Journal of Applied Life Sciences International
8(1): 1-8, 2016; Article no.JALSI.28698
ISSN: 2394-1103

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Vitamins C and E Alleviate Nephrotoxicity-induced by Potassium Bromate in Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both of the authors designed the study, wrote the protocol, supervised the work, carried out all laboratories work, performed the statistical analysis, managed the analyses of the study, wrote the manuscript and edited it. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/28698
Editor(s):
(1) Palanisamy Arulselvan, Institute of Bioscience, Universiti Putra Malaysia, Malaysia.
(2) Shahira M. Ezzat, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.
Reviewers:
(1) Marwa Monier Mahmoud Refaie, Minia University, Egypt.
(2) Ghada zaghloul Abbass Soliman, Biochem, NNI, Cairo, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/16211

Original Research Article

Received 31st July 2016
Accepted 9th September 2016
Published 16th September 2016

ABSTRACT

Potassium bromate ($KBrO_3$) is widely used in foods and water, in spite of its well-known oxidative cell and tissue damage. Therefore; vitamins C (vit C) and E (vit E) are examined to alleviate its nephrotoxicity. For this purpose 72 adult male albino rats were categorized into 6 groups. Group1 served as control; group 2 served 30 mg/Kg/ day vit C; group 3 served 300 mg/kg/day vit E; group 4 was injected intraperitoneally with $KBrO_3$ 20 mg/Kg/ dose twice weekly; and groups 5 and 6 received either vit C or E with $KBrO_3$ in the same scheme. After 4 weeks, kidney and serum were collected for analysis. $KBrO_3$-induced nephrotoxicity was evidenced by a significant increase in serum urea, creatinine and uric acid levels. Significant elevation in kidney injury molecule-1, interleukin-6, malondialdehyde levels and xanthine oxidase activity and a decrease in superoxide dismutase activity were noticed in $KBrO_3$-intoxicated rats. These changes were ameliorated in the vit C and E-treated groups through their antioxidant and anti-inflammatory properties.

Keywords: Vitamin C; vitamin E; potassium bromate; kidney injury molecule-1; interleukin-6; oxidative stress.

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1. INTRODUCTION

Potassium bromate ($KBrO_3$) is a food additive used primarily as a maturing agent to dough, a conditioner for fish paste and added to beer, cheese or fermented beverages. In addition, $KBrO_3$ is a constituent in cold-wave hair solutions [1]. It is also generated as a disinfectant in drinking water managed with ozone or chlorine [2].

Several reports on the assessment of $KBrO_3$ danger show that it is highly toxic as it causes lipid peroxidation and oxidative DNA damage in human and other mammals, which has induced by BrO_3^- in the presence of intracellular sulfhydryl compound such as reduced glutathione or cysteine [1].

$KBrO_3$ was known to cause renal damage on the basis of elevation in peroxynitrile ($ONOO^-$), reactive oxygen species (ROS), and 8-hydroxyguanosine levels in renal DNA induced oxidative stress [3]. Moreover; it is considered as a carcinogen, which induces chromosome aberration and 8-hydroxyguanosine generation and is capable of initiating renal tumorgenesis [4].

Vitamins as vitamin C (vit C) and E (vit E) are important components in the human diet. They exert protective effect against diseases such as cancer [5,6], cardiovascular diseases [7,8] and fatty liver [9] which may be attributed to its powerful antioxidant properties. As an antioxidant, they protect against the damaging effects of free radicals by scavenging lipid peroxy radicals and singlet oxygen [10]. Moreover; they stabilize the membrane and biological molecules such as DNA, proteins and lipids [11].

Thus, the aim of this work was to ascertain the role of vit C and E as antioxidants to inhibit the toxic effects of $KBrO_3$ on kidney.

2. MATERIALS AND METHODS

2.1 Chemicals

$KBrO_3$ used was imported from Alpha Chemica, India. Vit C was in the form of Cevarol oral drops (100 mg/ml) from UniPharma Company, Cairo, Egypt. While; vit E used from vitamin E soft gelatin capsules (1000 mg/ capsule) of Pharco Pharmaceuticals, Alexandria.

