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Boron as a protective agent in reducing paracetamol-induced testicular toxicity in rats: A biochemical perspective

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ABSTRACT

In the study, the protective effects of boron against paracetamol (PR)-induced toxicity in rat testicular tissue were investigated using various biochemical parameters. Rats were categorized into five groups and administered 50 and 100 mg/kg of boron (sodium pentaborate) orally for six days, followed by a single dosage of 1 g/kg of paracetamol to induce toxicity. Testicular tissues were assessed using ELISA and levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), cysteine-aspartic acid protease (Caspase-3), malondialdehyde (MDA), reduced glutathione (GSH), tumor necrosis factor-α (TNF-α), and interleukin-1 beta (IL-1β) were assessed. A significant decrease in SOD, CAT, GPx activities and GSH levels and an increase in MDA levels were observed in the PR group. Boron (B) treatment was found to increase antioxidant levels while decreasing lipid peroxidation, inflammation and apoptosis markers in paracetamol-induced testicular toxicity ($p<0.001$). The study concluded that boron may be effective in alleviating paracetamol-induced testicular damage.

1. Introduction

Paracetamol (PR, N-acetyl-p-aminophenol, APAP) is prescribed as an analgesic for headaches, dental pain, infections, neuralgia, myalgia, rheumatic pain, ear and sinus infections, surgical procedures, and traumas, as well as an antipyretic [1]. At therapeutic doses, APAP is primarily metabolized via glucuronidation and sulfation. A smaller fraction is converted by cytochrome P450 enzymes into the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). The NAPQI formed is then reduced by reduced glutathione (GSH) into a non-toxic, soluble mercapturic acid and excreted in the urine. However, in cases of paracetamol overdose, glutathione reserves are depleted and the accumulated NAPQI is not effectively removed. NAPQI is covalently bound to proteins, lipids, DNA, and other important macromolecules. Additionally, it has been reported that the NAPQI metabolite oxidizes sulfhydryl groups, disrupting the structure of proteins regulating Ca^{++} metabolism [2,3]. The toxic effects of NAPQI further exacerbate toxicity by increasing the production of reactive oxygen species (ROS), which lead to cell damage and inflammation. Although paracetamol is considered safe enough for use even in children, excessive or extended dosages of paracetamol can induce toxicity in organs such as the liver, kidneys, and testes, adversely affecting blood chemistry and fertility [4-6]. Exposure to potentially hazardous and therapeutic compounds in the environment might cause testicular tissue damage. The process of spermatogenesis includes various mechanisms that can be disrupted by toxic influences, leading to both functional and structural damage to sperm cells, as well as potential disturbances in the pituitary-hypothalamic and sex hormone balance. In the testes, Leydig cells, Sertoli cells, and germ cells are particularly vulnerable to toxic effects [7]. Massive amounts of paracetamol were reported to act on the male reproductive system by altering semen quality, specifically sperm morphology, thus affecting fertility. PR was assumed to limit testosterone synthesis, cause oxidative stress, stimulate spermatocyte death, diminish nitric oxide production, and inhibit prostaglandin synthesis [8]. Therapeutic approaches to such poisonings have recently become a focus of research and development. Researchers in the field of alternative medicine have placed great emphasis on minerals and herbal medicines. Boron and its derivatives have been used in many areas, from medicine to industry, since ancient times. Boron has recently been utilized in multiple health fields, facilitating new avenues for research with significant promise [2].

Boron is a crucial element found in nature in about 230 forms as crystal salts with sodium, magnesium, calcium, and as boric acid and various borate compounds (e.g., boric acid, borax). Borates are predominantly found in soil, rock, and water sources [9,10]. The importance of boron and its compounds is increasingly recognized in both health and science disciplines due to their detailed studies and economic

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value. Boron is essential for living organisms [11,12] and has been found to affect the metabolism of certain bioelements (Ca and P) [13,14] hormones (estrogen, testosterone, thyroid and insulin) [15], and vitamin D [16]. Additionally, it influences the activities of certain enzymes (aldehyde dehydrogenase, xanthine oxidase, etc.) [11], ROS [17], lipid peroxidation [17, 18], and has been found to enhance the capacities of other antioxidants [19]. In addition, boron and its derivatives have been identified as inhibitors of serine proteases, thereby contributing to various physiological processes, including apoptosis [20]. Moreover, boron supplementation has been reported to increase TNF-α level and strengthen the immune system in various animal breeds, reducing levels of inflammation markers [15-21,22]. Other studies utilizing boric acid or borax have also reported that boron contributes positively to antioxidant activity, reducing DNA damage and lowering lipid peroxidation levels [18,19]. A study examining how B affects reproductive performance, found that rats administered B to counteract the harmful impacts of bisphenol A had both oxidative stress and inflammation reduced [23]. In another study, it was reported that B attenuates apoptosis in cyclophosphamide-induced testicular damage due to its antioxidant and anti-apoptotic properties [24].

