Journal of Trace Elements in Medicine and Biology 24 (2010) 161–164

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

Biochemistry

The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats

Sinan Ince [a,*], Ismail Kucukkurt [b], Ibrahim Hakki Cigerci [c], A. Fatih Fidan [b], Abdullah Eryavuz [d]

[a] Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, 03200 Afyonkarahisar, Turkey
[b] Department of Biochemistry, 03200 Afyonkarahisar, Turkey
[c] Faculty of Science and Literatures, Department of Biology, 03200 Afyonkarahisar, Turkey
[d] Faculty of Veterinary Medicine, Department of Physiology, 03200 Afyonkarahisar, Turkey

ARTICLE INFO

Article history:
Received 15 September 2009
Accepted 7 January 2010

Keywords:
Borax
Boric acid
DNA damage
Lipid peroxidation
Oxidative stress

ABSTRACT

The aims of this study were to clarify the effects of high dietary supplementation with boric acid and borax, called boron (B) compounds, on lipid peroxidation (LPO), antioxidant activity, some vitamin levels, and DNA damage in rats. Thirty Sprague Dawley male rats were divided into three equal groups: the animals in the first group (control) were fed with a standard rodent diet containing 6.4 mg B/kg, and the animals in the experimental group were fed with a standard rodent diet added with a supra-nutritional amount of boric acid and borax (100 mg B/kg) throughout the experimental period of 28 days. The B compounds decreased malondialdehyde (MDA), DNA damage, the protein carbonyl content (PCO) level in blood, and glutathione (GSH) concentration in the liver, Cu–Zn superoxide dismutase (SOD), and catalase (CAT) activity in the kidney. The B compounds increased GSH concentration in blood and the vitamin C level in plasma. Consequently, our results demonstrate that B supplementation (100 mg/kg) in diet decreases LPO, and enhances the antioxidant defense mechanism and vitamin status. There are no differences in oxidant/antioxidant balance and biochemical parameters except for serum vitamin A and liver GSH concentration, between the boron compounds used in this study.

Introduction

Boron (B) compounds, most notably boric acid and borax, are used in the industrial sector for the preparation of disinfectants and drugs. However, B is primarily essential for plants, and some animals, and beneficial for humans in nutritional amounts, and is thus found in animal and human tissues at low concentrations [1]. B deficiency may occur in animals when their diet contains B at 0.3 mg/kg; the maximum tolerable level of B is 150 mg/kg diet [2].

B supplementation in animal and human nutrition may have important effects on various metabolic and physiological systems of the organism. Some studies have demonstrated that B has effects on the metabolism of minerals (Ca and P) [3], vitamin D [4], enzymes (aldehyde dehydrogenase, xanthine oxidase, cytochrome b_5 reductase) [4,5], hormones (insulin, estrogen, testosterone, T3, T4) [6,7], energy substrates (triglycerides, glucose) [8,9], and reactive oxygen species [10]. However, the biochemical mechanism of B is not yet completely known. Currently, two hypotheses have been advanced for the biochemical and physiological functions of B in animals, including humans. Firstly, B may play a role in cell-membrane functions that influence response to hormone action, trans-membrane signaling and trans-membrane movement of regulatory ions [11]. Secondly, B may act as a metabolic regulator in several enzymatic systems [12].

Oxygen free radicals are highly reactive and may cause damage in cells and tissues by interacting with cell membranes and organelles. Lipid peroxidation (LPO) is a ubiquitous phenomenon in the body under the influence of oxidative stress. Boron limits oxidative damage by enhancing the body's store of glutathione and its derivates, or by inducing other reactive oxygen species (ROS) neutralizing agents [13]. Garcia-Gonzalez et al. [14] suggested that B deficiency increases the concentration of superoxide dismutase (SOD), catalase (CAT), and peroxidase in Anabaena PCC 7119 heterocysts. In addition, Turkez et al. [10] observed that at low doses (15 mg/L) B compounds increased both SOD and CAT activities in erythrocytes versus control, while at high doses (500 mg/L) they decreased both SOD and CAT activities in erythrocytes. Pawa and Ali [15] found that when borax (4.0 mg/kg), as a B source, was administered orally to rats for three consecutive days at an interval of 24 h followed by the administration of thioacetamide (400 mg/kg), it offset the deleterious effects of thioacetamide by modulating the oxidative stress parameters; they suggested that boron could help to maintain the oxidant/antioxidant balance of the affected tissue. However,

* Corresponding author. Tel.: +90 2722281 312x130; fax: +90 2722281 349.
E-mail address: since@aku.edu.tr (S. Ince).

