Experimental and Toxicologic Pathology 64 (2012) 93–101

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

The effects of some boron compounds against heavy metal toxicity in human blood

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ARTICLE INFO

Article history:
Received 31 December 2009
Accepted 24 June 2010

Keywords:
Boron compounds
Genotoxicity
Heavy metal
Human blood culture
Oxidative stress

ABSTRACT

Heavy metals can accumulate in the environment and cause serious damages to ecosystems and human health. Boron is considered to be essential micronutrient with its well established biological functions and the antioxidant effects of boric acid (BA) are controversial. However, the potential of important boron compounds in cellular activities remains unexplored. Therefore, we aimed to assess the efficacies of some boron compounds (BA, borax, colemanite and ulexite) on the genotoxicity induced by heavy metals (arsenic trioxide, colloidal bismuth subcitrate, cadmium chloride, mercury chloride and lead chloride) in human blood cultures. For this aim, sister chromatid exchange (SCE) and micronuclei (MN) assays were performed to establish DNA damages in lymphocytes. Besides, oxidative stress was monitored by estimating the changes of main antioxidant enzyme activities and the levels of total glutathione (TGSH) in erythrocytes. The present study showed that heavy metal treatments increased the frequencies of SCE and MN and the plasma malondialdehyde (MDA) level; decreased the antioxidant enzyme activities and the level of TGSH compared to controls. Whereas, the tested boron compounds (5–20 ppm) significantly reduced the genotoxic effects induced by low doses of heavy metals. Our results revealed that the protective roles of boron compounds occurred with the effectiveness on their anti-oxidant capacity. In conclusion, these compounds could be useful in the development of functional food and raw materials of medicine.

1. Introduction

Arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are common environmental pollutants. These metals have no beneficial effects in humans, and there is no known homeostasis mechanism for them (Llobet et al., 2003). Among industrially used and mined heavy metals, bismuth (Bi) is one of the least toxic of the heavy metals. However, intoxication with Bi has occurred from its use in medicine (Lewis, 1996). The general population is exposed to heavy metals at several concentrations either voluntarily through supplementation or involuntarily through intake of contaminated food and water or contact with contaminated soil, dust, or air (Meeker et al., 2008). And exposure to these toxic metals is associated with many serious diseases including Alzheimer's (Mutter et al., 2007), Parkinson's (Guzeva et al., 2008), autoimmune (Ohsawa, 2009), digestive (Borges et al., 2008), heart (Saad et al., 2006) disorders and liver, kidney, stomach and lung cancers (Waalkes, 2000; Vahter, 2007). One of the major mechanisms behind metal toxicity has been attributed to oxidative stress (Flora et al., 2008). In fact, various studies connect heavy metals with oxidative DNA damage, since these metals may reduce the level of the main antioxidant compounds in several animal tissues by inactivating enzymes and other antioxidant molecules (Chater et al., 2008; Jihen et al., 2009).

On the other hand, boron, the fifth element in the periodic table, is a naturally occurring element. In the environment, boron is combined with oxygen and other elements in compounds called borates. Borates are widely found in nature, and are present in oceans, rocks and soils. There are several commercially important borates, including BA, borax (BX) and the minerals colemanite (COL) and ulexite (UX) (Cotton and Wilkinson, 1998). These compounds are widely used in industrial, agricultural, cosmetic, medical settings, household products and a numerous smaller applications (Fail et al., 1998; Pahl et al., 2005). The boron, a trace mineral for plants, animals and humans, has been shown to have apparent beneficial effects in humans at intakes commonly found in diets abundant in foods such as fruits and vegetables (Nielsen, 1996; Devirian and Volpe, 2003). Research findings suggest that physiological amounts of supplemental dietary boron (as BA) affect

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doi:10.1016/j.etp.2010.06.011

Table 1
The effects of As_2O_3, CBS, $CdCl_2$, $HgCl_2$ and $PbCl_2$ on oxidative stress and antioxidant capacity in human blood cultures.

Groups		SOD	CAT	GSH-Px	TGSH	MDA
Control		106.4 ± 11.6	267.9 ± 37.6	93.6 ± 10.4	58.1 ± 8.4	378.1 ± 33.4
As_2O_3	3 ppm	71.8 ± 7.5*	208.5 ± 26.5*	72.1 ± 8.2*	38.8 ± 5.5*	1052.5 ± 163.7*
	5 ppm	49.9 ± 6.4*	177.6 ± 23.2*	55.2 ± 6.5*	32.3 ± 5.1*	1952.3 ± 284.9*
CBS	7.5 ppm	102.5 ± 10.7	251.5 ± 34.9*	81.4 ± 9.2*	54.8 ± 7.1	430.7 ± 46.1
	15 ppm	89.3 ± 9.1*	238.2 ± 29.5*	70.3 ± 7.9*	42.2 ± 6.1*	785.0 ± 118.2*
$CdCl_2$	3 ppm	94.3 ± 8.9*	234.6 ± 27.5*	75.3 ± 8.4*	50.5 ± 6.2*	830.7 ± 119.4*
	5 ppm	83.2 ± 8.0*	202.7 ± 24.6*	62.4 ± 6.9*	41.7 ± 4.9*	1316.5 ± 238.6*
$HgCl_2$	6 ppm	77.4 ± 7.8*	200.7 ± 23.1*	70.5 ± 7.5*	44.2 ± 6.0*	915.5 ± 144.7*
	9 ppm	52.3 ± 6.5*	167.9 ± 22.7*	49.1 ± 5.8*	38.6 ± 4.7*	1327.3 ± 210.1*
$PbCl_2$	3 ppm	70.3 ± 7.1*	213.8 ± 29.6*	74.2 ± 8.3*	40.8 ± 5.9*	969.5 ± 137.6*
	5 ppm	45.8 ± 6.3*	181.5 ± 24.3*	59.3 ± 6.8*	35.2 ± 4.4*	1515.4 ± 235.7*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level. Unit definition for SOD (U/mL): one unit inhibits by 50% the maximum reduction of nitro blue tetrazolium at 25 °C, pH 7.0; for CAT (U/g Hb): one unit decomposes one micromole of hydrogen peroxide per minute at 25 °C, pH 7.0; for GSH-Px (U/mL): one unit catalyzes the oxidation by H_2O_2 of 1.0 μmol of reduced glutathione to oxidized glutathione per min at pH 7.0 at 25 °C; TGSH (mM/g Hb); MDA (μmol/L).

a wide range of metabolic parameters in animals (Hunt, 1998). It probably strengthens the antioxidant defense mechanism by a yet unknown mechanism (Pawa and Ali, 2006). Orally administered boron is rapidly and completely absorbed from the gastrointestinal tract into the blood stream (Usuda et al., 1998) and plays an important role in improving arthritis, plasma lipid profiles, brain function (Devirian and Volpe, 2003). A variety of boronated agents with hypolipidemic, anti-inflammatory or anticancer properties is also developed (Barranco et al., 2008). Moreover, the boron compounds show minimal potential for genotoxicity in bacteria and cultured mammalian cells (Moore and Expert Scientific Committee, 1997; Turkez et al., 2007). Thus, these compounds remain very interesting research topics due to equivocal and relatively unknown useful actions, roles in the treatment of various diseases, and interactions of other elements.

Since the complete avoidance of exposure to heavy metals is very difficult, chemoprevention is a popular strategy for protecting humans and animals from the risk of serious health problems by exposure to these toxic metals. Several antioxidants including vitamins (Appenroth and Winnefeld, 1998; Fahmy et al., 2008), N-acetyl-cystein (Ustundag and Duydu, 2007), taurine (Bosgelmez et al., 2008), bioflavonoids (Ponnusamy et al., 2008) were tested for their effectiveness in minimizing heavy metals-induced oxidative DNA damages. However, to the best of our knowledge, the effects of boron compounds against metal toxicities were not yet studied. Therefore, the present study was conducted to evaluate the efficacy of four main boron compounds; BA, BX, COL and UX in genotoxicity induced by arsenic trioxide (As_2O_3), cadmium chloride ($CdCl_2$), lead chloride ($PbCl_2$), colloidal bismuth subcitrate ($C_6H_5O_7Bi$) and mercury chloride ($HgCl_2$) exposures in peripheral human blood cultures. For this purpose, SCE and MN tests covering a wide range of induced genetic damage as primary DNA damage were performed on peripheral lymphocytes. Also, the oxidant-antioxidant status of blood cultures were assessed by measuring superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), TGSH and MDA.