In this study, its possible effects on testicular tissue against paracetamol damage were investigated. Limited studies exist on the effects of boron in mitigating paracetamol-induced testicular toxicity in rats. This study aims to examine the therapeutic dosage of boron in testicular tissue and its role in reproductive function, considering its antioxidant, anti-inflammatory, and antiapoptotic capabilities identified in prior research.

2. Materials and Methods

2.1. Materials

PR; A commercially available Parol® tablets (500 mg/tablet, Atabay Chemical Industry, Türkiye) were ground into powder using a porcelain mortar after which they were weighed, suspended in distilled water, and administered orally in a single dose of 1 g/kg to the groups receiving paracetamol [5,25,26].

Sodium pentaborate ($NaB_5O_8.5H_2O$) compound used as a boron source was commercially obtained from Kale Natural Company, Türkiye. Doses of 50-100 mg/kg were diluted in distilled water and given to rats orally [18,27].

The Experimental Animals Laboratory at Atatürk University provided 35 male Sprague Dawley rats (n=7) weighing 250 grams for this investigation. The animals were habituated for one week under regulated laboratory settings (temperature: 24±1°C, relative humidity: 45%±5, and a 12-hour light/dark cycle) before the experiment. Throughout the trial, the rats were fed standard laboratory pellets and given free access to water. Ethical approval for this research was received from the Atatürk University's Animal Experiments Local Committee for Ethics (approval number 114, dated 04.07.2022).

2.2. Methods

In this experiment, B and PR were administered orally to rats via gastric gavage. A single dose of paracetamol was administered to the rats only on the 6th day (30 minutes after boron and physiological serum administration). Experimental groups, treatment doses and durations are given in Table 1. At the end of the study, one day after the paracetamol administration (on the 7th day), the rats were anesthetized with mild sevoflurane, decapitated, and testicular tissue samples were collected. Tissues were washed with cold isotonic solution (0.9% NaCl) and stocked at -20°C until biochemical tests were performed.

2.3. Assay of Oxidants and Antioxidants

The effects of paracetamol and boron on testicular tissue were assessed by measuring MDA (Cat. No: YLA0029RA) and GSH (Cat. No: YLA0121RA) levels and the activities of enzymatic antioxidants SOD (Cat. No: YLA0115RA), CAT (Cat. No: YLA0123RA), and GPx (Cat. No: YLA0119RA). All these parameters were analyzed using YLBiont commercial ELISA kits (Shanghai, China) based on the sandwich enzyme-linked immunosorbent assay (ELISA) method.

2.4. Assay of Inflammation and Apoptosis

Paracetamol overdose is known to directly affect inflammation and apoptosis markers. The effects of inflammation and apoptosis were assessed by measuring TNF-α (Cat. No: YLA0118RA) and IL-1β (Cat. No: YLA0030RA) levels, as well as Caspase-3 (Cat. No: YLA0017RA) activity, using ELISA kits

Table 1. Experimental groups and treatment doses

Treatment	Dose	Duration	Drug and Chemicals
Control	No drugs	6 days	Physiological saline
PR	1 g/kg (single dose)	6 days (saline) 1 day (PR)	Physiological saline, PR on the day before sacrifice
B100	100 mg/kg/day	6 days	B100
PR+ B100	Boron: 100 mg/kg/day	6 days (B100) 1 day (PR)	B100, PR on day before sacrifice
PR+ B50	Boron: 50 mg/kg/day	6 days (B50) 1 day (PR)	B50, PR on day before sacrifice

Table 2. MDA, GSH levels and SOD, GPx, CAT activations of boron against paracetamol toxicity in testicular tissue

