THE EFFECT OF SODIUM TETRABORATE ON ANTIOXIDANT ENZYMES UNDER *IN VITRO* CONDITIONS

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ABSTRACT

Herein, the effects of sodium tetraborate on antioxidant enzyme activities from human blood cells under *in vitro* conditions were studied. Red blood cells were exposed to different concentrations of sodium tetraborate. Biochemical parameters, such as catalase (CAT), superoxide dismutase (SOD), glutathione-*S*-transferase (GST), glutathione reductase (GSH), glutathione peroxidase (GSH-Px) and glucose-6-phosphate dehydrogenase (G-6-PDH) were examined to determine oxidative stress. According to our findings, boron compounds at low concentrations were useful in supporting antioxidant enzyme activities in human red blood cells. Sodium tetraborate showed the effects of being neither activator nor inhibitor for all enzymes studied. The results of this study led us to conclude that there were no significant effects on antioxidant enzyme activities of the sodium tetraborate in the studied concentration ranges.

KEYWORDS:
Antioxidant enzymes, sodium tetraborate, boron.

1. INTRODUCTION

Boron is a ubiquitous element widely distributed in nature in the form of borates at low concentrations in soils and rocks, and is released by the natural weathering processes in the form of boric acid, which is water-soluble and biologically available [1, 2]. Boron compounds are very commonly used in a wide range of industrial applications, in a variety of ways. The production of boron compounds has substantially increased recently, as a result of increasing demand for these compounds in nuclear technology; in rocket engines as fuel; and in the production of heat-resistant materials, such as refractories and ceramics, high-quality steel, heat-resistant polymers, catalysts, etc. [3, 4].

It is a trace element playing an important role in hormonal metabolisms, cell membrane functions, and enzyme reactions. Being a dynamic trace element, boron has been observed to exert multiple effects. Although boron seems indispensable for growth and development in mammals, it has also been shown to adversely affect animal and plant systems at higher doses [5]. The biological essentiality of the element in plants is well established since 1920s [1, 6]. However, in animals, evidences have only recently been started accumulating, suggesting the role of boron in diseased conditions [7-9]. In animals and humans, boron has been shown to have important roles in calcium and magnesium metabolism and macro-mineral functions. Some effects have been seen in the normal activity of brain function and cognitive performance [5, 7]. Current knowledge about the toxic level of boron in humans needs to be improved. The limited data on this topic has only been obtained from human poisoning cases and toxicity studies on animals. Nielsen *et al.* (1987) [10] has shown that the metabolism of steroid hormones can be influenced by boron. In this study, low doses of boron resulted in an increase in the plasma concentration of both oestrogen and testosterone, as well as an increase in calcium retention in postmenopausal women [10].

So far, antioxidants have attracted much interest with respect to their protective effect against damage by free radicals that may be the cause for many diseases including cancer [11, 12]. Oxidative stress can lead to some cancers, artherosclerosis, and adverse effects of aging [12, 13]. However, the compounds with antioxidant properties contribute to protection of cells and tissues [12, 14]. Oxidative challenge is alleviated by antioxidant compounds, but more importantly, by induction of antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), glutathione-*S*-transferase (GST), glutathione reductase (GSH), glutathione peroxidase (GSH-Px), and glucose-6-phosphate dehydrogenase (G-6-PDH) [15, 16]. These enzymes form a defense complex against reactive oxygen species (ROS).

Undoubtedly, the knowledge of the oxidative changes caused by boron compounds is critical to the effective enzyme researches. In this context, the blood cells are potential vulnerable cells. So, oxidative studies of blood are pivotal, because such studies will serve to evaluate

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and improve the effects on enzyme activities of boron compounds. With this background in mind, *in vitro* human studies are also most important. Based on the above data, the actions on blood antioxidant defenses of sodium tetraborate against ROS are not identified yet. It is reported that oxidative stress caused by ROS damages DNA [17]. Therefore, the objective of this work was to study the effect of sodium tetraborate on antioxidant enzyme systems including catalase (CAT), superoxide dismutase (SOD), glutathione-*S*-transferase (GST), glutathione reductase (GSH), glutathione peroxidase (GSH-Px), and glucose-6-phosphate dehydrogenase (G-6-PDH) in the red blood cells of normal humans compared to those of workers exposed to boron dusts during mining and manufacturing processes in *in vitro* systems. The basic design of the study was to compare antioxidant enzyme activity between control and experimental groups. As a result, the effects of sodium tetraborate on antioxidant enzyme systems are reported between workers exposed to boron dusts during mining and manufacturing processes, and humans living in far regions from boron mining.