2. Materials and methods

2.1. Materials

H_3BO_3 (CAS No. 10043-35-3,), $CdCl_2$ (CAS No. 7790-78-5), and $C_6H_5O_7Bi$ (CAS No. 813-93-4) were purchased from Sigma Co. (St. Louis, MO, USA). $PbCl_2$ (CAS No. 7758-95-4) and $HgCl_2$ (CAS No. 7487-94-7) were from Merck (Darmstadt, Germany). As_2O_3 (CAS No. 1327-53-3) was from Fluka (USA). $Na_2B_4O_7{\cdot}10H_2O$ (CAS No. 1303-96-4), $Ca_2B_6O_{11}{\cdot}5H_2O$ (CAS No. 1318-33-8) and $NaCaB_5O_9{\cdot}8H_2O$ (CAS No. 1319-33-1) were also purchased from Eti Mine Works General Management (Turkey).

2.2. Experimental design and treatments

Human blood was obtained by veinpuncture from four non-smoking donors. Four different doses of BA, BX, COL and UX (5, 10, 15 and 20 ppm) were used. Two different concentrations of As_2O_3, $CdCl_2$, $PbCl_2$ (3 and 5 ppm), $C_6H_5O_7Bi$ (7.5 and 15 ppm) and $HgCl_2$ (6 and 9 ppm) were selected (concentrations in blood). The concentrations were selected according to the works of Rao et al. (2001), Bonacker et al. (2005), Geyikoglu and Turkez (2005), Turkez et al. (2007), Laparra et al. (2008) and Lewinska et al. (2008). The heavy metals and boron compounds were added to the cultures just before the incubation for cytogenetic and biochemical analysis. After the applications of BA, BX, COL, UX and heavy metals, separate and together, the blood was incubated for 1 h at 37 °C except for SCE and MN (see below). Besides, the control samples of each volunteer were incubated, and they treated equally as the samples, but without addition of boron compounds and As_2O_3, $CdCl_2$, $PbCl_2$, $C_6H_5O_7Bi$, $HgCl_2$.

2.3. Erythrocytes

Erythrocytes were obtained from heparinized blood samples by centrifugation (2500 × *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 with a ratio of 1:1 divided in appropriate aliquots and stored at −80 °C until further analysis.

2.4. Enzymatic activities

SOD activity was determined in erythrocytes 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 colored adrenochrome formation, the superoxide anion-radical is formed as an intermediate product. SOD activity was measured by monitoring the increase in the absorbance at 480 nm.

Eritrosit CAT activitiy 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

Table 2
MDA levels in human erythrocyte after concomitant treatments with boron and heavy metal compounds for 1 h.

Control Tested agents		378.1 ± 33.4 As_2O_3		378.1 ± 33.4 CBS		378.1 ± 33.4 $CdCl_2$		378.1 ± 33.4 $HgCl_2$		378.1 ± 33.4 $PbCl_2$	
		3 ppm	5 ppm	7.5 ppm	15 ppm	3 ppm	5 ppm	6 ppm	9 ppm	3 ppm	5 ppm
Boric acid	5 ppm	426.2 ± 52.8	1124.0 ± 170.1*	406.5 ± 35.6	438.2 ± 50.5	440.5 ± 57.6	772.7 ± 94.3*	450.7 ± 61.5	913.7 ± 138.2*	437.5 ± 57.1	926.0 ± 137.9*
	10 ppm	454.5 ± 49.1	1404.7 ± 214.8*	417.5 ± 43.4	479.0 ± 52.0*	519.2 ± 92.7*	1019.7 ± 152.9*	596.2 ± 102.1*	1267.7 ± 211.2*	496.2 ± 61.8*	1158.2 ± 169.5*
	15 ppm	689.7 ± 94.6*	1786.5 ± 241.3*	436.0 ± 61.8	554.5 ± 69.4*	576.0 ± 97.1*	904.5 ± 121.1*	777.0 ± 112.3*	1153.2 ± 148.5*	669.2 ± 107.8*	1443.7 ± 202.2*
	20 ppm	897.7 ± 121.1*	1998.5 ± 249.6*	487.7 ± 62.3*	618.5 ± 96.8*	639.5 ± 89.4*	1286.7 ± 206.2*	967.5 ± 153.9*	1397.5 ± 197.6*	909.2 ± 134.9*	1497.5 ± 179.8*
Borax (Tincal)	5 ppm	706.2 ± 116.4*	796.2 ± 126.5*	426.2 ± 55.9	451.0 ± 59.6	461.5 ± 61.5	914.7 ± 158.5*	491.2 ± 61.5*	1175.5 ± 145.5*	397.2 ± 64.8	1218.5 ± 161.5*
	10 ppm	784.7 ± 132.7*	1279.7 ± 161.2*	433.2 ± 52.4	517.2 ± 68.4*	556.7 ± 84.3*	1260.5 ± 173.8*	688.7 ± 121.5*	1043.2 ± 129.4*	725.5 ± 129.8*	1129.2 ± 157.1*
	15 ppm	1150.2 ± 166.3*	1665.0 ± 218.1*	496.5 ± 60.1*	594.2 ± 72.3*	689.5 ± 98.1*	1155.7 ± 142.5*	896.5 ± 132.2*	1260.7 ± 155.0*	890.2 ± 133.8*	1430.5 ± 178.9*
	20 ppm	1297.7 ± 169.1*	2027.5 ± 269.8*	489.5 ± 62.2*	694.7 ± 89.2*	752.7 ± 114.5*	1373.5 ± 179.4*	879.0 ± 125.5*	1406.5 ± 182.7*	798.5 ± 138.5*	1490.2 ± 208.7*
Colemanite	5 ppm	881.7 ± 140.5*	1473.7 ± 184.7*	423.2 ± 43.7	538.7 ± 69.4*	556.5 ± 80.9*	951.5 ± 170.3*	577.2 ± 90.1*	1026.5 ± 167.4*	526.5 ± 71.4*	1293.5 ± 157.6*
	10 ppm	1143.7 ± 180.6*	1567.5 ± 176.5*	442.7 ± 58.7	611.5 ± 94.1*	636.2 ± 96.7*	1290.2 ± 196.8*	733.7 ± 131.5*	1284.7 ± 188.3*	890.7 ± 141.7*	1441.0 ± 185.9*
	15 ppm	922.5 ± 155.8*	1870.2 ± 241.1*	512.0 ± 61.2*	689.7 ± 95.2*	768.7 ± 123.1*	1375.5 ± 201.5*	855.5 ± 121.8*	1271.7 ± 176.3*	1014.7 ± 150.5*	1607.7 ± 214.4*
	20 ppm	1184.2 ± 179.5*	2196.5 ± 271.8*	518.7 ± 64.5*	743.2 ± 101.6*	903.5 ± 165.9*	1458.7 ± 196.3*	987.5 ± 140.6*	1486.2 ± 207.0*	1157.5 ± 169.5*	1706.5 ± 219.8*
Ulexite	5 ppm	687.2 ± 119.3*	974.2 ± 151.3*	391.7 ± 46.2	554.2 ± 77.4*	459.0 ± 59.6	680.7 ± 103.6*	402.5 ± 61.5	503.0 ± 89.1*	418.7 ± 57.4	963.0 ± 136.7*
	10 ppm	431.5 ± 67.1	955.7 ± 138.8*	429.2 ± 53.7	621.7 ± 94.6*	542.7 ± 82.5*	986.5 ± 131.1*	421.7 ± 64.4	676.7 ± 116.8*	518.5 ± 74.2*	1208.7 ± 161.3*
	15 ppm	725.0 ± 126.1*	1113.2 ± 159.7*	441.5 ± 51.0	645.5 ± 89.1*	761.7 ± 108.3*	1179.7 ± 186.7*	669.7 ± 123.5*	998.2 ± 134.4*	687.0 ± 91.3*	1430.5 ± 176.5*
	20 ppm	1112.5 ± 173.4*	1459.5 ± 203.3*	437.7 ± 54.5	681.5 ± 94.4*	868.0 ± 115.1*	1328.7 ± 196.8*	864.5 ± 125.4*	1163.5 ± 126.5*	898.7 ± 124.4*	1569.2 ± 238.9*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

Table 3
SOD enzyme activities in human erythrocytes after the combined treatments of human lymphocytes with boron and heavy metal compounds for 1 h.