Parameter	Control	PR	B100	PR+B50	PR+B100
MDA (nmol/g)	17.12±0.61$^{###}$	74.73±4.11***	20.99±1.12$^{###}$	68.78±3.73***	49.17±3.04***/$^{###}$
SOD (ng/mg protein)	0.67±0.02$^{###}$	0.40±0.01***	0.78±0.07$^{###}$	0.44±0.01***/$^{\Delta\Delta\Delta}$	0.61±0.01$^{##}$
GSH (nmol/g)	9.84±0.26$^{###}$	7.60±0.14***	9.40±0.26$^{###}$	7.57±0.11***/$^{\Delta\Delta\Delta}$	8.89±0.14$^{###}$
GPx (ng/g protein)	5.48±0.27$^{###}$	4.15±0.06***	4.99±0.16$^{##}$	4.83±0.06	4.84±0.06
CAT (ng/g protein)	0.44±0.03$^{###}$	0.21±0.00***	0.23±0.01***	0.21±0.00***	0.22±0.00***

Statistical significance (Control vs. others: * p < 0.05, ** p < 0.01, *** p < 0.001; PR vs. others: $^{#}$p < 0.05, $^{##}$p < 0.01, $^{###}$p < 0.001; PR+B100 vs. PR+B50: $^{\Delta}$ p < 0.05, $^{\Delta\Delta}$ p < 0.01, $^{\Delta\Delta\Delta}$ p < 0.001) was analyzed using One Way ANOVA).

(YLBiont, Shanghai, China brand sandwich enzyme-linked immunosorbent assay).

2.5. Statistical Analysis

The statistical analyses in this study were carried out using SPSS software version 20.0 (IBM, USA). Group variations and significance levels for all metrics were assessed using a one-way analysis of variance (ANOVA), followed by Tukey's test for post-hoc multiple comparisons. The results are presented as mean ± SEM. A p-value of <0.05 was judged statistically significant.

3. Results

3.1. Level of MDA

MDA levels in testicular tissue were found to be significantly higher in the paracetamol group than in the control group. However, this elevated level was significantly reduced in groups receiving both boron dosages.

3.2. Antioxidant Enzymes and Reduced GSH

It was determined that the activity of SOD, an enzymatic antioxidant, decreased in the PR group compared to the control group (Table 2), and especially the 100 mg/kg dose of boron given together with paracetamol could increase the decreased activity (p<0.001). The GSH level of the PR group decreased compared to the control group. It was also determined that testicular GSH levels increased in the PR+B100 group compared to the PR group (p<0.001). It was concluded that there was a difference between the control group and the compared paracetamol rat groups in the testicular tissues examined in terms of GPx activation (p<0.001) and that boron could increase GPx activation in the applied groups (Table 2).

When CAT activity, an antioxidant responsible for breaking down H_2O_2 (hydrogen peroxide) in cellular structures, was examined, the PR group showed a decrease compared to the control group. However, no significant difference was observed in the boron-treated groups.

3.3. Analysis of Inflammatory Cytokines and Apoptosis

When the TNF-α cytokine level was examined, the level was significantly increased in the PR group compared to the control group. In the boron-applied groups, the level was significantly decreased compared to the PR group (p<0.001). The examination of TNF-α cytokine levels revealed a substantial rise in the PR group compared to the control group, while a significant drop was observed in the boron-treated groups relative to the PR group (p<0.001).

The IL-1β level, an inflammatory cytokine, was statistically significantly elevated in the PR group relative to the control group (Table 3). The boron treatment groups markedly diminished the elevation of IL-1ß levels relative to the PR toxicity group. Caspase-3 activity in the PR group was increased compared to the control group, and boron treatment decreased this level (p<0.001).

4. Discussion

This study explored the effects of boron (sodium pentaborate) in mitigating oxidative stress, inflammation, and apoptosis triggered by paracetamol-induced testicular damage in rats. Various biochemical markers were evaluated in testicular tissue to assess these effects.

Based on previous research, testicular damage was induced using a 1 g/kg dose of PR, a dosage commonly

Table 3. Inflammation (TNF-α, IL-1β) levels and apoptosis (Caspase-3) activation of boron against paracetamol toxicity in testicular tissue

Parameter	Control	PR	B100	PR+B50	PR+B100
TNF-α (µg/g)	82.23±3.30$^{###}$	135.75±8.00***	79.02±1.07$^{###}$	86.60±3.86$^{###}$	85.32±3.53$^{###}$
IL-1β (ng/g)	33.66±0.66$^{###}$	55.68± 3.04***	35.69±0.88$^{###}$	47.96±0.83***	45.07±0.74***/$^{###}$
Caspase-3 (ng/g)	0.72±0.01$^{###}$	0.95±0.01***	0.70±0.01$^{###}$	0.93±0.01***	0.85±0.02***/$^{##}$