2. MATERIALS AND METHODS

2.1 Materials

Sodium tetraborate ($Na_2B_4O_7.10H_2O$) was purchased from Eti Mine Works General Management (Bandirma, Turkey). All the other chemicals and reagents were of analytical reagent grade and purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). All the heparinized blood samples were taken from 50 healthy people, not exposed to any toxic agents and who did not smoke or drink, and from 50 workers exposed to boron dusts during mining and manufacturing. Their ages were in the rage of 25 to 35 years.

2.2 Enzyme activities

The blood samples were centrifuged at 2500×*g* for 15 min at 4 °C, and the plasma and buffer coat were removed. After the packed red cells were washed three times with potassium chloride (0.16 M KCl), the erythrocytes were hemolyzed with chilled water. The red blood cells were removed by centrifugation at 4 °C and 10000×*g* for 30 min. The blood samples were exposed to different concentrations of sodium tetraborate under *in vitro* conditions [18].

2.2.1 Catalase (CAT) activity

Catalase activity was determined by the method of Karabulut (2002) [19] and Çuvaç (2007) [20], assaying the hydrolyzation of H_2O_2 and decreasing absorbance at 240 nm over 5 min at 25 °C. In this method, into a 3-ml quartz cuvette, 2400 µL 0.05M phosphate buffer (pH 7.0), 500 µl 0.019 M H_2O_2 and 100 µL erythrocyte solution were added, and then, CAT activity was measured spectrophotometrically. CAT activity was expressed as mM of H_2O_2 reduced per min, using an extinction coefficient of ε_{240}= 0.0394 mM^{-1} cm^{-1} [19, 20].

2.3.2 Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by the method of Habdous (2003) [21]. It employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenolo)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. Using 1.10^{-4} M xanthine, 1.10^{-4} M INT, 0.05M phosphate buffer (pH 7.0) and xanthine oxidase, SOD activity was measured by monitoring the increase in the absorbance at 505 nm for 5 min, using an extinction coefficient of ε_{240}= 0.0394 mM^{-1} cm^{-1} [21].

2.2.3 Glutathione-*S*-transferase (GST) activity

Glutathione-*S*-transferase activity was determined by the method of Habig et al. (1974) [22] using 0.1 M glutathione (GSH), 30 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 0.05 M potassium phosphate buffer (pH 7). The method for the estimation of GST activity was based on the formation of 1-chloro-2,4-dinitrobenzene (CDNB)-conjugate in a reduced glutathione coupled reaction, in a reaction mixture consisting of the phosphate buffer, reduced glutathione and the enzyme source. The reaction was initiated by adding CDNB, and the rate of increase of product concentration was monitored by measuring the absorbance at 340 nm for 5 min at 37 °C in a spectrophotometer. The activity of GST was expressed as mM GSH-CDNB conjugate formed per min, using an extinction coefficient of 9.6 mM^{-1} cm^{-1} [22].

2.2.4 Glutathione reductase (GSH) activity

GSH activity was determined by the method of Beutler (1984) [23]. The assay system contained of 1 M Tris-EDTA buffer, pH 8.0, including 5 mM ethylenediaminetetraacetic acid (EDTA), 0.033 M GSSG and 2 mM NADPH. The activity was measured by monitoring the decrease in absorbance at 340 nm due to the oxidation of NADPH at 37 °C for 5 min. One enzyme unit is defined as the oxidation of one µmol NADPH per min under the assay conditions. The enzyme activity was calculated using a molar extinction coefficient of 6.22 M^{-1} cm^{-1} for NADPH at 340 nm [23].

