



Vitamins C and E as Effective Protectors against Potassium Bromate-induced Cardiac Injury 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.

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ABSTRACT

Potassium bromate (KBrO₃) is widely used in foods and water, in spite of its well-known oxidative cell and tissue damage. Therefore, vitamins C and E are examined to alleviate its cardiac injury. For this purpose 72 adult male albino rats were categorized into 6 groups. Group1 served as control; group 2 served 30 mg/Kg/ day vitamin C; group 3 served 300 mg/kg/day vitamin E; group 4 was injected intraperitoneally with KBrO₃ 20 mg/Kg/ dose twice weekly; and groups 5 and 6 received either vitamins C or E with KBrO₃ in the same scheme. After 4 weeks, heart and serum were collected for analysis. KBrO₃-induced cardiac injury was evidenced by a significant increase in serum aspartate transaminase (AST), creatine phosphokinase isoenzyme (CK-MB) and lactate dehydrogenase (LDH) activities and cardiac troponin I (cTnI) level. Significant reduction in cardiac collagen synthesis and elevation in matrix metalloproteinase-1 (MMP-1) and tumor necrosis factor- α (TNF- α) were noticed in KBrO₃-intoxicated rats. These changes were ameliorated in the vitamins C and E-treated groups through their antioxidant and anti-inflammatory properties.

Keywords: Vitamin C; vitamin E; potassium bromate; cardiac troponin I; collagen; tumor necrosis factor.

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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]. Oral doses of 185-385 mg/Kg body weight results in irreversible toxic effects on body systems. The LD50 of KBrO_3 was reported to be 157 mg/Kg body weight, while lower doses are associated with vomiting, diarrhea, nausea and abdominal pain [3-5].

These reactive oxygen species (ROS) is believed for the cardiotoxicity generation. Different cardiac oxidases especially xanthine oxidase, monoamine oxidase and NADH oxidase produce H_2O_2 , which directly and/or indirectly produce reactive hydroxyl radicals responsible for initiation and progression of myocardial injury. However, the myocardium has enzymatic and non-enzymatic system to neutralize free radicals [6]. But, due to the highly oxidative metabolism of KBrO_3 and fewer reactive cardiac antioxidant defenses, that heart is very sensitive to ROS induced damage [7].

Vitamins as vitamin C and E are important components in the human diet. They exert protective effect against diseases such as cancer [8,9], cardiovascular diseases [10,11] and fatty liver [12] 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 [13]. Moreover, they stabilize the membrane and biological molecules such as DNA, proteins and lipids [14].

Vitamin C is an effective antioxidant, acting to lessen oxidative stress that it acts as a reducing agent, donating electrons to various enzymatic and a few non-enzymatic reactions. The one- and two-electron oxidized forms of vitamin C, semidehydroascorbic acid and dehydroascorbic acid, respectively, can be reduced in the body by

glutathione and NADPH-dependent enzymatic mechanisms. The presence of glutathione in cells and extracellular fluids helps maintain ascorbate in a reduced state. Ascorbic acid performs numerous physiological functions in the human body. These functions include the synthesis of collagen, carnitine, and neurotransmitters; the synthesis and catabolism of tyrosine; and the metabolism of microsome [15].

Vitamin E has many biological functions. As an antioxidant, vitamin E acts as a peroxy radical scavenger, disabling the production of damaging free radicals in tissues, by reacting with them to form a tocopheryl radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state. As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage [16]. Other functions include enzymatic activities [17], gene expression [18], and neurological functions [19].

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

2. MATERIALS AND METHODS

2.1 Chemicals

KBrO_3 used was imported from Alpha Chemica, India. Vitamin C was in the form of Cevalol oral drops from UniPharma Company, Cairo, Egypt. While vitamin E used from vitamin E tablets 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 AIN [20] and tap water *ad libitum*.

