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Investigation of boron effect on trace elements and antioxidant capacity in paracetamol-induced nephrotoxicity model**

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ABSTRACT:

In this study, the effect of boron on the trace element and antioxidant capacity in paracetamol induced nephrotoxicity model was investigated. The main hypothesis on which the study is based has a unique value in terms of showing whether the antioxidant activity of boron has an effect on the trace elements and antioxidant capacity in our body. In the study, 56 albino Wistar rats were divided into 7 groups: Group 1 (Control); Group 2 (Paracetamol); Group 3 (Paracetamol + 50 mg/kg boron); Group 4 (Paracetamol + 100 mg/kg boron); Group 5 (Paracetamol + 200 mg/kg boron); Group 6 (Paracetamol + 140 mg/kg NAC); Group 7 (200 mg/kg boron). At the end of the research, tissue samples taken from rats were used for trace element analysis (Magnesium (Mg), Manganese (Mn), Cobalt (Co), Copper (Cu), Zinc (Zn), Selenium (Se), Malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS), superoxide dismutase activity (SOD), catalase activity (CAT) and glutathione peroxidase (GPx) analyses. Likewise, blood samples taken from the hearts of rats were examined for creatinine blood urea nitrogen (BUN) analyses. As a result of the findings, this study suggests that the boron may have antioxidant properties and may have a protective effect on trace elements and antioxidant capacity in paracetamol-induced nephrotoxicity.

Ratlarda parasetamol ile indüklenen nefrotoksisite modelinde eser elementler ve antioksidan kapasite üzerine borun etkisinin araştırılması

Özet

Bu çalışmada, parasetamol ile indüklenen nefrotoksisite modelinde borun eser element ve antioksidan kapasiteye etkisi araştırıldı. Çalışmanın amacı, borun antioksidan aktivitesinin, eser elementler ve vücudumuzdaki antioksidan kapasiteye etkisi olup olmadığını gösterme açısından benzersiz bir değere sahiptir. Çalışmada 56 albino Wistar rat 7 gruba ayrıldı: Grup 1 (Kontrol); Grup 2 (Parasetamol); Grup 3 (Parasetamol + 50 mg / kg bor); Grup 4 (Parasetamol + 100 mg / kg bor); Grup 5 (Parasetamol + 200 mg / kg bor); Grup 6 (Parasetamol + 140 mg / kg N-Asetilsistein (NAC)); Grup 7 (200 mg / kg bor). Araştırma sonunda, sıçanlardan alınan doku örnekleri iz element analizi için kullanılmıştır (Magnezyum (Mg), Manganez (Mn), Kobalt (Co), Bakır (Cu), Çinko (Zn), Selenyum (Se)), Malondialdehit (MDA), toplam antioksidan durumu (TAS), toplam oksidan durumu (TOS), süperoksit dismutaz aktivitesi (SOD), katalaz aktivitesi (CAT) ve glutatyon peroksidaz (GPx) analizleri. Aynı şekilde, sıçanların kalbinden alınan kan örnekleri de kreatinin kan üre azotu (BUN) analizleri için incelendi. Bulgular sonucunda bu çalışma, borun antioksidan özelliklere sahip olabileceğini ve parasetamol ile indüklenen nefrotoksisitede iz elementler ve antioksidan kapasite üzerinde koruyucu bir etkisi olabileceğini göstermektedir.

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1. Introduction

Paracetamol, one of the family of phenacetin, has been used widely since 1950 as analgesic and antipyretic agents (1). Paracetamol is considered to be a safe drug when taken in therapeutic doses, but overdose causes toxicity. Poisoning with paracetamol may cause liver and kidney toxicity (2). Paracetamol is mainly metabolized to sulphate and glucuronide metabolites. It is normally transformed into the highly reactive N-acetyl-p-benzoqui- nonimine (NAPQI) metabolite via CYP450. This metabolite is usually stabilized by glutathione (GSH) conjugation and is eliminated via kidney. However, in overdose paracetamol uptake, this mechanism becomes saturated and exceeds the capacity to detoxify NAPQI production. Excess NAPQI causes oxidative stress-related toxicity (3).

