Effect of boron on trace element level and oxidative stress in paracetamol induced hepatotoxicity model

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ABSTRACT

In this study, we have aimed to determine the relationship between the levels of total antioxidant-oxidant capacity, trace elements and protein carbonyl levels in paracetamol induced hepatotoxicity model and the effect of boron. We investigated the efficacy and antioxidant role of boron, which has the characteristics of protecting the oxidant/antioxidant system in tissues and increasing the glutathione depots, in reducing the negative effects of paracetamol which is known to cause widespread and high doses of hepatotoxic damage. Trace elements are essential substances for the human body and are found in small amounts. In addition to the role of cofactors in many enzymes, also required for function. The experimental animals used in our study were divided into seven groups: Control, 2 g/kg paracetamol, 2 g/kg paracetamol + 50 mg/kg boric acid, 2 g/kg paracetamol + 100 mg/kg boric acid, 2 g/kg paracetamol + 200 mg/kg boric acid, 2 g/kg paracetamol + 140 mg/kg N-acetylcysteine 200 mg/kg boric acid. As a result of the measurements, serum paracetamol aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels and analysis of tissue samples as a result of protein carbonyl (POC), total oxidant level (TOS) and oxidative stress index (OSI) levels increased, boron given groups significant decrease was observed. Paracetamol group protein thiol (P-SH), total antioxidant level (TAS) and trace element levels decreased while there was a significant ($p < 0.05$) increase in boron groups. Trace element levels (Mg, Cu, Zn, Se) were significantly lower in paracetamol groups compared to control group ($p < 0.05$) and Trace element levels (Mg, Cu, Zn, Se) were significantly ($p < 0.05$) increased boron supplement group compared to paracetemol group. As a result, we observed that the damage caused by the paracetamol to the oxidant / antioxidant balance decreased with boron supplementation and it can be said that the boron exhibits antioxidant properties based on its effects on different parameters.

1. Introduction

Paracetamol, (Acetaminophen, N-acetyl-p-aminophenol, N- (4-hydroxyphenyl) acetamide, APAP) is an analgesic and antipyretic drug that is used safely at therapeutic doses. Paracetamol is an alternative to aspirin with the same effect. Because paracetamol does not contain many of the side effects of aspirin. The fact that it is inexpensive and easily accessible and can be purchased without a prescription increased the use of paracetamol and brought with it the risk of toxicity [1,2]. Paracetamol is safe when used in therapeutic doses, but in children over the age of 6 years and in adults, 200 mg/kg is taken once or 10 grams of paracetamol is taken in 24 hours, resulting in hepatic and renal damage. Similar conditions can be seen in children under 6 years of age when taking paracetamol 200 mg / kg or more [1-3].

Paracetamol is mainly metabolized in the liver, via conjugation with glucuronic acid and sulphate, into two main nontoxic metabolites which are excreted via the urine. However, a small amount of paracetamol (%2-5) is metabolized by the cytochrome P450 (CYP450) enzyme system forming a highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which causes toxicity via an oxidative pathway [3-5]. NAPQI is a highly reactive molecule. At therapeutic doses, it reacts with reduced glutathione (GSH) to from a nontoxic conjugate that will be excreted [4,6,7]. Excessive NAPQI depletes hepatic GSH and covalently binds to cellular macromolecules, resulting in oxidative stress reactions, dysfunction of mitochondria and DNA damage. The toxic NAPQI that occurs after high doses causes the formation of reactive oxygen products and lipid peroxidation. GSH deficiency occurs and ultimately leads to impaired protein synthesis and intracellular calcium balance in hepatocytes These events ultimately may result in direct damage to hepatocytes, leading to cell death of hepatocytes [5,6,8]. Nowadays, various

studies have been carried out that boron element may have antioxidant effect and it has been reported that boron allows the continuity of oxidant / antioxidant balance in the body. Oxidative stress occurs when some reactive oxygen species such as superoxide radical (O_2^{-*}) are too large to detoxify in the cell. Reduced glutathione (GSH), one of the antioxidants that detoxify these reactive oxygen species, is reduced by oxidation in the formation of oxidative stress. Boron prevents oxidative damage by increasing the stocks of glutathione and 2 derivatives in the body, or by inducing agents which neutralize other reactive oxygen species. [9,10]. In addition, oxidized glutathione (GSSG) needs nicotinamide adenine dinucleotide phosphate (NADPH) in order to be re-reduced and to influence reactive oxygen species.. The importance of borun here is effective in regulating NADPH levels, and NADPH, which provides increased levels of reduced glutathione (GSH), is to reduce oxidative stress and oxidative stress-induced damage [9,10].