2.2 Animals and Treatment

Seventy two adult male albino rats “Sprague Dawely” 158-198 g were kept in stainless steel cages in the well-ventilated animal house of the Medical Research Center of the Faculty of Medicine, Ain Shams University from acclimatization (7 days) till the end of the experimental period (4 weeks). They had access to 12 h cycle of light/dark and provided with standard diet prepared by the American Institute of Nutrition (AIN) [12] and tap water *ad libitum.*

The animals were divided into the following six groups: Group 1, control; group 2, vit C; group 3, vit E; group 4, $KBrO_3$; group 5, $KBrO_3$+ vit C and group 6, $KBrO_3$+ vit E.

$KBrO_3$ was injected intraperitoneal at a toxic dose of 20 mg/Kg body weight/dose twice weekly prepared as 40 mg/ml distilled water [13]. The vit C was given orally in a high dose of 30 mg/Kg body weight/dose daily [11]. The vit E was given orally in a dose of 200 mg/Kg body weight/dose daily [9]. The animals were weighed weekly and the relative body weights gain were calculated at the end of the period of experiment. The protocol of this study was approved by the research ethical approval committee of the Medical Research Center, Ain Shams University.

2.3 Sample Collection

At the end of the experimental period, animals were sacrificed under ether anesthesia. Blood was collected from the hepatic portal vein, centrifuged (10 min, 3000 rpm, 4℃) for serum separation. Kidneys were excised, washed, dried and weighed for calculating their relative weights.

2.4 Serum Biochemical Assays

Creatinine, urea and uric acid concentrations were determined according to standard methods using diagnostic kits from BioSystems S.A. (Barcelona, Spain) according to Newman and Price [14], Fawcett and Scott [15] and Block and Geib [16], respectively.

2.5 Renal Biochemical Assays

Renal xanthine oxidase (XO) was extracted from tissues by 4 volumes of 0.05 M phosphate pH 7.5, and then centrifuged at 16000 x g for 10 min to get clear XO extract. Then it was used for

calorimetric determination using Abcam kit, UK according to Prajda and Weber [17].

Kidney injury molecule-1 (Kim-1) and Interleukin-6 (IL-6) were extracted by homogenizing the tissues in PBS on ice. The resulting suspension was subjected to two freeze-thaw cycles to further break the cell membrane. After that, the homogenates were centrifuged for 5 min at 5000 x g for extracting the supernatant which is used in the determination using chemiluminescent immunoassay kit, USA according to Ichimura et al. [18] and the enzyme-linked immunosorbent kit, Cloud-Clone Corp., USA according to Matsuda et al. [19], respectively.

Malondialdehyde (MDA) was extracted from tissues by homogenization in cold PBS buffer (with BHT 100 X). Then the homogenates were centrifuged at 13000 x g for 10 min. The resultant supernatants were used for the determination using Biovision kits (USA) Uchiyama and Mihara [20].

Superoxide dismutase (SOD) was extracted from tissue by homogenization in ice-cold 0.1 M Tris/Hcl, pH 7.4 containing 0.5% Triton X-100, 5 mM β-ME, 0.1 mg/ml PMSF. The homogenate was centrifuged at 14000 x g for 5 min at 4^{o}C. The resulted supernatants contain total SOD activity from cytosolic and mitochondria which was determined using Biovision kits (USA) according to Sun et al. [21].

2.6 Statistical Analysis

Results were expressed as mean ± Standard deviation (S.D.) of the mean. Differences among means were tested for statistical significance by one-way analysis of variance using SPSS package version 16. Statistical significance was considered when $P \leq 0.05$.

3. RESULTS

3.1 Effect of Vit C and E Administration on the Relative Body Weight Gain and Relative Kidney Weight in $KBrO_3$-toxicated Rats

The results of the present study showed that rats received vit C only showed significant ($P \leq 0.05$) reduction in relative weight gain as compared to control group. While; rats administered with vit E alone showed no significant change in relative weight gain as compared to control group as shown in (Table 1). On the other hand; administration of $KBrO_3$ caused significant reduction of relative body weight gain compared to control group. Furthermore; groups supplemented with vit C or E along with $KBrO_3$ showed significant ($P \leq 0.05$) increase in relative body weight gain compared to group treated $KBrO_3$ with only. Moreover; there was insignificant change in relative kidney weight between groups treated with vit C or E alone and those treated with $KBrO_3$ as compared to control group. However; rats administered $KBrO_3$ and treated with vit C or E showed significant ($P \leq 0.05$) increase in relative kidney weight comparing with the untreated group.

