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Investigation of Protecting Effect of Boric Acid against Mercury II Chloride Toxicity in Rat Brain Tissue

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Anahtar Kelimeler
Antioxidant Enzyme, Brain, Boric acid, $HgCl_2$, Rat

Abstract: In this study, the protective effects of boric acid (BA) in the prevention of brain damage caused by mercury II chloride ($HgCl_2$) in rats were investigated. In the experiment, 24 adult and Wistar albino male rats weighing roundly 200-300 g were used. Group I (Control, n=8): Isotonic saline (i.p), Group II ($HgCl_2$,n=8):(0.01g/kg)(oral), Group III ($HgCl_2$ + (BA) (n:8): $HgCl_2$(0.01g/kg) + BA (3.25mg/kg/day i.p.) group were administered. The rats in all groups were sacrificed at the end of the 10th day and their brain tissues were taken. Biochemical parameters including the enzyme activities of SOD, CAT and GSH-Px were measured. The enzyme activity of SOD was reduced in $HgCl_2$ treated group in comparison to the control group ($p<0.001$). Activity of the enzyme was increased after BA administration ($p<0.001$). CAT enzyme activity decreased in $HgCl_2$ and $HgCl_2$+BA administered groups with control ($p<0.001$). An increase in enzyme activity in BA group with $HgCl_2$ administered group was observed. GSH-Px enzyme activity decreased in $HgCl_2$ ($p<0.001$) and $HgCl_2$+BA ($p<0.05$) groups with control. However, an increase was found in BA administered group with $HgCl_2$ administered group ($p<0.001$). It is thought that antioxidant enzyme activities such as SOD, CAT and GSH-Px are significantly changed and BA may have a protective effect in the histopathological examination of brain tissue.

Civa II Klorürün Sıçan Beyin Dokusunda Oluşturduğu Toksisiteye Karşı Borik Asidin Koruyucu Etkisinin İncelenmesi

Keywords
Antioksidan Enzim, Beyin, Borik asit (BA), Civa II klorür ($HgCl_2$), Rat

Öz: Bu çalışmada, sıçanlarda civa II klorür'ün meydana getireceği beyin hasarının engellenmesinde borik asit (BA)'in koruyucu etkileri araştırıldı. Deneyde 200-300 gr ağırlığında, 24 adet yetişkin, Wistar albino cinsi erkek rat kullanıldı. Ratlar, Grup I (Kontrol,n:8):İzotonik serum uygulandı (i.p), Grup II (civa II klorür ($HgCl_2$),n:8):Oral yol ile (0.01 g/kg), Grup III ($HgCl_2$+BA,n:8): Oral yol ile $HgCl_2$ (0.01 g/kg/gün) + BA (3.25mg/kg/gün) (i.p) konsantrasyonda uygulaması yapıldı. Tüm gruplardaki ratlar 10. günün sonunda sakrifiye edilerek beyin dokuları alındı. Biyokimyasal parametrelerden SOD, CAT ve GSH-Px antioksidan enzim aktiviteleri ölçüldü. Ayrıca histopatolojik olarak değerlendirildi. SOD enzim aktivitesi değerlendirildiğinde; Hg uygulanan grupta kontrole oranla azaldığı ($p<0.001$) görüldü. BA, uygulanan grupta ise aktivitenin $HgCl_2$

grubu ile kıyaslandığında arttığı ($p<0.001$) tespit edildi. CAT enzim aktivitesi değerlendirildiğinde; kontrole oranla $HgCl_2$ ve $HgCl_2$+BA uygulanan gruplarda aktivitede azalma ($p<0.001$) olduğu görüldü. $HgCl_2$ uygulanan grupla karşılaştırıldığında BA uygulanan grupta enzim aktivitesinde artış belirlendi. GSH-Px enzim aktivitesi değerlendirildiğinde ise; kontrol grubu ile kıyaslandığında $HgCl_2$ ($p<0.001$) ve $HgCl_2$+BA ($p<0.05$) uygulanan gruplarda aktivitenin azaldığı görüldü. Ayrıca $HgCl_2$ uygulanan grup ile karşılaştırıldığında BA uygulanan grupta artış olduğu görüldü ($p<0.001$). SOD, CAT ve GSH-Px gibi antioksidan enzim aktivitelerinin anlamlı olarak değiştiği ve beyin dokusu histopatolojik incelemede BA'nın koruyucu etkisi olabileceği düşünülmektedir.