2.2.5 Glutathione peroxidase (GSH-Px) activity

Glutathione peroxidase activity was also measured according to Beutler method (1984) [23]. GSH-Px catalyses the oxidation of glutathione in the presence of tert-butyl hydroperoxide. Oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidized to NADP+. The assay system contained of 1 M Tris-EDTA buffer, pH 8.0, including 5 mM EDTA, 7 mM tert-butyl hydroperoxide, 0.1 M GSH, 0.01 M glutathione reductase, and 2 mM NADPH. The reduction in the absorbance of NADPH at 340 nm was measured. By measuring the absorbance change per min and by using the molar extinction coeffi-

cient (6.22 M^{-1} cm^{-1}) of NADPH, GSH-Px activity was calculated [23].

2.2.6 Glucose-6-phosphate dehydrogenase (G-6-PDH) activity

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity was measured spectrophotometrically at 37 °C [23]. Briefly, the enzyme sample was added after incubation of a mixture containing 1 M Tris-EDTA buffer (pH 8.0), 0.1M $MgCl_2$, 0.2 mM NADP+ and 6 mM glucose-6-phosphate. The activity was measured by monitoring the increase in absorption at 340 nm due to the reduction of NADP+ at 37 °C for 2 min. The enzyme activity was determined spectrophotometrically using a molar extinction coefficient value of 6.22 M^{-1} cm^{-1} [23].

2.3 Statistical analysis

Statistical analyses were performed using SPSS software (IBM SPSS Statistics 19). The data obtained from the experiments were expressed as mean and standard error values. The statistical differences among the control and experimental groups were evaluated by one-way ANOVA and Duncan post-hoc tests. A difference of $p < 0.05$ in the mean values was considered to be significant.

3. RESULTS AND DISCUSSION

Oxidative stress develops when the levels of antioxidants are lowered. Thus, the activities of antioxidant enzymes are important in cell defense. The antioxidant enzymes can be induced or inhibited in the blood cells exposed to different toxicants, and these enzymes play a main role in the defense of mammalian blood [24]. In the present study, the toxic effects of increasing concentrations of sodium tetraborate on antioxidant enzyme levels were investigated. Antioxidant enzymes like CAT, SOD, GSH-Px, GSH, GST and G-6-PDH form the first line of defense against ROS.

Figures 1-6 reveal the activities of antioxidant enzymes in human blood treated with sodium tetraborate under *in vitro* conditions. The activities of erythrocyte CAT, SOD, GSH-Px, GSH, GST and G-6-PDH were not changed under increasing sodium tetraborate concentration when compared to controls. Moreover, the activities of CAT ($p>0.05$), SOD ($p>0.05$), GST ($p>0.05$), GR ($p>0.05$), GSH-Px ($p>0.05$) and G-6-PD ($p>0.05$) do not show statistically significant modifications for any concentration (2, 4, 8, 17 and 33 ppm) of sodium tetraborate.

3.1 Catalase

Catalase disproportionates hydrogen peroxide, and protects membrane lipids and proteins from attack by peroxy radicals. As seen from Fig. 1, sodium tetraborate did not alter erythrocyte CAT activitiy. It was reported that boron compounds (boric acid, borax, colemanite and ulexite) have induced CAT activity [25]. Pawa and Ali (2006) [1] emphasized that liver CAT activity was decreased with borax treatment according to control group. On the contrary, liver CAT activity was increased by borax treatment after fulminant hepatic failure [1]. Zafar and Ali (2013) [26] determined that liver CAT activity remained unchanged in control and borax groups, but CAT activity was decreased in hepatocellular carcinoma.

3.2 Superoxide dismutase

Superoxide dismutase enzyme causes the formation of H_2O_2 while eliminating superoxide anions that are produced by environmental stresses [27, 28]. Endogenous H_2O_2 is converted to H_2O by catalase [28]. Figure 2 shows the effect of sodium tetraborate on SOD activity under *in vitro* conditons. As seen from Fig. 2, there is not a significant change in SOD activity. Similarly to our study, B supplementation (boric acid and borax) did not change SOD and CAT activities in the heart and liver [29]. However, this result is inconsistent with the findings reported by Turkez *et al.* (2007) [25] that, at low doses, B compounds increased the antioxidant enzyme activities of

