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# Modulating effects of the red alga *Alsidium corallinum* against potassium bromate- induced cardiotoxicity in adult mice

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## Abstract
Acute exposure to xenobiotics can cause cardiotoxicity. In this study, the modulatory effects of *Alsidium corallinum* (*A. corallinum*), a red alga, on the antioxidant defense mechanisms, oxidative stress and cellular redox status in the heart of mice treated with potassium bromate (KBrO3) were examined. Adult mice were divided into four groups: the first group served as control; the second group received in drinking water KBrO3 alone (0.5 g/L); the third group received both KBrO3 and *A. corallinum* ethanolic extract (7% of diet) and group IV received
*A. corallinum* ethanolic extract (7% of diet). The mice exposure to KBrO3 promoted oxidative stress with a rise in lipid peroxidation and advanced oxidation protein product levels, a decrease in antioxidant enzymatic and non-enzymatic levels such as glutathione, in addition to a decline in glutathione peroxidase, superoxide dismutase and catalase activities. The biochemical results were confirmed by the histopathological findings. Co-administration of *A. corallinum* improved the parameters cited above. These results suggested that *A. corallinum* had a protective effect against KBrO3- induced toxicity in adult mice.

**Keywords:** *Alsidium corallinum*, potassium bromate, heart, oxidative stress.

## Introduction
Potassium bromate (KBrO3) is an oxidizing agent, used in industries for the formation of hair solution and cosmetics. Excessive intake or exposure to it brings about the production of oxygen free species in living cells. Besides, KBrO3- contaminated ground water has severely affected the health of the populations of various regions in the world. In fact, it induces oxidative stress and results in carcinogenesis, or acts a as tumour promoter in carcinogen-initiated animals. KBrO3-mediated oxidative stress may lead to an enhancement of kidney cellular proliferation. It has been revealed that a single administration of KBrO3 has the potential to induce 8-hydroxydeoxyguanosine (8-OH-dG), marker of oxidative DNA damage in the kidneys of male rats. Indeed, 8-OH-dG is the major source of mutations and the initiating lesion in KBrO3 tumour induction [1]. Chromosomal aberrations induction and micronucleus formation have been reported both *in vivo* and *in vitro* [2]. Free radicals and reactive oxygen species (ROS) are generated during metabolism, respiration and exposure to KBrO3. ROS, which are products of xenobiotic metabolism, are capable of causing cellular damage leading to tissue injury. However, the biological effects of ROS are controlled by a wide range of antioxidants such as glutathione (GSH), tocopherols, carotenoids and both enzymatic and non-enzymatic antioxidant defence mechanisms.
Obviously, medicinal plants play an important part in the treatment of many degenerative disorders. So as to support an endogenous antioxidant enzymatic system, various antioxidant therapies are resorted to. Indeed, seaweeds have long been used as a foodstuff in Asia, and are considered as an under-exploited resource since they constitute invaluable potential sources of bioactive compounds, minerals, certain vitamins, and polysaccharides [3, 4]. It has now become evident that marine algae constitute a rich source of antioxidants [5-7]. Several studies have indicated that different kinds of marine algae, including the brown, green and red algae, have antioxidant and other biological activities of potential medicinal value [8, 9]. Seaweeds dietary antioxidants are believed to help prevent free-radical mediated diseases [10]. It is, therefore, essential to develop and utilize effective natural antioxidants in order to protect the human body from these radicals and, hence, hinder the development of chronic diseases [11].

The potential antioxidant compounds have recently been identified as some pigments and polyphenols. These compounds are widely found in plants or seaweeds, and are known to exhibit higher antioxidative activities [12].
To the best of our knowledge, there are no available data about KBrO3 induced toxicity in the heart and this work is the first investigation dealing with the protective effect of *A. corallinum* against this induced toxicity in adult mice. Therefore, our study, aimed at investigating the protective effects of the red alga *Alsidium corallinum* against KBrO3-induced oxidative damage in the heart of adult mice.

## Material and methods

### Chemicals and reagents

KBrO3 and all other chemicals required for biochemical assays were obtained from Sigma Chemicals Co. (St. Louis, USA).