Control Tested agents		106.4 ± 11.6 As_2O_3		106.4 ± 11.6 CBS		106.4 ± 11.6 $CdCl_2$		106.4 ± 11.6 $HgCl_2$		106.4 ± 11.6 $PbCl_2$	
		3 ppm	5 ppm	7.5 ppm	15 ppm	3 ppm	5 ppm	6 ppm	9 ppm	3 ppm	5 ppm
Boric acid	5 ppm	86.1 ± 9.3*	57.5 ± 6.6*	108.5 ± 12.2	103.8 ± 11.0	109.2 ± 12.3	89.2 ± 8.8*	86.2 ± 8.9*	65.9 ± 7.0*	83.5 ± 8.2*	51.2 ± 6.0*
	10 ppm	106.2 ± 11.8	50.6 ± 6.1*	109.2 ± 11.8	94.1 ± 9.2*	108.5 ± 12.0	87.6 ± 8.6*	93.5 ± 9.2*	69.3 ± 7.2*	99.7 ± 10.3	63.7 ± 6.8*
	15 ppm	91.7 ± 9.4*	44.7 ± 5.5*	105.3 ± 10.8	90.2 ± 8.6*	94.0 ± 9.2*	84.9 ± 8.7*	107.6 ± 11.7	66.2 ± 7.0*	109.2 ± 11.2	57.9 ± 6.6*
	20 ppm	84.0 ± 8.6*	40.8 ± 5.3*	103.1 ± 10.6	88.6 ± 8.4*	92.3 ± 9.0*	81.0 ± 8.4*	91.7 ± 8.9*	56.1 ± 6.2*	91.6 ± 9.4*	49.3 ± 5.1*
Borax (Tincal)	5 ppm	77.7 ± 7.6*	58.2 ± 6.3*	104.4 ± 9.7	93.2 ± 9.3*	110.8 ± 12.4	107.1 ± 12.1	102.4 ± 11.2	62.8 ± 6.7*	89.2 ± 9.3*	55.2 ± 5.8*
	10 ppm	75.5 ± 7.8*	50.7 ± 6.0*	102.5 ± 10.3	91.7 ± 8.9*	93.1 ± 9.4*	94.6 ± 9.0*	90.8 ± 8.9*	61.0 ± 6.6*	87.1 ± 9.4*	46.6 ± 5.3*
	15 ppm	70.3 ± 7.4*	50.4 ± 6.2*	99.8 ± 9.6	88.5 ± 8.4*	90.7 ± 8.8*	87.5 ± 8.8*	81.6 ± 8.4*	55.2 ± 5.9*	79.7 ± 8.7*	40.2 ± 5.0*
	20 ppm	66.2 ± 6.9*	48.6 ± 5.9*	92.4 ± 9.0*	84.1 ± 8.6*	88.3 ± 8.5*	80.6 ± 8.5*	76.3 ± 7.9*	49.2 ± 5.1*	71.0 ± 8.2*	36.7 ± 4.8*
Colemanite	5 ppm	80.5 ± 8.3*	51.3 ± 6.1*	104.4 ± 9.8	95.3 ± 9.1*	100.8 ± 11.4	85.2 ± 8.0*	86.7 ± 8.3*	60.2 ± 6.3*	100.2 ± 10.4	50.7 ± 5.8*
	10 ppm	77.2 ± 8.0*	45.2 ± 5.4*	101.6 ± 9.6	94.5 ± 9.0*	94.0 ± 8.9*	86.3 ± 8.2*	79.3 ± 8.0*	60.8 ± 6.1*	90.9 ± 9.3*	44.2 ± 5.3*
	15 ppm	74.1 ± 7.7*	40.8 ± 5.2*	99.4 ± 10.3	86.2 ± 8.8*	91.2 ± 8.7*	79.5 ± 7.8*	74.0 ± 7.7*	51.4 ± 6.0*	81.6 ± 8.7*	39.1 ± 4.9*
	20 ppm	66.2 ± 7.1*	39.1 ± 5.0*	91.1 ± 9.3*	80.3 ± 8.5*	88.8 ± 8.4*	76.3 ± 7.8*	71.9 ± 7.4*	47.3 ± 5.7*	77.5 ± 8.2*	37.0 ± 4.6*
Ulexite	5 ppm	77.9 ± 8.1*	68.6 ± 7.3*	110.3 ± 12.4	104.2 ± 10.9	109.5 ± 11.8	103.7 ± 11.1	98.8 ± 10.3	88.4 ± 8.7*	115.2 ± 12.7	59.7 ± 6.4*
	10 ppm	82.3 ± 8.3*	61.4 ± 6.8*	114.5 ± 13.1	91.5 ± 8.8*	93.5 ± 9.1*	93.6 ± 10.7*	113.5 ± 13.7	75.4 ± 7.3*	104.1 ± 10.9	57.3 ± 6.2*
	15 ppm	85.6 ± 8.9*	55.2 ± 6.1*	107.2 ± 10.4	87.7 ± 8.1*	91.7 ± 8.8*	89.3 ± 9.2*	105.8 ± 11.0	63.5 ± 6.7*	92.3 ± 9.5*	49.1 ± 5.5*
	20 ppm	80.4 ± 8.5*	49.4 ± 5.9*	103.7 ± 10.6	83.5 ± 7.9*	87.5 ± 8.6*	83.2 ± 8.1*	93.1 ± 9.1*	57.1 ± 6.1*	83.5 ± 8.8*	42.1 ± 5.1*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

Table 4
CAT enzyme activities in human erythrocytes after the combined treatments of human lymphocytes with boron and heavy metal compounds for 1 h.