1. INTRODUCTION

Recently, the damage caused by heavy metals to the ecological system has become almost commonplace. Since the ancient times, with the processing of metal ores, metals began to spread to the atmosphere and hydrosphere as a natural result of human activities [1]. Metals are naturally occurring elements of the earth's crust. Over 90 elements are present in the periodic table, and others, except 20, are characterized as metal, and 59 of these metals are classified as “heavy metals”[2]. Some of the heavy metals include arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and mercury (Hg) [3,4]. Hg is one of the most common environmental pollutants and is also used in industrial, pharmacological, agricultural and other areas [5,6]. Hg in the human body causes toxic effects on various vital organs[7]. Hg's toxicity in humans varies according to dose and exposure rate [8]. Hg has been reported to have serious effects on various organs by causing brain damage, liver dysfunction, kidney failure, gastrointestinal tract diseases and anomalies in central nervous system. [9-11]. Hg exposure promotes the generation of reactive oxygen species (ROS) [12]. Elevated levels of ROS causes oxidative stress leading to detrimental effects to diverse organs and tissues [13]. Antioxidant systems have been shown to play an significant role in Hg toxicity [14] [5].

Boric acid (BA) is a trace element that is important for plants, humans and animals due to its contribution to metabolic events [15,16]. BA, strengthens the antioxidant defense mechanism with an unknown mechanism [17]. BA is available in many consumer products including fabrics, wood, pesticides, many cosmetics and personal care products [18]. It has been reported that it plays an significant role in improving plasma lipid profiles and brain function [19,20]. The aim of this study was to examine the protective effects of BA in preventing the brain damage caused by mercury in rats.

2. MATERIALS AND METHODS

2.1.Chemicals

Boric acid (99.5 % purity) is obtained from Sigma-Aldrich (Germany) and $HgCl_2$ (99% purity) was purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals used were of analytical grade and were purchased from either Sigma or Merck (Darmstadt, Germany).

2.2. Experimental animals

A total of 24 adult male Wistar-albino rats (200-300 g) were used in the study. All animal experiments were carried out at the center of experimental animals by obtaining permission from Bingol University Animal Experiments Ethics Committee (BUHADEK, 21.02.2018 / Decision no: 2018/02). Rats were kept in cages in a controlled room, provided with a constant temperature of 20-22ºC as well as a 12-hour light-dark cycle (light from 07:00 to 19:00; dark from 19:00 to 07:00). Water and standard food were provided *ad libitum*. In this way, the rats were examined in their cages for a week and then experimental procedures had started.

2.3.Experimental Design

The rats were classified into three groups. Group I (Control, n:8): The rats in this group were given isotonic serum (i.p.), Group II ($HgCl_2$, n:8): rats in this group were given oral route $HgCl_2$ (0.01 g kg^{-1}), [21]**.** Group III ($HgCl_2$+BA, n:8): rats in this group were given oral route $HgCl_2$ (0.01 g kg^{-1}) + BA (3.25 mg kg^{-1}/day) (i.p) and brain tissues were obtained at the end of 10^{th} day for analysis [21-22]**.**

2.4. Preparation of tissue homogenate

Rats were anesthetized and sacrificed under anesthesia. Brain tissue was clean down 0.9 % NaCl and tissues were kept at -80 °C until analysis. Brain tissues (0.5 g) were homogenized in phosphate buffer. Following homogenization via homogenizer and glass-porcelain homogenizer for 8 minutes, samples were then centrifuged at 9500 rpm for 30 minutes [23]. All procedures were performed at 4 °C and then supernatants were collected for analysis. Supernatants were used to define the activity of the antioxidant enzyme.

2.5. Antioxidant enzymes estimation

SOD activity was determined according to Sun et al. (1988), at a wavelength of 505 nm [24]. CAT activity was measured according to Aebi (1984) at a wavelength of 240 nm [25]. The Activity of tissue GSH-Px was measured according to Paglia and Valentine (1967) at a wavelength of 340 nm[26]. The enzyme activities were expressed as EU mg^{-1}.

