Effects of Some Boron Compounds on Peripheral Human Blood

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Peripheral blood cultures were exposed to various doses (5 to 500 mg/L) of boron compounds. Sister-chromatid exchange, micronucleus and chromosomal aberration tests were applied to estimate the DNA damage, and biochemical parameters (superoxide dismutase, catalase, glutathione peroxidase, glutathione-*S*-transferase, glucose-6-phosphate dehydrogenase, total glutathione, malondialdehyde and total antioxidant capacity) were examined to determine oxidative stress. According to our findings, various boron compounds at low doses were useful in supporting antioxidant enzyme activities in human blood cultures. It was found that the boron compounds do not have genotoxic effects even in the highest concentrations, though in increasing doses they constitute oxidative stress. It is concluded that the tested boron compounds can be used safely, but it is necessary to consider the tissue damages which are likely to appear depending on the oxidative stress.

Key words: Boron Compounds, Genotoxicity, Oxidative Stress

Introduction

Boron is a trace mineral for plants, animals and humans (Nielsen, 1994). It also plays an important role in improving arthritis, plasma lipid profiles and brain function (Penland, 1994). It has been thought that boric acid and borax may not cause mutagenicity in humans since mutagenic effects were absent in bacterial and animal tests (NTP, 1987). For this reason, any *in vitro* or *in vivo* studies have not been performed in human tissues, whereas uncertainty of the effects of boron compounds on human health necessitates these researches. Recently, an *in vivo* study performed on boron-exposed (as boric acid) Chinese hamster ovary (CHO) notices dose-response increases in the frequencies of aberrant cells and chromosomal aberrations (Oliveira *et al*., 2005). On the other hand, the actions on tissue antioxidant defenses of boric acid against reactive oxygen species (ROS) are not adequately identified (Pawa and Ali, 2006). It is reported that oxidative stress caused by ROS damages DNA (DeFedericis *et al*., 2006). As a matter of fact, serious diseases, such as cancer and arteriosclerosis, occur when the survival mechanisms are unable to deal adequately with ROS in the cells (Dodd *et al*., 1997).

Undoubtly, the knowledge of the vascular changes caused by boron compounds is critical to the effective pharmacokinetic researches (Erica *et al.*, 2001). In this context, the blood cells are potential vulnerable cells (Laakso *et al*., 2001). So genetic and oxidative studies of blood are pivotal, because such studies will serve to evaluate and improve the therapeutic gain of boron compounds. With this background in mind, *in vitro* human studies are also most important. Unfortunately, the human epidemiology data are not compatible with the available data since they involved lower exposure levels than those used in the animal studies. Hovewer, basic toxicity information often provides valuable perspective for predicting potential risks to humans. Thus, limited conclusions can be drawn from animal studies due to the relative insensitivity of the end-points measured.

Based on the above data, this article was designed for describing for the first time genetic and oxidative effects in human blood due to the dose of different boron compounds (boric acid, borax, colemanite and ulexite) with an *in vitro* study. In the current study, the sister-chromatid exchange (SCE), micronucleus (MN), and chromosomal ab-

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erration (CA) tests covering a wide range of induced genetic damage as primary DNA damage were performed on peripheral lymphocytes. In addition, some oxidative parameters including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-*S*-transferase (GST), glucose-6-phosphate dehydrogenase (G-6-PDH), total glutathione (TGSH), malondialdehyde (MDA) and total antioxidant capacity (TAC), which are recently used to monitor the development and extent of damage due to oxidative stress, were investigated.

Materials and Methods

Experimental

Whole heparinized blood from ten healthy male non-smoking donors between age 24 and 30 with no history of exposure to any toxic agent was used in our experiments. Questionnaires were obtained from each blood donor to the evaluate exposure history, and, in addition, informed consent forms were signed by each donor. From all volunteers involved in this study, hematological and biochemical parameters were analyzed, and any pathology was not detected. The effects of the following agents were tested: boric acid (H_3BO_3, CAS No. 10043-35-3), borax ($Na_2B_4O_7 \cdot 10H_2O$, CAS No. 1303-96-4), colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$, CAS No. 1318-33-8) and ulexite ($NaCaB_5O_9 \cdot 8H_2O$, CAS No. 1319-33-1) (concentrations in blood 0, 5, 10, 20, 40, 50, 80, 100, 150, 200, 300, 400 and 500 mg/L). These investigations stem from the work of Türkez and Geyikoglu (2006). The compounds were purchased from Eti Mine Works General Management (Turkey). After supplementation of boron compounds, the blood was incubated for 1 h at 37 °C to adjust body conditions, except for testing SCE, MN and CA (see below). The control samples of each volunteer were incubated and treated equally as the samples, but without addition of boron compounds.