Control / Tested agents		267.9 ± 37.6 As_2O_3		267.9 ± 37.6 CBS		267.9 ± 37.6 $CdCl_2$		267.9 ± 37.6 $HgCl_2$		267.9 ± 37.6 $PbCl_2$	
		3 ppm	5 ppm	7.5 ppm	15 ppm	3 ppm	5 ppm	6 ppm	9 ppm	3 ppm	5 ppm
Boric acid	5 ppm	239.8 ± 32.7*	217.5 ± 26.6*	273.5 ± 43.6	269.2 ± 42.7	262.4 ± 33.1	241.4 ± 34.3*	237.5 ± 30.3*	193.7 ± 23.5*	245.4 ± 31.7*	208.7 ± 25.5*
	10 ppm	252.6 ± 35.1*	209.8 ± 24.8*	264.7 ± 41.0	249.1 ± 38.3*	275.5 ± 39.4	265.3 ± 37.1	248.7 ± 33.8*	216.4 ± 24.8*	233.7 ± 33.8*	201.6 ± 24.5*
	15 ppm	263.9 ± 39.3	214.2 ± 26.3*	259.1 ± 35.2	235.8 ± 30.7*	243.6 ± 30.7*	239.7 ± 32.0*	263.2 ± 34.3	208.5 ± 25.0*	252.8 ± 34.1*	193.8 ± 22.7*
	20 ppm	241.1 ± 31.5*	196.5 ± 24.6*	246.3 ± 31.8*	233.4 ± 29.4*	230.5 ± 25.4*	219.1 ± 27.6*	246.7 ± 31.7*	185.1 ± 23.6*	221.6 ± 30.5*	188.1 ± 21.8*
Borax (Tincal)	5 ppm	223.4 ± 29.6*	205.7 ± 25.7*	274.8 ± 39.6	265.5 ± 36.1	247.6 ± 34.3*	235.3 ± 32.8*	260.4 ± 35.9	209.3 ± 26.2*	240.8 ± 33.2*	203.2 ± 22.9*
	10 ppm	227.6 ± 30.9*	194.0 ± 24.3*	263.3 ± 35.7	253.4 ± 37.0*	242.7 ± 32.7*	231.8 ± 31.5*	238.5 ± 30.3*	191.2 ± 22.8*	229.1 ± 31.5*	189.5 ± 21.6*
	15 ppm	214.8 ± 26.3*	197.3 ± 25.9*	251.6 ± 36.3*	240.2 ± 32.9*	237.1 ± 31.4*	213.7 ± 26.1*	220.5 ± 27.3*	169.9 ± 23.2*	218.6 ± 26.3*	183.2 ± 24.5*
	20 ppm	203.6 ± 23.5*	180.7 ± 23.5*	245.6 ± 34.0*	231.6 ± 30.8*	230.9 ± 27.8*	206.5 ± 25.8*	207.6 ± 24.2*	159.8 ± 23.7*	207.6 ± 24.6*	178.4 ± 23.2*
Colemanite	5 ppm	221.2 ± 28.5*	203.8 ± 25.2*	261.8 ± 34.4	242.1 ± 33.2*	246.8 ± 36.2*	238.1 ± 29.5*	225.3 ± 26.1*	211.5 ± 24.8*	230.4 ± 31.1*	212.5 ± 26.4*
	10 ppm	209.8 ± 25.1*	191.6 ± 23.9*	250.3 ± 32.7*	236.5 ± 27.9*	244.1 ± 34.9*	229.0 ± 28.3*	221.8 ± 27.9*	200.9 ± 21.3*	226.8 ± 32.4*	203.0 ± 24.3*
	15 ppm	217.8 ± 28.2*	175.4 ± 21.8*	244.1 ± 33.2*	234.2 ± 29.1*	238.7 ± 33.6*	213.5 ± 23.6*	209.3 ± 24.5*	172.4 ± 20.7*	216.5 ± 28.9*	185.1 ± 25.3*
	20 ppm	196.7 ± 24.1*	168.8 ± 22.7*	240.6 ± 27.4*	229.5 ± 27.4*	231.5 ± 27.4*	198.2 ± 22.9*	198.4 ± 21.6*	151.2 ± 20.5*	208.8 ± 27.6*	171.5 ± 21.4*
Ulexite	5 ppm	234.2 ± 28.5*	209.8 ± 26.7*	282.5 ± 43.4	266.9 ± 36.2	264.7 ± 34.7	243.8 ± 36.4*	235.4 ± 31.4*	215.9 ± 27.4*	246.4 ± 32.5*	205.5 ± 24.0*
	10 ppm	265.5 ± 36.7	222.5 ± 29.3*	278.2 ± 40.9	260.5 ± 33.5	274.8 ± 38.5	262.4 ± 39.7	247.1 ± 32.9*	208.5 ± 26.7*	224.5 ± 29.8*	194.6 ± 25.4*
	15 ppm	274.8 ± 40.3	195.8 ± 25.3*	266.8 ± 36.8	248.9 ± 35.1*	241.5 ± 33.4*	234.5 ± 33.2*	271.3 ± 38.6	218.6 ± 25.3*	217.6 ± 27.8*	187.1 ± 22.3*
	20 ppm	249.1 ± 32.7*	178.1 ± 21.8*	260.1 ± 35.3	241.3 ± 32.6*	236.6 ± 31.2*	219.8 ± 25.8*	239.7 ± 31.5*	198.4 ± 24.8*	214.6 ± 27.1*	180.3 ± 23.3*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

Table 5
GSH-Px enzyme activities in human erythrocytes after the combined treatments of human lymphocytes with boron and heavy metal compounds for 1 h.

Control / Tested agents		93.6 ± 10.4 As_2O_3		93.6 ± 10.4 CBS		93.6 ± 10.4 $CdCl_2$		93.6 ± 10.4 $HgCl_2$		93.6 ± 10.4 $PbCl_2$	
		3 ppm	5 ppm	7.5 ppm	15 ppm	3 ppm	5 ppm	6 ppm	9 ppm	3 ppm	5 ppm
Boric acid	5 ppm	89.4 ± 9.6	69.1 ± 6.9*	96.2 ± 10.8	89.2 ± 9.9	87.6 ± 9.5	79.4 ± 8.4*	78.5 ± 8.9*	57.1 ± 6.4*	82.5 ± 9.1*	67.4 ± 7.6*
	10 ppm	95.5 ± 10.7	74.3 ± 8.3*	90.5 ± 10.3	82.9 ± 9.2*	91.1 ± 10.8	88.1 ± 9.5	89.1 ± 9.7	64.8 ± 7.3*	89.6 ± 9.9	79.1 ± 8.7*
	15 ppm	90.8 ± 10.3	55.4 ± 6.3*	93.6 ± 10.7	75.6 ± 8.5*	82.5 ± 9.1*	80.3 ± 9.2*	91.5 ± 10.3	60.2 ± 7.0*	80.4 ± 8.8*	77.5 ± 8.4*
	20 ppm	81.4 ± 9.3*	49.9 ± 6.5*	89.4 ± 9.7	71.7 ± 8.1*	74.1 ± 7.8*	72.6 ± 8.4*	84.3 ± 9.2*	55.3 ± 6.7*	76.1 ± 8.5*	63.1 ± 6.9*
Borax (Tincal)	5 ppm	74.1 ± 9.1*	61.1 ± 6.8*	92.4 ± 10.9	76.6 ± 8.6*	85.3 ± 9.7*	73.4 ± 8.3*	87.4 ± 9.3	60.3 ± 6.9*	87.9 ± 9.7	69.7 ± 7.7*
	10 ppm	79.4 ± 8.7*	62.2 ± 6.7*	87.1 ± 9.4	75.3 ± 8.4*	82.2 ± 9.5*	71.8 ± 8.2*	79.5 ± 8.6*	51.2 ± 6.8*	80.5 ± 8.8*	63.8 ± 7.3*
	15 ppm	70.5 ± 7.9*	57.4 ± 6.5*	80.2 ± 9.5*	69.2 ± 7.9*	76.4 ± 8.6*	64.2 ± 7.1*	66.3 ± 7.5*	50.1 ± 6.6*	80.2 ± 8.8*	57.1 ± 6.7*
	20 ppm	70.8 ± 8.1*	56.3 ± 6.4*	77.1 ± 8.6*	64.1 ± 7.3*	73.9 ± 8.7*	53.2 ± 6.1*	65.4 ± 7.2*	47.8 ± 5.9*	75.1 ± 8.1*	57.4 ± 6.9*
Colemanite	5 ppm	74.1 ± 8.1*	60.4 ± 7.4*	88.6 ± 9.6*	74.2 ± 7.7*	79.6 ± 8.7*	74.5 ± 8.5*	77.5 ± 8.7*	59.1 ± 7.1*	90.4 ± 10.1	68.3 ± 7.8*
	10 ppm	77.3 ± 8.7*	54.1 ± 5.8*	84.2 ± 9.3*	77.4 ± 8.0*	74.1 ± 7.8*	66.4 ± 7.1*	76.1 ± 8.6*	52.6 ± 6.4*	81.4 ± 9.2*	66.4 ± 7.5*
	15 ppm	66.1 ± 7.3*	47.4 ± 5.2*	77.8 ± 8.9*	69.2 ± 7.6*	70.5 ± 7.2*	61.3 ± 6.7*	71.3 ± 8.1*	47.7 ± 5.2*	74.5 ± 8.1*	58.6 ± 6.9*
	20 ppm	62.2 ± 6.9*	40.2 ± 5.4*	72.1 ± 8.4*	62.7 ± 7.4*	69.4 ± 7.2*	57.6 ± 6.6*	66.5 ± 7.4*	47.3 ± 5.3*	67.3 ± 7.2*	51.4 ± 6.5*
Ulexite	5 ppm	77.6 ± 8.9*	67.6 ± 7.5*	88.6 ± 9.7	80.1 ± 9.1*	90.2 ± 10.7	90.1 ± 10.4	72.6 ± 8.2*	66.7 ± 7.8*	89.7 ± 9.6	70.4 ± 8.1*
	10 ppm	89.5 ± 9.7	64.3 ± 7.7*	84.4 ± 9.6*	76.2 ± 8.7*	95.3 ± 11.2	89.2 ± 9.3	81.2 ± 8.9*	69.2 ± 7.4*	89.8 ± 9.7	70.6 ± 7.8*
	15 ppm	92.2 ± 10.5	62.7 ± 7.4*	81.1 ± 9.4*	78.7 ± 8.2*	83.5 ± 9.3*	81.6 ± 9.0*	89.6 ± 9.5	56.3 ± 6.8*	83.3 ± 9.4*	61.5 ± 7.1*
	20 ppm	82.8 ± 9.7*	59.3 ± 6.4*	76.9 ± 8.2*	71.2 ± 7.5*	79.1 ± 8.6*	71.7 ± 7.3*	88.4 ± 9.7	55.2 ± 6.6*	80.5 ± 8.6*	55.6 ± 6.4*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