Zinc, copper, manganese and selenium are inorganic compounds essential for life found in tissues in milligram per kilogram level or less [11]. They have also been described as substances that make up less than 0.01 percent of the dry weight of the body [12,13]. These micronutrients are known to specifically play roles in many physiological process; for example copper, manganese and zinc are an integral component of many metalloenzymes e.g. superoxide dismutase [14]. Zinc and copper have been reported to interact with one another in their antioxidant roles. Graetke & Chow [15] have identified a number of biomolecules capable of modulating the cellular effects of copper.

The aim of this study is to investigate the effect of boron in vivo on trace elements and oxidative stress caused by overdosing of paracetamol in paracetamol induced hepatotoxicity model and to contribute to the literature with information that will be used in other studies.

2. Materıals and methods

2.1. Materials

This study was carried out in the Scientific Analysis and Technological Application Center of Uşak University and in the Experimental Animals Research and Application Center of Afyon Kocatepe University (AKU).

2.2. Experimental animals

The part to be studied with experimental animals is planned as 24 hours. Rats were allowed to adapt to the animal facility at least 1 week before the experiments began. In this study, we used 70 male albino Wistar rats, obtained from the Afyon Kocatepe University (AKU) Experimental Animal Research and Application Canter. The animals weighed between 250-300 g and were kept under normal temperature conditions

(22-24ºC) in separate groups. The standard diet and water access of animals is provided until they are satisfied. The animal care and experimental protocols were approved by the Experimental Animal Ethics Committee Afyon Kocatepe University, (Afyonkarahisar, Turkey).

2.3. Preparation of chemicals

Application of paracetamol: In this study, 2 g/kg dose Phosphate buffer saline (PBS) 1% Carboxic methyl cellulose (CMC) solution was prepared for paracetamol rats. The prepared suspension was applied orally with gavage. The application was determined according to the related literature [7]. Four hours after the application of paracetamol, the rats in the whole group were given enough water and food until the end of the experiment.

Paracetamol doses were determined according to the literature [16].

Application of N-acetyl cysteine (NAC): In the study, 600 mg single tablet NAC prepared in 0.9% NaCl solution was given orally via gavage in appropriate doses.

Application of boron: Boric acid (H3BO3) as a boron source was obtained from tocris (cat no. 3177). Boric acid dissolved in serum physiological was administered to experimental animals by gavage at doses of 50, 100 and 200 mg/kg.

2.4. Method

The rats were divided in seven groups consisting of ten animals each. All groups were left hungry 24 hours before the experiment. Experiment groups and details are shown below.

Group I control: 2 ml PBS was given by gavage.

Group II (Paracetemol): At a dose of 2 g / kg, 2 ml of paracetamol solution was administered orally by gavage**.**

Group III (Paracetemol+ 50 mg boric acid): After administration of 2 g / kg oral paracetamol, 50 mg / kg boric acid was administered.

Group IV (Paracetemol + 100 mg boric acid): After administration of 2 g / kg oral paracetamol, 100 mg / kg boric acid was administered.

Group V (Paracetemol + 200 mg boric acid): After administration of 2 g / kg oral paracetamol, 200 mg / kg boric acid was applied.

Group VI (NAC + paracetamol): NAC 140 mg / kg + Paracetamol 2 g / kg was given. 140 mg / kg NAC (N-Acetyl Cysteine) was administered orally, 2 ml paracetamol was administered at a dose of 2 g/kg 1 hour after oral administration. After 12 hours, NAC application was repeated.

Group VII (200 mg boric acid): 200 mg / kg boric acid alone was applied.

Termination of the study

After administration of the drugs, the rats were allowed food for the following 24 h until they were sacrificed [(ketamin (65 mg / kg, i.p) - ksilazin (7 mg / kg, i.p)]. Blood sample were colleced under anesthesia. After sacrifice, the liver was removed immediately and kept at -80 ° C until the day of analysis.

2.5. Biochemical investigations

Analysis of liver tissue

The liver tissues were homogenized with tissue homogenizer by adding 5 ml ph 7,4 phosphate buffer for each sample. Homogenized tissue samples were taken into glass tubes and cooled Nüve NF 800R brand centrifuge was centrifuged at 4000 rpm at +4 OC for 10 min. The supernatant portion was taken and hidden to work later. For each analysis, the samples were solved and processed.