0946-672X/$ - see front matter
doi:10.1016/j.jtemb.2010.01.003

there is little research comparing the effects of different B sources on oxidative stress in tissues.

This study was conducted to investigate the effects of diet supplementation with boric acid and borax on LPO, antioxidant activity, some vitamin levels and DNA damage in rats.

Materials and methods

Materials

Chemicals

Boric acid (H_3BO_3) and borax ($Na_2B_4O_7 \cdot 10H_2O$) purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were used as test compounds. All the other chemicals and reagents were of analytical reagent grade and purchased from commercial sources.

Animals, diets, and experimental protocols

Thirty Sprague Dawley male rats (200–350 g) were purchased from the animal breeding laboratories of the Experimental Animal Research and Application Center (Afyon, Turkey) and divided into three groups consisting of 10 animals each. The animals were left for 2 days for acclimatization to animal room conditions. They were maintained under a 12-h light/12-h dark cycle at room temperature (25 ± 3 °C) and fed a standard rodent diet (Afyon Feed Industry, Afyonkarahisar, Turkey). Diet and water were provided to the animals ad libitum. The standard rodent diet was analyzed for B by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer 2100 DV, Waltham, Massachusetts, USA) after microwave (Berghof MWS-2, Eningen, Germany) digestion.

Boric acid and borax at 100 mg B/kg diet level were supplemented to the standard rodent diets of the boric acid and borax groups, respectively. This level of B is nontoxic for animals because when B compounds are given orally to animals for a short term, the LD_{50} values for boric acid and borax in laboratory animals (mice and rats) are in the range of approximately 400–700 mg B/kg of body weight [16,17]. The groups were fed for 4 weeks. The animals in the control group (control) were maintained on the standard rodent diet and water ad libitum, without administering any B compounds.

At the end of 4 weeks, blood samples were collected from all the rats after the animals were fasted overnight and sacrificed by anesthetizing with a combination of ketamine and xylasine HCl. Blood samples were obtained from each rat in the morning by using evacuated tubes (trace element-free vacutainer tubes) containing sodium citrate as anticoagulant and normal tubes. The blood samples were centrifuged at 3000*g* for 10 min at 4 °C, and then the plasma and serum were removed as completely as possible. Red blood cells were washed three times with 0.9% NaCl solution (phosphate buffer, pH: 7.2), and packed cells were hemolysed by adding an equal volume of cold distilled water.

The organs of the rats, including the liver, the kidney, and the heart, were removed immediately for examination of clinical biochemistry. Tissues were washed in ice-cold saline and homogenized 1:40 w/v in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. After centrifugation at 18000*g* for 15 min at 4 °C, the supernatant was extracted and kept at −30 °C in advance of assays.

The experimental protocols were approved by the Animal Care and Use Committee at Afyon Kocatepe University and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Methods

Biochemical analysis

Blood samples were separated into serum, plasma, and erythrocytes. Whole blood malondialdehyde (MDA) was estimated according to the method of Draper and Hardley [18], which is based on the coupling of MDA with thiobarbituric acid. Whole blood reduced glutathione (GSH) concentrations were assayed by the colorimetric method of Beutler et al. [19]. Erythrocytes were prepared according to Witterbourn et al. [20] and erythrocyte hemoglobin levels were determined as described by Fairbanks and Klee [21]. Cu–Zn SOD activity in erythrocytes was measured by the previously detailed method of Sun et al. [22]. CAT in erythrocytes was measured spectrophotometrically as described by Luck [23]. Plasma total antioxidant activity (TAA) and nitric oxide (NO_x) were measured according to a method proposed by Koracevic et al. [24] and Miranda et al. [25]. Endogenous lymphocyte DNA damage was analyzed by alkaline comet assay using a similar method described by Singh et al. [26]. The single-cell gel electrophoresis assay is a sensitive and powerful method for determining DNA strand breaks [26,27]. Protein carbonyl content (PCO) was measured by using the method of Levin et al. [28]. Serum vitamin A and β-carotene concentrations were estimated by the method of Suzuki and Katoh [29], and plasma vitamin C was measured by Kyaw [30]. The levels of MDA [31], GSH [19], SOD [22] and CAT [32] were also measured in liver, kidney, and heart tissue homogenates. Tissue protein content was assayed by the colorimetric method of Lowry et al. [33]. A Shimadzu UV-1601 visible spectrophotometer (Kyoto, Japan) was used for blood and tissue biochemical analysis.