### Animals and Treatment

Adult mice bought from the Central Pharmacy were used in the current study. The handling of the animals was approved by the Ethics Tunisian Committee for the Care and Use of Laboratory Animals. After a two- week acclimatization to laboratory conditions, the mice were divided into four groups of eight each: group I was used as negative control; group II received only KBrO3 in drinking water (0.5 g/L); group III received both KBrO3 (0.5 g/L) via their drinking water and 7 % alga ethanolic extract via their diet, and group IV, used as positive control, received via their diet 7 % alga ethanolic extract. Treatment was carried out over a 15- day period at the end of which the animals were killed by cervical decapitation to avoid stress. Hearts were dissected out, cleaned and weighed. Some samples were rinsed and homogenized (10% w/v) in an appropriate buffer (Ph: 7.4) and centrifuged at 7000 g for 20 minutes. The resulting supernatants were used for biochemical assays. Other samples immediately removed were cleaned and fixed in 10% formalin solution for histological studies.

### Protein quantification

Heart protein content was determined according to Lowry *et al*. [13] using bovine serum albumin as standard.

### Lipid peroxidation

Malondialdehyde (MDA) concentration was determined spectrophotometrically according to Draper and Hadley [14]. Its values were calculated using 1, 1, 3, 3- tetraethoxypropane as standard and expressed as nmoles of MDA/g of heart.

*Determination of advanced oxidation protein product (AOPP) levels*

AOPP levels were determined according to the method of Kayali *et al*. [15]. Each sample AOPP concentration was calculated using the extinction coefficient of 261 cm-1 mM-1 and the results were expressed as µmoles/mg of protein.

### Antioxidant enzyme activities determination

- Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich [16]. These activity units were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%, and the activity was expressed as units/mg of protein.
  Glutathione peroxidase (GPx) activity was measured according to Flohe and Gunzler [17], and expressed as nmoles of GSH oxidized/min/mg of protein.
- Catalase (CAT) activity was assayed by the method of Aebi [18]. The enzymatic reaction was initiated by adding a 20- ml aliquot of the homogenized tissue and the substrate (H2O2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of mmol H2O2 consumed/min/mg of protein.

### Heart GSH levels

Heart GSH contents were measured at 412 nm using the method of Ellman *et al*. [19], modified by Jollow *et al*. [20]. Total heart GSH levels were expressed as µg/g of tissue.

### Histopathological studies

Some heart samples, intended for histological examination by light microscopy, were immediately fixed in formalin solution (10%) and processed in a series of graded ethanol solutions, then embedded in paraffin, serially sectioned at 5 mm and stained with hematoxylin–eosin. Six slides were prepared from each heart. All sections were evaluated for the degree of heart injury.

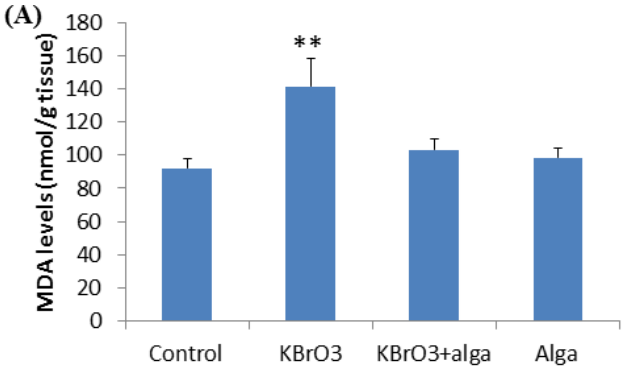
### Statistical analysis

The values of each parameter are expressed as the mean ± standard deviation (x± SD). Duncan's multiple range tests provided mean comparisons with the level of statistical significance set at $P<0.05$. Statistical analyses were performed using SPSS for Windows (Version 17.0).

## Results

### Estimation of lipid peroxidation

Our results revealed a significant increase in heart lipid peroxidation, in KBrO3-treated mice, as evidenced by MDA levels. In fact, in the heart homogenate of KBrO3-treated mice, MDA levels significantly increased by 53% compared to those of control group. Supplementation of *A.corallinum* to the diet of (KBrO3+*A.corallinum*) group decreased MDA contents in the heart when compared to KBrO3 group. In positive controls (*A.corallinum* group), heart MDA contents were not significantly changed, when compared to negative controls (Figure 1).



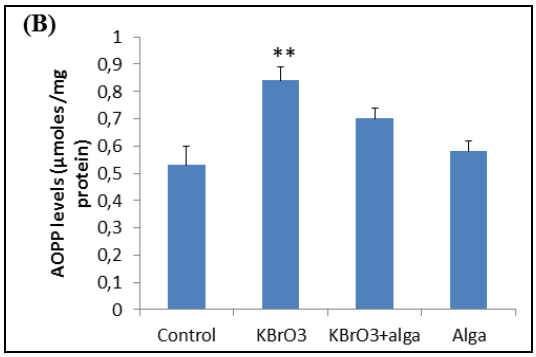



**Fig 1:** Malonaldialdehyde (MDA) and Advanced oxidation protein products (AOPP) levels in the heart of adult mice controls or treated during 15 days with $KBrO_3$, $KBrO_3$ +A. corallinum alone. Values are expressed as means ± S.D for 10 animals in each group. Comparisons are made between treated vs control group; **P < 0.01.

