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Investigation of the Protective Effect of Boric Acid Against Hepatotoxic and Nephrotoxic Injury Induced by Acrylamide in Rats

Investigación del Efecto Protector del Ácido Bórico contra Lesiones Hepatotóxicas y Nefrotóxicas Inducidas por Acrilamida en Ratas

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SUMMARY: To investigate if the administration of boric acid (BA) would exert any protective effect against possible nephrotoxicity and hepatotoxicity induced by the exposure to acrylamide (ACR) in rats. In our study, we used a total of 28 rats that were divided into four equal groups. Group 1: the control group which was not treated with any procedure. Group 2: the ACR group that was administered ACR 50 mg/kg/day via intraperitoneal (i.p) route for 14 days. Group 3: the BA group that was administered BA 200 mg/kg/day via gavage via peroral (p.o) route for 14 days. Group 4: the ACR+BA group that was administered BA simultaneously with ACR. Total antioxidant and oxidant (TAS/TOS) capacities were measured in all groups at the end of the experiment. In addition, the specimens obtained were evaluated with histopathological examination. Studies showed that the ACR and ACr+BA groups were not significantly different in terms of hepatic TAS level while the TOS level was higher in the ACR group than the ACR+BA group. The groups did not show any significant difference regarding renal TAS and TOS levels. In the histopathological examination of the hepatic tissue, the histopathological injury score of the ACR group was significantly higher than those of the other groups whereas it was significantly lower in the ACR+BA group than the ACR group. Our study concluded that Boric acid had a protective effect against acrylamide-induced hepatotoxicity, but not against nephrotoxicity.

KEY WORDS: Hepatotoxicity; Nephrotoxicity; Acrylamide; Boric Acid.

INTRODUCTION

Acrylamide (ACR) is a water soluble, highly reactive compound that is formulated as C3H5NO (Tyl & Friedman, 2003). It is known to be widely utilized in cosmetics, waste water treatment, and textile industry (Tareke *et al*., 2002). It is found in high amounts in food products with carbohydrate content that are processed at high temperatures, such as fried or baked foods (Kunnel *et al*., 2019). Children and the young are under greater risk because they consume food such as fried potatoes, potato chips, and biscuits (Kadawathagedara *et al*., 2018). Different studies on ACR toxicity have shown that it induces neurotoxicity (Tabeshpour *et al*., 2020), causes hepatotoxicity (Yousef & El-Demerdash, 2006), and is carcinogenic (Hogervorst *et al*., 2010).

Boric acid (H3BO3) is an essential micro nutrient or mineral substance that is naturally found in vegetables and some food. Boron is absorbed from the gastrointestinal system (Sogut *et al*., 2015) and when solved in water, it is converted to a weak form, boric acid (BA) (Dinca & Scorei, 2013). It is known that compounds containing boron possess some in vitro and in vivo biological activities (Benderdour *et al*.,1998). Studies have also shown that it has anti-cancer, anti-inflammatory , antioxidant, hepatoprotective, and anti-genotoxic effects (Ince *et al*., 2014).

In the present study, we biochemically and histopathologically evaluated the effects of BA against hepatoxic and nephrotoxic injury induced by ACR.

MATERIAL AND METHOD

This study was approved by the local ethics committee with the protocol number of 2022/21. Our study used 28 Wistar Albino male rats with a mean weight of 320 to 400 grams.

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Supply of chemicals. Acrylamide was purchased from Sigma Alrich and Boric asit from Tekkim. The rats were divided into 4 groups, each containing 7 rats. Group 1 (n=7) was the Control group that was not treated during the experiment. Group 2 (n=7) was the ACR group that was administered ACR 50 mg/kg/day via intraperitoneal (i.p) route. Group 3 (n=7) was the BA group that was administered BA 200 mg/kg/day via gavage via peroral (p.o) route. Group 4 (n=7) was the ACR+BA group that was administered ACR 50 mg/kg/day via i.p in addition to BA 200 mg/kg/day administered via p.o route.