Statistical analysis of data

The data obtained from the animal experiments were expressed as mean and standard error ($\pm$SEM). The statistical differences among the control, boric acid, and borax groups were evaluated by one-way ANOVA and Duncan post-hoc tests using the SPSS computer software program. A difference of $p < 0.05$ in the mean values was considered significant.

Results

The mean level of blood MDA ($p < 0.001$) was decreased and the mean level of blood GSH ($p < 0.05$) was increased by boric acid and borax supplementation compared to the control. There were no differences in the erythrocyte SOD and CAT activity levels among the groups (Table 1). The mean levels of SOD kidney ($p = 0.001$) and CAT kidney ($p < 0.001$) were decreased in the boric acid and borax groups compared to the control. Borax supplementation decreased ($p < 0.001$) liver GSH concentration compared to the control and boric acid groups (Table 1).

There were no differences in the mean of the TAA and NO_x levels among the groups. However, the vitamin A level was higher ($p < 0.05$) in the boric acid than in the borax group. The vitamin C level was higher in the boric acid and borax groups compared with the control ($p < 0.001$). There were no differences in β-carotene levels among the groups. DNA damage and PCO levels decreased in the boric acid and borax groups ($p < 0.05$) compared to the control (Table 2).

Discussion

In the present study, the rats in the all groups were fed with the same commercial standard rodent diet and the diet contained

Table 1
Effects of boric acid and borax on malondialdehyde (MDA), glutathione (GSH), Cu–Zn superoxide dismutase (SOD) and catalase (CAT) in blood, erythrocyte and tissues.

Parameters (n:10)	Sample	Control	Boric acid	Borax	P value
MDA (nmol/mL)	Blood	8.88 ± 0.52^{a}	5.94 ± 0.46^{b}	5.75 ± 0.18^{b}	0.000
MDA (nmol/g tissue)	Liver	1.99 ± 0.49	1.74 ± 0.24	1.69 ± 0.16	0.769
	Kidney	2.97 ± 0.19	3.81 ± 0.15	3.50 ± 0.90	0.090
	Heart	1.66 ± 0.60	1.20 ± 0.81	1.44 ± 0.36	0.617
SOD (U/mg Hb)	Erythrocyte	0.36 ± 0.03	0.35 ± 0.06	0.34 ± 0.07	0.971
SOD (U/μg protein)	Liver	0.10 ± 0.03	0.07 ± 0.05	0.03 ± 0.006	0.431
	Kidney	0.28 ± 0.04^{a}	0.09 ± 0.02^{b}	0.13 ± 0.02^{b}	0.001
	Heart	0.50 ± 0.20	0.25 ± 0.02	0.28 ± 0.10	0.332
CAT (k/g Hb)	Erythrocyte	27.39 ± 5.68	27.01 ± 7.61	18.49 ± 2.58	0.471
CAT (k/μg protein)	Liver	5.57 ± 0.41	5.53 ± 1.14	4.49 ± 0.53	0.559
	Kidney	3.70 ± 0.56^{a}	1.25 ± 0.17^{b}	1.64 ± 0.21^{b}	0.000
	Heart	0.95 ± 0.46	0.72 ± 0.13	0.27 ± 0.06	0.187
GSH (mg/dL)	Blood	28.04 ± 9.8^{b}	48.64 ± 11.13^{a}	54.24 ± 8.56^{a}	0.015
GSH (mg/g tissue)	Liver	17.1 ± 0.4^{a}	16.6 ± 0.5^{a}	14.2 ± 0.4^{b}	0.000
	Kidney	14.6 ± 0.6	15.2 ± 0.6	15.3 ± 0.8	0.720
	Heart	15.3 ± 0.3	14.3 ± 0.7	14.0 ± 0.3	0.209

[a,b] In the same line values with different letters show statistically significant differences in blood MDA, GSH liver, CAT kidney ($p < 0.001$), SOD kidney ($p = 0.001$) and blood GSH ($p < 0.05$).

Table 2
Effects of boric acid and borax on plasma total antioxidant activity (TAA), nitric oxide (NO_x), vitamin C, serum vitamin A, β-carotene, blood DNA damage and protein carbonyl content (PCO) in the groups.