Table 6
The amount of TGSH in human erythrocyte after concomitant treatments with boron and heavy metal compounds for 1 h.

Control / Tested agents		58.1 ± 8.4 As_2O_3		58.1 ± 8.4 CBS		58.1 ± 8.4 $CdCl_2$		58.1 ± 8.4 $HgCl_2$		58.1 ± 8.4 $PbCl_2$	
		3 ppm	5 ppm	7.5 ppm	15 ppm	3 ppm	5 ppm	6 ppm	9 ppm	3 ppm	5 ppm
Boric acid	5 ppm	53.7 ± 7.8	39.1 ± 5.8*	59.8 ± 8.9	51.2 ± 7.2	60.5 ± 8.1	51.7 ± 6.7	62.1 ± 8.4	52.7 ± 6.9	57.4 ± 6.4	43.4 ± 5.2*
	10 ppm	59.7 ± 9.2	37.7 ± 4.7*	54.4 ± 6.8	45.5 ± 5.9*	57.3 ± 7.4	48.5 ± 6.4*	54.4 ± 7.1	47.5 ± 6.1*	56.8 ± 7.4	42.7 ± 5.5*
	15 ppm	46.4 ± 6.2*	33.1 ± 5.4*	51.2 ± 6.9	41.2 ± 5.3*	46.6 ± 6.6*	44.6 ± 5.8*	46.9 ± 6.3*	41.3 ± 4.2*	45.5 ± 5.6*	37.5 ± 5.2*
	20 ppm	40.3 ± 5.2*	37.4 ± 4.8*	44.1 ± 5.8*	46.5 ± 6.2*	48.8 ± 5.3*	41.8 ± 5.9*	43.6 ± 5.8*	36.1 ± 4.4*	41.2 ± 5.1*	38.2 ± 4.8*
Borax (Tincal)	5 ppm	46.4 ± 6.3*	35.6 ± 4.8*	54.1 ± 7.1	41.1 ± 6.6*	53.9 ± 6.8	52.9 ± 7.1	51.9 ± 7.2	42.9 ± 5.6*	51.2 ± 6.5	40.2 ± 5.1*
	10 ppm	41.8 ± 5.2*	31.5 ± 5.2*	58.4 ± 8.2	43.4 ± 5.9*	50.2 ± 6.4*	47.1 ± 5.9*	44.1 ± 6.1*	41.6 ± 5.2*	44.1 ± 6.2*	34.1 ± 4.5*
	15 ppm	39.4 ± 4.7*	36.5 ± 5.1*	42.1 ± 6.9*	46.2 ± 6.1*	47.3 ± 5.5*	37.7 ± 5.2*	41.1 ± 5.4*	33.8 ± 6.1*	41.5 ± 4.7*	32.1 ± 4.4*
	20 ppm	37.2 ± 5.3*	29.3 ± 4.7*	46.3 ± 8.2*	39.8 ± 5.6*	47.2 ± 5.6*	39.7 ± 5.1*	42.5 ± 5.3*	37.6 ± 5.4*	38.2 ± 5.3*	31.5 ± 4.8*
Colemanite	5 ppm	40.2 ± 5.6*	30.7 ± 5.2*	54.2 ± 7.5	42.7 ± 6.1*	47.4 ± 6.2*	43.6 ± 5.7*	47.2 ± 6.1*	40.7 ± 4.8*	51.1 ± 6.5	41.8 ± 5.6*
	10 ppm	37.2 ± 4.9*	34.4 ± 4.1*	55.8 ± 7.3	44.5 ± 5.7*	41.8 ± 4.2*	42.5 ± 5.9*	41.1 ± 5.8*	36.6 ± 4.9*	45.5 ± 5.6*	39.4 ± 4.6*
	15 ppm	39.5 ± 5.1*	31.7 ± 5.4*	49.9 ± 6.1*	42.3 ± 5.1*	43.6 ± 5.2*	41.2 ± 5.7*	38.2 ± 4.4*	40.2 ± 5.2*	40.1 ± 5.3*	37.5 ± 4.6*
	20 ppm	35.1 ± 6.2*	34.1 ± 5.2*	42.2 ± 6.3*	42.7 ± 5.8*	42.6 ± 5.5*	36.2 ± 5.3*	38.9 ± 5.6*	38.9 ± 5.2*	37.4 ± 4.7*	34.2 ± 4.3*
Ulexite	5 ppm	55.7 ± 9.2	52.4 ± 6.1	61.2 ± 8.4	45.3 ± 6.5*	56.1 ± 6.2	52.4 ± 6.3	51.2 ± 6.2	44.2 ± 5.8*	54.3 ± 6.1	42.9 ± 6.1*
	10 ppm	47.2 ± 7.1*	44.2 ± 5.7*	59.7 ± 7.6	40.3 ± 4.6*	48.4 ± 6.1*	45.1 ± 5.8*	43.5 ± 5.7*	41.1 ± 5.6*	46.6 ± 5.9*	41.1 ± 5.5*
	15 ppm	41.2 ± 6.1*	37.8 ± 4.2*	54.9 ± 6.7	37.1 ± 5.7*	40.1 ± 5.1*	37.5 ± 5.9*	37.9 ± 5.1*	36.7 ± 4.3*	40.9 ± 5.2*	34.9 ± 4.7*
	20 ppm	39.6 ± 5.5*	39.4 ± 5.4*	46.8 ± 6.9*	39.2 ± 5.4*	44.2 ± 5.6*	40.7 ± 5.6*	40.6 ± 5.5*	38.5 ± 5.1*	41.2 ± 5.4*	37.4 ± 5.8*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

Table 7
The effects of As_2O_3, CBS, $CdCl_2$, $HgCl_2$ and $PbCl_2$ on SCE and MN rates in human blood cultures.

