Inhibitory effect of bee venom against potassium bromate causing genetic toxicity and biochemical alterations in mice

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Background/aim
Bee venom (BV) therapy is a highly effective treatment, capable of improving one's health. The present study attempts to assess the effect of BV on the toxicity of oral administration of potassium bromate ($KBrO_3$) which has been widely used in food and cosmetic industries.
Materials and methods
Sixty adult male mice were gavaged with $KBrO_3$ at two doses (100 and 200 mg/kg body weight) for 10 days. Afterwards, BV at a dose of 120 μg/kg body weight was injected subcutaneously three times per week for two successive weeks. The genetic study was performed using chromosomal aberration and micronucleus formation in the bone marrow, DNA fragmentation in liver cells and by sperm analysis. In addition, serum biochemical markers such as catalase and malondialdehyde, kidney, and liver functions were assessed.
Results
The results have shown that $KBrO_3$ caused DNA damage that represented the increase in the frequencies of chromosome abnormalities, micronuclei formation, percentage of DNA fragmentation, and sperm morphological abnormalities. Meanwhile, the results showed that $KBrO_3$ exhibited severe toxicity for antioxidant activities for liver and kidney functions. Conversely, BV significantly decreased the frequencies of DNA damage in all aforementioned parameters induced by $KBrO_3$. In addition, it improved the antioxidant activities and the function of the liver and kidneys.
Conclusion
BV has a potent ameliorating effect against the $KBrO_3$ hazard impacts in animal tissues especially at higher doses. This observation indicated that BV could be a potential therapeutic agent in the treatment of $KBrO_3$ risk.

Keywords:
bee venom, chromosome aberrations, DNA fragmentation, liver and kidney functions, mice, micronucleus test, potassium bromate

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Introduction

Potassium bromate ($KBrO_3$) is used in making bread for giving strength and elasticity to the dough during the baking process. It is also used as food additive and in cheese production, beer making, and is commonly added to fish paste products [1]. It is also important in pharmaceutical and cosmetic industries and is a constituent of cold wave hair solutions [2]. In addition, $KBrO_3$ found as a byproduct of ozone disinfection in drinking water samples [3].

$KBrO_3$ has been classified by the International Agency for Research on Cancer as a carcinogenic compound [4,5], where it causes renal tumors [6], mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid in rats [7]. Administration of $KBrO_3$ to rats was found to induce oxidative stress and reduced the antioxidant power of rat blood [8]. $KBrO_3$ is highly injurious to tissues especially the central nervous system tissues [9]. It causes primary DNA oxidative damage and increases 8-hydroxydeoxyguanosine DNA adduct levels, a representative marker of oxidative DNA modification, *in vivo* and *in vitro* [10]. It also caused structural chromosomal aberrations in the bone marrow cells of rats [11]. In addition, $KBrO_3$ caused micronuclei in peripheral blood reticulocytes in mice and rats [12,13] and in the cells of the glandular part of stomach and liver of rats [14].

Bee venom (BV) is a natural toxin produced by the honey bee and it has a prime role of defense for the bee colony and protects bees from external predators by a complex mixture of substances [15,16]. It

contains different peptides including melittin, apamin, adolapamin, and mast cell degranulating peptide [17,18]; the major component is melittin which is about 50% of its dry weight [19]. BV also contains biologically active amines, nonpeptide components as well as enzymes, which is composed of hyaluronidase acid, phosphomonoesterase, α-D-glucosidase, phospholipase A_2, and lysophospholipase [20]. Many recent studies have demonstrated that BV has radioprotective [21], antimutagenic [22], anti-inflammatory [23], antinociceptive [24], and anticancer [18,25] activities on various types of cancerous cells, such as the liver [26], bone [27], prostate, breast, cervical, and renal cancer cells [19,28]. Therefore, in recent years the popularity of the BV as an alternative therapy in the treatment of many diseases has been increased.

The present work aims to assess the effect of BV on genetic toxicity and biochemical alterations induced by $KBrO_3$ in mice. The genetic study will include chromosomal aberration and micronucleus assays in the bone marrow, DNA fragmentation in liver cells, and sperm physical characters. In addition, the biochemical study will include assessment of liver and kidney functions as well as the malondialdehyde (MDA) content and catalase (CAT) enzyme in albino mice.

Materials and methods

Chemicals used

BV obtained from VACSERA Co (Dokki, Cairo, Egypt).

$KBrO_3$ obtained from El-Gomhouria Company (Cairo, Egypt), in the form of a white powder.

Animals

Male Swiss albino mice (*Mus musculus*) 3 months of age, weighing 25–30 g were obtained from the National Research Centre (Cairo, Egypt). These mice were maintained under controlled conditions of temperature (25±2°C) and light (12 light : 12 dark). Mice were provided with standard diet and tap water. They were acclimatized to laboratory conditions for at least 7 days before the experiment.

Ethical consideration

All animals received human care in compliance with the guidelines of Animal Care and Use Committee of the National Research Centre and the National Institute of Health (NIH publication 86-23 revised 1985).

Experimental protocol

This experiment was conducted on 60 Swiss albino mice divided equally into six groups.

(1) Control group (group 1): this group served as a negative control group and received saline solution at a dose of 120 μg/kg body weight.
(2) BV group (group 2): these animals were subcutaneously injected with BV at a dose of (120 μg/kg body weight) three times per week for two successive weeks according to Alvarez-Fischer *et al* [29].
(3) Low-dose $KBrO_3$ group (group 3): these animals were injected with $KBrO_3$ at a dose of 100 mg/kg body weight orally for 10 days.
(4) High-dose $KBrO_3$ group (group 4): these animals were orally gavaged $KBrO_3$ (200 mg/kg body weight) for 10 days. The doses of $KBrO_3$ used in the current study were adjusted according to Kurokawa *et al.* [4].
(5) Low-dose $KBrO_3$+BV group (group 5): animals orally gavaged $KBrO_3$ as in group 3, then BV was injected subcutaneously at a dose of 120 μg/kg body weight three times per week for two successive weeks.
(6) High-dose $KBrO_3$+BV group (group 6): animals received a high dose of $KBrO_3$ orally as in group 4, and then the BV was injected subcutaneously at a dose of 120 μg/kg body weight three times per week for two successive weeks.