3.2 Effect of Vit C and E Administration on the Kidney Function Tests in $KBrO_3$-toxicated Rats

Treatment of rats with vit C or E alone did not cause any statistical variation in the level of serum creatinine when compared with the control group (Table 2), while; there was a significant ($P \leq 0.05$) reduction in serum urea and uric acid levels compared to control group. On the other hand, the group treated with $KBrO_3$ alone had a significant ($P \leq 0.05$) elevation of serum creatinine, urea, and uric acid levels compared with control group indicating nephrotoxicity. However; these increased serum levels were decreased significantly ($P \leq 0.05$) by the co-administration of vit C or E compared with the group treated with $KBrO_3$.

3.3 Effect of Vit C and E Administration on the Renal Oxidative Stress in $KBrO_3$-toxicated Rats

Supplementation of vit C or E alone exhibited the antioxidant activity of SOD in kidney tissue by a significant elevation ($P \leq 0.05$) of its activity level compared to control group (Table 3). Vit E alone showed significant reduction of kidney MDA level compared to control. While, vit C alone showed no effect on kidney MDA level compared with control group. In $KBrO_3$ treated group, the activity level of antioxidant enzyme, SOD in kidney tissue showed a significant ($P \leq 0.05$) decrease as compared with control group. While, there was significant ($P \leq 0.05$) elevation in renal MDA level indicating lipid peroxidation caused by $KBrO_3$ administration. Furthermore, the groups administered $KBrO_3$ along with vit C or E had significant ($P \leq 0.05$) increase in renal SOD activity and significant ($P \leq 0.05$) decrease in MDA level as compared with $KBrO_3$ toxicated group.

Table 1. Effect of $KBrO_3$ intoxication and its treatment with vit C or E on relative body weight gain and relative kidney weight in experimental rats (mean ±S.D.)

Parameters / Groups	Relative body weight gain (%)	Relative kidney weight (%)
Control	17.07± 2.77[a]	0.84 ± 0.07[a]
Vit C	12.61± 5.25[c]	0.84 ± 0.05[a]
Vit E	17.09± 3.41[a]	0.82 ± 0.04[a]
$KBrO_3$	7.78 ± 2.6[b]	0.89 ± 0.07[a]
$KBrO_3$ + vit C	14.60± 3.21[c]	0.94 ± 0.04[b]
$KBrO_3$ + vit E	15.91± 2.64[c]	0.93 ± 0.02[b]

There are no significant difference between means have the same letters in the same column

Table 2. Effect of $KBrO_3$ intoxication and its treatment with vit C or E on kidney function tests in experimental rats (mean ± S.D.)

Parameters / Groups	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Serum uric acid (mg/dl)
Control	0.47 ± 0.02[a]	27.50 ± 0.33[a]	3.36 ± 0.04[a]
Vit C	0.44 ± 0.03[a]	23.00 ± 0.91[b]	3.02 ± 0.09[b]
Vit E	0.50 ± 0.03[a]	25.62 ± 0.70[b]	2.30 ± 0.03[b]
$KBrO_3$	1.70 ± 0.06[b]	40.75 ± 0.96[c]	5.42 ± 0.12[c]
$KBrO_3$ + vit C	1.08 ± 0.03[c]	32.25 ± 0.36[d]	3.79 ± 0.02[d]
$KBrO_3$ + vit E	0.79 ± 0.01[c]	32.00 ± 1.06[d]	3.51 ± 0.07[d]

There are no significant difference between means have the same letters in the same column

Table 3. Effect of $KBrO_3$ intoxication and its treatment with vit C or E on renal oxidative stress in experimental rats (mean ± S.D.)