Boron is an important nutrient that needs to be taken from outside by the human body because it is needed in small amounts and can not be synthesized in cells. Until 1981, it was thought that boron had no effect on people. Later studies showed that boron is an indispensable element for many treatments and it is effective in oxidant antioxidant balance in the body. Boron increases the amount of glutathione and its derivatives in the body or induces antioxidants against reactive oxygen species and shows protective effect against oxidative damage (4).

This study suggests that the boron may have a protective effect on the trace elements and antioxidant capacity in paracetamol-induced nephrotoxicity and if the data are supported by further research, it is thought that boron will provide a positive contribution to the literature in terms of showing that it may be a new antioxidant agent that can be used in the paracetamol toxicity.

2. Material and Methods

Experimental animals:

56 male, albino, Wistar rats weighing 180 grams to 200 grams rats which were used grown in Afyon Kocatepe University Experimental Animal Research and Application Center were used. One week before starting the experiment, the animals were adapted to the laboratory to prevent them from being affected negatively by stress. The animals were fed with water and feed (ad libitum) for the duration of the experiment and they were housed in normal laboratory temperature (22-24 ºC) for 12 h light / dark lighting. All interventions during the study were performed in accordance with the rules declared by Uşak University Local Animal Ethics Committee and with the approval of 2017 / 02-01. Animals were cared for at the Afyon Kocatepe University Experimental Animal Research and Application Center.

Chemicals used ın the experiment:

Paracetamol: In the study, a dose of 2 g / kg per paracetamol was prepared in a 1% Carboxy Methyl Cellulose (CMC) solution of Phosphate buffer saline (PBS), corresponding to 2 ml. The prepared suspension was administered orally via the gastric catheter. Paracetamol doses were determined according to the related literature (5,6).

N-Acetyl Cysteine (NAC): In the study, 600 mg single tablet NAC (Mentopin, Hermesarzneimittel Gmbh, Munich-Germany) prepared in 0.9% NaCl solution was given orally with gavage. NAC is an antidote to paracetamol poisoning due to its ability to increase glutathione available for NAPQI conjugation, and NAC is usually given to patients for the treatment of paracetamol toxicity. NAC group was used as a positive control group.

Boron application: Boric acid (H_3BO_3) 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. Boron doses were determined according to the related literature (7).

Ketamine and Xylazine: In the study, animals were given 65 mg / kg ketamine and 7 mg / kg xylazine intraperitoneally for euthanasia.

Experimental plan:

Rats were divided into 7 groups (n=8, for each group) and were subjected to the following experimental protocols.

Group 1 (Control): Control group.

Group 2 (Paracetamol): 2 g / kg paracetamol was administered orally via gavage.

Group 3 (Paracetamol + 50 boron): 2 g / kg of paracetamol and then 50 mg / kg of boric acid were administered orally via gavage.

Group 4 (Paracetamol + 100 boron): After administration of 2 g / kg oral paracetamol, 100 mg / kg boric acid was administered orally by gavage.

Group 5 (Paracetamol + 200 boron): After administration of 2 g / kg oral paracetamol, 200 mg / kg boric acid was administered orally by gavage.

Group 6 (Paracetamol + NAC): After administration of 140 mg / kg oral NAC, 2 g / kg oral paracetamol was administered. NAC application was repeated one hour and 12 hours after paracetamol administration (2 g/kg paracetamol + 140 mg/kg NAC + 2 g/kg paracetamol)

Group 7 (200 boron): 200 mg / kg boric acid was administered orally via gavage.

Collecting tissue and blood samples:

The animals were given water and feed until they were saturated during the experiment. After applications, the animals were sacrificed under anesthesia with ketamine (65 mg / kg, i.p) and xylazine (7 mg / kg, i.p) and blood samples were taken from their hearts. At the same time, one of the kidneys of the animals in the whole groups was stored in phosphate buffer at -80^{o}C until analysis time.