Determination of protein carbonyl groups (PCO) in tissue

Protein carbonyl groups in tissue samples Levine et al. studied according to the modified spectrophotometric method [17].

When the carbonyl groups are combined with the DNP, a colored hydroxy is formed and the absorption of the hydrase is studied at a wavelength of 360 nm.

Procedure:

500 μl sample was mixed with 20% tricloroaceticacitance (TCA). The samples were centrifuged at 4000 rpm for 15 minutes and supernatant poured. Pelet was mixed with 500 μl DNP and kept at room temperature in the dark for 1 hour. Each 10 min was vortexed to treat the pellet with DNP. Then it was mixed with 500 μl of 20% TCA and kept at room temperature for 2-3 minutes. After centrifugation at 4000 rpm for 3 min, the supernatant was poured and the same procedure was repeated three times with 10% TCA. The precipitate was dissolved in 2 ml of 1 M NaOH at 370 C for 30 minutes. The absorbance of the sample was studied on the Shimadzu UV-mini 1240 spectrophotometer at a wavelength of 360 nm against the NaOH blank.

The results were given as μmol / mg protein using εmax = 22000 M-1.cm-1

Determination of protein sulfhydryl (–Sh) groups

Protein-SH groups were determined according to the spectrophotometric method of Koster et al. [18]. Protein –SH groups are reduced by 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and released a chromophore (5-merkapto-2-nitrobenzoic acid) by creating a disulfide bond. The absorption of the chromophore formed was determined to be 412 nm wavelength.

Procedure:

150 μL phosphate buffer was added on 10 μL sample and 40 μL DTNP (1% sodium citrate) was added and kept at 370 C for 5 min. The absorbance of the sample was read in the ELISA reader against reactive blank at 412 nm wavelength.

The results were given as μmol/mg protein using Emax = 13600 $M^{-1}.cm^{-1}$

Total antioxidant status (TAS)

Total antioxidant status (TAS) in tissue samples was measured by using Rel Assay brand commercial kits. The measurement method is based on the fact that all the antioxidant molecules in the sample are deoxorised in proportion to the total concentration of the antioxidant molecules in the colour as a result of reduction of the colour ABTS cationic radicals. Trolox is a water soluble analog of vitamin E, which is used as the calibrator. Results are expressed as mmol Trolox Equivalent/L [19].

Total oxidant status (TOS)

The total oxidant status of samples (TOS) was measured using Rel assay brand commercial kits. The measurement was performed by colorimetric method based on oxidation of oxidant molecules to ferrous ion to ferric ion [20]. Results were expressed as μmol H_2O_2 Equivalent/L.

Calculation of oxidative stress index (OSI)

Oxidative stress index (OSI), an indicator of oxidative stress, is expressed as the percentage of total oxidant status/level (tos) levels to total antioxidant status/level (TAS) levels. When calculating the Oxidative Stress Index (OSI) of the samples, TAS levels are converted to μmol units. The results were expressed in Arbitrary Units (AU).

Determination of trace element (Mg, Cu, Mn, Zn, Co, Se) levels

When preparing the sample, dilution process was applied with 3% HNO3 (nitric acid). 1 ml sample + 9 ml 3% HNO3 (nitric acid) was added to the samples which were diluted 1/10. Then, centrifugation was performed at 4000 rpm for 10 min +4 ° C and the supernatant was filtered and prepared for Thermo Scientific Neslab ThermoFlex2500 ICP-MS device. The nitric acid solution was used to remove organic substances from the samples. After data entry of standards such as 0.01, 0.05, 0.1, 0.5, 1 and 5 ppm, the samples were given to the device for reading and the results were evaluated as ppm.

AST ALT measurement

Bloods taken to the biochemistry tube were centrifuged at 4000 rpm for 10 minutes at +4 ° C and stored at -80 °C until analysis. AST and ALT analysis were performed in 8 ml gel tubes (Lot No: 7163845) at Uşak Medical Park hospital biochemistry laboratory. Then AST and ALT levels were measured in Abbott C4100 brand integrated autoanalyser biochemistry instrument.

2.6. Statistical analysis

The data were analyzed using SPSS-18 computer program and the results were given as mean ± standard deviation (SD). After the homogeneity of the groups was tested, Tukey HSD and Duncan were used in one-way ANOVA test to find differences between groups. P>0.05 değeri istatistiksel açıdan anlamlı kabul edildi. Differences were considered statistically significant at P<0.05.