Parameters (n:10)	Control	Boric acid	Borax	P value
TAA (mmol/L)	3.60 ± 0.57	4.02 ± 0.25	4.27 ± 0.48	0.571
NO_x (μmol/L)	10.92 ± 2.46	11.75 ± 3.24	13.13 ± 1.67	0.873
Vitamin A (μg/dL)	27.76 ± 0.72^{ab}	34.22 ± 1.07^{a}	23.21 ± 3.91^{b}	0.015
β-carotene (μg/dL)	4.37 ± 0.31	4.92 ± 0.41	3.54 ± 0.60	0.126
Vitamin C (mg/dL)	0.15 ± 0.05^{b}	0.27 ± 0.02^{a}	0.25 ± 0.02^{a}	0.000
DNA damage (AU)	25.5 ± 2.77^{a}	$22.75 \pm 2{,}60^{b}$	21.87 ± 2.47^{b}	0.030
PCO (nmol/mL)	2.09 ± 0.16^{a}	1.82 ± 0.27^{b}	1.85 ± 0.19^{b}	0.036

[a,b] In the same line values with different letters show statistically significant differences in vitamin C ($p < 0.001$), vitamin A, DNA damage and PCO ($p < 0.05$).

6.4 mg B/kg. Hunt [12] reported that the physiological amount of B in animal diet is 3 mg/kg, and its deficient level is ≤ 0.3 mg/kg. Nielsen [34] found depressed growth in rats fed with a diet containing 0.3–0.4 mg/kg B. If those reports are applied to our study, then the amount of B (6.4 mg/kg diet) in the standard rodent diet in the present study was sufficient for animals.

Oxidative stress is originally defined as the disequilibrium between prooxidants and antioxidants in biological systems [35]. Increased oxidative stress refers to an imbalance between the intracellular production of free radicals and the cellular defense mechanisms, and MDA is one of the most important oxidative stress markers. Until now, no studies have been done to compare boric acid with borax supplementation in animal diet, as boron sources at high but nontoxic levels, in terms of their effects on oxidant/antioxidant balance. In the present study, both boric acid and borax supplementation significantly decreased MDA concentration in blood, which shows that there is no difference in the decreased free radical damage in cells between boric acid and borax supplementation. Turkez et al. [10] reported that B did not alter MDA concentration at low doses (5–50 mg/L) but increased it at high doses (500 mg/L) in human peripheral blood cultures exposed to various doses (5–500 mg/L) of B compounds. Our data indicate that diet supplementation with 100 mg B/kg has a protective effect against oxidative damage in blood. However, Mohora et al. [36] reported that 80 mg/kg boric acid supplementation in diet increased the MDA level in rat liver tissue. In our study, both boric acid and borax numerically decreased MDA concentration in the liver and the heart, but increased it in the kidney. The increased LPO in the kidney may be due to the utilization of excess energy because B is excreted primarily in urine [12].

Oxidative stress develops when the levels of antioxidants are lowered. Thus, the activities of antioxidant enzymes (SOD, CAT, GSH) are important in cell defense [22,37]. In the present study, erythrocyte SOD and CAT activities were not affected by both boric acid and borax supplementation in the diet of rats. Similarly, B supplementation unchanged SOD and CAT activities in the heart and the liver, but decreased in the kidney because of the elimination of B through the kidney. This result supported the results reported by Mohora et al. [36] that liver SOD activity was not affected by boric acid in rats. Turkez et al. [10] reported that B compounds at low concentrations increased the GSH level in erythrocytes. In the present study, B supplementation in diet enhanced the GSH level in blood, with a concomitant decrease in lipid peroxidation. This result is consistent with those of Hunt and Idso [13]. However, B supplementation decreased the GSH level in the liver. Generally, the consumption of high energy, associated with GSH depletion, is exhibited in the liver, which is the largest GSH reservoir [15]. This result supported the findings reported by Mohora et al. [36] that the GSH level decreased in rat liver tissue after 90 days of treatment with 40 mg/kg boric acid.

Antioxidant capacity is an important factor in all physiological standards, and for the performance of humans and all animals [39,40]. This study showed that dietary B supplementation did not alter the plasma antioxidant capacity of rats when compared to control animals. However, this result is inconsistent with the findings reported by Turkez et al. [10] that, at low doses, B compounds increased TAA in humans. The discrepancy between our study and that of Turkez et al. [10] may be attributed to the procedures used to measure TAA. Turkez et al. [10] determined TAA in erythrocytes under in vitro conditions while we measured it in plasma, which contains many nonspecific antioxidants such as urea, uric acid, and proteins. High production of NO has been suggested as a cause of tissue injury [40]. In the present study, B supplementation had no effect on plasma NO_x, which may indicate that B supplementation does not affect NO production.