Trace element, total antioxidant status (TAS), total oxidant status (TOS), malondialdehyde (MDA), dismutase activity (SOD), catalase activity (CAT) and glutathione peroxidase (GPx) analyses were performed with tissue samples taken from animals. Blood samples were used for creatinine and blood urea nitrogen (BUN) analyses.

Biochemical analyses:

Trace element analysis: Inductively coupled plasma mass spectrometry (ICP-MS) (Neslab Thermoflex 2500, Thermoscientific) was used for trace element analysis. The analyzes were carried out at Uşak University Scientific Analysis Technological Application and Research Center. The results were expressed in ppm unit.

Analysis of total antioxidant status (TAS): For the TAS analysis, the Rel Assay Diagnostics Total Antioxidant Status Assay Kit (Lot #: DR16069A) was used and the results were obtained by colorimetric method.

Analysis of total oxidant status (TOS): The Rel Assay Kit (Lot #: DR16080O) was used for the TOS analysis and the results were obtained by colorimetric method.

Analysis of malondialdehyde (MDA): In the acidic medium, thiobarbituric acid (TBA) reacts with MDA at high temperature and pink chromogen is formed. The resulting pink color amounts were read spectrophotometrically at 532 nm. Results were given in nmol / mg protein units.

Analysis of dismutase activity (SOD): For the analysis of SOD, an ELISA kit was used using rat specific double antibody sandwich ELISA method. (SunRed Biotechnology Company, Rat (SOD) ELISA Kit, Lot #: 201802). Results were given in ng / ml.

Analysis of catalase activity (CAT): For the analysis of CAT, an ELISA kit was used using rat specific double antibody sandwich ELISA method (SunRed Biotechnology Company, Rat (CAT) ELISA Kit, Lot #:201802). Results were given in ng / ml.

Analysis of glutathione peroxidase (gpx): For the analysis of GPx, an ELISA kit was used using rat specific double antibody sandwich ELISA method (SunRed Biotechnology Company, Rat (GPx) ELISA Kit, Lot #:201802). Results were given in ng / ml.

Analysis of creatinine and blood urea nitrogen (BUN): Bloods taken to the anticoagulant tube were centrifuged at 4000 rpm for 10 minutes. Serum physiological (Isotonic Sodium chloride solution, Mediflex, Eczacıbaşı, 1000 ml) was washed and centrifuged. The samples were placed in auto analyzer (Abbott Architect ci4100) and urea and creatinine analysis were automatically calculated by the instrument. This analysis was performed at Medical Park Uşak Hospital.

Statistical Analysis

Data were statistically analyzed by using SPSS-18 ANOVA and stated as means and standard deviation. P <0.05 was considered to be significant. The significance of the difference between the groups was made by Post Hoc Multiple Comparative Tests using Duncan technique in One-Way ANOVA test.

3. Results

Biochemical analysis for kidney tissue

The results of MDA, SOD, CAT, GPx, TAS, TOS in kidney tissue are shown in Table 1. According to results, MDA levels and TOS levels were significantly higher in the paracetamol group than the control group (p <0.05) and boron groups were significantly lower compared to paracetamol groups (p <0.05). The levels of SOD, CAT, GPx and TAS were significantly lower in the paracetamol group compared to the control group (p <0.05) and boron groups were significantly higher compared to paracetamol groups (p <0.05).

Table 1: MDA, TAS, TOS, CAT, SOD and GPx levels of groups.