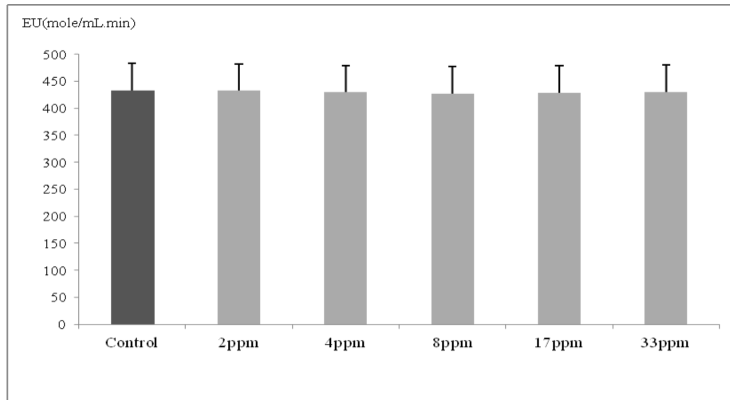

FIGURE 1 - The effect of sodium tetraborate on CAT activity.

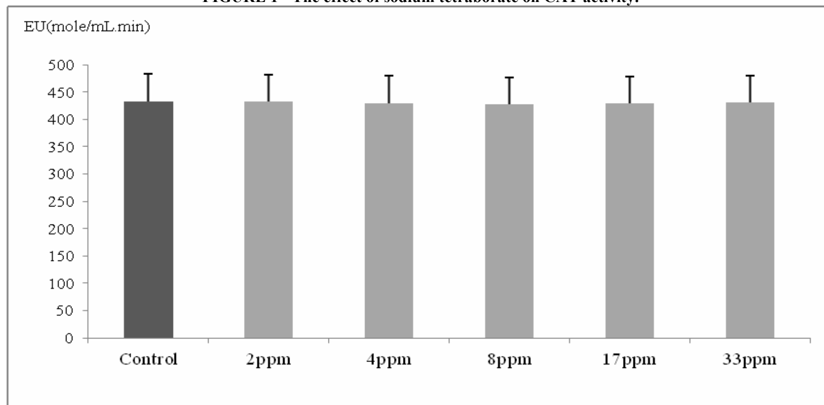

FIGURE 2 - The effect of sodium tetraborate on SOD activity.

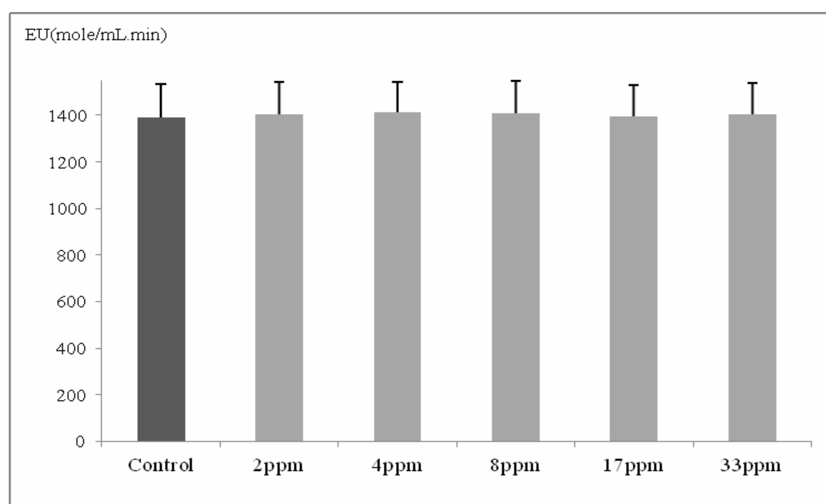

FIGURE 3 - The effect of sodium tetraborate on GST activity.

erythrocytes. In Turkez's study, not only boric acid but also borax (sodium tetraborate decahydrate), ulexite and colemanite have caused significant increases in SOD activity [25]. Also, Nielsen (1989) [30] reported that B supplementation increased erthrocyte SOD activity in men and postmenopausal women. Zafar and Ali [26] studied the effect of borax on liver antioxidant enzymes. In this study, Zafar and Ali have given thioacetamide (TAA) to rats in order to cause hepatocellular carcinoma (HCC). This study showed that, after HCC, the rat liver SOD activity, after treatment with borax during 122 days, was increased in comparison with the HCC group. In spite of that, SOD activity of borax group and the control groups was not changing [26].