Groups		SCE/cell	MN/1000 cell
Control		4.5 ± 1.4	3.6 ± 0.8
As_2O_3	3 ppm	9.7 ± 1.9*	11.3 ± 1.9*
	5 ppm	16.7 ± 2.8*	17.6 ± 2.3*
CBS	7.5 ppm	6.7 ± 2.0*	6.9 ± 0.9*
	15 ppm	8.9 ± 2.3*	10.8 ± 1.3*
$CdCl_2$	3 ppm	7.8 ± 1.7*	9.8 ± 1.3*
	5 ppm	11.6 ± 2.2*	14.3 ± 2.7*
$HgCl_2$	6 ppm	9.5 ± 2.3*	10.8 ± 1.9*
	9 ppm	18.3 ± 2.5*	17.3 ± 2.9*
$PbCl_2$	3 ppm	8.9 ± 2.0*	11.8 ± 2.3*
	5 ppm	15.4 ± 2.6*	15.9 ± 3.1*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

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 × *g*) and estimation of activity was made with 1% haemolysates. One unit of catalase activity was defined as the activity required for degrading 1 μmol hydrogen peroxide in 60 s.

GSH-Px activity was measured in erythrocytes 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 hemoglobin. Conversion of NADPH was monitored continuously in a spectrophotometer at 340 nm for 3 min at 25 °C.

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

The content of plasma MDA was measured by the thiobarbutiric 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.

2.5. 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 6.5 mL of culture medium (Chromosome Medium B; Biochrom, Berlin) with 5 μg/mL of phytohemagglutinin (Biochrom). With the aim of providing successive visualization of SCEs, 5-bromo-2'-deoxyuridine (BrdU) (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 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 fluorescence plus Giemsa (FPG) procedure. For each treatment condition, well-spread twenty second division metaphases containing 42–46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per cell.

The MN test was performed by adding cytochalasin B (Sigma; final concentration 6 μg/mL) after 44 h of culture. 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 slides 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.

2.6. Statistical analysis

Statistical analysis was performed using SPSS software (version 13.0, SPSS, Chicago, IL, USA). The two-tailed Student's *t*-test was used to compare SCE and MN frequencies and between treated and control groups. For statistical analysis of biochemical data Duncan's test was used. Statistical decisions were made with a significance level of 0.05.

3. Results

The treatments with 5, 10, 15 and 20 ppm of BA, BX, COL and UX do not affect the MDA level of blood. Moreover, these compounds lead considerable positive alterations on antioxidant enzyme activities and TGSH level ($P < 0.05$). Our findings are similar to a previous study findings (Turkez et al., 2007). Therefore, the data are not presented. The effects on oxidative stress of As_2O_3, CBS, $CdCl_2$, $HgCl_2$, $PbCl_2$ alone are shown in Table 1. In present study, the concentrations of plasma MDA is found to be significantly elevated in the all heavy metal-treated groups compared to the control groups. Moreover, the activities of major enzymes of the antioxidant defense system, namely SOD, CAT, GSH-Px activities and levels of TGSH in erythrocytes are significantly decreased as due to metal exposures.

Tables 2–6 present the results of simultaneous and concomitant applications of boron compounds on the heavy metals. Noteworthy, the increases in the MDA level are inhibited after treatments of BA, UX (5 and 10 ppm) and BX (5 ppm) on the heavy metals ($P < 0.05$) (Table 2).

Moreover, the applications of BA and UX (5, 10 and 15 ppm), BX (5 and 10 ppm), COL (5 ppm) are found to significantly elevate the decreased activities of SOD, CAT and GSH-Px by low doses of metal compounds. Also, the low doses of BA and UX (5 and 10 ppm) are showed to be effective against high doses of CBS and $CdCl_2$ above-mentioned enzyme activities (Tables 3–5).

It can be seen from Table 6 that the decrease in the TGSH levels in erythrocytes induced by heavy metals can be prevented by the addition of BA (5 and 10 ppm), BX, COL and UX (5 ppm). But only 5 ppm of BA (for CBS, $CdCl_2$ and $HgCl_2$), BX (for $CdCl_2$) and UX (for As_2O_3 and $CdCl_2$) restore the TGSH level as compared to high doses of heavy metals alone applied groups.

Our results indicate that all BA, BX, COL and UX concentrations (5, 10, 15 and 20 ppm) do not cause any genotoxic effect in human lymphocytes compared with controls (data not shown). In contrast, all heavy metals (except for 7.5 ppm CBS) exhibit significant increases in SCE and MN rates compared to those in control cultures (Table 7).

After the combined treatments with boron and metal compounds, the boron compounds (BA, BX and UX) elicit a statistically significant reduction in metals-induced SCE, MN and CA frequencies (Tables 8 and 9).

Table 8
The SCE rates after the combined treatment of human lymphocytes with boron and metal compounds.

Control / Tested agents		4.5 ± 1.4 As_2O_3		4.5 ± 1.4 CBS		4.5 ± 1.4 $CdCl_2$		4.5 ± 1.4 $HgCl_2$		4.5 ± 1.4 $PbCl_2$	
		3 ppm	5 ppm	7.5 ppm	15 ppm	3 ppm	5 ppm	6 ppm	9 ppm	3 ppm	5 ppm
Boric acid	5 ppm	6.9 ± 1.6*	12.9 ± 2.6*	4.4 ± 1.6	5.7 ± 1.4	5.6 ± 1.5	8.6 ± 1.9*	6.8 ± 1.6*	13.4 ± 2.4*	5.5 ± 1.5	10.3 ± 2.0*
	10 ppm	6.1 ± 1.5	11.8 ± 2.1*	4.6 ± 1.5	6.6 ± 1.7*	6.3 ± 1.6	9.4 ± 1.8*	6.9 ± 1.7*	14.1 ± 2.5*	5.2 ± 1.6	8.7 ± 1.8*
	15 ppm	6.5 ± 1.7	14.2 ± 2.6*	5.8 ± 1.2	7.4 ± 1.6*	7.1 ± 1.7*	10.9 ± 2.1*	7.5 ± 1.9*	16.7 ± 2.8*	5.8 ± 1.5	12.4 ± 2.6*
	20 ppm	7.4 ± 1.8*	17.3 ± 2.5*	6.8 ± 1.5*	8.8 ± 2.0*	7.6 ± 1.5*	11.7 ± 2.0*	8.8 ± 1.8*	19.4 ± 2.7*	7.0 ± 1.7*	12.2 ± 2.5*
Borax (Tincal)	5 ppm	7.7 ± 1.9*	12.6 ± 2.2**	4.7 ± 1.5	6.9 ± 1.8*	6.7 ± 1.5*	8.9 ± 1.8*	7.4 ± 1.5*	14.8 ± 2.3*	6.2 ± 1.6	11.4 ± 2.4*
	10 ppm	8.3 ± 1.6*	13.9 ± 2.1*	6.2 ± 1.6	7.6 ± 1.6**	7.1 ± 1.7*	9.9 ± 1.7*	7.9 ± 1.6*	15.3 ± 2.6*	6.9 ± 1.7*	14.4 ± 2.6*
	15 ppm	9.2 ± 1.9*	16.5 ± 2.4**	6.7 ± 1.7*	8.5 ± 1.9*	7.8 ± 1.6*	11.1 ± 2.0*	9.3 ± 1.8*	17.7 ± 2.6*	7.9 ± 1.8*	13.9 ± 2.5*
	20 ppm	10.7 ± 2.0*	17.2 ± 2.1*	7.1 ± 1.6*	8.8 ± 2.1*	8.6 ± 1.8*	12.4 ± 1.9*	10.5 ± 1.9*	19.4 ± 2.9*	9.2 ± 2.0*	15.8 ± 2.7*
Colemanite	5 ppm	8.5 ± 1.9*	13.8 ± 2.7*	6.6 ± 1.3*	7.8 ± 1.8*	7.4 ± 1.5*	10.7 ± 2.1*	7.2 ± 1.7*	15.7 ± 2.8*	7.4 ± 1.5*	13.7 ± 2.2*
	10 ppm	9.6 ± 2.4*	16.4 ± 2.4*	6.8 ± 1.5*	8.4 ± 1.6*	7.9 ± 1.6*	11.5 ± 2.2*	8.7 ± 1.9*	14.8 ± 2.7*	7.7 ± 1.7*	14.8 ± 2.5*
	15 ppm	10.6 ± 2.1*	19.4 ± 2.3*	7.1 ± 1.7*	9.3 ± 1.9*	8.4 ± 1.7*	13.7 ± 2.4*	9.8 ± 2.0*	18.9 ± 2.9*	8.6 ± 1.9*	15.9 ± 2.9*
	20 ppm	11.5 ± 2.3*	18.8 ± 2.7*	7.6 ± 1.6*	10.7 ± 2.1*	8.9 ± 1.8*	12.6 ± 1.9*	11.3 ± 2.3*	20.2 ± 2.6*	9.5 ± 2.1*	17.3 ± 2.7*
Ulexite	5 ppm	7.3 ± 1.7*	11.7 ± 2.1*	4.5 ± 1.5	6.1 ± 1.3	5.8 ± 1.4	6.4 ± 1.5	6.1 ± 1.7	10.6 ± 2.1*	6.3 ± 1.7	11.1 ± 2.0*
	10 ppm	6.4 ± 1.6	9.7 ± 1.9*	4.2 ± 1.6	6.7 ± 1.8*	6.9 ± 1.6*	7.5 ± 1.8*	5.4 ± 1.2	12.5 ± 2.4*	7.4 ± 1.9*	10.7 ± 1.9*
	15 ppm	5.7 ± 1.3	13.4 ± 2.1*	4.6 ± 1.6	7.6 ± 1.7*	7.6 ± 1.8*	8.6 ± 1.7*	6.8 ± 1.8*	14.8 ± 2.7*	8.2 ± 1.8*	12.9 ± 2.2*
	20 ppm	6.1 ± 1.5	15.5 ± 2.6**	4.7 ± 1.4	8.0 ± 1.7*	7.8 ± 1.6*	10.4 ± 1.9*	8.1 ± 1.7*	15.4 ± 2.4*	7.9 ± 1.7*	13.8 ± 2.6*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