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

KBrO₃ was injected intraperitoneal at a toxic dose of 20 mg/Kg body weight/dose twice weekly prepared as 40 mg/ml distilled water [21]. The vitamin C was given orally in a high dose of 30 mg/Kg body weight/dose daily [14]. The vitamin E was given orally in a dose of 200 mg/Kg body weight/dose daily [12]. The animals were weighed weekly and the change in body weight was calculated at the end of the experimental period.

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°C) for serum separation. Hearts were excised, washed, dried and weighed for calculating its relative weight.

2.4 Serum Biochemical Assays

Creatine kinase isoenzyme-MB (CK-MB), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities were determined according to standard methods using diagnostic kits from BioSystems S.A. (Barcelona, Spain) according to Reitman and Frankel [22], Buhl and Jackson [23] and Dawson et al. [24], respectively. Assessment of serum cardiac troponin I (cTnI) level was carried out by enzyme-linked immunosorbent assay using kit purchased from Cloud-Clone Corp., USA according to Apple et al. [25].

2.5 Cardiac Biochemical Assays

Cardiac collagen was extracted from 100 mg tissue by using PBS. The homogenized solution (1 ml) was stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 X g, 2-8°C. The supernatant was removed for assay by the

quantitative sandwich immunoassay technique ELISA kit (Cusabio, USA) according to Neuman and Logan [26].

Cardiac matrix metalloproteinase-1 (MMP-1) and tumor necrosis factor- α (TNF- α) were extracted by rinsing the tissue in 5-10 ml PBS with a glass homogenizer on ice. The resulting suspension was subjected to two freeze-thaw cycles to further break the cell membranes. Then the homogenates were centrifuged for 5 minutes at 5000 X g and the supernatants were removed for assay by enzyme-linked immunosorbent assay kits (Cloud-Clone Corp., USA) according to Zhang et al. [27] and Engelmann et al. [28], respectively.

2.6 Statistical Analysis

Results were expressed as mean \pm 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 Vitamins C and E Administration on the Body Weight Gain and Relative Heart Weight in KBrO₃-toxicated Rats

The results of this study indicate that vitamin C or E administration alone did not affect the body weight gain and relative heart weight of rats as compared to control group (Table 1). KBrO₃ treatment decreased significantly ($P \leq 0.05$) body weight gain with no change in relative heart weight. Furthermore, the vitamins treatments of KBrO₃ showed significant ($P \leq 0.05$) improvement in body weight gain compared to the toxicated group.

Table 1. Effect of KBrO₃ intoxication and its treatment with vitamins C or E on body weight gain and relative heart weight in experimental rats (mean \pm S.D.)

Parameters Groups	Body weight gain (g)	Relative heart weight (%)
Control	36.0 \pm 2.6 ^{ac}	0.41 \pm 0.01 ^a
Vitamin C	27.0 \pm 4.2 ^a	0.40 \pm 0.01 ^{ad}
Vitamin E	37.0 \pm 3.0 ^{ac}	0.42 \pm 0.01 ^{ab}
KBrO ₃	16.0 \pm 2.0 ^b	0.43 \pm 0.02 ^{ac}
KBrO ₃ \pm vitamin C	31.0 \pm 2.8 ^{ad}	0.36 \pm 0.01 ^d
KBrO ₃ \pm vitamin E	35.0 \pm 2.3 ^{ad}	0.39 \pm 0.02 ^{ad}

There are no significant difference between means have the same letters in the same column ($P \leq 0.05$)

3.2 Effect of Vitamins C and E Administration on the Cardiac Enzyme Activities in KBrO₃-Toxicated Rats

Significant alterations in serum cTnI, LDH, CK-MB, and AST activities were seen in rats treated with KBrO₃ as compared to control group (Table 2). Supplementations of vitamins C or E alone did not affect serum cTnI level as compared to control group. While their administration to rats treated with KBrO₃ showed significant ($P \leq 0.05$) serum reduction compared to KBrO₃ treated group indicating tissue improvement.