Statistical significance (Control vs. others: * p < 0.05, ** p < 0.01, *** p < 0.001; PR vs. others: $^{#}$ p < 0.05, $^{##}$ p < 0.01, $^{###}$ p < 0.001) was analyzed using One Way ANOVA).

referenced in experimental models [5,25]. High doses of paracetamol, frequently used for its analgesic and antipyretic properties, are known to generate reactive oxygen species (ROS), deplete antioxidant defenses, and impair liver, kidney, and testicular function [1,28]. Oxidative stress arises when the equilibrium between antioxidants and pro-oxidants is disrupted. Under such conditions, antioxidant enzymes like SOD, CAT, GSH and GPx decline in activity, whereas MDA levels, indicative of lipid peroxidation, increase [29, 30]. GSH serves a vital role in maintaining cellular redox balance and shielding enzymatic antioxidants. Its depletion by NAPQI, a toxic paracetamol metabolite, is a key driver of tissue necrosis and apoptosis [31-33]. Experimental findings from Muhammad et al. demonstrated that 500 mg/kg of paracetamol elevated MDA levels in rat testicular tissue while reducing GPx and SOD activity [34]. Other studies further reinforce that paracetamol induces oxidative damage and compromises antioxidant defenses [35-37].

Research on boron supplementation highlights its contributions to bone integrity, cognitive function, endocrine balance, mineral metabolism, inflammatory regulation, and antioxidant mechanisms [13,38-41]. A study evaluating borax administration (200 mg/kg for 6 days) against cyclophosphamide-induced toxicity found that boron supplementation reduced MDA levels while enhancing GSH content in testicular tissue [24]. Another investigation reported that although plasma CAT and SOD activities remained unchanged in boron-supplemented goats, glutathione reductase activity and sperm quality improved in the treated group [42]. However, excessive boron intake has also been linked to potential testicular toxicity in certain studies [43,44]. In the present study, paracetamol exposure led to a notable reduction in antioxidant enzyme activity and an increase in MDA levels, reflecting heightened cellular damage. Pre-treatment with boron, particularly at a dose of 100 mg/kg, exhibited a protective effect by reversing these alterations.

Apoptosis, a programmed cell death mechanism, is crucial for removing dysfunctional or damaged cells and is implicated in various inflammatory, neurodegenerative, and autoimmune disorders [45-47]. Caspase-3, an essential apoptotic enzyme, facilitates cytoskeletal breakdown, promoting cell death [48]. Immune system regulators such as TNF-α and interleukins (IL-1β, IL-2, IL-4, IL-6, IL-10) play essential roles in host defense. However, their excessive secretion can result in cytotoxicity, tissue injury, and septic complications [42, 49, 50]. Testicular cells, including Leydig, Sertoli, and germ cells, are particularly vulnerable to environmental and toxic stressors [37,51]. Numerous studies suggest that boron, administered at varying concentrations, can mitigate inflammatory and apoptotic responses in testicular tissue [42,52].

Findings from this study demonstrate that TNF-α, IL-1β levels, and Caspase-3 activity were significantly increased in paracetamol-exposed testicular tissue. However, these markers were significantly reduced in the groups receiving boron pre-treatment at doses of 50 and 100 mg.

5. Conclusions

This study demonstrates that paracetamol administration induces oxidative stress, inflammation, and apoptosis in rat testicular tissue, as evidenced by decreased SOD, CAT, GPx, and GSH levels, alongside increased MDA, TNF-α, IL-1β, and Caspase-3 activity. These biochemical alterations indicate heightened lipid peroxidation, weakened antioxidant defenses, and enhanced apoptotic activity, leading to testicular damage.

Conversely, boron supplementation at 50 mg/kg and 100 mg/kg doses exhibited protective effects against paracetamol-induced toxicity. Specifically, boron pre-treatment restored antioxidant enzyme activity, reduced oxidative stress markers, and suppressed inflammatory cytokine levels, particularly at the 100 mg/kg dose. These findings suggest that boron may play a crucial role in mitigating testicular toxicity through its antioxidant, anti-inflammatory, and anti-apoptotic properties.

While these results highlight the therapeutic potential of boron, further investigations are necessary to elucidate its long-term safety, optimal dosage, and precise mechanisms of action in reproductive health. Future studies should explore molecular pathways involved in boron's protective effects and evaluate its applicability in clinical settings.

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7. Conflict of Interest

The authors have no conflict of interest.

8. Author Contribution Statement

Esra Aktas Senocak: Methodology, data analysis and editing, visualization, and writing draft.

Necati Utlu: Methodology, project management, sourcing, review writing and editing.

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