflict of Interest

The authors declare no conflict of interest.

References

mutation detects oxidative mutagens. Proc Natl Acad Sci USA 79: 7445-7449.


