



Original Research

## Boric acid protects against cyclophosphamide-induced oxidative stress and renal damage in rats

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**Abstract:** The worldwide increase in the rate of cancer incidence also leads to a significant increase in the use of chemotherapeutic agents. Unfortunately, the optimal clinical usefulness of these agents is heavily restricted by a high incidence of several organ toxicity and as well reason oxidative stress and bring about changing in antioxidant status. Kidney toxicity is a side effect often encountered with cyclophosphamide (CPM) which is commonly used in most cancer chemotherapy. The present study aims on the assessment of the probable defensive efficacy of Boric acid (BA) against CPM-induced oxidative stress and renal damage in rats. Based upon our investigation results with oxidative stress markers and light microscopic findings, it can be concluded that BA significantly reduced CPM induced oxidative stress and renal damage. As far as we know, high dose (200 mg/kg) of BA are the first study on the prevention of kidney damage caused by CPM. It is thought that the renoprotective effects of BA may be due to an increase in the activity of the antioxidant protection system and also inhibition of lipid peroxidation.

**Key words:** Boric acid; Cyclophosphamide; Renoprotective; Lipid peroxidation; Oxidative stress; Nephrotoxicity.

### Introduction

The kidney is a vital organ that has important tasks in organizing many biological processes including elimination of metabolic waste products and in the regulation of extracellular fluids, electrolyte composition and acid-base balance. The toxic effects of drugs on the kidney are both widespread and an expected fact, which can disrupt any or all of the kidney function (1). Numerous different drugs, chemicals and certain metals cause acute and chronic kidney damage. CPM is an anticancer drug known to cause kidney damage in humans and animals (2). Depending on the use of CPM, tubular area necrosis and fibrosis, enlargement of glomeruli and inflammation occur, which causes kidney damage. Sufficient information about the pathogenic mechanism of CPM-induced kidney damage is not available in the literature. However, kidney damage may be associated with oxidative stress, as the metabolites of CPM cause the formation of ROS. Many studies have shown that antioxidants protect cells against harmful effects of drugs and chemical substances (3).

BA is a naturally occurring trace element with antioxidant properties. Apart from its traditional use in health services, many areas including industry, agriculture are also widely used (4). In experimental studies, two hypotheses have been raised about the biochemical and physiological role of BA in living organisms. In principle, BA can act both in response to hormone action and cell-membrane functions that affect both transmembrane signals and the movement of regulatory ions (5). On the other hand, BA may play a role as a metabolic manager in some enzymatic systems. At the same time, BA increases the amount of reduced glutathione

in the body, reducing the effects of oxidative damage and inhibiting the production of other ROS (6). In light of the above information, this study is to investigate the potential protective effect of BA on oxidative stress and kidney damage caused by CPM in rats.

### Materials and Methods

99% pure boric acid, a boron compound, and CPM (Sigma-Aldrich, Darmstadt, Germany) were purchased from a commercial company. BA and CPM were implemented as i.p..

### Experimental Protocol

In this study, twenty four Sprague Dawley male rats weighing 180-240 gr were used. Rats were kept in controlled laboratory conditions where they were fed with pellets and tap water. Randomly divided into 4 groups each containing 6 rats. The rats were kept in the auto control chambers at  $45 \pm 50\%$  humidity, at a temperature of  $22 \pm 2^\circ \text{C}$ , for 12 hours in daylight and in the dark. All animal procedures monitored in this study were approved by Eskişehir Osmangazi University Animal Welfare Committee (Ethics Committee No: 2018/648-1). BA and CPM dissolved in distilled water. Control group animals were treated with 0.5 mL of saline for 6 days. On CPM group animals, CPM (200 mg / kg) was administered on the fourth day of the experiment (7). BA group animals received BA (200 mg / kg) for 6 days. Animals belonging to BA + CPM group were treated with BA for 6 days and CPM with BA on the fourth day of the experiment. On the seventh day of the experiment, animals in all experimental groups were anesthetized to evaluate blood and kidney tissues (8).

## Light Microscopic Evaluation

Kidney tissues were insert small pieces and permanent in Bouin's solution. Latering dehydration in mounting series of ethanol, tissue samples were shined in xylene, and next fixed in paraffin and 5–6  $\mu$ m thick sections were prepared. Sections were marked with Haematoxylin–Eosin (H–E) and Masson's trichrome (Masson).