At the end of each experiment two portions of blood samples were collected, one in heparinized tubes to obtain plasma and the other portion was allowed in tubes for 30 min to clot and centrifuged at 5000 rpm for 10 min to obtain serum for biochemical analysis. The bone marrow and liver samples were collected for genetic analysis; moreover, sperms of mice were collected from the epididymis, then prepared and analyzed for sperm count and morphology.

Methods

Chromosomal aberration assay

Chromosomal aberration assay was performed as per the method of Schmid [30] and modified by Aron *et al.* [31]. Mice were sacrificed by cervical dislocation and the bone marrow was obtained from both femora. Routine preparations of metaphases were made and stained with Giemsa. Chromosomal aberration was calculated by counting 50 metaphase spreads/animal.

Micronucleus assay

The micronucleus assay was carried out in bone marrow, according to the method of Schmid [30].

A total of 1000 polychromatic erythrocytes were scored per animal for the evaluation of the frequencies of micronucleated polychromatic erythrocytes.

Sperm analysis

Sperms were prepared and analyzed according to the protocols of Wyrobek and Bruce [32].

Sperm morphology

A drop of sperm suspension was smeared onto a slide, left to dry and then stained with eosin A; the slides were washed in water and air dried again. The smears were microscopically analyzed at a magnification of ×1000 for observation of abnormalities [32].

Sperm counts

Sperm counts were performed visually using a hamocytometer. The count was repeated three times for each sample to minimize error [32].

DNA fragmentation assay

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA according to Lu *et al.* [33]. In brief, liver tissues were homogenized, washed overnight at 37°C in PBS, and lysed in 0.5 ml of DNA extraction buffer (50 mM Tris-HCl, 10 mM EDTA. 0.5% Triton, and 100 μg/ml proteinase K, pH 8.0). The lysate was then incubated with 100 μg/ml DNase-free RNase for 2 h at 37°C, followed by three extractions of an equal volume of phenol/chloroform (1 : 1 v/v) and a subsequent re-extraction with chloroform by centrifuging at 15 000 rpm for 5 min at 4°C. The extracted DNA was precipitated in two volume of ice-cold 100% ethanol with 1/10 volume of 3 M sodium acetate, pH 5.2 at −20°C for 1 h, followed by centrifuging at 15 000 rpm for 15 min at 4°C. After washing with 70% ethanol, the DNA pellet was air dried and dissolved in 10 mM Tris-HCl/1 mM EDTA, pH 8.0. The DNA was then electrophoresed on 1.5% agarose gel and stained with ethidium bromide in Tris/acetate/EDTA (TAE) buffer (pH 8.5, 2 mM EDTA, and 40 mM Tris-acetate). A 100-bp DNA ladder (Invitrogen, Waltham, Massachusetts, USA) was included as a molecular size marker and DNA fragments were visualized and photographed by exposing the gels to ultraviolet transillumination.

Biochemical study

The quantitative measurements of MDA and CAT enzyme were done in the plasma according to the method of Ohkama *et al.* [34] and Aebi [35], respectively, using kits of Biodiagnostic Co. (Dokki, Giza, Egypt).

The kidney function was measured in the serum to determine the levels of urea according to Bartles and Bohmer [36] and creatinine according to Fowcelt and Scott [37], using kits of Biodiagnostic Co.. The estimation of the levels of glutamic oxaloacetic transaminase and glutamic pyruvate transaminase activities were done according to IFCC [38], the protein determined according to Gornall *et al.* [39]. In addition, the determination of total bilirubin was performed according to Water and Gerade [40] and alkaline phosphatase according to the Scandinavian Society [41] using kits of Biodiagnostic Co..

Statistical analysis

Statistical analysis was carried out with statistical package for the social sciences software, version 22 (IBM SPSS). Data were analyzed using one-way analysis of variance followed by Duncan's multiple test at a significant level of P value less than or equal to 0.05. The data are represented by mean±SE.

Results

Chromosomal aberrations

The results regarding the protective effects of BV against the $KBrO_3$-induced chromosomal aberrations in male mice are summarized in Table 1. There were many different structural chromosomal aberrations such as gap, break, deletion, centric fusion, centromeric attenuation, and endomitosis in addition to numerical aberrations such as aneuploidy and polyploidy. Our data has shown that there was no significant difference in the frequencies of chromosomal aberrations between BV and the negative control group for most type of aberrations. Low and high doses of $KBrO_3$ showed a significant increase ($P \leq 0.05$) in chromosomal aberrations comparing with vehicle control. In contrast, a significant reduction in chromosomal aberrations was observed in animals treated with BV after $KBrO_3$ and tends to control in most aberrations in low dose. Aberrations in high dose also decreased but did not reach the control group.

Micronucleus test

Figure 1 represents the mouse bone marrow micronucleus test (×1000) with Giemsa stain showing none and micronucleated polychromatic erythrocytes. The result has shown that there was no statistically significant difference in the number of micronuclei between BV and control groups. The number of micronuclei significantly increased ($P \leq 0.05$) by treatment with low and high doses of $KBrO_3$ compared with the control group. In addition,

Table 1 Effect of potassium bromate and bee venom on chromosomal aberrations of mice bone marrow