Collection and staining of tissue samples. At the end of the study all animals were weighed and administered general anesthesia using intraperitoneal Ketamine HCl (Ketalar, Pfizer Inc, USA) (90 mg/kg) + Xylazine HCl (Rompun, Bayer Health Care AG, Germany) (10 mg/ kg). An abdominal incision was made from the midline and the abdominal cavity was opened. Animal's welfare was preserved by checking the animal's skin or finger squeeze movement to check the adequacy of sedation. The animals were sacrificed by taking blood from intracardiac cavity via exsanguination. The blood samples were centrifuged at 300 rom for 10 minutes to separate serum that was sent to the biochemistry laboratory for biochemical analyses. The specimens that were collected for histopathological studies were put in 10 % formaldehyde and sent to the Histology / Embryology laboratory.

Biochemical analyses. Measurement of Total Antioxidant and Oxidant Capacities Kits purchased from Rel Assay Diagnostics (Gaziantep, Turkey) were used to study TAS and TOS capacity in the hepatic and renal tissues taken from all rats. TAS and TOS capacities were studied using the automatic measurement method developed by Erel (2004, 2005).

Hepatic and renal function tests. After the sera of the blood samples taken to determine hepatic function tests were separated, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels were studied. Renal function tests were studied by measuring blood urea nitrogen (BUN) and creatinine levels.

Inflammatory markers. After the plasma of the blood samples was separated, Interleukin-1 beta (IL-1b), Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels were determined with ELISA kits.

Histopathological studies. Scoring of the changes in the renal and hepatic tissues that underwent routine histological examination was based on the scoring criteria of Camargo Jr. *et al*. (1997) and Chatterjee *et al*. (2000):

For hepatic tissue:
- Grade 0: minimum injury or uninjured
- Grade 1: mild injury; cytoplasmic vacuoles and pycnosis
- Grade 2: moderate injury; cytoplasmic vacuoles, hepatocellular swelling without necrosis, irregularity of intercellular margins, sinusoidal dilation and congestion,
- Grade 3: moderate or severe injury; necrosis, cytoplasmic hypereosinophilia, diffuse sinusoidal dilation and congestion,
- Grade 4: severe injury; necrosis, disruption and bleeding of hepatic cords, disruption of tissue integrity.

For renal tissue:
- Grade 0: uninjured renal tissue
- Grade 1: tubular cell swelling, loss of margins, nuclear decline and loss of 1/3 of tubular integrity.
- Grade 2: In addition to the findings of Grade 1, more severe nuclear decline, and loss of 2-3 of tubular integrity.
- Grade 3: More severe nuclear decline, and loss of more than 2/3 of tubular integrity (Chatterjee *et al*., 2000).

Statistical analysis. Statistical analysis of the data was carried out using SPSS for Windows version 20 (SPSS Inc., Chicago, IL, USA) software package. Normality of data distribution was tested with Shapiro Wilk test. Normally distributed variables were compared using One-sided Analysis of Variance (ANOVA), and non-normally distributed variables with Mann Whitney-U Test. Level of significance was set at $p<0.05$.

RESULTS

Biochemical analysis

TAS and TOS Capacities. TAS capacity in the hepatic tissue was the highest in the BA group ($p<0.05$). There was no significant difference between the ACR group and the ACR+BA group with respect to TAS capacity ($p>0.05$).

ACR group had the highest TOS level in the hepatic tissue compared with the other groups ($p<0.05$), and the ACR group's TOS level was significantly higher than the ACR+BA group ($p<0.05$) (Table I).

There was no significant difference between the study groups in terms of TAS and TOS capacities in the renal tissue. ($p>0.05$) (Table II).

Inflammatory markers. TNF-a level was significantly higher in the hepatic tissue of the ACR group than the other

groups ($p<0.05$) while it was significantly lower in the ACR+BA group than the ACR group ($p<0.05$). IL-1b level showed no significant difference between the ACR group and the ACR+BA group ($p>0.05$) (Table I).