Table 9
The MN rates after the combined treatment of human lymphocytes with boron and metal compounds.

Control / Tested agents		3.6 ± 0.8 As_2O_3		3.6 ± 0.8 CBS		3.6 ± 0.8 $CdCl_2$		3.6 ± 0.8 $HgCl_2$		3.6 ± 0.8 $PbCl_2$	
		3 ppm	5 ppm	7.5 ppm	15 ppm	3 ppm	5 ppm	6 ppm	9 ppm	3 ppm	5 ppm
Boric acid	5 ppm	4.5 ± 1.0	12.4 ± 1.9*	3.7 ± 1.4	8.3 ± 1.3*	4.6 ± 1.3	9.6 ± 1.8*	7.6 ± 1.5*	14.6 ± 2.3*	3.3 ± 1.2	9.8 ± 1.7*
	10 ppm	3.4 ± 0.6	12.8 ± 1.6*	5.2 ± 0.8*	9.2 ± 0.7*	5.1 ± 0.8*	10.7 ± 1.6*	8.3 ± 0.9*	13.3 ± 1.6*	3.7 ± 1.5	10.4 ± 2.1*
	15 ppm	5.2 ± 1.1*	14.6 ± 1.8*	4.8 ± 1.5	11.6 ± 1.9*	6.8 ± 1.6*	12.9 ± 2.7*	9.6 ± 1.7*	15.9 ± 2.5*	4.4 ± 1.1	11.6 ± 1.4*
	20 ppm	6.9 ± 1.3*	15.9 ± 2.1*	6.6 ± 1.1*	10.7 ± 1.4*	8.3 ± 1.4*	12.5 ± 2.1*	10.5 ± 1.4*	17.1 ± 2.2*	6.7 ± 1.4*	13.5 ± 1.8*
Borax (Tincal)	5 ppm	4.8 ± 1.2	15.4 ± 3.2*	5.3 ± 0.9*	8.8 ± 1.0*	7.2 ± 1.6*	11.8 ± 2.8*	5.6 ± 1.3	12.7 ± 2.6*	8.6 ± 2.0*	13.4 ± 2.5*
	10 ppm	6.7 ± 1.6*	15.8 ± 1.9*	7.0 ± 1.5*	9.6 ± 1.8*	8.4 ± 1.1*	13.5 ± 1.9*	8.1 ± 1.6*	15.8 ± 3.1*	10.3 ± 1.6*	14.8 ± 2.1*
	15 ppm	7.8 ± 1.3*	17.3 ± 2.3*	7.6 ± 1.3*	10.3 ± 2.1*	10.6 ± 1.5*	14.7 ± 2.6*	8.8 ± 1.3*	17.9 ± 4.2*	11.2 ± 1.9*	16.1 ± 3.0*
	20 ppm	10.3 ± 1.5*	18.7 ± 2.6*	8.1 ± 1.6*	10.8 ± 1.4*	9.8 ± 1.4*	14.1 ± 2.1*	9.7 ± 1.7*	17.5 ± 2.6*	12.5 ± 2.2*	17.2 ± 2.8*
Colemanite	5 ppm	8.9 ± 2.0*	16.2 ± 2.4*	5.0 ± 1.5*	8.6 ± 1.2*	6.7 ± 0.9*	13.8 ± 2.5*	9.4 ± 1.5*	15.8 ± 1.8*	10.8 ± 1.6*	15.7 ± 1.8*
	10 ppm	12.4 ± 2.6*	16.9 ± 3.1*	6.5 ± 1.3*	10.5 ± 1.6*	7.9 ± 1.6*	15.9 ± 3.1*	9.1 ± 0.9*	14.5 ± 2.1*	9.7 ± 1.4*	15.2 ± 1.3*
	15 ppm	11.8 ± 1.7*	15.7 ± 2.4*	8.3 ± 1.4*	11.7 ± 2.7*	8.9 ± 1.7*	14.6 ± 2.4*	10.6 ± 1.7*	17.9 ± 2.3*	12.7 ± 3.1*	16.8 ± 2.4*
	20 ppm	12.0 ± 1.5*	17.8 ± 2.7*	8.9 ± 1.7*	11.1 ± 2.2*	10.9 ± 2.3*	15.1 ± 1.8*	11.4 ± 1.9*	18.6 ± 2.8*	13.5 ± 2.7*	17.5 ± 1.9*
Ulexite	5 ppm	4.4 ± 0.9	13.5 ± 2.3*	3.9 ± 0.7	7.6 ± 1.6*	5.7 ± 1.1	8.6 ± 1.9*	6.7 ± 1.4*	11.9 ± 2.2*	4.5 ± 1.2	11.8 ± 1.6*
	10 ppm	4.7 ± 1.3	12.5 ± 1.9*	5.5 ± 1.6*	8.7 ± 1.8*	7.8 ± 1.6*	10.6 ± 1.8*	5.8 ± 1.2*	11.6 ± 1.9*	8.8 ± 1.6*	13.7 ± 2.2*
	15 ppm	6.9 ± 1.8*	14.9 ± 2.7*	6.8 ± 0.9*	8.1 ± 1.3*	8.8 ± 1.4*	12.4 ± 2.1*	7.3 ± 1.4*	13.3 ± 2.2*	10.8 ± 2.3*	15.3 ± 3.1*
	20 ppm	7.1 ± 1.4*	14.3 ± 2.4*	7.1 ± 1.3*	8.5 ± 1.8*	8.2 ± 1.3*	11.7 ± 1.9*	9.8 ± 1.6*	14.5 ± 1.8*	12.1 ± 2.6*	16.4 ± 2.7*

Values are presented as mean ± S.D.; $n = 4$.
* Means significantly different from the control at the $P < 0.05$ level.