2.6. Histopathological Analysis of the Brain

10 weeks old 24 Wistar albino strain rats in 3 groups (n:8) were used in this study. Firstly, all rats were anesthetized with (i.p) of 10 mg kg^{-1} xylazine and 60 mg kg^{-1} ketamine and euthanasia was applied by decapitation under deep anesthesia [27]. After that, necropsy was applied properly to modified Virchow's technique in all rats and the brain tissues were taken immediately [28]. Brain tissue samples were put and fixed in 10% buffered formaldehyde solution and treated as routine protocol of dehydrated in ascending grades of ethanol, cleared in xylene and then embedded in paraffin [29]. Paraffin embedded tissue example sectioned at 5 µm thicknesses by rotary microtome (Leica, RM2125). The slides were stained with hematoxylin & eosin (H&E) for histopathological investigation [30]. Recent the slides were examined and photographed by using light microscope with imaging system (Leica, DM2500/DFC295) for detection of neuronal degeneration and loss in frontal cortex of brain.

2.7. Statistical Analysis

Data were analyzed with SPSS, Windows 15.0 (SPSS, Inc., Chicago, IL, ABD). Results were statistically analyzed with post hoc Least Significance Difference Test (LSD) and one-way ANOVA. Results were presented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Biochemical findings

In the study, when the levels of rat brain antioxidant enzyme activity were evaluated, it was seen that SOD enzyme activity decreased in the $HgCl_2$ group compared to control group ($p<0.001$). In the $HgCl_2$+BA treated group, the enzyme activity was increased compared to $HgCl_2$ group ($p<0.001$) (Figure I).When the enzyme activity of CAT, which is one of the most important defense enzymes that converts hydrogen peroxide in water and oxygen to all living beings, is considered; it was observed that there was a decrease in activity ($p<0.001$) in groups with $HgCl_2$ and $HgCl_2$ + BA compared to control. When compared with $HgCl_2$ treated group, an increased enzyme activity was observed in the $HgCl_2$+BA treated group (Figure II). When the GSH-Px enzyme activity, which has an significant role in defense mechanism against oxidative stress is evaluated; it was observed that the activity was decreased in $HgCl_2$ ($p<0.001$) and $HgCl_2$ + BA ($p<0.05$) treated groups in comparision to control group. The activity of the enzyme was higher in $HgCl_2$+BA treated group in comparisipn to the $HgCl_2$ group ($p<0.001$) (Figure III). Based on these findings, it could be concluded that, BA is an important antioxidant in preventing the loss of enzyme activity due to heavy metal damage.

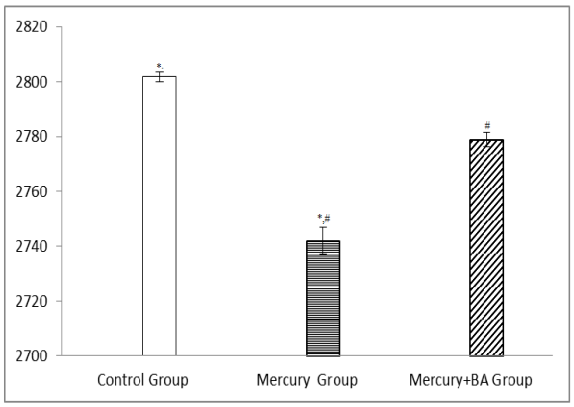


Figure 1. Superoxide dismutase (SOD) levels in brain tissue of groups (EU mg^{-1}) (*,# $p<0.001$)

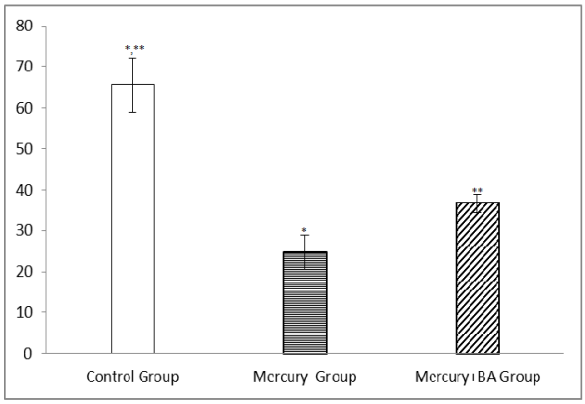


Figure 2. Catalase (CAT) levels in brain tissue of groups (EU mg^{-1}) (*,**$p<0.001$)