TNF-α level in the renal tissue was similar in the ACR group and the ACR+BA group ($p>0.05$). IL-1b levels were similar across the study groups ($p>0.05$) (Table II).

Hepatic and Renal Function Tests. When AST, ALT, and LDH levels, which we used to evaluate hepatic function, were analyzed, it was observed that the ACR and ACR+ BA groups did not show any significant difference regarding serum AST level ($p>0.05$) whereas both groups had a significantly higher AST level than the BA and control groups ($p<0.05$). ALT and LDH levels, on the other hand, were significantly lower in the ACR+BA group than the ACR group ($p<0.05$) (Table III).

Table I. Mean ± Standard deviation values of the statistical analysis of the biochemically analyzed hepatic tissue.

Group	TAS	TOS	TNF-α	IL-1_
Control	1.05±0.20b,c	201.88±21.45	515.88±196.99b	714.66±143.38b
ACR	0.44±0.27a,c	380.19±149.59a	2742.98±617.75a,c	1420.23±216.27a,c
BA	2.63±0.80a,b	190.78±27.53b	589.15±195.35a,b	637.57±210.02b
ACR+BA	0.76±0.20c	179.19±39.79b	973.13±218.49b	989.27±321.13

TAS – total antioxidant status (mmol H2O2 equivalent/L); TOS – total oxidant status (mmol Trolox equivalent/L); TNF-a – tumor necrosis factor- alpha (pg/mL); IL-1b – interleukin-1 beta (pg/mL). a: Significant difference with the Control group; b; Significant difference with the ACR group; c: Significant difference with the BA group.

Table II. Mean ± Standard deviation values of the statistical analysis of the biochemically analyzed renal tissue.

Group	TAS	TOS	TNF-α	IL-1α
Control	1.50±0.48	25.32±6.60	595.59±173.55	686.53±205.44
ACR	1.41±0.32	54.34±23.63	1287.92±309.77a,c	887.37±343.93
BA	1.96±0.33	26.88±17.62	518.13±232.11a	726.48±154.53
ACR+BA	1.76±0.51	53.44±47.47	817.94±102.19a,b	762.91±249.26

TAS – total antioxidant status (mmol H2O2 equivalent/L); TOS – total oxidant status (mmol Trolox equivalent/L); TNF-a – tumor necrosis factor- alpha (pg/mL); IL-1b – interleukin-1 beta (pg/mL). a: Significant difference with the Control group; b; Significant difference with the ACR group; c: Significant difference with the BA group.

Table III. Mean ± Standard deviation values of the statistical analysis of the biochemically analyzed hepatic and renal tissue function tests.

Group	AST (μ/l)	ALT (μ/l)	LDH (μ/l)	Urea (mg/dl)	Cre (mg/dl)
Control	61.42±22.46b	47.00±14.74b	187.71±118.83b	47.85±7.69b	0.93±0.32b
ACR	451.71±296.89a,c	283.00±61.77a,c	1187.85±263.61a,c	136.14±45.82a,c	2.59±0.95a,c
BA	71.57±14.57b	44.71±10.99b	324.85±201.32b	48.00±10.23b	0.94±0.32b
ACR+BA	251.85±170.08a,c	139.14±37.45a,b,c	571.00±314.33b	121.71±44.53a,c	2.12±1.12a

AST – aspartate aminotransferase; ALT – alanine aminotransferase; LDH – lactate dehydrogenase. a: Significant difference with the Control group; b; Significant difference with the ACR group; c: Significant difference with the BA group.

There was no significant difference between urea and creatinine levels, indicators of renal function, of the ACR and ACR + BA groups ($p>0.05$) (Table III).

Histopathological analysis. Statistical analysis of the histopathological injury of the hepatic tissues (Fig. 1): Hepatic injury score of the ACR group was significantly higher than that of the ACR+BA group ($p<0.05$).