Treatment	Structural aberrations							Numerical aberrations			Total aberrations
	Gap	Break	Deletion	Centric fusion	Endomitosis	Centromeric attenuation	Total	Aneuploidy	Polyploidy	Total	
Control	1.25 ±0.25^{d}	2.0±0.41^{b}	2.0±0.41^{cd}	0.75±0.25^{cd}	1.0±0.41^{c}	2.50±0.64^{cd}	9.50±1.19^{d}	1.25±0.48^{b}	1.25±0.48^{c}	2.50±0.87^{c}	12.0±1.87^{d}
Bee venom	1.0±0.41^{d}	0.5±0.29^{c}	1.0±0.41^{d}	0.0^{d}	1.50±0.29^{c}	2.0±0.41^{d}	6.0±1.08^{e}	2.0±0.41^{b}	0.50±0.29^{c}	2.50±0.50^{c}	8.50±1.44^{d}
Low-dose potassium bromate	4.0 ±0.40^{bc}	3.0 ±0.41^{ab}	3.75 ±0.25^{ab}	2.0±0.41^{ab}	3.0±0.41^{ab}	4.50±0.29^{ab}	20.25 ±1.10^{b}	4.75±0.25^{a}	3.0±0.41^{b}	7.75 ±0.48^{ab}	28.0±1.58^{b}
High-dose potassium bromate	6.25 ±0.48^{a}	4.0±0.41^{a}	4.25 ±0.48^{a}	2.50±0.29^{a}	3.75±0.25^{a}	5.0±0.41^{a}	25.75 ±0.85^{a}	4.75±0.48^{a}	4.25 ±0.48^{a}	9.0±0.91^{a}	34.75±1.70^{a}
Low-dose potassium bromate+bee venom	3.0±0.40^{c}	2.25 ±0.25^{b}	3.0±0.41^{bc}	1.50±0.29^{bc}	1.75±0.25^{c}	3.50±0.29^{bc}	15.0±0.70^{c}	3.50±0.29^{a}	2.75 ±0.25^{b}	6.25 ±0.48^{b}	21.25±0.95^{c}
High-dose potassium bromated+bee venom	4.75 ±0.48^{b}	2.75 ±0.25^{b}	3.5±0.28^{ab}	1.75±0.25^{ab}	2.75±0.25^{b}	4.25±0.25^{ab}	19.75 ±0.48^{b}	4.25±0.48^{a}	3.25 ±0.25^{ab}	7.50 ±0.64^{ab}	27.25±0.94^{b}

All values are expressed as mean±SE. All values with different letters are significantly different at *P* value less than or equal to 0.05.

Figure 1

Photograph of mouse bone marrow micronucleus test. Arrow (a) represents normal cell and arrow (b) represents micronucleated polychromatic erythrocytes.

there was a marked increase in the number of micronuclei in high dose of $KBrO_3$ than low dose. In contrast, groups treated with $KBrO_3$ followed by BV injection showed a significant decrease ($P \leq 0.05$) in the micronucleus frequency compared with $KBrO_3$-treated groups (Fig. 2).

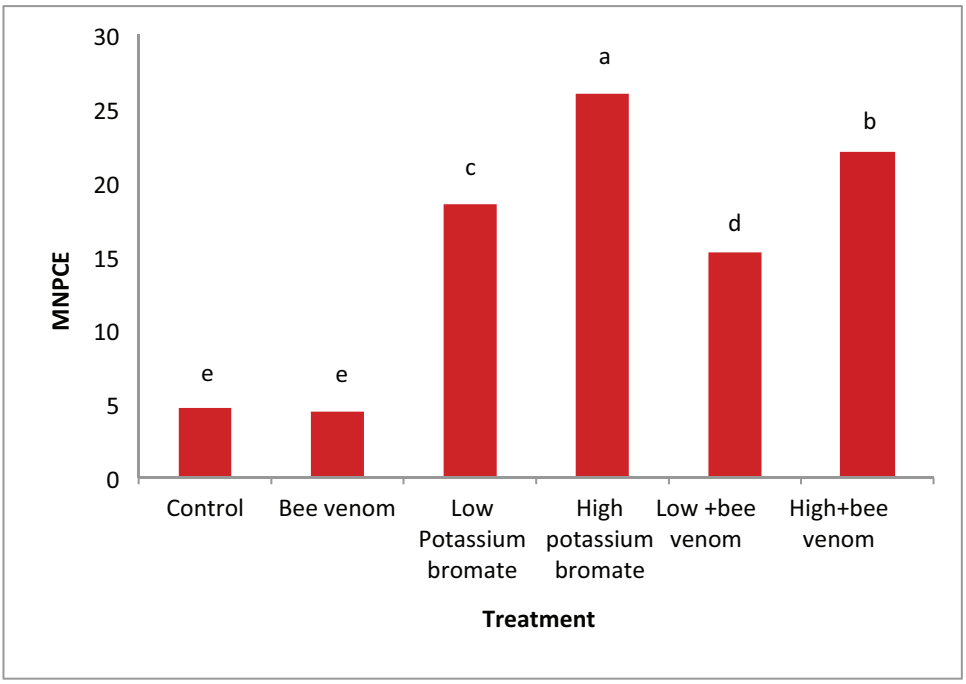
Sperm morphology and count

The results of sperm morphology analysis are presented in Table 2. There are different types of sperm abnormalities included amorphous, without hock, small, big, and banana heads, in addition to coiled tail. There was no significant difference between BV and the control group in all types of abnormalities. Nevertheless, $KBrO_3$ at two doses was significantly increased in most types of abnormalities ($P \leq 0.05$) compared with the vehicle control. It was also observed that treatment with BV after injection with $KBrO_3$ significantly decreased ($P \leq 0.05$) the most common types of abnormalities for both low and high doses comparing with that injected $KBrO_3$ only. Meanwhile, sperm count significantly decreased ($P \leq 0.05$) in $KBrO_3$-treated mice as compared with control. In contrast, mice injected with $KBrO_3$ and supplemented with BV encountered significantly increased percentage of sperm count compared with $KBrO_3$-treated mice (Fig. 3).

DNA fragmentation

Figure 4 represents the DNA fragmentation patterns in the liver of the control and treated groups of mice. The results demonstrated that $KBrO_3$ induced a ladder-like pattern in low and high dose of $KBrO_3$ (lanes 3 and 4) compared with the control and BV groups (lanes 1 and 2), while the group treated with

Figure 2



Effect of potassium bromate and bee venom on the frequencies of micronucleated polychromatic erythrocytes (MNPCE) in male mice. Values with different letters are significantly different at *P* value less than or equal to 0.05.