Erythrocytes

Erythrocytes were obtained from heparinized blood samples by centrifugation ($2500 \times g$, for 20 min) at 4 °C. The red cells were then washed three times with 5 volumes of phosphate buffered saline (PBS; 150 mmol L^{-1} NaCl, 1.9 mmol L^{-1} NaH_2PO_4, 8.1 mmol L^{-1} Na_2HPO_4, pH 7.4), and divided 1:1 in appropriate aliquots and stored at −80 °C until further analysis.

Enzymatic activities

SOD activity was determined by the method of Misra and Fridovich (1972), which is based on the ability of superoxide dismutase to inhibit the process of epinephrine self-oxidation in alkaline medium. In the reaction of coloured adrenochrome formation, the superoxide radical anion is formed as an intermediate product. SOD activity was measured by monitoring the increase in the absorbance at 480 nm.

CAT was determined by the method of Aebi (1984). To 3 mL H_2O_2 (54 mM H_2O_2 in 50 mM phosphate buffer, pH 7.0), 5 μL of a catalase solution were added and the decrease in H_2O_2 was measured spectrophotometrically (Beckman DU 500, USA) at 240 nm for 60 s at 25 °C. In the erythrocyte preparations, haemolysates were centrifuged ($9000 \times g$) and estimation of activity was made with 1% haemolysates. One unit of catalase activity was defined as the activity required to degrade 1 μmol of H_2O_2 in 60 s.

GSH-Px activity was measured using hydrogen peroxide as substrate (Carlberg and Mannervik, 1972). Potassium azide was added to inhibit catalase. Potassium ferricyanide was added to inhibit the pseudo-peroxidase activity of haemoglobin. Conversion of NADPH was monitored continuously in a spectrophotometer at 340 nm for 3 min at 25 °C.

GST activity was determined by the method of Habig *et al.* (1974), using 1 mM glutathione (GSH), 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 0.1 M potassium phosphate buffer (pH 6.5). The rate of increase of product concentration was monitored by measuring the absorbance at 340 nm for 3–5 min at 25 °C in a spectrophotometer. Within this period, the rate of reaction was linear with time.

G-6-PDH activity was determined according to the protocol of Sigma, No. 345-UV. G-6-PDH (Sigma) catalyzes the first step in the pentose phosphate cycle to 6-phospho-gluconate (6PG), spectrophotometrically observable as an increase in the absorbance at 340 nm.

TGSH was measured according to the method of Akerboom and Sies (1981). The method is based on the catalytic action of glutathione reductase in a system, in which GSH undergoes sequential oxidation by DTNB and reduction by NADPH. The measure of the concentration of glutathione in samples equals to the velocity of

increase of absorbance. Glutathione content was calculated from a calibration curve made with oxidized glutathione (GSSG).

The content of MDA was measured by the thiobarbituric acid (TBA) method which was modified from methods of Satoh (1978) and Yagi (1984). Peroxidation was determined as the production of MDA which in combination with TBA forms a pink chromogen compound whose absorbance at 532 nm was measured.

The automated TEAC (Trolox equivalent antioxidant capacity) assay was carried out using an automated analyzer (Olympus AU 2700, Mishima, Japan) with commercially available kits (Total Antioxidant Status, Randox Laboratories, Ardmore, UK). It was thus sometimes called the Randox-TAS assay in this study. In this assay, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) is incubated with metmyoglobin and H_2O_2 to produce the ABTS radical cation. This species is blue-green. Antioxidants present in the sample cause a reduction in absorption proportional to their concentration (Prior and Cao, 1999; Erel, 2004).