Statistical analysis of the histopathological injury of the renal tissues (Fig. 2): Renal injury was significantly higher in the ACR+BA group than both the BA group and the Control group ($p<0.05$).

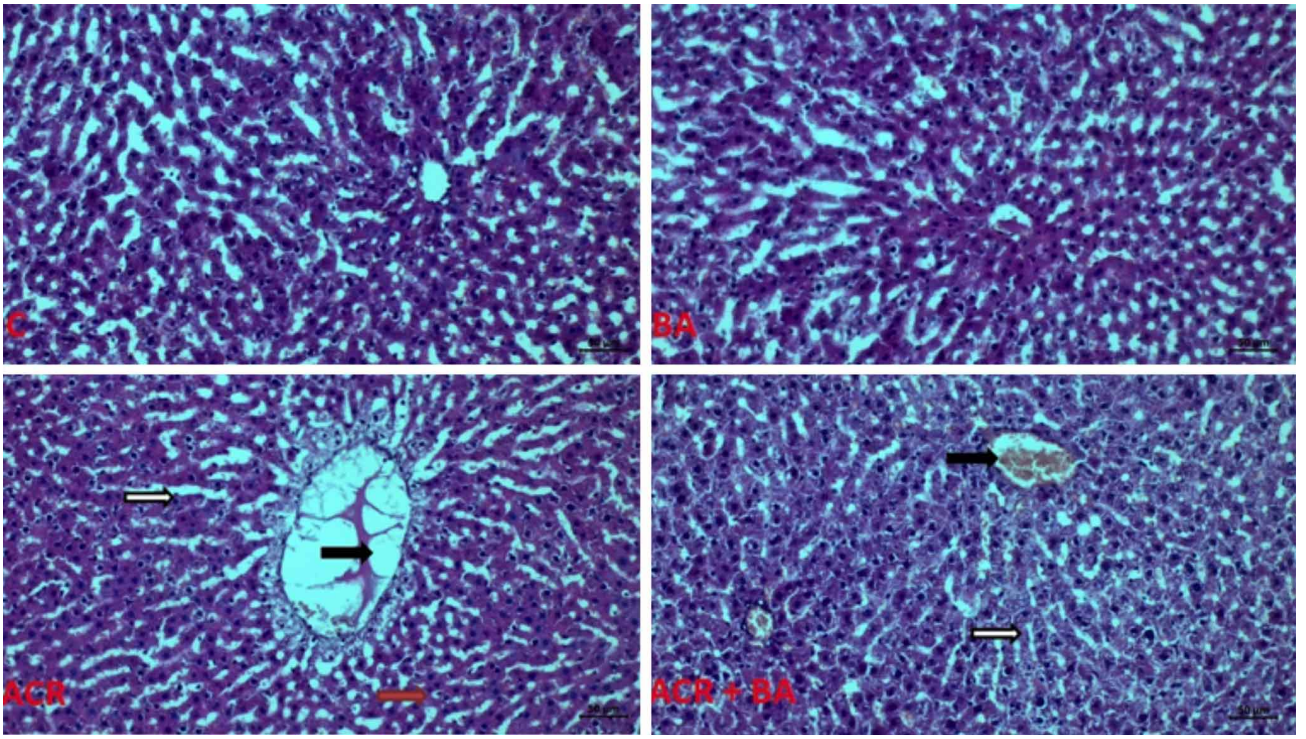

Fig. 1. (C): Normal histological view of the hepatic tissue of the control group (H&E x 400). (BA): Normal histological view of the hepatic tissue of the Boric acid group (H&E x 400). (ACR): Histological view of the hepatic tissue of the Acrylamide group (black arrow: congestion, red arrow: pycnosis, white arrow: sinusoidal dilation) (H&E x 400). (ACR+BA): histological view of the hepatic tissue of the Acrylamide + Boric acid group (black arrow: congestion, white arrow: normal sinusoidal view) (H&E x 400).