## Biochemistry assays

The serums were obtained from the blood samples by centrifugating for 10 min at 3000 rpm. Serum GSH, MDA, GPx, CAT, NOx, BUN and Cre levels were analyzed with an automated biochemical auto-analyzer (HITACHI-917).

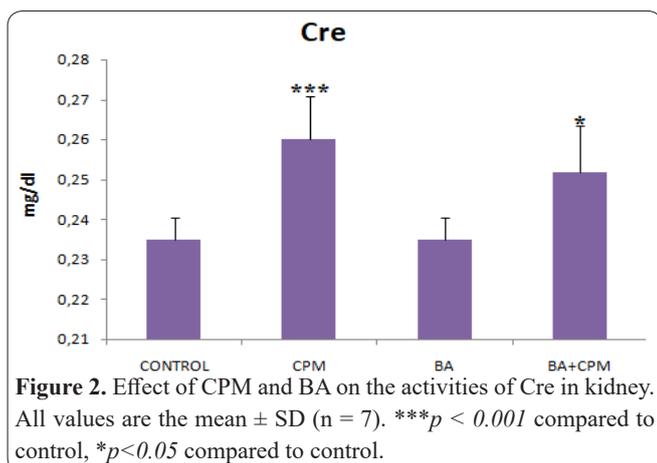
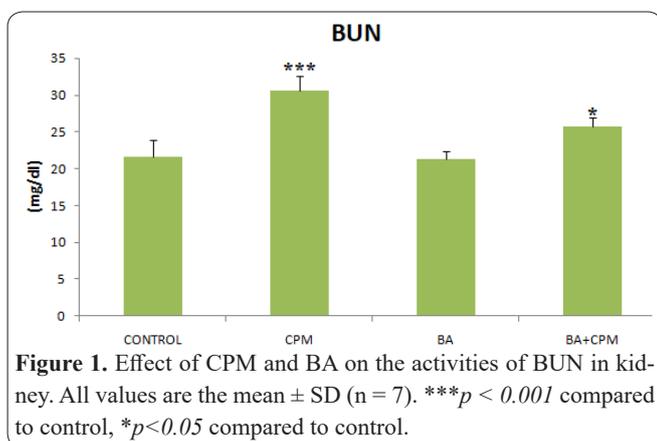
## Statistical analysis

The results were expressed as the mean  $\pm$  standard error of the mean. Statistical analysis was performed followed by one-way analysis of variance (ANOVA) With Tukey's multiple interval test,  $p < 0.001$  was accepted statistically. Triplicate samples taken from each animal were taken and the results were expressed as an average for each animal.

## Results

### BA guards renal function

BUN and serum Cre are markers of renal dysfunction. As seen in Figure 1-2, levels of serum BUN and Cre were considerably ( $p < 0.001$ ) increased in the CPM group when compared to the control and BA groups. BUN and Cre values in the BA + CPM group, which had been pre-protected with BA for 3 days, decreased



significantly in comparison with the group given CPM only ( $p < 0.05$ ).

### BA is protective against oxidative stress

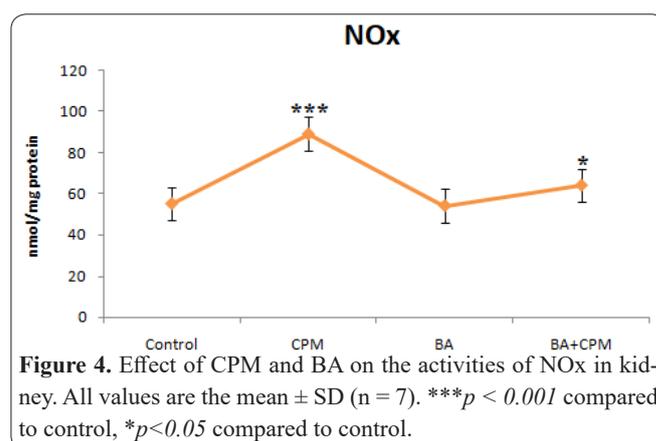
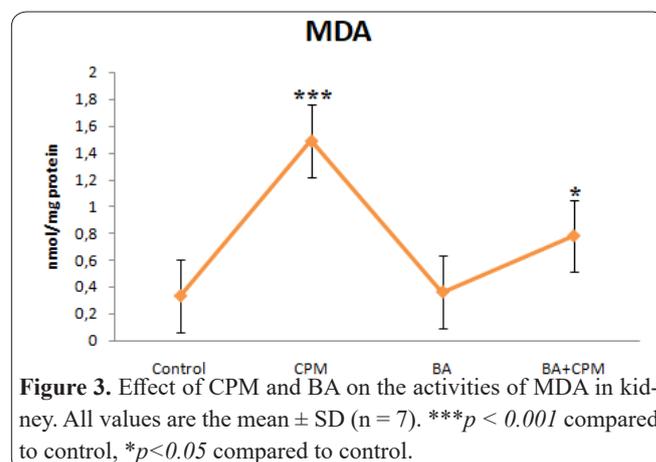
The effects of BA on MDA and NOx, known as oxidative stress parameters, levels were shown in Figure 3. The level of MDA, indication of lipid peroxidation, was notably ( $p < 0.001$ ) increased in the CPM group compared to the control and BA groups. However, MDA level in the BA+CPM group considerably decreased in comparison with the group only given CPM ( $p < 0.05$ ). The level of NOx was significantly ( $p < 0.001$ ) increased in the CPM group compared to the control and BA groups (Figure 4). But, NO level in the BA+CPM group considerably decreased in comparison with the group only given CPM ( $p < 0.05$ ).