Non-enzymatic antioxidant compounds such as vitamin C and β-carotene minimize the damaging effects of ROS in the body [37]. β-carotene is a quencher of singlet oxygen and has the ability to react directly with the peroxyl radicals involved in LPO [41,42]. In the present study, B supplementation had no effect on serum β-carotene and vitamin A concentrations. However, the rats fed with

boric acid had significantly higher vitamin A concentration than the rats fed with borax. This result is probably due to the effect of low blood B concentration on the conversion of β-carotene to vitamin A in the liver, because B in borax is less absorbed from the gastrointestinal tract than B in boric acid [12,38]. Our results indicate that boric acid as a B source is more beneficial for vitamin A concentration in the blood than borax. Vitamin C is a major water-soluble antioxidant and acts as the first defense against ROS in whole blood and plasma [43]. B supplementation increased plasma vitamin C concentration in this study. This could explain the decrease in LPO levels in blood due to B supplementation.

DNA damage induced by free radicals is of great importance because it initiates and promotes carcinogenesis [44]; DNA is exposed to attack by free radicals approximately 10.000 times a day [45,46]. B supplementation decreased DNA damage in this study. Our results provide the first data about the effects of B supplementation in the form of boric acid or borax in the diet of animals on DNA damage using comet assay on rats. Free radicals react with proteins and modify amino acid residues by oxidation, nitrosation, and carbonylation [27]. The plasma PCO significantly decreased in the boric acid and borax groups compared to the control in this study. This result shows the positive effect of B on proteins against oxidation in rats and may explain with the antioxidant activities and depletion of free radicals generation of B.

Our results demonstrate that B supplementation (100 mg/kg) in diet decreases LPO, and enhances the antioxidant defense mechanism and vitamin status. There are no differences in oxidant/antioxidant balance and biochemical parameters, except for serum vitamin A and liver GSH concentration, between the boron compounds used in this study. Therefore, the application of boron compounds in rat increased livability which, in turn, might be enhance life span.