3.3 Glutathione S-transferase (GST)

GST plays a vital role in prevention of oxidative damage by conjugating reactive species, and by detoxifying lipid peroxidation products [31]. From Fig. 3, the current study well establishes that sodium tetraborate does not lead to the induction of oxidative stress. Herein, GST activity was not changed by sodium tetraborate under *in vitro* conditions. Similarly, Pawa and Ali [1] reported that there was no significant difference in GST activity between control and borax groups. However, GST activity increased in rats receiving borax, followed by fulminant hepatic failure [1]. GST, which increased to 180% in HCC rats, decreased significantly after the boron treatment (118%), but GST activity did not show any other change between control and borax groups [26]. Turkez *et al.* [25] confirmed that GST activity was induced by 15 mg/L boron compounds (boric acid, borox, colemanite and ulexite), but GST activity was reduced with high doses.

3.4 Glutathione reductase (GSH)

GSH is a unique cellular tripeptide that plays a vital role in maintaining the oxidant/antioxidant balance in the tissue that is essential for normal cellular function [1]. GSH is also a major component of red blood cells that plays a central role in the antioxidant defense of cells [32, 33]. The tripeptide counteracts the damaging effect of the peroxides and hydroperoxides produced as a result of the oxidative stress. Severe depletion of GSH that is considered as the consequence or the cause of oxidative stress, affects the activity levels of various enzymes involved in its recycling, such as glutathione reductase and glucose 6-phosphate dehydrogenase. Glutathione reductase helps to regenerate reduced glutathione from the oxidized glutathione, at the expense of NADPH produced in a reaction catalyzed by glucose 6-phosphate dehydrogenase [1]. As seen in Fig. 4, the fact that the activity of GSH does not change with increasing sodium tetraborate concentrations, sodium tetraborate has not any toxic effect on this enzyme. This enzyme involved in the glutathione turnover does appear to be normalizing, suggesting that boron could help to maintain the oxidant/antioxidant balance of the affected tissue. It should be noted that boron itself is not an antioxidant, but is reported to strengthen the antioxidant defense system of the tissue [34, 37]. In another study, it was found that GSH activity significantly decreased fulminant hepatic failure (FHF) rat group, but GSH activity increased when animals were given borax following FHF. However, GSH activity of control and borax groups was close to each other [1].

3.5 Glutathione peroxidase

GSH-Px and GST protect DNA and lipids of the cell against peroxidation products [36]. In general, the reduction in the activity of GSH-Px may be due to decrease in the availability of the substrate (GSH), and also due to ROS induced alterations in its protein structure [37]. Figure 5 shows the effect of sodium tetraborate on GSH-Px activity. GSH-Px activity did not alter by exposing to sodium tetraborate. Mohora *et al.* (2002) [38] established that rat liver GSH-Px activity increased at low boric acid concentration, contrary to the control, but at high concentration, it decreased [38]. Pawa and Ali [1] emphasized that GSH-Px activity was unchanged between borax and control groups, but GSH-Px activity was reduced in fulminant hepatic failure, contrary to control group [1]. It has also been reported that boron had no effect on GSH concentration, GSH peroxidase and GSSG reductase activities, or ratio of GSSG to GSH [39].

3.6 G-6-PDH activity

G-6-PDH catalyzes the first step of the pentose phosphate metabolic pathway, which is an exclusive source of NADPH in the red blood cells [40]. It was recorded that the produced NADPH provides the regeneration of reduced glutathione, which prevents haemoglobin denaturation, preserves the integrity of the red blood cell membrane sulfhydryl groups, and detoxifies peroxides and oxygen-

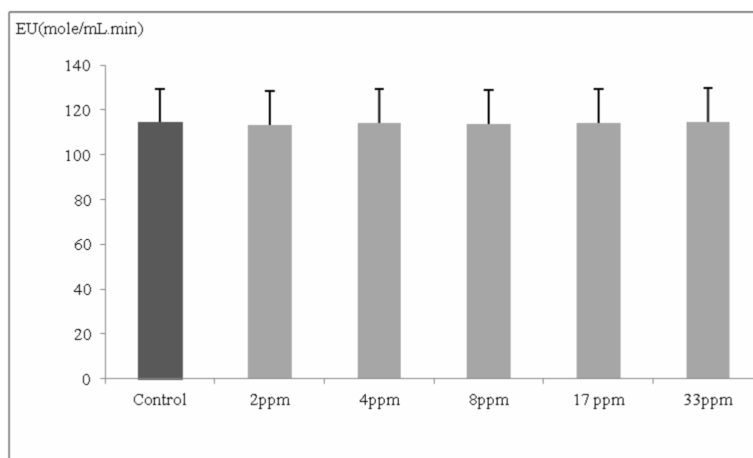

FIGURE 4 - The effect of sodium tetraborate on GR activity.