Table 2 Effect of potassium bromate and bee venom on sperm morphology of mice epididymis

Treatment	Head abnormalities						Tail abnormalities	Total abnormalities
	Amorphous	Without hock	Small	Big	Banana	Total	Coiled	
Control	5.0±0.58^{e}	5.33 ±0.33^{e}	0.67 ±0.33^{e}	0.33 ±0.33^{bc}	0.0^{c}	11.33 ±0.33^{e}	0.67±0.33^{c}	12.0±0.58^{e}
Bee venom	4.67±0.33^{e}	5.0±0.57^{e}	0.33 ±0.33^{e}	0.0^{c}	0.0^{c}	10.0 ±0.85^{e}	0.67±0.66^{c}	10.67±0.33^{e}
Low-dose potassium bromate	18.0±0.58^{c}	15.33 ±0.33^{c}	9.33 ±0.66^{c}	1.0 ±0.58^{abc}	0.67 ±0.33^{bc}	39.67 ±0.33^{c}	3.67±0.33^{b}	43.0±1.53^{c}
High-dose potassium bromate	26.0±0.58^{a}	21.67 ±0.88^{a}	16.67 ±0.88^{a}	1.67 ±0.33^{a}	1.67 ±0.33^{a}	62.0 ±1.15^{a}	8.0±0.58^{a}	65.67±0.33^{a}
Low-dose potassium bromate +bee venom	15.33 ±0.67^{d}	12.0 ±0.58^{d}	6.33 ±0.33^{d}	0.33 ±0.33^{bc}	0.33 ±0.33^{c}	34.33 ±0.67^{d}	3.0±0.58^{b}	37.33±0.88^{d}
High-dose potassium bromated +bee venom	21.33 ±0.33^{b}	17.67 ±0.33^{b}	12.67 ±0.33^{b}	1.33 ±0.33^{ab}	1.33 ±0.33^{ab}	54.33 ±1.20^{b}	4.0±0.58^{b}	58.33±1.20^{b}

All values are expressed as mean±SE. All values with different letters are significantly different at *P* value less than or equal to 0.05.

BV after injection with $KBrO_3$ showed less smearing of DNA close to the control group (lanes 5 and 6).

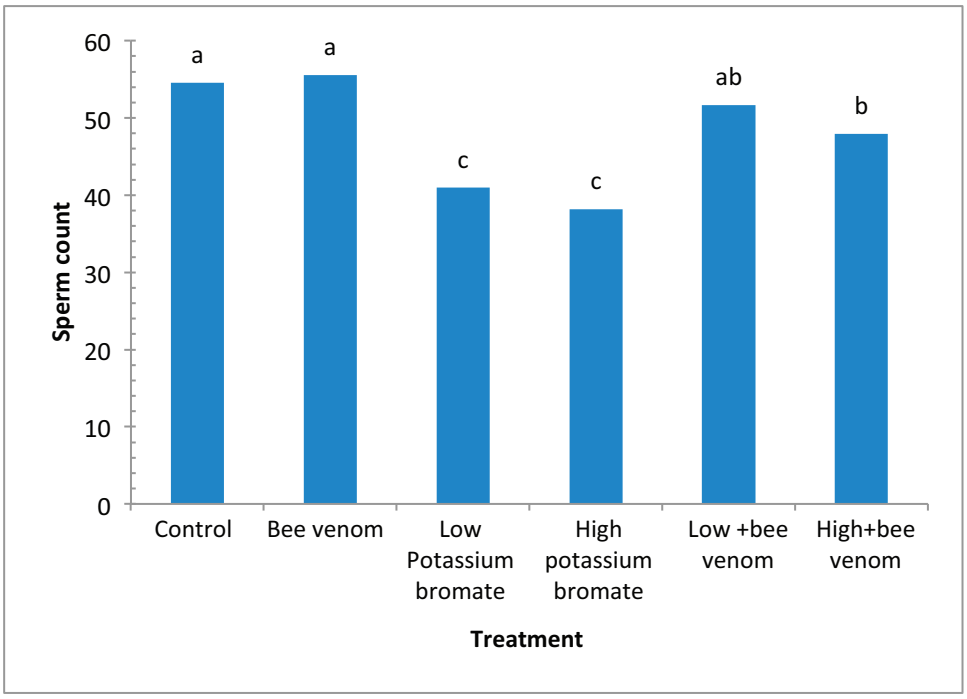
Determination of catalase activity and malondialdehyde

Mice treated with $KBrO_3$ at low and high doses showed significant decrease of CAT activity, while MDA values were significantly increased ($P \leq 0.05$) when compared with the untreated groups (Table 3). In contrast, treatment with BV after $KBrO_3$ induced significant increase in CAT activity. Conversely, the activity of MDA significantly decreased ($P \leq 0.05$) for both low-dose and high-dose groups compared with that injected with $KBrO_3$ only.