3. Results

3.1. Results of TAS from liver tissue

Total antioxidant values were significantly lower in the PARA (paracetemol) group than in the PARA + 50 BA (boric acid), PARA + 100 BA, PARA + 200 BA, PARA + NAC, and 200 BA groups (P <0.05). The results are shown in Table 1. TAS values of the PARA + NAC group were significantly lower than the control, PARA + 50 BA, PARA + 100 BA, PARA + 200 BA, 200 BA groups and significantly higher than the PARA group (P <0.05). There was no significant difference between the control, PARA + 50 BA, PARA + 100 BA, PARA + 200 BA and 200 BA groups.

3.2. Results of TOS from liver tissue

Total oxidant values as shown in Table 1, the TOS values of the PARA group were significantly higher than the control, PARA + 100 BA, PARA + 200 BA and 200 BA groups (P <0.05). PARA + NAC group TOS values were higher than PARA + 100 BA, PARA + 200 BA and 200 BA groups and significantly lower than PARA group (P <0.05). There was no significant difference between control, PARA + 100 BA, PARA + 200 BA and 200 BA groups. Also, there was no significant difference between PARA + 50 BA and PARA + NAC groups.

3.3. Results of OSI from liver tissue

OSI levels as shown in Table 1, OSI values of the PARA group were significantly higher than the control, PARA + 100 BA, PARA + 200 BA and 200 BA groups (P <0.05). PARA + NAC group OSI values were no significant difference found than PARA + 100 BA, PARA + 200 BA and 200 BA groups and significantly higher than PARA group (P <0.05).

3.4. Results of PCO from liver tissue

PCO values as shown in Table 1, the PCO values in the PARA group were significantly higher than the control, PARA + 50 BA, PARA + 100 BA, PARA + 200 BA, PARA + NAC and 200 BA groups (P<0.05). PCO values of PARA + 100 BA, PARA + 200 BA group were significantly higher than the PARA + NAC group (P<0.05) and it was found to be significantly lower than the PARA group (P<0.05). There was no significant difference between control, PARA + 50 BA, PARA + 200 BA and 200 BA groups. Also there was no significant difference between PARA + 100 BA group and PARA + 200 BA.

3.5. Results of protein-SH from liver tissue

Protein-SH values as shown in Table 1, protein-SH values in PARA group were found to be significantly lower (P<0.05) than control, PARA + 50 BA, PARA + 100 BA, PARA + 200 BA and PARA + NAC groups. PARA + NAC group protein-SH values were lower than PARA + 100 BA and PARA + 200 BA group and significantly higher than PARA group (P<0.05). No significant difference was found between control and PARA + 200 BA groups. In addition, there was no significant difference between PARA + 50 BA group and PARA + NAC group.

Table 1. TAS, TOS, PCO, TOS, OSI and -SH values in liver.

Groups	Liver TAS (mmol Trolox Ekivalent/L)	Liver TOS (µmol H_2O_2 Ekivalent/L)	Liver OSI Arbutrary Units	Liver PCO nmol/mg protein	Liver –SH µmol/mg protein
Control	1,27±0,68[a]	20,01±3,72[a]	0,91±0,17[a]	83,40±4,55[a]	360,20±20,81[a]
Paracetemol	0,60±0,23[c]	69,75±3,21[c]	2,07±0,54[c]	441,50±33,57[c]	101,60±4,69[b]
Paracetamol+50mg/kg boric acid	1,24±0,86[a]	30,34±4,45[b]	1,11±0,22[a]	106,70±34,24[a]	193,90±6,79[c]
Paracetemol+100 mg/kg boric acid	1,17±0,70[a,b]	23,02±2,98[a]	1,01±0,32[a,b]	150,90±27,33[b]	255,80±18,66[a,c]
Paracetemol+200 mg/kg boric acid	1,20±0,96[a,b]	19,05±2,61[a]	1,94±0,48[c]	150,90±27,36[b]	346,00±25,25[a]
Paracetemol+NAC	1,04±0,69[b]	27,97±3,04[b]	1,05±0,43[a,b]	109,00±35,57[a]	110,90±8,99[c]
Boric acid 200 mg/kg	1,24±0,13[a]	21,32±2,21[a]	1,54±0,58[b,c]	89,50±8,97[a]	136,40±28,32[b,c]

[a,b,c]: Means in the same column by the same letter are not significantly different according to the One Way ANOVA-Duncan and Tukey-HSD test (P<0.05). Values are mean ± SD, n=10.