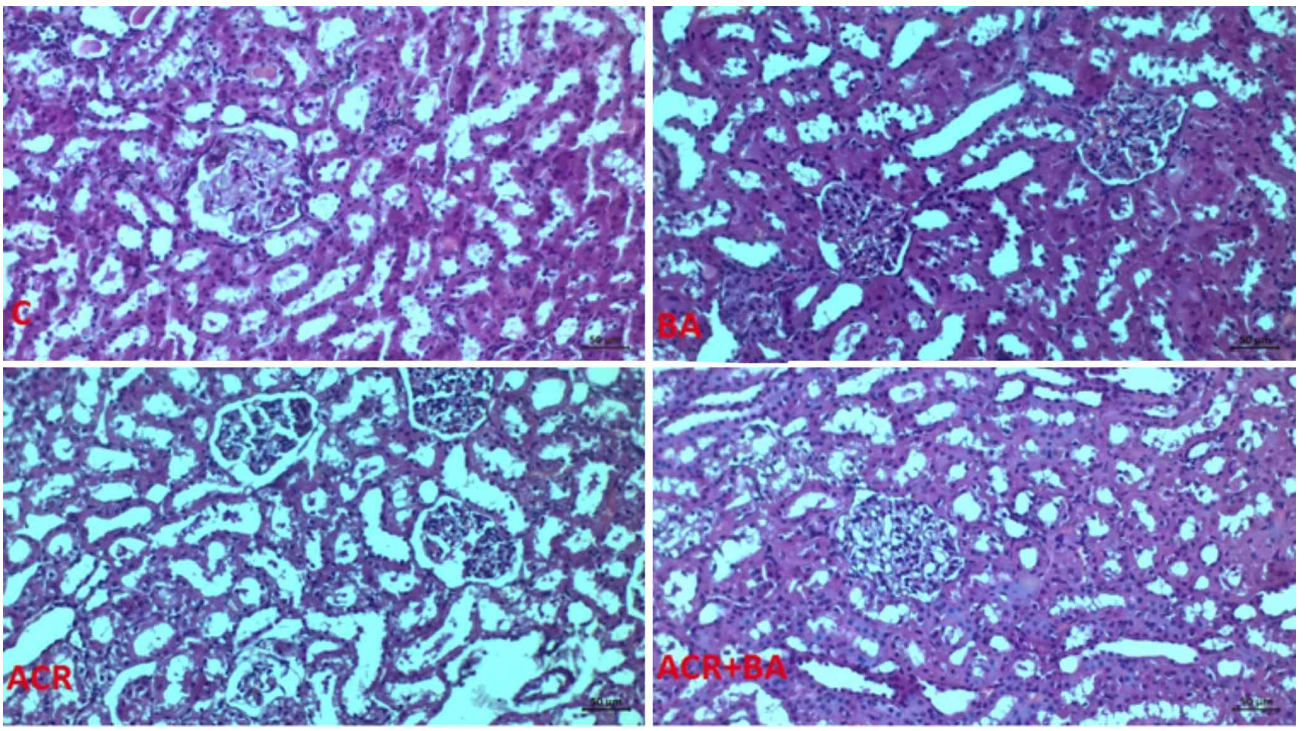

Fig. 2. (C): Histological view of the renal tissue of the control group (H&E x 400). (BA): Normal histological view of the renal tissue of the Boric acid group (H&E x 400). (ACR): Histological view of the renal tissue of the Acrylamide group (H&E x 400). (ACR+BA): Histological view of the renal tissue of the Acrylamide+ Boric acid group (H&E x 400).

DISCUSSION

Liver is a vital organ that plays a role in the detoxification of xenobiotics, environmental pollutants, and chemical deugs (Gu & Manautou, 2012). ACR is a toxic substance that forms particularlyin burned and boiling oil (Sirot *et al*., 2012). Studies have demonstrated that ACR increases oxidative stress, thereby causing elevated hepatic function tests (such as ALT, AST) and histopathological changes (Rizk *et al*., 2018). Similarly, we observed that ACR caused intense histopathological changes in hepatic tissue and adversely affected hepatic enzymes. BA is known as an antioxidant and anti-inflammatory agent that is widely used to protect mitochondrial membrane, promote wound healing, prevent oxidative stress, reduce toxic effects of heavy metals, and treat inflammatory disorders (Sogut *et al*., 2015).