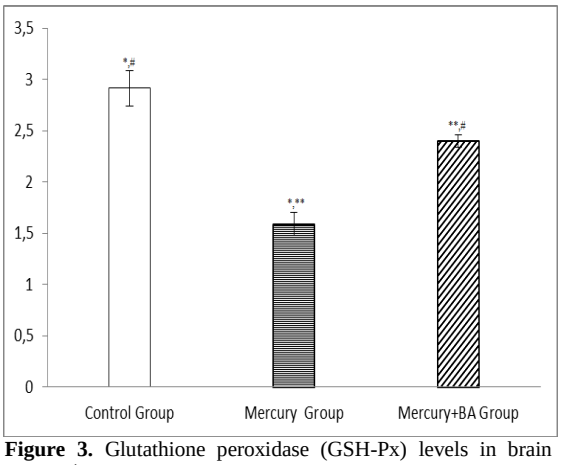


Figure 3. Glutathione peroxidase (GSH-Px) levels in brain tissue (EU mg^{-1}) (*,**$p<0.001$,#:$p<0.05$)

3.2. Histopathologic Analysis results

3.2.1. Frontal cortex of brain

No significant histopathologic lesions were observed in control group and the frontal cortexes of brains were showed normal histological structure (Figure 4–A, B). In the $HgCl_2$ administrated group in frontal cortexes of brains, perineural vacuolization and satellitosis, indication of neuronophagia, and congestion of blood vessels were found most frequently lesions. On the other hand, necrosis of some of pyramidal neurons with pyknotic nuclei, neuronal shrinkage and microgliosis were observed in the frontal cortexes of brains (Figure 4-C, D). In addition to this, in the frontal cortexes of brains

of $HgCl_2$+BA treated rats (Group-3) slight histopathological findings, which were less than $HgCl_2$ administrated rats, were observed in the sections There were congestion of some blood vessels and rarely neuronal shrinkage in the frontal cortexes of brains of $HgCl_2$+BA treated rats (Figure 4 – E, F).

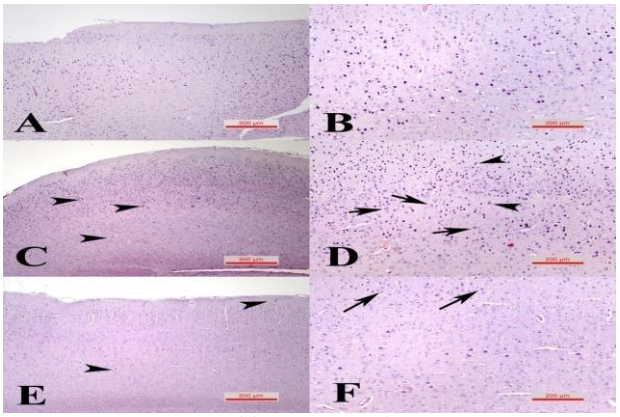


Figure 4. Histopathology of frontal cortex of brain with H&E staining. Normal histological structure of frontal cortex, x40 magnification (A); and x100 magnification (B) in control group (Group-1).Congestion of blood vessels (arrow heads) x40 magnification (C); perineural vacuolization and satellitosis (arrows); necrosis of pyramidal neurons with pyknotic nuclei and microgliosis (arrow heads) x100 magnification (D) in frontal cortex of $HgCl_2$ administered group (Group-2). Congestion of blood vessels (arrow heads) x40 magnification (E); neuronal shrinkage (arrows)x100 magnification (F) in frontal cortex of boric acid treatment group (Group-3).