Determination of kidney function and protein content

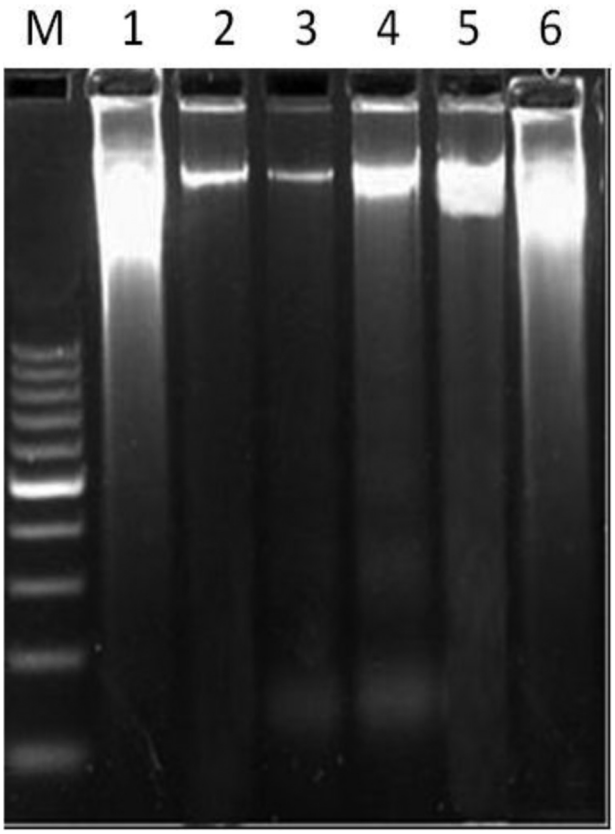
The level of urea and creatinine of $KBrO_3$-treated group (low and high doses) was significantly increased ($P \leq 0.05$) when compared with the control group (Table 4). In contrast, there was significant decrease in protein content as compared with the control. Treatment with BV after $KBrO_3$ injection caused significant decrease of urea and creatinine levels and significant increase of protein

Figure 3



Effect of potassium bromate and bee venom on the sperm count of mice. Values with different letters are significantly different at *P* value less than or equal to 0.05.

Figure 4



Agarose gel electrophoresis of DNA fragmentation. M, marker (100 bp); lane 1, control; lane 2, bee venom; lane 3, potassium bromate ($KBrO_3$) (low dose); lane 4, potassium bromate (high dose); lane 5, bee venom+low-dose $KBrO_3$, lane 6, bee venom+high-dose $KBrO_3$.

Table 3 Effect of potassium bromate and bee venom on plasma malondialdehyde and catalase in mice

Treatment	MDA (nmol/ml)	Catalase (U/l)
Control	7.09±0.09^{c}	795.30 ±3.40^{a}
Bee venom	7.07±0.07^{c}	796.56 ±2.94^{a}
Low-dose potassium bromate	11.98±0.30^{b}	490.78 ±4.57^{d}
High-dose potassium bromate	15.50±0.20^{a}	409.82 ±3.16^{e}
Low-dose potassium bromate+bee venom	7.22±0.13^{c}	676.84 ±4.70^{b}
High-dose potassium bromate+bee venom	7.47±0.11^{c}	639.49 ±4.71^{c}

All values are expressed as mean±SE. MDA, malondialdehyde. All values with different letters are significantly different at *P* value less than or equal to 0.05.

content compared with that of $KBrO_3$-treated animals.

Determination of liver function

Table 5 represents the effect of BV and $KBrO_3$ on liver function. Significant elevation ($P \leq 0.05$) in liver function marker (glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, bilirubin, and alkaline phosphatase) levels was observed in mice treated with low and high doses of $KBrO_3$ compared with the control group. However, mice treated with BV after low and high dose showed a

Table 4 Effect of potassium bromate and bee venom on protein and kidney function tests in serum of mice

Treatment	Kidney function	
	Urea (mg/dl)	Creatinine (mg/dl)
Control	22.71±0.25^{d}	0.58±0.01^{c}
Bee venom	22.68±0.24^{d}	0.59±0.0^{c}
Low-dose potassium bromate	48.11±0.01^{b}	0.99±0.03^{b}
High-dose potassium bromate	59.27±0.41^{a}	1.12±0.03^{a}
Low-dose potassium bromate+bee venom	23.15±0.20^{cd}	0.60±0.0^{c}
High-dose potassium bromate+bee venom	23.87±0.30^{c}	0.62±0.0^{c}

All values are expressed as mean±SE. All values with different letters are significantly different at *P* value less than or equal to 0.05.

Table 5 Effect of potassium bromate and bee venom on liver function tests in serum of mice

Treatment	Liver function				Total protein (g/dl)
	GOT (U/g)	GPT (U/g)	Total bilirubin (mg/dl)	Alkaline phosphatase (U/g)	
Control	50.34±0.23^{e}	54.16±0.95^{d}	0.29±0.01^{d}	151.85±1.05^{c}	7.95±0.17^{a}
Bee venom	51.56±1.53^{de}	52.68±0.46^{d}	0.29±0.13^{d}	151.57±1.47^{c}	7.89±0.14^{a}
Low-dose potassium bromate	64.07±0.38^{b}	74.06±0.39^{b}	0.59±0.01^{b}	414.04±7.70^{b}	5.19±0.11^{c}
High-dose potassium bromate	79.23±0.46^{a}	83.38±0.44^{a}	0.70±0.01^{a}	509.48±5.35^{a}	4.22±0.11^{d}
Low-dose potassium bromate+bee venom	53.38±0.30^{d}	56.16±0.36^{c}	0.30±0.01^{cd}	156.20±0.56^{c}	7.67±0.17^{a}
High-dose potassium bromate+bee venom	55.81±0.25^{c}	56.74±0.20^{c}	0.31±0.01^{c}	157.94±0.63^{c}	7.09±0.09^{b}

All values are expressed as mean±S.E. GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvate transaminase. All values with different letters are significantly different at *P* value less than or equal to 0.05.

significant decrease in liver function marker levels when compared with that injected with $KBrO_3$ only.

Discussion

Previous reports have confirmed that $KBrO_3$ can induce multiple organ toxicity mainly in the kidney and carcinogenic and, mutagenic effects in human and experimental animals [42,43]. Animal and human studies have shown that BV may protect against oxidative stress and it is not genotoxic and do not produce oxidative damage at low concentrations [44].

According to genetic analysis, $KBrO_3$ induced significant increases in frequencies of chromosomal aberrations and DNA fragmentation, which is similar to the results reported by Speit *et al.* [45], Kaya and Topaktaş [46] where they found that $KBrO_3$ increased the number of chromosomal aberrations and DNA strand breaks. Moreover, $KBrO_3$ in drinking water increased the number of aberrant metaphase cells in rat bone marrow [11]. The oxidative stress induced by bromate has been postulated to play a role in its genotoxcicty through the lysosome damage mechanism that precedes oxidative DNA damage [47].