### BA strengthens antioxidant system

The effect of BA on the activity of antioxidant enzymes including CAT, GSH and GPx is shown in Table 1. As seen in Table 1, the activity of CAT, GSH and GPx were considerably ( $p < 0.001$ ) decreased in the CPM group compared to the control and BA groups. In contrast, CAT, GSH and GPx levels in the BA+CPM group considerably increased when compared to the group just given CPM.

### BA protects kidney from harmful effects of CPM

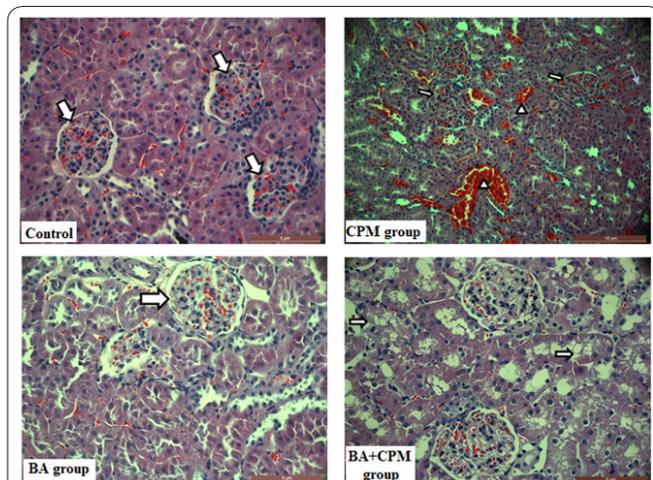
Histopathologic examination of the kidney in the control and BA groups showed normal architecture, that is to say glomerular structure, small capillary area, proximal convoluted tubules and epithelial cells in distal convoluted tubules were normal, and the boundary of the visceral layer and



**Table 1.** GSH, GPx and CAT levels of the samples taken from rats

Groups	GSH (nmol/mg protein)	GPx (nmol/mg protein)	CAT (U/mg protein)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Control	38,2 $\pm$ 2,25	7,3 $\pm$ 0,25	38,3 $\pm$ 1,01
CPM	25,79 $\pm$ 1,37 <sup>a</sup>	4,1 $\pm$ 0,13 <sup>a</sup>	18,1 $\pm$ 0,25 <sup>a</sup>
BA	38,71 $\pm$ 1,23	7,5 $\pm$ 0,14	39,5 $\pm$ 0,07
BA+CPM	32,3 $\pm$ 1,02 <sup>b</sup>	6,7 $\pm$ 0,41 <sup>b</sup>	24,7 $\pm$ 0,17 <sup>b</sup>

<sup>a</sup>;  $p < 0,001$ , <sup>b</sup>;  $p < 0,05$ .



**Figure 5.** Histopathological observations showing effects of BA on CPM-induced renal toxicity changes in kidney. Thin arrow: bleeding foci, Head Arrow: vascular congestion, arrow: constriction in Bowman interval (H&E).

the parietal layer of the renal capsule were shined. In the CPM group, glomerular atrophy and fragmentation, prominent enlargement of the capsule cavities, damage to the parietal layer of the visceral layer and renal capsule, vacuolization in several inflammatory epithelial cells, turbidity in the structure of epithelial cells and cell fragments in the tubules were present. In the kidney sections of the group given CPM with BA significant improvement was seen in Figure 5.