Mahmood *et al*. (2015) administered ACR at a dose of 25-50 mg/kg/day via oral route and observed an increase in AST and ALT levels. We administered ACR at a dose of 50 mg/kg/day via intraperitoneal route and caused increased AST, ALT, and LDH levels, which subsequently startes to normalize with the administration of BA.

Parameters showing antioxidant and oxidant capacities (TAS/TOS) are an important diagnostic tool in toxicology studies (El-Beltagi & Ahmed, 2016). It has been reported that an exposure to ACR caused injury by increasing MDA levels in organs like brain and liver where oxidative stress plays an important role (Karimani *et al*., 2019). In a study where hepatic ischemia and reperfusion (I/R) was formed, free radicals which were formed were reduced after the administration of BA (Ozek *et al*., 2018). Similarly, we observed that BA increased TAS level and ACR increased TOS level. When BA was administered after ACR, on the other hand, there occurred a significant decrease in TOS level compared with the ACR group.

Elhelaly *et al*. (2019) observed that when ACR was administered (20 mg/kg/day), it caused increased serum TNF-a, IL-1b and IL-6 levels. The same study also reported that ACR exerted harmful effects on renal tissues by altering antioxidant enzyme systems (Elhelaly *et al*., 2019). We also demonstrated that ACR caused detrimental effects on renal tissue but BA had no protective role against those effects. Bedir *et al*., (2021) demonstrated that ACR, a prooxidant agent, affected glomerular tubules and interstitial tissues. Similarly, we observed that ACR caused histopathological changes in renal tissues and BA had no favorable effect to mitigate those histopathological changes.

CONCLUSION

According to the results of our study, we determined that boric acid showed a protective effect against acrylamide-induced hepatotoxicity but not against nephrotoxicity. However, more comprehensive studies using different dose and durations are needed in this field.

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RESUMEN: El objetivo de este estudio fue investigar si la administración de ácido bórico (BA) ejercería algún efecto protector frente a la posible nefrotoxicidad y hepatotoxicidad inducida por la exposición a acrilamida (ACR) en ratas. En nuestro estudio, utilizamos un total de 28 ratas que se dividieron en cuatro grupos iguales. Grupo 1: grupo control que no fue tratado. Grupo 2: grupo ACR al que se le administró ACR 50 mg/kg/día por vía intraperitoneal (i.p) durante 14 días. Grupo 3: grupo BA al que se le administró BA 200 mg/kg/día por sonda por vía peroral (p.o) durante 14 días. Grupo 4: grupo ACR+BA al que se administró BA simultáneamente con ACR. Las capacidades antioxidantes y oxidantes totales (TAS/TOS) se midieron en todos los grupos al final del experimento. Además, los especímenes obtenidos fueron evaluados con examen histopatológico. Los estudios demostraron que los grupos ACR y ACr+BA no fueron significativamente diferentes en términos del nivel hepático de TAS, mientras que el nivel de TOS fue mayor en el grupo ACR que en el grupo ACR+BA. Los grupos no mostraron ninguna diferencia significativa con respecto a los niveles renales de TAS y TOS. En el examen histopatológico del tejido hepático, la puntuación de lesión histopatológica del grupo ACR fue significativamente mayor que la de los otros grupos, mientras que fue significativamente menor en el grupo ACR+BA que en el grupo ACR. Nuestro estudio concluyó que el ácido bórico tiene un efecto protector contra la hepatotoxicidad inducida por acrilamida, pero no contra la nefrotoxicidad.

PALABRAS CLAVE: Hepatotoxicidad; Nefrotoxicidad; Acrilamida; Ácido bórico.

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