Regarding our results of micronucleus test, there was a marked increase in the number of micronuclei in high doses of $KBrO_3$ than low doses which is in accordance with the study of Awogi *et al.* [12] where it has been reported that bromate increased the incidence of micronuclei, a sign of DNA damage, in the blood of MS/Ae and CD-1 mice strains. Also bromate elevated the frequency of cells with micronuclei in V79 Chinese hamsters [45]. Similarly, the number of micronuclei was significantly increased in F344 rats intraperitoneally injected with bromate [13]. Moreover, we have investigated the effect of $KBrO_3$ on sperm morphology and the results showed that $KBrO_3$ significantly increased the different types of sperm abnormalities. Our results is in coincidence with a previous study by Adil *et al.* [48] who found that the exposure of prepubertal rats to $KBrO_3$ delay their growth, causes testicular hypoplasia, and deteriorate spermatogenesis, which lead to sterility in the future. A significant reduction was detected in the testes weight of rats fed with $KBrO_3$ [49]. Another previous study has shown significant decrease in epididymal sperm density in Sprague–Dawley male rats treated with bromate [50].

The biochemical analysis has shown that due to the oxidative stress exerted by the administration of $KBrO_3$, a significant decrease of CAT activity and significant increase in MDA were recorded which is in line with the previous work of Edwards and Fuller [51]. Other study reported that the mice group treated with bromate showed high levels of MAD and low levels of CAT [52]. Parallel to these, in male rats treated with

bromate, a decrease in the activity of CAT enzyme was recorded [53].

In addition, $KBrO_3$ affected the kidney and liver functions, which are inconsistent with a former study carried out by Omer *et al.* [54], whereas treatment with $KBrO_3$ caused a reduction in the total protein in Wistar albino rats. Mice treated with $KBrO_3$ showed an increase in the urea and creatinine level and a decrease in protein level [55]. Elevated levels of creatinine in the plasma confirmed that $KBrO_3$ intake causes severe kidney damage [56]. The hepatotoxic effect of $KBrO_3$ referred to the reduction in an important antioxidant molecule glutathione, which can be used by many organs, including kidney and liver to resist the induced oxidative stress [57].

In contrast, the result of the present study shows that the intake of BV after $KBrO_3$ recorded inhibitory effects against genotoxicity induced by $KBrO_3$. This result was confirmed by several studies, which recorded the antiapoptotic effect of BV against the apoptosis induced by actinomycin D in normal hepatocyte cells [28]. Treatment with BV combined with bleomycin reduced DNA fragmentation compared with bleomycin alone [58]. Lee *et al.* [59] stated that the potentially reduced genotoxicity of BV in normal cells is due to its high reactivity against cell membrane which results in cell killing without reaching the DNA and exerting genotoxic effects and also it activates the forkhead subfamily transcription factors (FKHR and FKHR1), which have the ability to repair damaged DNA. In addition, a significant increase in hepatic GSH content following the administration of BV could be explained by the increased hepatic nitric oxide which protects cells from oxidative stress [60].

Regarding biochemical study, the results have shown that BV had significantly decreased the elevation of liver biomarkers, representing the hepato-protective effect of BV, which is in agreement with the results recorded by El-Bassiony *et al.* [26] and Merit [61], where they found that the injection of BV at different concentrations decreased the levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. The hepato-protective effect of BV is exerted by inhibiting the secretion of proinflammatory cytokines, and decreasing the elevated serum aminotransferase enzymes injury [28].

Moreover, the present study demonstrated that kidney function markers, including urea and creatinine, were decreased following BV treatment. BV has protective effects on renal tubular injury (epithelial necrosis) [62]. Treatment with BV significantly increased serum protein and albumin levels [26]. Merit [61] recorded that injection with BV at different concentrations reduced the levels of serum parameters (albumin, globulin, and urea) of carcinogenic rats.

In addition, the treatment with BV in this study significantly decreased the level of MDA associated with increased CAT level in liver tissue which is similar to the results recorded by Abdel-Rahman *et al.* [63]. This is attributed to the potent antioxidant property of BV that leads to a decrease in the levels of reactive oxygen species, which appears with the observations of BV affecting superoxide dismutase, glutathione, and CAT [64].

Conclusion

The results of the genetic and biochemical analysis revealed that BV has a potent ameliorating effect against high doses of $KBrO_3$. This observation indicated the BV could be a potential therapeutic agent in the treatment of toxic effects of $KBrO_3$. Further studies regarding the therapeutic effect of BV on $KBrO_3$ toxicity in other animals and other organs are needed.

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Conflicts of interest

There are no conflicts of interest.

References

1 Ahmed MK, Mahmood R. Protective effect of taurine against potassium bromate-induced hemoglobin oxidation, oxidative stress, and impairment of antioxidant defense system in blood. Environ Toxicol 2014; 31:304–313.

2 Oloyede OB, Sunmonu TO. Potassium bromate content of selected bread samples in Ilorin, Central Nigeria and its effect on some enzymes of rat liver and kidney. Food Chem Toxicol 2009; 47:2067–2070.

3 Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003; 189:41–54.

4 Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y. Toxicity and carcinogenicity of potassium bromate – a new renal carcinogen. Environ Health Perspect 1990; 87:309–335.

5 De Angelo AB, George MH, Kilburn SR, Moor TM, Wolf DC. Carcinogenecity of potassium bromate administered in the drinking water to male B6C3F1mice and F344/N rats. Toxicol Pathol 1998; 26:587–594.

6 Ali BH, Al Za'abi M, Karaca T, Al Suleimani Y, Al Baluhi KA, Manoj P, *et al.* Potassium bromate-induced kidney damage in rats and the effect of gum acacia thereon. Am J Transl Res 2018; 10:126–137.