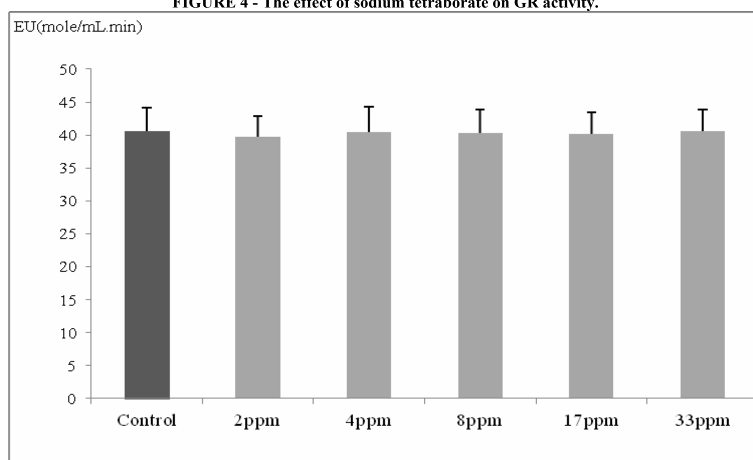

FIGURE 5 - The effect of sodium tetraborate on GSH-Px activity.

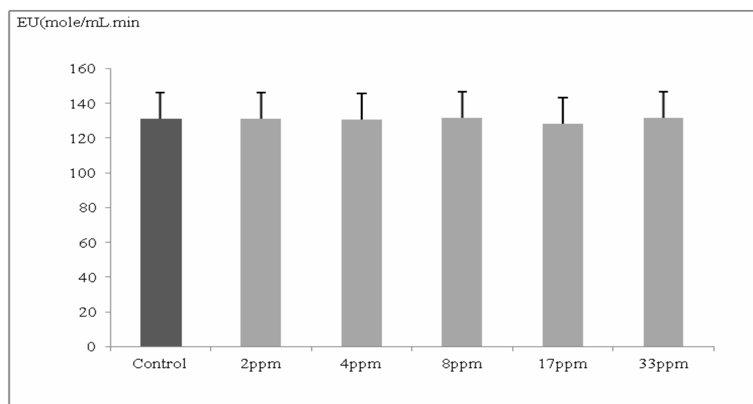

FIGURE 6 - The effect of sodium tetraborate on G6PD activity.

free radicals in the red blood cell [41]. The main toxic effects of increasing doses of involved boron compounds decreased antioxidant enzyme levels. Figure 6 shows the effect of sodium tetraborate on G-6-PDH under *in vitro* conditions. The results show that there is not any important change in G-6-PDH activity. On the other hand, Ku *et al*. (1993) [42] found that some compounds showed nega- tive effects on the activities of GST, GSH-Px, G-6-PDH enzymes and the glutathione levels. This decreasing in the enzyme activity might be due to selenium. However, they also said that the decreasing trend in the activities of antioxidant enzymes, which are independent from selenium, reveals that boron compounds could be influential, not only interacting with selenium but also in other respects. Furthermore, it is possible that boron compounds can increase the activities of antioxidant enzymes by inducing the accumulation of cAMP at low doses [42].

4. CONCLUSIONS

There is no difference in red blood cell antioxidant levels between control and experimental groups. The response of antioxidant levels in control and experimental groups at increasing concentrations of sodium tetraborate treatment does not change. Our data show that antioxidant enzyme activities were not consistently related to increasing sodium tetraborate concentrations. Sodium tetraborate may not be a risk factor in red blood cells for antioxidant enzymes. It could be concluded that sodium tetraborate is not an oxidant and has no effect on antioxidants, such as CAT, GSH-Px, GST, SOD, G-6-PDH and XOD, demonstrated with an *in vitro* model. In summary, boron compounds appear to offer benefits in the support of antioxidant defense capacity. Sodium tetraborate acts neither as activator nor inhibitor for all enzymes studied.

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