Cytogenetic methods

Human peripheral blood lymphocyte cultures were set up according to a slight modification of the protocol described by Evans and O'Riordan (1975). A 0.5 mL aliquot of heparinized blood was cultured in 6 mL of culture medium (Chromosome Medium B; Biochrom, Berlin) with 5 μg/mL of phytohaemagglutinin (Biochrom). Above-mentioned doses of boron compounds were added into the culture tubes. In addition, for each individual, lymphocyte cultures without boron compounds were studied as a control group. With the aim of providing successive visualization of SCEs, 5-bromo-2′-deoxyuridine (Sigma; final concentration 20 μM) was added at culture initation. The cultures were incubated in complete darkness for 72 h at 37 °C.

Exactly 70 h and 30 min after beginning of the incubations, demecolcine (*N*-diacetyl-*N*-methylcolchicine, Sigma) was added to the cultures to achieve a final concentration of 0.5 μg/L. After hypotonic treatment (0.075 M KCl), followed by three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v), centrifugation, and resuspension, the cell suspension was dropped onto chilled, grease-free microscopic slides, air-dried, aged, and then differentially stained for the inspection of the SCE rate according to the fluorescence plus Giemsa (FPG) procedure (Perry and Wolff, 1974). For each treatment condition, well-spread thirty second division metaphases containing 42–46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per cell.

For analysis of structural chromosomal aberrations (chromatid or chromosome gap and chromatid or chromosome break) parallel cultures were carried out without bromodeoxyuridine (BrdU) for 72 h. Treatments were similar as described in the sister-chromatid exchanges analysis. 2 h prior to harvesting 0.1 ml of colchicine (0.2 μg/mL) was added to the culture flask. Hypotonic treatment and fixation were performed in the same way as in the SCE analysis. To prepare slides, 3–5 drops of the fixed cell suspension were dropped on a clean slide and air-dried. The slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. 30 Well-spread metaphases were analyzed for each treatment to detect the presence of chromosomal aberrations. Criteria to classify the different types of aberrations were in accordance with the recommendation of EHC 46 for environmental monitoring of human populations (IPCS, 1985).

The MN test was performed by adding cytochalasin B (Sigma; final concentration 6 μg/mL) after 44 h of culture (Fenech and Morley, 1985). At the end of the 72-h incubation period, the lymphocytes were fixed with ice-cold methanol/acetic acid (1:1, v/v). The fixed cells were put directly on silides using a cytospin, and stained with Giemsa solution. All slides were coded before scoring. The criteria for scoring micronuclei were as described by Fenech (1993). At least 1000 binucleated lymphocytes were examined per concentration for the presence of one, two or more micronuclei.

Statistical analysis

Statistical analyses were performed using SPSS software (version 11.5, SPSS, Chicago, IL, USA). The two-tailed Student's *t*-test was used to compare SCE and CA frequencies between treated and control groups. The statistical analysis of MN frequencies was also performed by use of the χ^2 test. For the statistical analysis of biochemical data Duncan's test was used which is obligatory for data presented in work because of high interindi-

vidual differences. Statistical decisions were made with a significance level of 0.05.

Results

The boron compounds (until concentrations of 20 mg/L) caused significant increases in the antioxidant enzyme activities (SOD, CAT, GSH-Px, GST and G-6-PDH) of erythrocytes. Also, the levels of TGSH and TAC significantly increased. On the contrary, all these studied parameters decreased with increasing concentrations of the test compounds (Tables I and II).

80 mg/L boric acid and borax, 40 mg/L colemanite and 100 mg/L ulexite did not affect the MDA levels in blood. However, the results obtained with

Table I. SOD, CAT, GSH-Px, GST and G-6-PDH enzyme activities in human erythrocytes incubated with boron compounds at different concentrations for 1 h. Values given are means ± standard deviation; $n = 10$. Statistical analyses were performed as indicated in the text. * Significantly different from control at the $p < 0.05$ level.
Unit definitions: For SOD: One unit inhibits by 50% the maximum reduction of nitro blue tetrazolium at 25 °C, pH 7.0; for CAT: one unit decomposes 1 μmol of H_2O_2 per minute at 25 °C, pH 7.0; for GSH-Px: one unit catalyzes the oxidation by H_2O_2 of 1.0 μmol of reduced glutathione to oxidized glutathione per min at 25 °C, pH 7.0; for G-6-PDH: one unit reduces 1 μmol of NAD per minute at 37 °C, pH 7.8, using glucose-6-phosphate as substrate.