3.6. AST and ALT results in serum

Serum AST and ALT results are shown in Table 2. According to our results, AST and ALT values were significantly higher (P <0.05) in the paracetamol group compared to the control group. These findings confirm that there is hepatotoxicity in the samples.

3.7. Elements measurement results in liver tissue

The trace element measurements in liver are given in Table 3. Trace element levels were significantly lower in paracetamol groups compared to control group (p<0.05).

4. Discussion

In this study, it was aimed to determine the effect of boron element on both trace element (Mg, Zn, Mn, Cu, Co, SE) and PCO, protein-SH, TAS, TOS, OSI, AST, ALT by creating parasetamol induced hepatotoxicity model. Paracetamol is a safe, effective analgesic and antipyretic drug when administered at the therapeutic dose range. However, an increase in the amount of reactive molecule NAPQI is observed when taken in high doses [21-23]. Normally, NAPQI is detoxified by GSH and excreted through bile. When the amount of NAPQI increases, it easily reacts with proteins, DNA and unsaturated lipids by consuming GSH stores in the liver [23, 24]. It causes hepatotoxicity by binding to intracellular proteins. Reduction or depletion of GSH levels inadequate to defend the body against the effects of free radicals. Eventually, oxidative stress occurs and the antioxidant defense system causes deterioration [11]. The deterioration of the antioxidant system and increased ROS levels affect the formation of hepatotoxic damage [21].

ROS, which occurs in parasetamole induced hepatotoxicity model, causes various problems in cell mechanisms by affecting macromolecules such as protein, lipid, nucleic acids [25,26]. The formation of ROS leads to lipid peroxidation and carbohydrate oxidation, and the products of these reactions lead to protein modification. As a result of protein modifications, PCO levels increase and protein-SH levels decrease. Protection of the antioxidant defense system plays an important role in preventing the damage caused by reactive oxygen species and preventing the emergence of oxidative stress [25,27].

The deterioration of the antioxidant defense mechanism induces reactive oxygen formation and causes

Table 2. AST and ALT levels in serum.

Groups	AST (U/L)	ALT (U/L)
Control	99,30±6,46[a]	24,20±2,65[a]
Paracetemol	160,70±17,12[b]	68,50±3,83[b]
Paracetamol+50mg/kg boric acid	138,40±19,42[c,d]	36,50±6,22[c,d]
Paracetemol+100 mg/kg Boric acid	104,60±13,64[a]	31,20±3,39[a,c,d]
Paracetemol+200 mg/kg Boric acid	107,50±16,63 [d]	35,20±7,30[c,d]
Paracetemol+NAC	105,30±9,14[a]	23,00±2,01[a]
Boric acid 200 mg/kg	100,70±9,95[a]	24,60±2,75[a]

[a,b,c]: Means in the same column by the same letter are not significantly different according to the one way ANOVA-Duncan test and Tukey-HSD (P<0.05). Values are mean ± SD, n=10.

Table 3. Trace element levels in liver tissue.

Groups	Mg ppm	Zn ppm	Cu ppm	Se ppm
Groups	11,35±0,92[a]	5,97±0,81[a]	0,45±0,03[a]	4,67±0,29[a]
Control	4,88±0,51[d]	1,68±0,39[b]	0,24±0,09[b]	2,41±0,24[c]
Paracetemol	9,87±0,79[a]	1,77±0,37[b]	0,30±0,03[c]	3,70±0,17[b]
Paracetamol+50mg/kg boric acid	11,20±1,11[a,b,c]	2,46±0,15[c]	0,32±0,01[c]	4,44±0,30[a]
Paracetemol+100 mg/kg boric acid	10,77±0,91[a,b,c]	1,64±0,20b	0,42±0,02[a]	4,41±0,21[a]
Paracetemol+200 mg/kg boric acid	11,45±0,79[c]	1,54±0,14[b]	0,33±0,01[c]	4,52±0,16[a]
Paracetemol+NAC	11,86±1,23[b]	5,59±1,53[a]	0,44±0,04a	4,62±0,48[a]

[a,b,c]: Means in the same column by the same letter are not significantly different according to the one way ANOVA-Duncan and Tukey-HSD test (P<0.05). Values are mean ± SD, n=10.

oxidation of proteins by some mechanisms. There was a close relationship between protein damage and PCO levels. These PCO groups are a marker in determining the structural changes in proteins and the antioxidant system [26,28,29]. In addition, the loss of the protein-SH groups, which have the property of breaking the oxidation chain and showing antioxidant properties, has also helped to determine the damage to proteins [29,30].