Tablo 1: *Grupların MDA, TAS, TOS, CAT, SOD ve GPx seviyeleri.*

	MDA (nmol/mg protein)	SOD (ng/ml protein)	CAT (ng/ml protein)	GPx (ng/ml protein)	TAS (mmol Trolox Equivalent/L)	TOS (µmol H_2O_2 Equivalent/L)
Control	$0,30 \pm 0,06^{a}$	$1,80 \pm 0,10^{a}$	$1,67 \pm 0,17^{a}$	$1,53 \pm 0,41^{a}$	$0,52 \pm 0,11^{a}$	$18,87 \pm 4,32^{a}$
Paracetamol	$0,87 \pm 0,10^{c}$	$1,17 \pm 0,31^{e}$	$1,18 \pm 0,07^{d}$	$0,67 \pm 0,41^{c}$	$0,03 \pm 0,01^{b}$	$70,0 \pm 4,95^{d}$
Paracetamol + 50 boron	$0,44 \pm 0,12^{a,b}$	$1,26 \pm 0,17^{d,e}$	$1,27 \pm 0,25^{c,d}$	$0,90 \pm 0,31^{b,c}$	$0,06 \pm 0,02^{b}$	$29,62 \pm 6,47^{c}$
Paracetamol + 100 boron	$0,46 \pm 0,24^{a,b}$	$1,71 \pm 0,14^{a,b}$	$1,46 \pm 0,15^{b,c}$	$1,08 \pm 0,37^{b}$	$0,16 \pm 0,08^{b}$	$25,25 \pm 6,40^{b,c}$
Paracetamol + 200 boron	$0,42 \pm 0,26^{a,b}$	$1,42 \pm 0,17^{c,d}$	$1,37 \pm 0,26^{c,d}$	$0,81 \pm 0,34^{b,c}$	$0,48 \pm 0,05^{a}$	$19,25 \pm 4,52^{a}$
Paracetamol + NAC	$0,55 \pm 0,38^{b}$	$1,57 \pm 0,25^{b,c}$	$1,31 \pm 0,24^{c,d}$	$1,09 \pm 0,20^{b}$	$0,40 \pm 0,02^{a}$	$27,87 \pm 6,08^{c}$
200 Boron	$0,28 \pm 0,09^{a}$	$1,89 \pm 0,24^{a}$	$1,60 \pm 0,18^{a,b,d}$	$1,11 \pm 0,10^{b}$	$0,50 \pm 0,12^{a}$	$22,75 \pm 3,84^{a,b}$

^{a,b,c,d} *: In the same column values with different letters show statistically significant differences in MDA, TAS, TOS, CAT, SOD, GPx levels (p<0.05).*

Creatinine and blood urea nitrogen (BUN) analysis results:

Serum BUN and creatinine levels are given in table 2 and table 3, respectively. According to our results, the BUN and creatinine levels of the paracetamol group were significantly higher than the control group ($p < 0.05$). These levels were found to be significantly lower at boron groups ($p < 0.05$) than the paracetamol group.

Table 2: BUN levels of groups

Tablo 2: *Grupların BUN seviyeleri*

	BUN [ul/L]
Control	$18,12 \pm 1,95^{a}$
Paracetamol	$29,62 \pm 4,24^{b}$
Paracetamol + 50 boron	$20,62 \pm 5,47^{a}$
Paracetamol + 100 boron	$20,62 \pm 3,15^{a}$
Paracetamol + 200 boron	$20,37 \pm 4,59^{a}$
Paracetamol + NAC	$20,50 \pm 5,92^{a}$
200 Boron	$18,37 \pm 1,68^{a}$

The difference between groups with different letters is statistically significant *(p<0,05).*

Table 3: Creatinine levels of groups

Tablo 3: *Grupların Kreatinin seviyeleri*

	Creatinine [ul/L]
Control	$0,37 \pm 0,05^{a}$
Paracetamol	$0,84 \pm 0,05^{d}$
Paracetamol + 50 boron	$0,82 \pm 0,03^{d}$
Paracetamol + 100 boron	$0,64 \pm 0,02^{c}$
Paracetamol + 200 boron	$0,50 \pm 0,06^{b}$
Paracetamol + NAC	$0,47 \pm 0,10^{b}$
200 Boron	$0,44 \pm 0,05^{b}$

The difference between groups with different letters is statistically significant *(p<0,05).*

Trace element results:

The trace element results of kidney tissue are given in Table 4. According to the data obtained, it was noted that the trace element levels in the paracetamol group were significantly lower than the control group ($p < 0.05$).