4. Discussion

Experimental investigations have repeatedly shown that erythrocytes are particularly sensitive to oxidative stress (Guemouri et al., 1991; Karaman et al., 2009). It is also interesting that BA can change the oxidative metabolism in animal systems (Kelly, 1997). However, it has not been replied that change of oxidative metabolism appears if as a result of the induction of oxidative stress or the supporting of antioxidant capacity (Hunt and Idso, 1999). At this context, the present investigation findings provide evidence for findings of our previous study. It is clearly proven that low doses of BA and other boron compounds (BX, UX and COL) (5–20 ppm) alone can increase the antioxidant capacity by increasing the enzyme activities in erythrocytes (Turkez et al., 2007). As known, the blood cells are potential vulnerable cells (Laakso et al., 2001). Undoubtly, antioxidant enzymes have main role in the defense of mammalian blood (Prasad et al., 2006). And, the knowledge of the vascular changes caused by boron compounds is critical to the effective pharmacokinetic researches (Erica et al., 2001). Therefore, genetic and oxidative studies will serve to evaluate and improve the therapeutic gain of boron compounds in blood tissue.

The heavy metals have adversely effects on red blood cells and peripheral white blood cells (Kolusari et al., 2008; Mendez-Gomez et al., 2008). However, the toxic effects of metals remain uncertain in blood tissue. Recent findings from studies may provide some insight. The data suggest that the oxidative stress is involved in the onset of metal toxicity in different cells (Esrefoglu et al., 2007; Kolusari et al., 2008). This suggestion is also supported by our findings. And, it is established that the order of heavy metal-induced oxidative status is As > Pb > Hg > Cd > Bi in plasma. Thus, the present study reveals that there is an increased oxidative stress in blood with heavy metals exposures as due to MDA level, and this situation may be associated with the increased SCE and MN frequencies in lymphocytes. Indeed, the increase in MDA after metal treatments is an important sign of oxidative stress and elevated lipid peroxidation in a variety of lipid systems, such as plasma, organs and cell membranes (Kasperczyk et al., 2009). Therefore, the most pronounced effects can arise in proliferating cells (Bengtsson et al., 2001). Thus, SCEs occur spontaneously in proliferating cells and is regarded as a manifestation of damage to the genome. It has been also commonly used as a test of mutagenicity in order to evaluate cytogenetic responses to chemical and heavy metal exposures in both *in vivo* and *in vitro* studies (Gebel et al., 1996; Ergene et al., 2007). Again, MN assay provides a measure of both chromosome breakage and chromosome loss or non-disjunction in clastogenic and aneugenic events, respectively (Karaman et al., 2009).

This work is noteworthy because almost the all boron compounds (BA, BX, UX and COL) present usefullness against different metal toxicities in human blood. The observation that, MDA level in plasma is significantly decreased by effects of boron compounds (5 and 10 ppm). And the order of effectiveness in preventing metal toxicity is BA > UX > BX > COL. It is reported that the metals are found mainly accumulated in the nucleus and, to a minor extent, in the nucleolus and in the cytoplasmic ribosomes. It is argued that this accumulation is indicative of the interaction with nucleic acids, especially with DNA (Kopf-Maier and Krahl, 1983). According to our findings, the main toxic effects of increasing doses of metal compounds involve decreased antioxidant enzyme levels. On the contrary, boron compounds lead to decreased SCE and MN formations in lymphocytes together the increasing activities of antioxidant enzymes in erythrocytes. In this point, the ability of specific enzymes to modulate the toxicity of metals is indisputable. As a matter of fact, the oxidative stress develops when the levels of antioxidants are lowered (Tapiero et al., 2004). And it is emphasized that the antioxidants (both enzymatic and non-enzymatic) can provide protection in mammalian cells against deleterious metal-

induced free radical attacks (Valko et al., 2005). In fact, SOD has a central role against to 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 is recorded that SOD activity in human erythrocytes has increased by boron supplement (as BA) (Nielsen, 1989). CAT activity protects against peroxide radicals lipids and proteins in erythrocyte membranes (Hunt and Idso, 1999). Our results also emphasize that there is a significant increase in activity of GSH-Px. GSH-Px can protect DNA and lipids of the cell against to peroxidation products (Bukowska, 2004).

As regards antioxidant and anti-genotoxic effects of the boron compounds in present study, UX compound provides an important protection against to As toxicity. This compound interferes with As(III). As known, the most toxic of arsenic species is As(III) and it exerts its cellular toxicity by binding to key sulfhydryl groups, which results in enzyme inhibition (Lee and Ho, 1994; Peraza et al., 1998). For this reason, the antioxidants may be used to reduce the incidence of SCEs induced by As in cultured human lymphocytes (Nair et al., 2004). At this context, glutathione is a major component of erythrocytes. Also, it is a thiol peptide and antioxidant that plays an important role in many xenobiotic detoxification reactions including arsenic detoxification (Lee and Ho, 1994). The fact that in present study is observed a significant interaction as due to glutathione level between boron compounds (BA and UX) and arsenic. As a matter of fact, UX (5 ppm) increases decreased glutathione level against As(III) (3 ppm). Moreover, the induction of glutathione by UX occurs even at high dose of As(III) (5 ppm). It is reported that the oxidative stress inversely related to intracellular glutathione levels and is exacerbated by glutathione depletion (Bray and Taylor, 1993). Because, glutathione also plays an important role in the antioxidant enzyme activities (Meister, 1983).

According to previous reports, CBS shows toxic effects in blood (Geyikoglu and Turkez, 2005; Geyikoglu et al., 2007). Our results obtained from testing CBS in this study are similar in some respects to what we see with arsenic, suggesting possible parallels in their toxicity mechanisms. And, the responses are also consistent with a genotoxic response to oxidative damage. Thus, the protective role of BA (5 ppm) is associated with increasing glutathione against the high dose of CBS (15 ppm).

Again, BA, UX and BX appear to be most effective for high Cd toxicity and BA for high Hg toxicity because of enzymatic activities and increased the TGSH level. Based on above-mentioned data, one common concept that has emerged is the role of the antioxidant preservative of the boron compounds in the metals-induced genotoxicity. Nevertheless, it is noteworthy that there is a different protection as related to the variety of compounds against metal-induced negative alterations. This is not surprising because many factors may also be suspected to be related to the metal carcinogenesis. These parameters are mineral deficiencies, interactions to metals of trace elements (Herman and Geraldine, 2009). As known, these metabolic disturbances may also be attributed oxidative stress in erythrocytes (Serban et al., 1994). For example, Cd decreases micronutrients as iron, calcium, and copper in plasma (Devirian and Volpe, 2003). On the other hand, dietary calcium deficiency increases lead toxicity in critical tissues as blood (Six and Goyer, 1970). Probably, mercury–selenium interactions cause toxicity and increasing permeability of the plasma membranes to calcium (Nath et al., 1996). At this point, boron-mineral interactions may not be ignored through transport in blood tissue of minerals. Because, boron (as BA) interferes with the metabolism and transport of calcium, magnesium, copper ions, selenium and glucose (Obreza, 2004). Again, BA may regulate membrane permeability (Bolanos et al., 2004). Although the exact mechanism is known, it is suggested that these parameters may be changed under the influence of tested boron compounds. At this context, these compounds need to be elucidated for these phenomena against metal toxicities.

In conclusion, this study firstly reveals that boron compounds provide an important protection against metal-induced damages in blood tissue. As regards to the genotoxicity associated with different metal toxicities, present results offer new antioxidant roles interfering oxidative DNA damage into the protective effects of the boron compounds and also suggest potential new directions for further study on the biological effects of the these compounds.

Acknowledgements

This investigation was supported part by National Boron Research Institute (BOREN) (Grant number: Ç0134). We are also grateful to all volunteers for the blood samples. The authors are also grateful to Dr. Savas Yesilyurt and Gokhan Yuksel for his proofreading the article.

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