References

[1] Environmental Protection Agency. Toxiclogical review of boron and compounds. Integrated Risk Information System (IRIS) Washington DC, 2004; EPA/635/04/052.
[2] McDowell LR. Boron. In: Cunha TJ, editor. Minerals in animal and human nutrition. London: Academic Press; 1992. p. 367–8.
[3] Meacham SL, Taper LJ, Volpe SL. Effects of boron supplementation on bone mineral density and dietary, blood, and urinary calcium, phosphorus, magnesium, and boron in female athletes. Environ Health Perspect 1994;102:79–82.
[4] Hunt CD. Biochemical effects of physiological amounts of dietary boron. J Trace Elem Exp Med 1996;9:185–213.
[5] Devirian TA, Volpe SL. The physiological effects of dietary boron. Crit Rev Food Sci Nutr 2003;43:219–31.
[6] Nielsen FH, Hunt CD, Mullen LM, Hunt JR. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. Faseb J 1987;1:394–7.
[7] Armstrong TA, Spears JW, Lloyd KE. Inflammatory response, growth, and thyroid hormone concentrations are affected by long-term boron supplementation in gilts. J Anim Sci 2001;79:1549–56.
[8] Hunt CD. Boron homeostasis in the cholecalciferol-deficient chick. Proc Natl Acad Sci 1988;42:60.
[9] Eren M, Kocaoglu-Güclü B, Uyanik F, Karabulut N. The effects of dietary boron supplementation on performance, carcass composition and serum lipids in Japanese quails. J Anim Vet Adv 2006;5:1105–8.
[10] Turkez H, Geyikoglu F, Tatar A, Keles S, Özkanc A. Effects of some boron compounds on peripheral human blood. Z Naturforsch 2007;62:889–96.
[11] Nielsen FH. Nutritional requirements for boron, silicon, vanadium, nickel and arsenic: current knowledge and speculation. Faseb J 1991;5:2661–7.
[12] Hunt CD. The biochemical effects of physiologic amount of dietary boron in animal nutrition models. Environ Health Perspect 1994;102:35–43.
[13] Hunt CD, Idso JP. Dietary boron as a phsiological regulator of normal inflammatory response: a review and current research progress. J Trace Elem Exp Med 1999;12:221–33.
[14] Garcia-Gonzalez M, Mateo P, Bonilla I. Boron requirement for envelope structure and function in Anabaena PCC 7119 heterocysts. J Exp Bot 1991;42:925–9.
[15] Pawa S, Ali S. Boron ameliorates fulminant hepatic failure by counteracting the changes associated with the oxidative stress. Chem-Biol Interact 2006;160:89–98.
[16] Pfeiffer CC, Hallman LF, Gersh I. Boric acid ointment: a study of possible intoxication in the treatment of burns. J Am Med Assoc 1945;128:266–74.
[17] Weir RJ, Fisher RS. Toxicologic studies on borax and boric acid. Toxicol Appl Pharmacol 1972;23:351–64.
[18] Draper HH, Hardley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421–31.
[19] Beutler E, Dubon OB, Kelly M. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882–8.
[20] Witterbourn CC, Hawkins RE, Brain M, Carrel W. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1975;55:337–41.
[21] Fairbanks VF, Klee GG. Biochemical aspect of haematology. In: Tiez NW, editor. Fundamentals of clinical chemistry. Philadelphia: WB Saunders; 1987. p. 803–4.
[22] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497–500.
[23] Luck H. Catalase. In: Bergmeyer HU, editor. Methods in analysis. London: Academic Press; 1955.
[24] Koracevic F, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. Clin Pathol 2001;54:356–61.
[25] Miranda KM, Espey MG, Wink AD. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001;5:62–71.
[26] Singh PN, Mccoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.
[27] Kocyigit A, Keles H, Selek S, Guzel S, Celik H, Erel O. Increased DNA damage and oxidative stress in patients with cutaneous leishmaniasis. Mutat Res 2005;585:71–8.
[28] Levin RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–78.
[29] Suzuki J, Katoh NA. Simple and cheap methods for measuring serum vitamin-A in cattle using only a spectrophotometer. Nippon Juigaku Zasshi 1990;52:1281–3.
[30] Kyaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. Clin Chim Acta 1978;86:153–7.
[31] Ohkawa H, Ohishi N, Yagi Y. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.
[32] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
[33] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[34] Nielsen F.H. Trace element metabolism in man and animals-5 Abstracts. Aberdeen, Scotland; 1984. p. 26.
[35] Sies H. Oxidative stress: introductory remarks. In: Sies H, editor. Oxidative stress. London: Academic Press; 1985. p. 1–8.
[36] Mohora M, Boghianu L, Muscurel C, Duta C, Dumitrache C. Effects of boric acid on redox status in the rat liver. Romanian J Biophys 2002;12:77–82.
[37] Scandalios JG. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz J Med Biol Res 2005;38:995–1014.
[38] Nielsen F.H. Dietary boron affects variables associated with copper metabolism in humans. In: Anke M, Baumann W, Braunlich H, Brückner C, Groppel B, Grün M, editors. 6th international trace element symposium, Karl-Marx-Universitat, Leipzig and Friedrich-Schiller-Universitat, Jena, Germany, 1989. p. 1106–11.
[39] Draper HH, McGirr LG, Hadley M. The metabolism of malondialdehyde. Lipids 1986;21:305–7.
[40] Bohloli M, Uzun H, Aytac E, Toklu AS, Paksoy M, Durak H, et al. Hyperbaric oxygen (HBO) therapy after partial hepatectomy: an experimental study on oxidative stress in rats. Scand J Lab Anim Sci 2007;34:131–40.
[41] Krinsky NI. Carotenoids as antioxidants. Nutrition 2001;17:815–7.
[42] Paiva SAR, R.M. Russell. β-carotene and other carotenoids as antioxidants. J Am Coll Nutr 1999;18:426–33.
[43] Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE, et al. Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. AIDS 1998;12:1653–9.
[44] Saravan R, Pugalendi KV. Assessment of the pharmacological effect of silymarin on ethanol induced DNA damage by sindle cell gel electrophoresis. Indian J Pharmacol 2005;37:261–2.
[45] Halliwell B, Dizdaroglu M. The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. Free Radical Res Commun 1992;16:75–87.
[46] Aruoma OI, Halliwell B, Dizdaroglu M. Iron ion-dependent modification of bases in DNA by the superoxide radical-generating system hypoxanthine/xanthine oxidase. J Biol Chem 1989;264:13024–8.