### Protein Oxidative Damage Marker

Figure 2 shows the levels of AOPP indices of protein oxidative damage in the heart tissue of normal and experimental animals. In KBrO3 group, a significant increase of AOPP levels in the heart tissue of adult mice (35%) was observed when compared to controls. Co- treatment of mice with *A. corallinum* resulted in a marked decrease in heart AOPP levels, when compared with KBrO3 group.

### Non-enzymatic antioxidant levels in the heart

GSH level was determined in the heart homogenates. A significant decrease of GSH level by 34% was evident in mice exposed to KBrO3 (Table 1). Supplementation of *A. corallinum* ameliorated the levels of this parameter in (KBrO3+*A.corallinum*) group when compared to KBrO3 group.

### Enzymatic antioxidant status in the heart

Antioxidant enzyme activities of CAT, SOD and GPx of control and treated groups are represented in Table 1. KBrO3 treatment led to a significant decrease in CAT, SOD and GPx by 46%, 43% and 42% in KBrO3 group, respectively, compared to those of control. Treatment with *A. corallinum* restored the levels of enzymatic antioxidants to near normal values when compared with KBrO3-treated mice.

**Table 1:** Enzymatic antioxidant activities (glutathione peroxidase, catalase and superoxide dismutase) and the glutathione levels in the heart of adult mice controls or treated during 15 days with KBrO3, KBrO3+ *A.corallinum* and *A.corallinum* alone.

| Parameters and treatments | GPX | GSH | Catalase | SOD |
|---|---|---|---|---|
| Control | 6.94±1.75 | 357.2±48.14 | 7.92±0.88 | 93.29±6.04 |
| $KBrO_3$ | 3.96±1.62*** | 233.65±33.7*** | 4.21±0.54*** | 52.44±5.87*** |
| $KBrO_3$+alga | 5.15±0.69 | 297.6±24.2 | 4.87±0.34 | 64.33±8.38 |
| Alga | 6.50±0.41 | 321.57±26.6 | 7.19±0.27 | 87.42±6.24 |

SOD: unit/mg protein.
GPx: nmoles of GSH oxidized/min/mg protein. CAT: nmol H2O2/min/mg protein.
GSH: µg/g tissue

### Heart histopathology

Light microscopic examination indicated a normal structure of the heart in the controls (Fig. 2A). Exposure to KBrO3 induced degenerative changes in this organ. KBrO3 intoxication caused significant damages in skeletal cardiac muscle as well as vascular congestion (Fig. 2B). The severe heart damages significantly decreased when *A. corallinum* was added in the diet of KBrO3 treated mice compared with those treated only with KBrO3 (Fig. 2C).

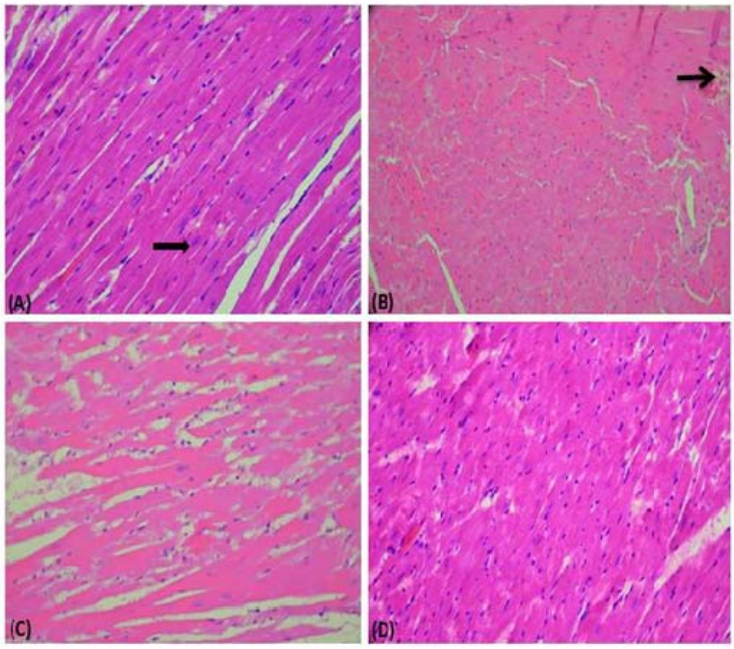



**Fig 2:** Histological findings in heart tissue of adult mice from the four experimental groups: (A) Control group; (B) $KBrO_3$ + A. group; (C) $KBrO_3$ + A. corallinum; (D) A. corallinum.
Hematoxylin-eosin, X 400. Arrows indicate: ➡ normal cardiac muscle fibers: ➡ vascular congestion.