3.3 Effect of Vitamins C and E Administration on Collagen Synthesis and Inflammation in KBrO₃-Toxicated Rats

Treatment of rats with KBrO₃ led to marked ($P \leq 0.05$) elevation in heart collagen, MMP-1, and TNF- α levels indicating cardiac injury (Table 3). Moreover, vitamins C or E supplementation alone did not have any effect on these parameters which were similar to control. Furthermore, their administration to rats treated with KBrO₃ showed significant ($P \leq 0.05$) decrease of collagen, MMP-1 and TNF- α levels compared to the KBrO₃ treated group.

4. DISCUSSION

Previous studies have reported that oxidative stress plays an important role in the pathophysiology of KBrO₃-mediated damage [7,14,29]. The oxidative stress of KBrO₃ promotes oxidative cell damages and injuries in different tissues and organs through the production of ROS which react with protein, lipids and nucleic acids [30]. As there is a negative balance between oxidative stress damage and antioxidants; the heart tissue is very sensitive to ROS damage induced by KBrO₃ because of its high oxidative metabolism and weak antioxidant defense [14]. However antioxidants are molecules that can prevent or reduce the extent of oxidative destruction of biomolecules [31]. El-Deeb and Abd-El-Hafez [14] concluded that there were morphological changes of mitochondria induced by ROS produced from KBrO₃. These alterations lead to impair the ability of mitochondria to synthesize ATP and to carry out their wide range of metabolic functions. Moreover myofibrillar injury were arisen as a secondary event of the mitochondrial dysfunction, which could lead to an imbalance in calcium uptake and loss of ATP production, resulting in disturbance of normal myofibrillar structure and function causing altered myofilament formation and renewal [32].

Table 2. Effect of KBrO₃ intoxication and its treatment with vitamins C or E on serum cTnI, LDH, CK-MB, and AST activities in experimental rats (mean \pm S.D.)

Parameters	cTnI (Pg/ml)	LDH (U/L)	CK-MB (U/L)	AST (U/ml)
Control	411.76 \pm 6.65 ^a	1818 \pm 15.7 ^a	1135 \pm 3.37 ^a	115.12 \pm 0.29 ^a
Vitamin C	395.3a9 \pm 3.60 ^a	1688 \pm 16.8 ^b	1531 \pm 4.36 ^b	138.00 \pm 1.60 ^b
Vitamin E	356.78 \pm 6.35 ^a	1526 \pm 8.06 ^c	1263 \pm 4.41 ^c	134.00 \pm 0.32 ^c
KBrO ₃	825.04 \pm 8.70 ^b	3490 \pm 11.5 ^d	2298 \pm 5.87 ^d	187.12 \pm 1.48 ^d
KBrO ₃ \pm vitamin C	690.33 \pm 3.03 ^c	3205 \pm 10.4 ^e	1782 \pm 7.51 ^e	168.75 \pm 2.38 ^e
KBrO ₃ \pm vitamin E	648.48 \pm 2.59 ^d	3121 \pm 9.52 ^f	1666 \pm 11.30 ^f	152.12 \pm 0.72 ^f

There are no significant difference between means have the same letters in the same column ($P \leq 0.05$)

Table 3. Effect of KBrO₃ intoxication and its treatment with vitamins C or E on heart collagen, MMP-1, and TNF- α levels in experimental rats (mean \pm S.D.)

Parameters	Collagen (pg/100 mg)	MMP-1 (ng/100 mg)	TNF- α (pg/100 mg)
Control	318.23 \pm 5.25 ^b	0.19 \pm 0.01 ^a	69.85 \pm 1.30 ^a
Vitamin C	346.68 \pm 1.52 ^a	0.17 \pm 0.01 ^a	67.76 \pm 0.59 ^a
Vitamin E	336.06 \pm 5.23 ^a	0.22 \pm 0.01 ^a	72.11 \pm 0.93 ^a
KBrO ₃	843.40 \pm 10.40 ^c	4.90 \pm 0.15 ^c	136.96 \pm 1.98 ^b
KBrO ₃ \pm vitamin C	767.99 \pm 4.66 ^d	3.92 \pm 0.13 ^d	124.84 \pm 0.92 ^c
KBrO ₃ \pm vitamin E	746.22 \pm 1.32 ^e	3.82 \pm 0.12 ^d	111.97 \pm 0.74 ^d