In our study, PCO values of PARA group increased significantly compared to control group. The PARA + NAC and PARA + 50 BA groups significantly decreased the PCO values in the rising PARA group. Although PARA + 100 BA and PARA + 200 BA groups significantly decreased PCO values in increasing PARA group, they were not as effective as PARA + NAC and PARA + 50 BA groups.

Protein-SH levels, which are an important marker for antioxidant defense mechanism, showed a significant decrease in PARA group compared to control group. The PARA + NAC group significantly increased the low protein-SH levels in the PARA group. PARA + 50 BA, PARA + 100 BA and PARA + 200 BA groups significantly increased the low protein-SH levels in the PARA group. The group that brought the protein-SH levels closer to the control group was PARA + 200 BA group.

As a result of some studies, it has been reported that there is a connection between trace element level and oxidative stress. Some trace elements such as Mg and Zn cause an increase in ROS and MDA and oxidative damage [31]. Some of the trace elements (Mg, Zn, Cu, Se) act as a cofactor in the structure of the antioxidant enzyme and affect the function of the antioxidant defense system. In this study, the effectiveness of these elements was investigated in ICP-MS [32].

PARA group Mg, Zn, Cu and Se levels were significantly lower than the control group. In terms of the Se values between the groups, it was observed that the low Se values in the PARA group were significantly increased in the PARA + NAC, PARA + 100 BA and PARA + 200 BA groups. Se is an antioxidant for the organism by taking part in the structure of glutathione peroxidase (GPx) which is active in preventing free radical damage [33,34]. In our study, increasing the Se values of boric acid administered groups may be associated with the effect of boron on glutathione level, which has antioxidant properties.

The PARA + NAC group significantly increased the Mg level in the low level PARA group. Similarly, it can be said that PARA + 50 BA, PARA + 100 BA and PARA + 200 BA groups significantly increase Mg levels and positively affect the antioxidant system. In paracetamol-induced hepatotoxicity model, increase in Mg levels of boric acid treated groups may be attributed to function as cofactors of enzymes involved in antioxidant defense system.

It was observed that Zn values in the low PARA group increased statistically only in the PARA + 100 BA group. Although not statistically significant, PARA + 50 BA group Zn values are higher than PARA group. Zn is a cofactor of sod, one of the important enzymes of the antioxidant system, and participates in the detoxification of free radicals by various mechanisms. [35]. It can be said that boron has a positive effect on antioxidant defense system by considering at the increase in Zn values of boric acid applied groups. Since Zn affects the antioxidant system by protecting sulfhydryl groups against oxidation, it can be considered significant that the boron increases the Zn values. When Cu values were examined, it was observed that PARA group was lower than control group. The low Cu levels of PARA group increased the PARA + 50 BA, PARA + 100 BA and PARA + 200 BA groups significantly. Cu is also a trace element that functions to protect the organism against free radicals. [36,37]. Due to the increase in Cu levels in boric acid treated groups, the protective effect of boron in antioxidant system can be mentioned.

Trace elements are known to specifically play roles in many physiological process; for example copper, manganese and zinc are an integral component of many metalloenzymes e.g. superoxide dismutase [14]. Moreover, being an integral part, copper is bound to other proteins and released to play important catalytic roles in detoxification of reactive species [38]. Smith *et al*. [39] have also established that copper deficiency may increase susceptibility to oxidative damage.

The effects of boron, which has been widely used in many different areas up to now, are of great importance in the field of health. Boron has a wide range of functions in our body and has been instrumental in regulating antioxidant defense mechanisms by increasing enzyme levels such as sod, cat and GSH-Px [10]. Boron affects the regulation of NADPH levels. NADPH is a molecule that has a positive effect on glutathione (GSH) levels. GSH is an antioxidant that protects cells from oxidative stress-induced damage and reduces the sulfydryl groups of proteins [40,41]. In this study, it has been observed that boric acid has a positive effect on the trace element level such as Mg, Se, Cu, Zn which is involved in many antioxidant enzymes, it helps to decrease high protein carbonyl content and low levels of protein-SH levels. In conclusion, it can be said that high dose of paracetamol affects free radical formation and increases PCO levels and decreases protein-SH levels.

Conclusion

The function of boron can be concluded that it has a protective effect in the antioxidant defense system by bringing these parameters closer to normal values.

Acknowledgement

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