Table 4: Co, Cu, Se, Mg, Zn and Mn levels of groups

Tablo 4: *Grupların Co, Cu, Se, Mg, Zn ve Mn seviyeleri*

	Co	Cu	Se	Mg	Zn	Mn
Control	0,010 ± 0,001^{a}	0,42 ± 0,05^{a,b}	1,63 ± 0,39^{b,c}	21,96 ± 1,35^{a}	6,96 ± 2,33^{a}	0,10 ± 0,012^{a}
Paracetamol	0,008 ± 0,001^{c}	0,29 ± 0,05^{d}	0,93 ± 0,27^{d}	17,02 ± 1,01^{d}	4,70 ± 0,64^{d}	0,07 ± 0,004^{b}
Paracetamol + 50 boron	0,008 ± 0,003^{b,c}	0,35 ± 0,02^{c}	1,50 ± 0,14^{c}	18,13 ± 1,68^{c,d}	5,20 ± 0,14^{c,d}	0,09 ± 0,007^{a}
Paracetamol + 100 boron	0,009 ± 0,003^{d}	0,41 ± 0,07^{a,b}	1,51 ± 0,47^{c}	18,01 ± 3,32^{c,d}	5,27 ± 0,75^{c,d}	0,09 ± 0,018^{a}
Paracetamol + 200 boron	0,009 ± 0,004^{b}	0,47 ± 0,06^{a}	1,94 ± 0,42^{a,b}	20,5 ± 2,31^{a,b}	5,12 ± 0,30^{a,b}	0,09 ± 0,015^{a}
Paracetamol + NAC	0,011 ± 0,002^{a}	0,41 ± 0,04^{b}	2,03 ± 0,29^{a}	19,92 ± 1,61^{b,c}	4,96 ± 0,10^{b,c}	0,09 ± 0,025^{a}
Boron	0,010± 0,001^{a}	0,43 ± 0,05^{a,b}	2,01 ± 0,19^{a}	19,46 ± 0,70^{b,c}	5,89 ± 0,38^{b,c}	0,09 ± 0,016^{a}

The difference between groups with different letters is statistically significant *($p<0,05$).*

4. Discussion and Conclusion

In this study, the effects of boron on trace elements and antioxidant capacity in paracetamol-induced nephrotoxicity model in rats were investigated. Paracetamol is a non-steroidal anti-inflammatory drug with a strong analgesic and antipyretic properties. Paracetamol is a drug that is very reliable in therapeutic doses and has little side effects and is therefore widely used throughout the world. Because of its widespread use, high safety profile and ease of access without over-the-counter access, the risk of toxicity is high. It has been reported that overdose of paracetamol may cause severe hepatotoxicity and nephrotoxicity (8). Paracetamol toxicity begins with symptoms of nausea and vomiting 12-24 hours after taking the drug. 24-48 hours later, the patient feels better but in 48-72 hours, biochemical changes begin in the body.

When paracetamol is used in therapeutic doses, sixty percent is converted to glucuronic acid with the help of the transferase enzyme, while thirty-five percent is converted to sulfuric acid by the help of the sulfonyl transferase enzyme. Two percent is excreted in the urine. A small amount of paracetamol is converted to NAPQI by the cytochrome P-450 enzyme.

Since NAPQI is a reactive electrophilic molecule, it covalently binds to the cysteine moieties of intracellular proteins and forms chelates 3- (cysteine-S-yl). These chelates cause tissue damage (9). At therapeutic doses, NAPQI is reacted with glutathione and excreted via the bile. However, overdose intake increases the amount of NAPQI and depletes the glutathione depots in the liver. The released NAPQI is covalently bound to the cysteine moieties of intracellular proteins and this mechanism is considered the main pathway for paracetamol-induced hepatotoxicity (10). Overdose intake is known to cause damage to the kidney as paracetamol is excreted through the kidney after being metabolized in the liver. NAPQI and p-aminophenol formed by N-acetylation in the renal cortex may accumulate due to depletion of glutathione depots. Kidney damage can be caused by NAPQI binding to membranes and sulfhydryl proteins and covalent binding of p-aminophenol to renal macromolecules (11). The nephrotoxic effects of paracetamol may vary depending on the acute or chronic intake. Acute overdose paracetamol intake (10-15 g) may lead to acute

toxicity with proximal tubule damage and necrosis, while very low doses (5000-1000 mg) may cause renal damage resulting in analgesic nephropathy (12). Most studies conducted with paracetamol toxicity indicate that glutathione, which is a natural antioxidant in the body, decreases excessively due to toxic substances and causes weakening of the antioxidant defense system and consequently causing damage (13).