There are no significant difference between means have the same letters in the same column ($P \leq 0.05$)

Four weeks of KBrO_3 intraperitoneal injection induced an elevation in the serum activities of AST, CK-MB and LDH and the level of cTnI due to their leakage from damaged myocytes as a consequence of peroxidation [33], are sensitive indicators of cardiac injury [34]. The measures of AST, CK-MB and LDH activities are important of both early and late phases of cardiac injury especially during clinical follow-up [35]. Serum CK-MB activity is considered as a sensitive indicator at an early stage of myocardial ischemia, while elevated levels of LDH is proportional to the extent of injury to myocardial tissues [36]. Cardiac troponins (cTn) (T, I and C) are established as gold standard blood biomarkers with high sensitivity and specificity for myocardial degeneration in man [37,38]. These contractile proteins are released from myocardium proportional to the degree of tissue injury and myocyte membranes distribution. The cTn bind to the thin myofilament through tropomyosin (TnT) and mediates both calcium activation (TnC) and inhibition (TnI) of thick and thin myofilament sliding to produce contraction [39]. As a consequence of cardiac membrane damage, the functionally early releasable unbound TnI is leaked (approximately 3% of total cTnI) [40] and T pool (cytosolic cTnT fraction of approximately 5%) [41]. This relatively early release of cTnI could be the result of rapid myofibrillar breakdown or may reflect the presence of free cTnI within the sarcoplasm [42].

As a consequence of the ROS generated from KBrO_3 intoxication, that the cardiac structure integrity was greatly affected. This was revealed from the decreased level of cardiac collagen synthesis accompanied with increased activity of MMP-1 and level of $\text{TNF-}\alpha$ in the KBrO_3 toxicated rats. The myocardial structural integrity is determined by the fibrillar collagen which is regulated by balance between synthesis and degradation [43]. ROS effect on collagen metabolism in cardiac fibroblasts is taken place by affecting both synthesis and the activity of degradative enzymes. ROS decreased collagenase-sensitive [^3H] proline incorporation and the abundance of mRNA for procollagenase $\alpha_1(\text{I})$, $\alpha_2(\text{I})$ and $\alpha_1(\text{III})$. On the other hand, ROS increased the total activity of extracellular MMP-1 through the transcriptional and posttranscriptional levels. The posttranscriptional is activated by ROS through the latent proenzymes [44]. Moreover El-Deeb and Abd-El-Hafez [14] concluded that KBrO_3 toxicity induced cardiac extracellular matrix (ECM) expansion and remodeling as a result of

the increased fibroblasts activity which leads to fibrosis. In addition, fibroblasts produce a number of cytokines, peptides and enzymes among which MMP and their inhibitors directly impact of ECM turnover and homeostasis. However these alterations were arrested from the start in the vitamin C treated rats.

The $\text{TNF-}\alpha$ is one of the inflammatory cytokines, that is increased as a result of KBrO_3 induced ROS. Oxidative stress is known to activate p-38 mitogen which activates protein kinase and nuclear factor kappa B and thus plays a role in the sequence of signaling events involved in the production of myocardial $\text{TNF-}\alpha$ [45]. Previously, it was concluded that $\text{TNF-}\alpha$ exerted potent effect on the collagen metabolism resulting in a depletion of fibrillar collagen [46].

Therefore the administration of either of the vitamins C or E parallel to KBrO_3 maintained the regulation between collagen synthesis and degradation and inflammation indices through their antioxidant capacities as previously reported by John et al. [47], Gupta et al. [48], El-Demerdash et al. [49], Bao et al. [50] and El-Deeb and Abd-El-Hafez [14]. That each of vitamins C or E administered to the toxicated rats increased cardiac collagen level and decreased both of the cardiac MMP-1 and $\text{TNF-}\alpha$.

5. CONCLUSION

The present study indicates that administration of each of the vitamins C or E has a protective effect on cardiac 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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