The brain is a vital part of the organism that works as a system of coordination and regulation for body parts [31]. The accumulation of serious metals in the brain area may interfere with the synthesis of specific enzymes responsible for brain functions and, in turn, produce neurological disorders, including Alzheimer's disease and encephalopathy [32]**.** $HgCl_2$ has been regarded as a highly toxic metal by humans for many years [6]. Both biological and toxicological impact of $HgCl_2$ depends on form of $HgCl_2$. Inorganic $HgCl_2$ ingested in the diet is weakly immersed throughout the intestines and mostly excreted with stools [33]**.** People may be exposed to $HgCl_2$ by contaminated water and food [34]. Genotoxic effects of inorganic and organic forms of metal were defined in many *in vitro* and *in vivo* studies [35-37]. Organic forms are generally more toxic, while inorganic forms are more abundant in the environment [38]. One of the main mechanisms responsible for $HgCl_2$ toxicity is oxidative stress [39-41]**.** Oxidative stress can trigger a protective antioxidant system that avoids or ameliorates neuro toxicity in the brain [32]**.** The use of antioxidants in various disorders associated with oxidative stress is becoming widespread [31]. Antioxidants are very important in eliminating ROS, which causes oxidative stress in the body [42]. BA is a weakly acidic, white crystalline structure found in nature (minerals, seawater). BA has various uses as a protective and industrial agent [43]. It is stated that boron can regulate oxidant-antioxidant balance of various tissues [44]. It has been reported that BA is used as an anti-inflammatory agent and is involved in wound healing, prevention of oxidative stress, elimination of toxic effects of heavy metals and regulation of mitochondrial membrane potential [45]. Enzymatic and non-enzymatic antioxidants play a significant role in the prevention of injuries caused by ROS [46]. SOD is an enzyme that converts superoxide ions into H_2O_2. GSH-Px and catalase metabolize H_2O_2 into water. The protection of the balance between ROS and antioxidant enzymes is therefore very important and can serve as an important mechanism to prevent damage caused by oxidative stress [47]. In this study, SOD enzyme activity was decreased in HgCl2 group compared to control group ($p<0.001$). In the HgCl2+BA treated rats, the enzyme activity was increased compared to the $HgCl_2$ group ($p<0.001$). It was observed that there was a decrease in $HgCl_2$+ BA group compared to control but this decrease was not statistically important (Figure 1). Evaluation of CAT enzyme activity revealed that; $HgCl_2$ and $HgCl_2$ + BA groups showed decreased activity as compared to the control ($p<0.001$). A rise in enzyme activity was detected in the BA treated group compared to the $HgCl_2$ group (Figure 2).

The antioxidant and antiapoptotic effect of boric acid on hepatoxicity in chronic alcohol-fed rats were examined in a different study. It was revealed that SOD and CAT enzyme activity were increased in alcohol + BA treatment groups in comparison to alcohol treated group[45].

In another study, the effect of BA on oxidative stress in rats with fetal alcohol syndrome was investigated. The results revealed that activity of SOD and CAT were increased in the alcohol + BA group compared to the alcohol administered group[46]. When GSH-Px enzyme activity was evaluated; When compared with the control group activity was decreased in $HgCl_2$ ($p<0.001$) and $HgCl_2$+ BA ($p<0.05$) administered groups. There was also an increase in the BA treated group compared to the $HgCl_2$ administered group ($p<0.001$) (Figure 2). Different studies indicated that GSH-Px enzyme activity increases with BA [45-48]**.** The results show that antioxidant effect in these tissues may be related to the increase in SOD, CAT, GSH-Px activity. In a study, it was found that low dose of BA may protect brain against the pathological effects of $AlCl_3$ in the rat brain. Moreover; it was also found that low BA dosage had a protective effect on the intense neuronal damage induced by $AlCl_3$ using stereological examination [22]. In addition to these, *in vivo* and *in vitro* studies have shown that compounds containing BA and BA have a protective role against many cancers [22,49]**.** Despite the presence of several studies, the study of the protective effect of BA on neuronal damage induced by heavy metals are inadequate [50]. Mercury readily penetrates the placental and the blood–brain barrier [51-53], In a research, it was demonstrated that *in vitro* cultured oligodendrocytes are sensitive to the toxic effects of mercury [54]. In a different study, it was determined that mercury and boric acid have an inhibition effect on some metabolic enzymes [55]. In addition, in another study examining the effects of various heavy metals on one of the important metabolic enzymes, it was reported that some heavy metals increase enzyme activity and some cause enzyme inhibition [56]. In our study neuronal degeneration found in group-2 and group-3 was

observed to be different severe degree in each, however no microscopic lesion was observed in group-1 (Figure 4). This suggests that in the BA administration, the prevention and treatment of neuronal damage in brain is partially effective. Limited neurodegenerative changes were observed microscopically in the group-3, and these changes may refer that boric acid did have partial influence on protection or treatment effects of cerebral neurons. In this study, we examined the effects of BA on cerebrocortical neurons damage in rat models. Future research require further explorations of the effects of boric acid at various dosages and long time periods.

4. CONCLUSION

As a result; significant rise in antioxidant enzyme levels such as SOD, CAT, GSH-Px and positive effects in histopathological evaluation suggest that BA may be a potential protective agent for the brain and our results may contribute to further studies.

5. ETHICAL CONSIDERATIONS

Study was approved by Animal Experiments Local Ethical Committee (BUHADEK,21.02.2018 /2018-02, Decision no: 02-04).

Conflıcts Of Interest

The authors declare that they have no conflict of interest.

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