## Discussion

CPM is a widely used anticancer and immunosuppressive drug. Notwithstanding its therapeutic effects, CPM may have certain deleterious side effects and so cause damage to certain organs like the liver (9) brain (10), lung, kidney (3) and bone marrow (11). The toxic property of CPM are associated with two active metabolites, phosphoramid and acrolein (12). While phosphoramid possesses antineoplastic activity, acrolein may cause nephrotoxicity (13), lipid peroxidation, structural change and antioxidant activity alteration. (14). For this reason, research into finding a drug or substance to be used as a complementary healer with CPM will help reduce CPM-induced kidney damage. Several studies have confirmed that CPM induces kidney toxicity by rising the oxidative stress specifically (3,13,15). One of the studies related to toxicity caused by CPM emphasized that 200 mg / kg CPM causes pathological symptoms, such as glomerular degeneration, hypercellularity and shrinkage in the kidney tissue (16). Goudarzi *et al.* (7) reported in one of their studies that 200 mg/kg CPM caused a rise in serum BUN and Cre levels. Apart from destruction of the renal function, MDA and NO levels

had increased while GSH, SOD, GPx and CAT activities had decreased. In the same study, researchers reported significant injuries to the kidney (haemorrhage, tubular necrosis/degeneration, tubular dilatation, and leukocyte cell leukaemia). In another study, Koss and Lavin (17) investigated adverse effects of CPM on various organs of the rat. They reported that a single dose of CPM (200 mg/kg) caused necrosis of tubular epithelium in experimental animals. In similar study Sadeghi *et al* (18) investigated protective effects of hydroalcoholic extract of *Lavandula officinalis L.* in renal toxicity induced by CPM (200 mg/kg). They emphasized that CPM caused a rise in Cre and BUN, MDA levels and a significant drop in GSH, CAT, and SOD levels. In parallel with the studies mentioned above, in the kidney sections just given CPM were observed hemorrhage foci, vascular congestion, restriction in the Bowman capsule and glomerular damage. On the other hand, biochemical data showed that In CPM experimental group, BUN, Cre and MDA levels increased while GSH, GPx, NOx and CAT levels decreased. Our results were in line with the literature.

BA is a naturally occurring trace element with its antioxidant properties. The antioxidant mechanism of BA has not yet been fully elucidated (19); however, it is a well-known component of cell membrane functions and enzymatic reactions (20, 21). BA reduces oxidative stress and scavenges ROS (22). Experimental studies have also shown that BA has protective effects against oxidative stress, hepatotoxicity, pancreatic injury, genotoxicity and cardiotoxicity (8). Furthermore, BA administration dropped prostate cancer cell proliferation by reducing intracellular  $Ca^{+2}$  reserves and signalling, which are important in apoptosome formation (23). BA might also have an indirect defensive effect on cell death, that is, apoptosis. It increases anti-oxidant levels (most likely by preventing GSH reduction) and restrictions inflammatory processes by reducing intracellular ROS and  $Ca^{+2}$  levels and lastly, reduced TNF- $\alpha$  and ROS levels prevent apoptotic cell death (24). In a previous study, BA supplementation (200 mg/kg) was shown to reduce lipid peroxidation (MDA and NO) by increasing antioxidant activity such as GSH, SOD and CAT and, and it was reported that no toxic effect could be observed in the group administered 200 mg/kg BA alone (8). In another study, 100 mg/ kg/day BA was also used in doses and once not revealed to be poisonous to rodents administered for a short time (4 weeks or less) (25). Coban *et al.* (26) reported that BA guarded the liver, kidney and brain tissues of rats against malathion-induced cellular damage. Sogut *et al.* (27) revealed that MDA levels increased significantly in alcohol-induced tissue damage, while GSH, GPx, and CAT levels decreased. In the group given 100 mg/kg BA together with

alcohol, there was a significant decrease in the MDA level, while an increase was observed in the GSH, CAT and GPx levels. Our biochemical and histological findings are consistent with those of the studies mentioned above (Figure 1 and Tables 1). In conclusion, our results suggest that BA has protective effects on nephrotoxicity, apoptosis and oxidative stress caused by CPM (Figures 1-5 and Table 1). The results of current study revealed that the renoprotective effects of BA might be linked to enhancing the activity of the antioxidant guard system and inhibiting lipid peroxidation as well. Likewise, BA preserves cells and increases proliferation after the tissue has been damaged in rats.

### Interest conflict

None

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