Parameters / Groups	Renal superoxide dismutase (U/100 mg)	Renal malondialdehyde (nmol/mg)
Control	7.75 ± 0.10[a]	5.58 ± 0.06[a]
Vit C	8.87 ± 0.07[b]	5.36 ± 0.06[a]
Vit E	8.90 ± 0.04[b]	5.23 ± 0.12[b]
$KBrO_3$	4.99 ± 0.04[c]	12.08 ± 0.08[c]
$KBrO_3$ + vit C	6.35 ± 0.11[d]	10.66 ± 0.07[d]
$KBrO_3$ + vit E	6.99 ± 0.03[d]	10.03 ± 0.04[d]

There are no significant difference between means have the same letters in the same column

3.4 Effect of vit C and E Administration on the Kidney Injury and Inflammation in $KBrO_3$-toxicated Rats

The results showed that administration of vit C or E alone significantly ($P \leq 0.05$) reduced the levels of Kim-1 and IL-6 and XO activity in kidney tissue compared with control group (Table 4). However; the kidney levels of Kim-1 and IL-6 and activity of XO were significantly ($P \leq 0.05$) elevated in $KBrO_3$ treated group as compared to control group revealing its inflammatory and cellular injury effects. Moreover; vit C and E supplementation alleviated the toxicity induced by bromate that was manifested by the significant ($P \leq 0.05$) reduction in Kim-1 and IL-6 levels and XO activity in kidney tissue.

4. DISCUSSION

The present study revealed that $KBrO_3$ administration caused marked increase in the serum levels of urea, creatinine and uric acid and relative kidney weight and relative body weight gain. These results indicate that the $KBrO_3$ induced acute kidney injury similarly to those previously noted [3,4,22]. Hence; bromate is rapidly absorbed from the gastrointestinal tract, at least in part unchanged, so that its toxicity appears in a few hours after ingestion. The pathophysiology of $KBrO_3$-mediated kidney damage is related to the generated ROS as emphasized by the elevated renal MDA level and decreased SOD activity.

Table 4. Effect of $KBrO_3$ intoxication and its treatment with vit C or E on kidney injury and inflammation in experimental rats (mean ± S.D.)

Parameters / Groups	Kidney injury molecule-1 (Pg/100 mg)	Renal xanthine oxidase (mU/100 mg)	Renal interleukin-6 (Pg/100 mg)
Control	353.89 ±5.87[a]	5.57 ± 0.07[a]	47.71 ± 2.35[a]
Vit C	341.15 ± 4.29[b]	5.20 ± 0.05[b]	41.63 ± 0.81[b]
Vit E	290.76 ± 2.61[b]	4.85 ± 0.03[b]	37.88 ± 1.15[b]
$KBrO_3$	869.01 ± 3.35[c]	9.01 ± 0.08[c]	108.12 ± 2.51[c]
$KBrO_3$ + vit C	809.24 ± 3.56[d]	8.54 ± 0.01[d]	89.39 ± 0.87[d]
$KBrO_3$ + vit E	777.70 ± 4.73[d]	7.63 ± 0.06[d]	82.02 ± 0.47[d]

There are no significant difference between means have the same letters in the same column

MDA, the final metabolite of lipid peroxidation, is utilized not only as an available parameter of oxidative stress but also to translate ROS into active chemicals and to magnify the function of ROS through the chain reaction, including cellular metabolism and functional impairment [23]. Moreover; $ONOO^-$ is contributed to $KBrO_3$-induced oxidative stress [1]. $ONOO^-$ can be formed much faster than the reaction of nitric oxide (NO) with hem compounds, that the rate is comparable to the rate of reaction of superoxide anion with SOD [3]. So that in this study, the reduction in the renal SOD activity in the $KBrO_3$-treated group can be due to the accumulated superoxide anion-induced by ROS generated from it.

Blood uric acid levels are controlled by both the rates of production and excretion and the rate of enzymatic and non-enzymatic degradation. Since, blood uric acid is frequently used for the elimination of excess ROS [24], that the elevated serum uric acid seems to be a response to $KBrO_3$-induced oxidative stress for the enhancement of antioxidant capacity. The increased blood uric acid would result in hyperuricemia and gout, and uric acid is apt to accumulate in kidney tissue causing kidney failure [25].

XO is considered as the indicator of DNA catabolism that it plays a role in purine metabolism. It catalyzes the conversion reactions of hypoxanthine to xanthine and then to uric acid, the last reactions in the purine catabolism, with by-product of toxic superoxide radical. In this regard, it is a key enzyme between purine and free radical metabolism. There is growing evidence that superoxide radicals generated by XO are primarily responsible for the cellular deterioration associated with several conditions [26]. The present study demonstrated high XO activity following $KBrO_3$ treatment which may indicate that there might be possible cell and nucleic acids destruction because of $KBrO_3$ toxicity in renal tissue.