7 Wolf DC, Crosby LM, George MH, Kilburn SR, Moore TM, Miller RT, DeAngelo AB. Time- and dose-dependent development of potassium bromate-induced tumors in male Fischer 344 rats. Toxicol Pathol 1998; 26:724–729.

8 Ahmed MK, Mahmood R. Oral administration of potassium bromate, a major water disinfection byproduct, induces oxidative stress and impairs the antioxidant power of rat blood. Chemosphere 2012; 87:750–756.

9 Ajarem J, Altoom NG, Allam AA, Maodaa SN, Abdel-Maksoud MA, Billy Chow KC. Oral administration of potassium bromate induces neurobehavioral changes, alters cerebral neurotransmitters level and impairs brain tissue of swiss mice. Behav Brain Funct 2016; 12:12–14.

10 Lee YS, Choi JY, Patrk MK, Choi EM, Kasai H, Chung MH. Induction of OH8Gua glycosylase in rat kidneys by potassium bromate (KBrO3), a renal oxidative carcinogen. Mutat Res 1996; 364:227–233.

11 Starek A, Starek-⊠wiechowicz B. Toxicological properties of potassium bromate. J Pharma Reports 2016; 1:1–9.

12 Awogi T, Murata K, Uejima M, Kuwahara T, Asanami S, Shimono K, Morita T. Induction of micronucleated reticulocytes by potassium bromate and potassium chromate in CD-1 male mice. Mutat Res 1992; 278:181–185.

13 Sai K, Hayashi M, Takagi A, Hasegawa R, Sofuni T, Kurokawa Y. Effects of antioxidants on induction of micronuclei in rat peripheral blood reticulocytes by potassium bromate. Mutat Res 1992; 269:113–118.

14 Okada E, Fujiishi Y, Narumi K, Kado S, Wako Y, Kawasako K, Kaneko K, Ohyama W. Evaluation of repeated dose micronucleus assays of the liver and gastrointestinal tract using potassium bromate: a report of the collaborative study by CSGMT/JEMS. MMS. Mutat Res 2015; 780:94–99.

15 An HJ, Kim KH, Lee WR, Kim JY, Lee SJ, Pak SC, *et al.* Anti-fibrotic effect of natural toxin bee venom on animal model of unilateral ureteral obstruction. Toxins 2015; 7:1917–1928.

16 Orsolic N. Bee venom in cancer therapy. Cancer Metastasis Rev 2012; 31:173–194.

17 Moreno M, Giralt E. Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan. Toxins 2015; 7:1126–1150.

18 Saleh R. Bee venom therapy for cancer: a literature review. Int J Sci Eng Res 2017; 8:590–597.

19 Park JH, Kum YS, Lee TI, Kim SJ, Lee WR, Kim BI, *et al.* Melittin attenuates liver injury in thioacetamide-treated mice through modulating inflammation and fibrogenesis. Exp Biol Med (Maywood) 2011; 236:1306–1313.

20 Han SM, Park KK, Nicholls YM, Macfarlane N, Duncan G. Effects of honeybee (Apis mellifera) venom on keratinocyte migration in vitro. Pharm Mag 2013; 9:220–226.

21 Shafiga T, Elmar B. Radioprotective action of venom of honey bee Apis mellifera Caucasica. Int J Environ Agri Biotechnol 2017; 2:2288–2292.

22 Varanda EA, Monti R, Tavares DC. Inhibitory effect of propolis and bee venom on the mutagenicity of some direct- and indirect-acting mutagens. Teratog Carcinog Mutagen 1999; 19:403–413.

23 Park YC, Koh PS, Seo BK, Lee JW, Cho NS, Park HS, *et al.* Long-term effectiveness of bee venom acupuncture and physiotherapy in the treatment of adhesive capsulitis: a one-year follow-up analysis of a previous randomized controlled trial. J Altern Complement Med 2014; 20:919–924.

24 Yoon H, Kim MJ, Yoon I, Li DX, Bae H, Kim SK. Nicotinic acetylcholine receptors mediate the suppressive effect of an injection of diluted bee venom into the GV3 acupoint on oxaliplatin-induced neuropathic cold allodynia in rats. Biol Pharm Bull 2015; 33:710–714.

25 Huh JE, Baek YH, Lee MH, Choi DY, Park DS, Lee V. Bee venom inhibits tumor angiogenesis and metastasis by inhibiting tyrosine phosphorylation of VEGFR-2 in LLC-tumor-bearing mice. Cancer Lett 2010; 292:98–110.

26 El-Bassiony MN, Mahfouz HM, Hussein AS, El-Hamamy MM, Abdel Daim MM, Bufo SA. Effect of honey bee venom on cancer in rats model. J Entomol 2016; 13:72–83.

27 Chu ST, Cheng HH, Huang CJ, Chang HC, Chi CC, Su HH, *et al.* Phospholipase A2-independent Ca2+ entry and subsequent apoptosis induced by melittin in human MG63 osteosarcoma cells. Life Sci 2007; 80:364–369.

28 Park JH, Kim KH, Kim SJ, Lee WR, Lee KG, Park KK. Bee venom protects hepatocytes from tumor necrosis factor-α and actinomycin D. Arch Pharmacal Res 2010; 33:215–223.

29 Alvarez-Fischer D, Noelker C, Vulinovic F, Grunewald A, Chevarin C, Klein C, *et al.* Bee venom and its component apamin as neuroprotective agents in a Parkinson disease mouse model. PLoS ONE 2013; 8:1700.

30 Schmid W. The micronucleus test. Mutat Res 1975; 31:9–15.

31 Aron CS, Sorg R, Zimmer D. The mouse bone marrow micronucleus test: evaluation of 21 drug candidates. Mutat Res 1989; 223:129–140.

32 Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. Proc Nat Acad Sci 1975; 72:4425–4429.

33 Lu T, Xu Y, Mericle MT, Mellgren RL. Participation of the conventional calpains in apoptosis. Biochimica Biophysica Acta 2002; 1590:16–26.