	Dose [mg/L]	Boric acid	Borax	Colemanite	Ulexite
SOD activity [U/mL]	Control	98.10 ± 6.75	98.10 ± 6.75[c]	98.10 ± 6.75	98.10 ± 6.75
	15	105.50 ± 6.77*	104.50 ± 6.68*	104.10 ± 5.19*	119.10 ± 7.99*
	50	91.90 ± 5.80*	92.60 ± 3.95	87.50 ± 5.21*	93.60 ± 4.47
	500	72.90 ± 6.48*	61.78 ± 4.14*	59.70 ± 6.43*	74.60 ± 5.94*
CAT activity [U/g Hb]	Control	278.90 ± 17.16	278.90 ± 17.16	278.90 ± 17.16	278.90 ± 17.16
	15	299.70 ± 21.76	301.20 ± 22.30*	298.40 ± 22.23	303.20 ± 22.38*
	50	286.80 ± 21.58	274.80 ± 20.95	260.70 ± 20.90	272.70 ± 20.07
	500	248.90 ± 21.65*	241.70 ± 20.36*	238.80 ± 20.75*	246.60 ± 20.57*
GSH-Px activity [U/L]	Control	9866.40 ± 558.98	9866.40 ± 558.98	9866.40 ± 558.98	9866.40 ± 558.98
	15	10475.60 ± 598.54*	10487.60 ± 584.56*	10370.60 ± 587.20*	12920.50 ± 642.76*
	50	9397.20 ± 519.21	9253.10 ± 512.18*	9277.60 ± 519.71*	9231.90 ± 525.94*
	500	7925.40 ± 463.74*	7476.30 ± 466.35*	7390.50 ± 469.71*	8004.80 ± 458.47*
GST activity [μmol/g Hb]	Control	29.13 ± 3.29	29.13 ± 3.29	29.13 ± 3.29	29.13 ± 3.29
	15	40.03 ± 4.25*	37.86 ± 3.71*	32.40 ± 3.57	40.41 ± 4.69*
	50	27.39 ± 4.07	25.62 ± 4.68	26.82 ± 4.48	28.36 ± 3.58
	500	21.70 ± 3.56*	19.76 ± 3.89*	16.89 ± 3.45*	24.41 ± 3.47*
G-6-PDH activity [mU/g Hb]	Control	15.11 ± 0.76	15.11 ± 0.76	15.11 ± 0.76	15.11 ± 0.76
	15	15.93 ± 0.72*	15.90 ± 0.79*	15.81 ± 0.72*	17.13 ± 0.91*
	50	15.19 ± 0.67	14.34 ± 0.61*	14.01 ± 0.74*	15.18 ± 0.47
	500	9.30 ± 0.62*	9.05 ± 0.61*	8.21 ± 0.62*	10.14 ± 0.63*

Table II. Amount of TGSH, TAC capacity and MDA levels in human erythrocytes incubated with boron compounds at different concentrations for 1 h. Abbrevations are as in Table I.

	Dose [mg/L]	Boric acid	Borax	Colemanite	Ulexite
TGSH level [mM/g Hb]	Control	56148.20 ± 3818.58	56148.20 ± 3818.58	56148.20 ± 3818.58	56148.20 ± 3818.58
	15	62708.60 ± 4642.51*	60395.10 ± 4561.57*	59035.90 ± 3058.69	64435.70 ± 4324.27*
	50	54198.80 ± 3926.09	53719.20 ± 3518.62	51888.50 ± 3229.96*	55223.30 ± 4357.81
	500	43224.50 ± 3507.77*	45609.10 ± 3975.66*	41045.70 ± 3147.68*	45827.50 ± 3669.60*
TAC [μmol/L]	Control	7.86 ± 0.39	7.86 ± 0.39	7.86 ± 0.39	7.86 ± 0.39
	15	8.19 ± 0.26*	8.22 ± 0.45*	7.73 ± 0.46	9.47 ± 0.60*
	50	7.32 ± 0.35*	7.21 ± 0.34*	7.07 ± 0.36*	7.52 ± 0.37
	500	6.06 ± 0.28*	5.86 ± 0.41*	5.60 ± 0.38*	6.19 ± 0.26*
MDA level [μmol/L]	Control	355.30 ± 21.32	355.30 ± 21.32	355.30 ± 21.32	355.30 ± 21.32
	15	354.10 ± 21.91	358.80 ± 24.30	362.70 ± 24.92	348.30 ± 26.07
	50	365.30 ± 23.42	372.90 ± 24.05	382.40 ± 25.29*	356.20 ± 26.04
	500	396.90 ± 25.18*	399.50 ± 25.66*	406.30 ± 23.65*	379.80 ± 27.56*