## Discussion

Despite the fact that several antioxidants exhibited a promising capacity in reducing KBrO3-induced toxicity, to date none has been shown to act selectively at the toxicity site: the heart. The major objective of this work was to assess the potential protective effect of *A. corallinum* against KBrO3-induced cardiotoxicity since this has not yet been investigated. In this study, it was observed that a volume of 0.5 g/L of KBrO3 induced cardiotoxicity in mice, evidenced by lipid peroxidation increase shown in the MDA level, compared to the controls. Evidence also suggests formation of oxygen free radicals, which can cause cell damage via lipid peroxidation [21]. Free radicals increased production could also lead to protein-protein cross-linkage formation and protein oxidation, resulting in protein fragmentation and modification of amino acid side chains [22]. In fact, heart homogenates of KBrO3-treated mice showed a significant protein oxidation degree observed through an increase in AOPP level, compared to the controls. According to Pham-Huy *et al*. [23] oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases including cardiovascular disease like atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma. On the other hand, co-treatment with *A. corallinum* was able to lower significantly lipid peroxidation and protein oxidation levels induced by KBrO3.

Therefore, this alga protective effect could be attributed to its antioxidant properties demonstrated by DPPH inhibition and FRAP assay, and by the presence of antioxidants such as phenolic compounds [24]. Our results showed that CAT, SOD and GPx tissue activities and GSH level in heart tissue decreased significantly in KBrO3-treated animals, compared to the controls, proving as a result an impairment of antioxidant status due to KBrO3. However, in *A. corallinum*-treated animals, their activities were restored to normal values, compared to the controls. This could be due to the presence of antioxidants such as polyphenols, flavonoids and β-carotene in *A. corallinum* [25, 26]. As a matter of fact, recent researches have shown that the antioxidants of plant origin with free-radical scavenging properties could have great importance as therapeutic agents in several diseases caused due to oxidative stress [23, 27]. The results of this study indicated that *A. corallinum* can protect the cardiac myocytes against KBrO3-induced oxidative stress damages, and confirm earlier findings showing that the induction of oxidative stress and lipid peroxidation are among the basic mechanisms responsible for cardiotoxicity [25].

Currently there has been an increased interest globally to identify antioxidant compounds from plant sources. Actually, plants produce large amount of antioxidants to prevent the oxidative stress, which they are pharmacologically potent and have low or no side effects for use in protective medicine and the food [28]. Earlier investigations have demonstrated that *A. corallinum* exhibits an antioxidant property in various oxidative conditions causing tissue injury [24, 25]. *A. corallinum* extract has been found to be effective in free radical-induced KBrO3 lipid peroxidation and protein oxiadtion, playing a protective role in the major organs, including the liver and kidney [24, 26]. It has been also well established that keompferol, one of the major compounds of *A. corallinum*, possesses significant antioxidant and radical scavenging properties [29].

So as to substantiate the biochemical findings, a histological examination of the heart was undertaken. Cardiac tissue histopathological examination revealed that KBrO3 treatment caused abnormal ultrastructural changes, such as vacuolization, hemorrhage and apoptosis. In mice treated with *A. corallinum*, these KBrO3-induced histological changes were minimal, suggesting protection from cellular damage by KBrO3. The current study demonstrated that *A. corallinum* could inhibit ultrastructural alterations induced by KBrO3 in the heart. The reversal of KBrO3-induced ultrastructural changes and the restoration of antioxidant status by *A. corallinum* proved that *A.corallinum* could improve the KBrO3-induced myocardial damage. In fact, it was shown that *A. corallinum* preserved the normal myocardial structure. This result corroborates our hypothesis that *A. corallinum* provides protection against KBrO3- induced cardiotoxicity.

## Conclusion

To conclude, our study demonstrates, for the first time, that *A. corallinum* ameliorates KBrO3-induced cardiotoxicity in mice. This effect could be attributed to the antioxidant activities of this alga, and to the presence of antioxidant components such as polyphenols, flavonoids, polysaccharides and β-carotene.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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