Since paracetamol is primarily metabolized in the liver, overdose intake first causes hepatotoxicity. The kidneys are affected in the advanced stages of liver damage or rarely alone without liver damage. Therefore, there are few studies investigating the association of paracetamol with hepatotoxicity, while there are few studies investigating the relationship between paracetamol and nephrotoxicity. Toxicity in the kidney occurs in the form of acute tubular necrosis. It has been observed that 1-2% of patients with overdose paracetamol intake develop renal failure (14). The mechanism of paracetamol-induced toxicity in the kidney is still not fully elucidated. In overdose paracetamol, glutathione and sulfation reactions reach saturation and this activates the cytochrome P-450 enzyme system. CYP2E1 isoenzyme is responsible for biotransformation in the kidney. Toxic metabolites such as NAPQI and p-aminophenol are formed by Prostaglandin synthetase, N-deacetylase enzymes and cytochrome P-450 enzyme systems (15). These toxic metabolites combine with sulfhydryl and glutathione in cellular proteins to form conjugates. The depletion of glutathione is thought to lead to oxidative stress. As a result, it is thought that it causes the activation of lysosomal enzymes and caspases and causes damage to the hemostasis and damage to the kidney function by damaging the tissue (16).

In paracetamol-induced kidney damage, it is thought to be the key task of NAPQI which is formed with the help of CYP-450 enzyme systems. These metabolites combine with sulfhydryl and glutathione in cellular proteins to form conjugates, and these conjugates increase oxidative stress and are thought to be one of the possible toxicity mechanisms (17). The increase in oxidative stress and therefore the weakening of the antioxidant defense system is also important in paracetamol-induced kidney toxicity. For this reason, CAT, SOD, GPx and TAS were investigated as antioxidant parameters and MDA, TOS, BUN and creatinine were investigated as oxidative parameters. In addition, the effect of boron on trace element levels was investigated.

Lipid peroxidation is one of the most important mechanisms that cause paracetamol-related toxicity. Lipid peroxidation is associated with free oxygen radicals. MDA is the final product of lipid peroxidation and is the most common marker. Therefore, in tissues exposed to oxidative stress due to increased free radicals, increase in MDA level is observed. For this reason, MDA level can be considered as a biomarker for oxidative stress (18). In many studies in the literature, it has been reported that paracetamol toxicity increases the MDA level and successful treatments reduce these elevated MDA levels and prevent paracetamol toxicity. In the study, Zhao et al. were investigated the protective effect of rhein in rats on paracetamol induced hepatic and renal toxicity. It was stated that the MDA level of the paracetamol group was higher than the control group and this increased value was decreased in the treated group (19). Cheng-chin et al. were studied the protective effect of S-allyl and S-propyl in paracetamol-induced hepatotoxicity and they indicated that MDA level of paracetamol group is higher than the control group and this increased value was decreased in the treated group (20). Yapar et al. were investigated the protective effect of L-Carnitine in paracetamol-induced hepatotoxicity and stated that MDA level of paracetamol group is higher than the control group and this increased value was decreased in the treated group (21,22). In this study, it was observed that MDA levels were increased statistically significant in the paracetamol group due to nephrotoxicity, and boron application has significantly decreased these levels to a level close to the control group. This result supports the fact that the boron reduces the level of MDA in paracetamol-induced nephrotoxicity and has protective properties.