Therefore; it is important to find effective scavenger of active oxygen radicals. In this study, vit C and E are effective in attenuating $KBrO_3$-mediated kidney damage as reflected by reduction in serum urea, creatinine and uric acid also ameliorating the renal oxidative stress and XO activity. As stated previously that each of vit C and E had protective effects against nephrotoxicity induced by several chemicals. Ocak et al. [27] suggested that vit E, as well as vit C could be useful for reducing the detrimental effects on vancomycin-induced toxicity in kidneys by decreasing the blood urea nitrogen and renal MDA and NO levels increased by vancomycin. In addition; Ashrafi et al. [28] demonstrated that vit C reduced the levels of blood urea nitrogen and serum creatinine in rats treated from renal toxicity induced by cisplatin, but the reduction was not significant. Moreover; Kadhodaee et al. [29] demonstrated that antioxidant vit C and E had a role in preservation of renal SOD activities in gentamicin-induced nephrotoxicity.

Kim-1 is sensitive general renal injury or early repair biomarker which was detected in urine and tissue samples in animals exposed to nephrotoxicants. Kim-1 gene product is a type 1 membrane protein with an ectodomain that contains immunoglobulin and highly O-glycosylated mucin subdomains with multiple N-glycosylated sites. The structure of Kim-1 suggests that it may be involved in surface adhesion interaction of the proximal tubule epithelial cells in postischemic kidney [18]. Kim-1 is a phototypical member of a larger family of proteins that may function as an extracellular sensor or a receptor for adhesion/signaling in variety of processes involving cell-cell or cell-pathogen interactions [30]. Moreover; Van

Klinken et al. [31] concluded that Kim-1 might alter cellular adhesion and/or modulate interactions between this injured epithelial cell and the luminal contents which include casts, debris and viable epithelial cells that have become dislodged from the basement membrane. So that; Kim-1 is considered to be an adhesion and/or protective molecule for the cell surface [18].

As a consequence of the renal oxidative stress and nephrotoxicity recorded in this study, the renal IL-6 level is elevated in the rats toxicated with $KBrO_3$. Hence; Kim-1 is an immunoglobulin superfamily cell surface molecule expressed on epithelial cells after injury, that its increased expression on injured epithelial cells transforms the epithelial cells into phagocytes which can clear apoptotic and necrotic cells. Phagocytosis of dead cells mediated by Kim-1 up regulation may influence the inflammatory response in acute kidney injury [32]. Many cytokines are released by leukocytes and renal tubular cells into the injured kidney which are important components of both the initiation and extension of inflammation in acute kidney injury. The proinflammatory cytokines/chemokines IL-6 is increased in the kidney in ischemic acute kidney injury [33].

The present study demonstrated high level of renal Kim-1 and IL-6 in the $KBrO_3$ toxicated rats. This result emphasized that the renal oxidative stress injury induced by $KBrO_3$ is achieved to nephrotoxicity. However; these levels decreased in co-administration of vit C as well as vit E treated rats due to its antioxidant effect and the chelating action of ROS which leads to an improvement in renal injury. As previously reported that vit C protects against oxidative damage [4,11,34]. In addition vit E exerted its protective effect against oxidative stress as reported by John et al. [35], El-Demerdash et al. [36] and Gupta et al. [34]. Jenkins et al. [37] demonstrated that vit E significantly ameliorated renal fibrosis nephrotoxicity including the tubulointerstitial fibrosis score and prostanoids induced by cyclosporine. Also; vit E inhibited the expression of cyclooxygenase-I, hemeoxygenase-I, TGF-b and osteopontin-mRNA. In addition; Kadkhodaee et al. [38] concluded that co-administration of vit C and E had beneficial effects on renal preservation in gentamicin-induced nephrotoxicity by the preservation of glomerular filtration rate and renal glutathione content and the prevention of increasing urinary lactate dehydrogenase, N-acetyle-β-D-glucosaminidase and alkaline phosphatase activities. Moreover; vit C had a beneficial protective effect and repair structural and functional damages to glomeruli and renal tubules by engaging the existent antioxidative potential at the level of renal tissue [39].

5. CONCLUSION

The present study indicates that administration of each of the vit C or E has a protective effect on renal injury induced by $KBrO_3$, which is related to their anti-oxidative and anti-inflammatory properties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16211