Table III. Influence of boric acid, borax, colemanite and ulexite on SCE, MN and CA frequency in cultures of human peripheral lymphocytes. Values given are means ± standard deviation; $n = 10$. Statistical analyses were performed as indicated in the text. All values are not significantly different from the control group at the $p < 0.05$ level.

Test substance	Concentration	SCE/cell ± S.D.	MN/1000 cell	CA/cell ± S.D.
Boric acid	Control	6.30 ± 1.03	1.82	0.18 ± 0.03
	15 mg/L	6.34 ± 1.10	1.94	0.15 ± 0.02
	50 mg/L	6.68 ± 0.81	1.90	0.15 ± 0.02
	300 mg/L	6.57 ± 1.02	1.91	0.20 ± 0.04
Borax	Control	6.30 ± 1.03	1.82	0.18 ± 0.03
	15 mg/L	6.43 ± 0.96	1.92	0.15 ± 0.02
	50 mg/L	6.81 ± 0.79	1.83	0.20 ± 0.04
	300 mg/L	6.71 ± 0.92	1.87	0.20 ± 0.04
Colemanite	Control	6.30 ± 1.03	1.82	0.18 ± 0.03
	15 mg/L	6.61 ± 1.07	1.84	0.18 ± 0.03
	50 mg/L	6.80 ± 0.86	1.89	0.20 ± 0.04
	300 mg/L	6.80 ± 0.96	1.87	0.20 ± 0.04
Ulexite	Control	6.30 ± 1.03	1.82	0.18 ± 0.03
	15 mg/L	6.43 ± 1.11	1.85	0.15 ± 0.02
	50 mg/L	6.50 ± 0.70	1.91	0.15 ± 0.02
	300 mg/L	6.32 ± 1.00	1.57	0.15 ± 0.02

these compounds showed that MDA levels significantly increased at higher concentrations than the above-mentioned ones (Table II).

Table III presents the genotoxic evaluation of boron compounds in lymphocyte cultures. According to the SCE, MN and CA assays, these compounds have no genotoxic potential. As a matter of fact, the frequencies of SCEs and CAs did not change compared with the controls. Moreover, the rates of MN were similar to control values. The cultures exposed to 400 mg/L borax and colemanite were found to be sterile. Similarly, 500 mg/L of boric acid, borax, colemanite and ulexite caused sterility in lymphocytes.

Discussion

Antioxidant enzymes play a main role in the defence of mammalian blood, and these enzymes can be induced or inhibited in the blood cells exposed to different toxicants (Afaq *et al*., 1998; Prasad *et al*., 2006). In this study, there is considerable evidence that boron compounds (boric acid, borax, ulexite and colemanite) influence the antioxidant enzyme activities as related to the dose not leading to any genetic damage in human blood.