SOD is an antioxidant enzyme that catalyzes superoxide radical to hydrogen peroxide and dioxygen (23). It is an important antioxidant defense mechanism because it forms the first defense line against reactive oxygen species. Since antioxidant enzymes such as SOD and CAT are easily inactive in the case of oxidative stress, a decrease at enzyme levels in paracetamol toxicity may occur (24). In a study by Xin et al., SOD levels of paracetamol-induced toxicity group were decreased compared to control group (25). Hua et al. were investigated the mitochondrial protective effects of Picroside II in paracetamol induced hepatotoxicity in rats and indicated that the SOD level of the paracetamol group was decreased compared to the control group and the decreasing SOD values were increased in the groups given Picroside II. In this study, it was observed that SOD levels were decreased statistically significant in the paracetamol

group and boron application was increased these decreasing levels statistically close to the control group. This result supports the fact that the boron was increased the level of decreased SOD in paracetamol-induced nephrotoxicity and has protective properties.

GSH, one of the most common tripeptides in the liver, prevents the formation of free radicals such as hydrogen peroxide and superoxide radicals and protects against oxidative damage. Abdul Hamid et al. were studied the effect of Zingiber zerumbet extract on paracetamol-induced nephrotoxicity and indicated the oxidative damage with the reduction of GSH and GPx levels (27). Marc et al. were investigated the protective effect of L-Carnitine in paracetamol-induced toxicity in rats and reported that the GSH level of the paracetamol group was lower than the control group and this decreasing value was increased in the treated group (22). Fallah et al. were indicated that the GSH level of the toxication group was lower than that of the control group, but the GSH level was increased in the treatment group with Taraxacum officinale roots. GPx inhibits the formation of hydrogen peroxide by converting oxidized glutathione to reduced glutathione. GPx uses GSH as a substrate and GPx is found partly in the cell membrane and contains selenium-containing metalloenzymes (29). In the study of Balasubramaniyan et al., it was reported that the GPx level of the group with ethanolic toxicity was lower than the control group (30). In the study of Sumanth and Rana, it was observed that the GPx level of the toxicity group was much lower compared to the control group, while the GPx level of the group of alcohol extracts from Taraxacum officinale roots increased significantly compared to the toxicity group (31). In this study, it was observed that GPx levels were decreased statistically significant in the paracetamol treated group and boron application was increased these decreasing levels statistically significant close to the control group. This result supports the protective effect of boron in the paracetamol-induced nephrotoxicity.

CAT is an enzymatic antioxidant with high activity in the liver. It prevents the formation of highly reactive hydroxyl radicals by decomposing hydrogen peroxide into water and oxygen (32). Sumanth and Rana were indicated that the CAT level of the group given Taraxacum officinale was increased significantly compared to the toxicity group, while the toxicity group has been reported to show a significant reduction in CAT level compared to the control group (31). In a study conducted by Parmar et al., it was shown that CAT levels of hepatotoxicity groups were significantly decreased compared to the control group (33). In this study, it was observed that CAT levels were decreased statistically significant in the paracetamol group, and boron application was increased these levels to a level close to the control group statistically significant. This result supports the improvement in paracetamol-induced nephrotoxicity of the borate, which leads to a decrease in CAT level, which decreases as a result of increased oxidative stress in paracetamol-induced nephrotoxicity. This result supports the protective effect of boron, which provides an improvement in CAT level with decreasing oxidative stress.

Trace elements are inorganic molecules that are important for life and require a milligram amount per day for humans. Seven trace elements of vital importance in the human body; iron, selenium, zinc, molybdenum, copper, iodine and cobalt. Although the activity of boron and chrome has been defined for human life, its chemical functions have not been fully explained. Magnesium is the fourth most common cation in the human body, and the second most common cation in the cell after potassium is required for the functionality of many enzymes important for the body. It is involved in energy production and DNA synthesis in cells. Zinc is involved in the formation of DNA from the developmental stages of the mother's womb and is of great importance for body development. Manganese is more present in mitochondria as it is involved in cellular energy production. Cobalt is one of the parts of vitamin B12 and its deficiency can cause anemia. Copper is involved in the structure of many enzymes and cofactors and is involved in muscle, vein and tendon development. Selenium is also involved in the structure of the glutathione peroxidase enzyme (34). In chronic renal failure or dialysis treatment, abnormal changes in trace element levels may occur and may lead to clinical abnormalities. Fluctuations in the trace element level may be due to malnutrition, changes in gastrointestinal absorption or drug treatment (35). Cobalt, strontium, arsenic, silicon, cesium, molybdenum, chromium and mercury levels tend to increase in the reduction of renal functions, while elements such as selenium, bromine, zinc and rubidium tend to decrease. However, many data in different studies are not completely consistent with each other. It has been shown that there is a connection between renal failure and some trace elements (36). In this study, trace element levels (Magnesium, Zinc, Manganese, Cobalt, Copper and Selenium) were significantly decreased in the paracetamol group, and boron application increased these decreasing levels statistically significant to the level close to the control group. This result

