

# Role of endothelin-1 antagonist; bosentan, against cisplatin-induced nephrotoxicity in male and female rats

Zahra Jokar<sup>1,2</sup>, Mehdi Nematbakhsh<sup>2,3,4</sup>, Maryam Moeini<sup>1</sup>, Ardeshir Talebi<sup>1</sup>

<sup>1</sup>Water and Electrolytes Research Center, <sup>3</sup>Department of Physiology, Isfahan University of Medical Sciences, <sup>4</sup>Isfahan MN Institute of Basic and Applied Sciences Research, Isfahan, <sup>2</sup>Department of Physiology, Islamic Azad University, Jahrom Branch, Jahrom, Iran

## Abstract

**Background:** Cisplatin (CP) is a chemotherapy drug, with the major side effect of nephrotoxicity. The level of endothelin-1 (ET-1) increases during nephrotoxicity, which is accompanied with vasoconstrictive properties. Bosentan (BOS) is a nonselective ET-1 receptor antagonist, having vasodilatory and anti-hypertension effects. The purpose of this study was to investigate the renoprotective effect of BOS against CP-induced nephrotoxicity in male and female rats.

**Materials and Methods:** Male and female rats were divided into six groups; groups 1–3 and 4–6 were male and female rats, respectively. Animals in groups 1 and 4 were considered as negative control and groups 2 and 5 considered as positive control groups received BOS (30 mg/kg/day) alone and CP (2.5 mg/kg/day) alone, respectively, for 1-week. The animals in groups 3 and 6 were treated with both CP and BOS. Finally, serum parameters were measured, and the kidney tissue was subjected to staining to evaluate tissue damage.

**Results:** The serum levels of blood urea nitrogen and creatinine, kidney tissue damage score and kidney weight elevated, and body weight significantly decreased in both CP alone and in CP plus BOS-treated groups when compared with the control groups ( $P < 0.05$ ), while BOS did not ameliorate these parameters neither in males nor in females. No significant differences were observed in serum levels of nitrite and malondialdehyde between the groups, but kidney tissue level of nitrite decreased significantly in CP alone and CP plus BOS-treated groups ( $P < 0.05$ ).

**Conclusion:** Renoprotective effect of BOS, as ET-1 blocker, was not observed against CP-induced nephrotoxicity neither in male nor in female rats. This is while BOS promoted the severity of injuries in females.

**Key Words:** Bosentan, cisplatin, gender, nephrotoxicity, rat

## Address for correspondence:

Prof. Mehdi Nematbakhsh, Department of Physiology, Water and Electrolytes Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

E-mail: [nematbakhsh@med.mui.ac.ir](mailto:nematbakhsh@med.mui.ac.ir)

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## INTRODUCTION

Cisplatin (CP) is one of most common anticancer drug in clinic, used for treatment of solid tumors.<sup>[1,2]</sup> However, it is accompanied with side effects such as hearing disturbance,<sup>[3]</sup> hepatotoxicity,<sup>[4]</sup> and testicular and renal toxicity.<sup>[5]</sup> CP accumulates in kidneys,<sup>[6]</sup> resulting in nephrotoxicity that involves inflammation,<sup>[7]</sup> oxidative stress, apoptosis, and

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necrosis in kidney tubules.<sup>[8]</sup> Nephrotoxicity causes elevation in serum levels of creatinine (Cr) and blood urea nitrogen (BUN).<sup>[9,10]</sup> The endothelin (ET) axis includes ET-1 which decreases glomerular filtration rate (GFR)<sup>[11]</sup> and renal blood flow,<sup>[12]</sup> ET-2, and ET-3 isoforms while ET-1 shows a pivotal role in regulation of vascular tone and has two receptors as ET<sub>A</sub> and ET<sub>B</sub>.<sup>[13]</sup> Both receptors have been identified on vascular smooth muscle cells and mediates vasoconstriction where the ET<sub>B</sub> receptor has been identified on endothelial cells and mediates vasodilatation.<sup>[14]</sup> Selective and nonselective antagonists of ET are commonly used in different diseases such as cardiovascular disorders,<sup>[15]</sup> acute renal failure,<sup>[16]</sup> and pulmonary hypertension.<sup>[17]</sup> Bosentan (BOS) was recognized as a dual ET receptor antagonist that is shown to act as a vasodilator and improve heart failure and reduce renal dysfunction in experimental models.<sup>[18]</sup> Gender difference in renal function related to renin angiotensin system is reported,<sup>[19,20]</sup> while the function of ET is reported to be gender related also.<sup>[21]</sup> It has been shown that females and males exhibit different responses against aminoglycosides,<sup>[22]</sup> and some studies have been investigated the effects of agents such as Vitamin E,<sup>[23]</sup> L-arginine,<sup>[24]</sup> and angiotensin II receptor type 1 blocker; losartan<sup>[25]</sup> against CP-induced nephrotoxicity in both genders. Based on the induction of renal expression of ET by CP<sup>[11]</sup> and the reports on gender related CP-induced nephrotoxicity,<sup>[26-28]</sup> we hypothesized that ET<sub>A</sub> antagonist may prevent CP-induced renal toxicity. To test this hypothesis, co-administration of CP and BOS in rats was performed, and the results related to renal function were compared with the control groups.