34 Ohkama H, Ohishi W, Yag K. Colorimetric method for research only. Anal Biochem 1979; 95:351.

35 Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121–126.

36 Bartles H, Bohmer M. Colorimetric kinetic method to determine creatinine in serum or plasma. Clin Chem Acta 1972; 37:193–198.

37 Fowcelt JK, Scott JE. A rapid and precise method for the determination of urea. J Clin Pathol 1960; 13:156–159.

38 IFCC. Colorimetric determination of glotamic oxale acetic transaminase (GOT) or glotamic pyruivate transaminase (GPT) activity. J Clin Chem Clin Biochem 1980; 18:521.

39 Gornall AG, Bardawil CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem 1949; 177:751–766.

40 Water M, Gerade H. Coloremitric method for research only. Micro Chem 1970; 15:231–236.

41 Kieding R, Horder M, Gerhardt W, Pitkanen E, Tenhunen R, Stromme J, *et al.* Scandinavian society clinical chemistry and clinical physiology recommended method for determination of four enzymes in blood. Scand J Clin Lab Invest 1974; 93:306.

42 Ahmed MK, Khan AA, Ali SN, Mahmood R. Chemoprotective effect of taurine on potassium bromate-induced DNA damage, DNA-protein cross-linking and oxidative stress in rat intestine. PLoS ONE 2015; 10:1–16.

43 Kujawska M, Ignatowicz E, Ewertowska M, Adamska T, Markowski J, Jodynis-Liebert J. Attenuation of KBr O3-induced renal and hepatic toxicity by cloudy apple juice in rat. Phytother Res 2013; 27:1214–1219.

44 Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (Apis mellifera) against 915-MHz microwave radiation-induced DNA damage in Wistar rat lymphocytes: in vitro study. Int J Toxicol 2009; 28:88–98.

45 Speit G, Haupter S, Schutz P, Kreis P. Comparative evaluation of the genotoxic properties of potassium bromate and potassium superoxide in V79 Chinese hamster cells. Mutat Res 1999; 439:213–221.

46 Kaya FF, Topaktaş M. Genotoxic effects of potassium bromate on human peripheral lymphocytes in vitro. Mutat Res 2007; 626:48–52.

47 Zhang Y, Jiang L, Jiang L, Geng C, Li L, Shao J, Zhong L. Possible involvement of oxidative stress in potassium bromate-induced genotoxicity in human HepG2 cells. Chem Biol Interact 2011; 189:186–191.

48 Adil SE, Thoyba FF, Eiman MA, Ahmed AG. Effects of potassium bromate on male rat growth and testicular histology. Asian Pac J Reprod 2016; 5:376–380.

49 Nwonum CO, Irokanul EO, Iji CE, Alejolow OO, Adetunji CO. Effect of Thaumatococcus daniellii leaf rat-feed on potassium bromate induced testicular toxicity. Asian Pac J Reprod 2016; 5:500–505.

50 Kaiser LB, Wolfe GW, Lanning L, Chapin RE, Klinefelter G, Hunter ES. Short term reproductive and developmental effects of sodium bromate in S-D rats when admi-nistered in the drinking water (Abstract). Toxicologist 1996; 30:121–122.

51 Edwards CJ, Fuller J. Oxidative stress in erythrocytes. Comp Haematol Int 1996; 6:24–31.

52 Ali WSH, Al-Firdous FA. Effect of consumption of kiwi fruit on potassium bromate induced oxidative stress in rats. Aust J Basic Appl Sci 2012; 6:519–524.

53 Wahba HMA, Ibrahim TAA. Protective effect of flaxseed oil and vitamin E on potassium bromate-induced oxidative stress in male rat. Int J Curr Microbiol App Sci 2013; 2:299–309.

54 Omer R, Abuelgasim AI, Elmahdi B. Effect of potassium bromated on liver and blood constituents of Wistar Albino Rat. Am J Food Technol 2008; 3:310–314.

55 Stuti M, D'souza D. Effects of potassium bromate on the kidney and hematological parameters of Swiss albino mice. Bioscan 2013; 8:1011–1014.

56 Altoom NG, Ajarem J, Allam AA, Maodaa SN, Adel-Maksoud MA. Deleterious effects of potassium bromate administration on renal and hepatic tissues of Swiss mice. Saudi J Biol Sci 2018; 25:278–284.

57 Oyewo OO, Onyije FM, Awoniran PO. Hepatotoxic effect of potassium bromate on the liver of wistar rats. J Morphol Sci 2013; 30:107–114.

58 Abd-Elhakim YM, Khalil SR, Awad A, AL-Ayadhi LY. Combined cytogenotoxic effects of bee venom and bleomycin on rat lymphocytes: an in vitro study. BioMed Res Int 2014; 2014:9.

59 Lee YJ, Kang SJ, Kim BM, Kim YJ, Woo HD, Chung HW. Cytotoxicity of honeybee (Apis mellifera) venom in normal human lymphocytes and HL-60 cells. Chem Biol Interact 2007; 169:189–197.

60 Hassanein NMA, Hegab AM. Bee venom – lead acetate toxicity interaction. Aust J Basic Appl Sci 2010; 4:2206–2221.

61 Merit BM. Biochemical studies on some products of honeybee [MD thesis]. Cairo: Cairo University; 2006.

62 Kim H, Lee G, Park S, Chung H, Lee H, Kim J, *et al.* Bee venom mitigates cisplatin-induced nephrotoxicity by regulating CD4(+) CD25(+) Foxp3(+) regulatory T cells in mice. Evid Based Complement Alternat Med 2013; 2013:1–10.

63 Abdel-Rahman MI, Elebiary AS, Hafez SS, Mohammed HE, Abdel Moneim AE. Therapeutic activity of bee-stings therapy in rheumatoid arthritis causes inflammation and oxidative stress in female patients. Int J Res Ayurveda Pharm 2013; 4:316–321.

64 Hegazi AG. Medical importance of bee products. Clin Microbiol 2012; 12: 136–146.