It was pointed out that boric acid can change the oxidative metabolism in animal systems (Kelly, 1997). However, it has not been replied that a change of oxidative metabolism appears as a result of the induction of oxidative stress or the supporting of antioxidant capacity (Hunt and Idso, 1999). The present study clearly proved that low doses of various boron compounds increased the antioxidant capacity by increasing the enzyme activities (5 to 20 mg/L). Moreover, these compounds did not affect the MDA level of blood. It is thought that the increase in the MDA level is an important sign of oxidative stress (Kim *et al*., 2006). Likewise, oxidative stress develops when the levels of antioxidants are lowered. So the activities of antioxidant enzymes (SOD, CAT, GSH-Px, GST, GR ve G-6-PDH) have importance in the cell defense (Tapiero *et al*., 2004). SOD has a central role against oxidative damage. This enzyme catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen (Kakarla *et al*., 2005). Endogenous H_2O_2 may be converted to H_2O by catalase (Svistunenko, 2005). It was recorded that the SOD activity in human erythrocytes increases with boron supplement (as boric acid) (Nielsen, 1989). In our study, not only boric acid but also borax, ulexite and colemanite have caused significant increases in the SOD activity. This is also the first report that boron compounds have induced CAT activity. According to data collected in experiments, the CAT activity protects lipids and proteins in erythrocyte membranes against peroxide radicals (Hunt and Idso, 1999). Again, the present results emphasize that increases in the levels of the other antioxidant enzymes such as GSH-Px, GST and G-6-PDH are associated with the increases in the quantity of glutathione. Glutathione is a major

component of erythrocytes. It has an important role in the antioxidant enzyme activities abovementioned (Meister, 1983). GSH-Px and GST also protect DNA and lipids of the cell against peroxidation products (Bukowska, 2004). 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 (Altikat *et al.*, 2002). And it is 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-free radicals in the red blood cell (Weksler *et al.*, 1990). Thus, it has been concluded that the tested boron compounds have an enhancing effect on the antioxidant defense system in cells. Our findings are also supported by the increase in TAC capacity.

Noteworthy, the main toxic effects of increasing doses of boron compounds involve decreased antioxidant enzyme levels. These compounds showed negative effects on the activities of GST, GSH-Px, G-6-PDH enzymes and the glutathione levels. It was reported that the consumption of glutathione causes significant decreases in the activity of G-6-PDH and GSH-Px. For this reason, oxidative stress could develop (Efferth *et al.*, 2000). It is also the fact that boron compounds demonstrate adverse effects on GSH-Px at levels lower than those associated with other enzymes. At this point, we establish a clear dose-response relationship between boron compounds and this enzyme. The effect of boron (as boric acid) on the activity of the glutathione reductase enzyme in the plasma of mallard duck was due to selenium (Hoffman *et al.*, 1992). Similarly, there is an apparent effect of selenium in the GSH-Px activity (Al-Saleh and Al-Doush, 1997). However, we can not say that the effects in antioxidant enzymes of boron compounds were solely due to selenium. Because, the decreases in the activities of other antioxidant enzymes, which are independent from selenium, reveal that boron compounds could be influential not only interacting with selenium but also in other respects. As a matter of fact, Hall *et al.* (1980) indicated that boric acid and borax could change the hydrolytic enzyme activities through cAMP. A similar aspect may be acceptable for SOD, CAT, GST and G-6-PDH in our study hence activities of antioxidant enzymes increased as dependent on increases in the cAMP amount (Sugino *et al.*, 2002). Thus, it is possible that boron compounds can increase the activities of antioxidant enzymes by inducing the accumulation of cAMP at low doses. Likewise, the increasing doses of boric acid (three or five times) could diminish the amount of ATP in sperm cultures (Ku *et al.*, 1993). Since the formation of cAMP depends on the amount of ATP (Cheung, 1972), in the current study, high doses of boron compounds might have also prevented the activities of antioxidant enzymes by diminishing the ATP amount. But this suggested mechanism needs biochemical research related to the dose for each compound. Again, our results show that boric acid, borax, colemanite and ulexite do not lead to DNA damages in lymphocytes. It was reported that boric acid and borax do not have mutagenic effects in bacterial and animal tests (NTP, 1987). However, oxidative stress was caused by colemanite, borax, boric acid, ulexite in decreasing order in the present study. For this reason, blood cells may suffer, because oxidative stress could cause irreversible modifications of protein SH groups (Hooiveld *et al.*, 1998).

In summary, boron compounds appear to offer benefits in the support of antioxidant defense capacity (with increasing enzyme activities, TGSH and TAC, and constant MDA levels). Again in this study, boric acid, borax, ulexite and colemanite do not show genotoxic effects, though they form oxidative stress at increasing doses (with decreasing antioxidant enzyme activities, TGSH and TAC, and increasing MDA levels). In conclusion, boron compounds can be used safely, however, it will also be useful to take into consideration the cell damages, which are likely to develop depending on the oxidative stress.

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