supports the improvement of boron in the paracetamol-induced nephrotoxicity in the paracetamol-induced nephrotoxicity.

While TAS is a marker of antioxidant defense mechanism, TOS is a parameter that indicates the total number of oxidants. In this study, TAS and TOS levels were investigated as oxidative damage markers. The level of TAS showing antioxidant activity in the paracetamol group was found to be significantly lower than the control group, whereas the level of TOS indicating oxidative activity was found to be significantly higher than the control group, suggesting that oxidative damage may be responsible for paracetamol-induced nephrotoxicity. However, further studies are needed to fully understand the mechanism of development of oxidative damage. In the paracetamol group, TAS levels were decreased statistically significant and boron application was statistically significant increased these levels to a level close to the control group. It was observed that the TAS levels of the paracetamol + 200 boron group were found to be close to the control group statistically significant. This result supports the protective effect of boron in paracetamol-induced nephrotoxicity, which provides improvement in the level of TAS. It was observed that TOS levels were increased statistically significant in the paracetamol group and boron application was decreased statistically significant these levels to a level close to the control group. While the TOS levels of the paracetamol + 50 boron group and the TOS values of the Paracetamol + NAC group were close to each other, it was observed that the TOS level of the paracetamol + 200 boron group was found to be closest to the control group statistically. This result supports the protective effect of boron in paracetamol-induced nephrotoxicity, which provides improvement in the level of TOS.

Recent research has shown that boron is probably a necessary element for human and human health and that boron plays an important role in the synthesis and metabolism of various reactions. Karabağ Çoban et al. were indicated that organophosphate (OP) compounds cause oxidative stress and changes in the antioxidant status of the organism and animals. They were also stated that with the sufficient amount of boron, animals are protected against OP insects. They were indicated that boron application of OP-induced oxidative stress and its effects on enzyme activity provide correction in negative changes and develop antioxidant defense mechanism (37). In another study, it was reported that boron application had the same effect on endotoxin-induced oxidative stress (38). In a study of rodent model, it was shown that 40 mg / L of boron could increase the antioxidant capacity of the spleens and improve splenic tissue structure (39). In a recent study, it was reported that boron application leads to low oxidative stress in subjects exposed to chronic alcohol consumption (42). Boron application is thought to decrease oxidative stress by increasing glutathione reserves neutralizing oxidants (40). Karabağ Çoban et al. were stated that boron application increases the GSH levels and protects against the toxic effects of malathion (43). In a study on a neonatal necrotizing enterocolitis rat model, it has been shown that boron application increases the antioxidant level (41). It is also thought that the boron increases the level of antioxidant capacity by reducing intracellular ROS and Ca^{+2} ion levels (16) (18). In this study, paracetamol induced nephrotoxicity was composed and the level of free radicals was increased and oxidative stress was induced. Accordingly, trace elements, TAS, CAT, SOD and GPx levels were decreased statistically significant, while MDA, TOS, BUN and creatinine levels were increased. Compared to the control group, a significant decrease in free radical levels and a marked increase in decreased antioxidant capacity were observed in paracetamol + boron groups.

In conclusion, this study suggests that the antioxidant properties of the boron may support the protective effect of paracetamol-induced nephrotoxicity on trace elements and antioxidant capacity. This study has contributed positively to the literature in terms of showing that the boron may be a new antioxidant agent that can be used in paracetamol toxicity if the data is supported by further research.

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