## MATERIALS AND METHODS

### Animals

A total of 40 adult male (weighing  $205.7 \pm 3.5$  g) and female (weighing  $190.1 \pm 2.9$  g) Wistar rats (Animal Center, Isfahan University of Medical Sciences, Isfahan, Iran) were used in this study. The animals were housed under standard conditions with 12-h light/12-h dark cycle and had free access to water and food. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Isfahan University of Medical Sciences.

### Drugs

BOS, DMSO, and CP were purchased from Osvah Pharmaceutical Co. (OSVE) (Tehran, Iran), Merck (Germany), and EBEWE Pharma Ges.M.B.H (Australia), respectively.

## Experimental protocol

Wistar rats were randomly assigned to six groups and were treated for 7 days. Groups 1 (male,  $n = 7$ ) and 4 (female,  $n = 7$ ) received BOS (30 mg/kg/day ip). Groups 2 (male,  $n = 7$ ) and 5 (female,  $n = 7$ ) received CP (2.5 mg/kg/day ip). Groups 3 (male,  $n = 5$ ) and 6 (female,  $n = 7$ ) received both BOS and CP. BOS was dissolved in DMSO and was injected 1-h before CP administration every day. At the end of day 7, the rats were anesthetized with chloral hydrate, and blood samples were taken by heart puncture. All animals were sacrificed, and the kidneys were immediately removed and weighed. The left kidney was kept in formalin 10% for pathological investigations. The right kidney was homogenized and centrifuged to measure biochemical parameters.

## Measurements

The levels of serum Cr and BUN were measured using RA1000 auto-analyzer (Technicon, Ireland) and Pars Azmoon (Tehran, Iran) kits. The kidney and serum levels of nitrite were determined using an assay kit that involves the Griess reaction. Kidney tissue and serum levels of malondialdehyde (MDA) were measured by the manual method.<sup>[29]</sup>

## Histopathological procedures

The removed kidney was fixed in 10% formalin solution and prepared for histopathological staining. Hematoxylin and eosin stain was applied to determine the kidney injury; including tubular dilation and swelling, and debris in the luminal cell with inflammation. We scored the damage as kidney tissue damage score (KTDS) in the range of 1–4 while zero was considered for normal tissue without damage.

## Statistical analysis

The data are presented as mean  $\pm$  standard error of the mean. ANOVA with least significant difference as *post-hoc* was applied to compare the groups in terms of quantitative parameters. The Student's *t*-test was applied to find the statistical difference between genders. The data for KTDS were compared between the groups using the Kruskal–Wallis and Mann–Whitney tests.

## RESULTS

### Effect of BOS on serum levels of BUN, Cr, kidney weight, and KTDS

The results indicated that the serum levels of BUN and Cr significantly increased in CP alone-treated (positive control group) and CP plus BOS-treated groups when compared with negative control group in both genders ( $P < 0.05$ ). In addition, BOS did not ameliorate these parameters neither

in males nor in females [Figure 1], although kidney weight (KW) and KTDS were significantly different between female and male rats ( $P < 0.05$ ). The sample images of kidney tissues are demonstrated in Figure 2.

Effect of BOS on serum and kidney tissue levels of MDA and nitrite.

No significant differences were observed in serum and kidney tissue levels of MDA and the serum level of nitrite between the groups while kidney tissue level of nitrite significantly decreased in CP alone and CP plus BOS-treated groups [Table 1].

Effect of BOS on testis and uterus weight.

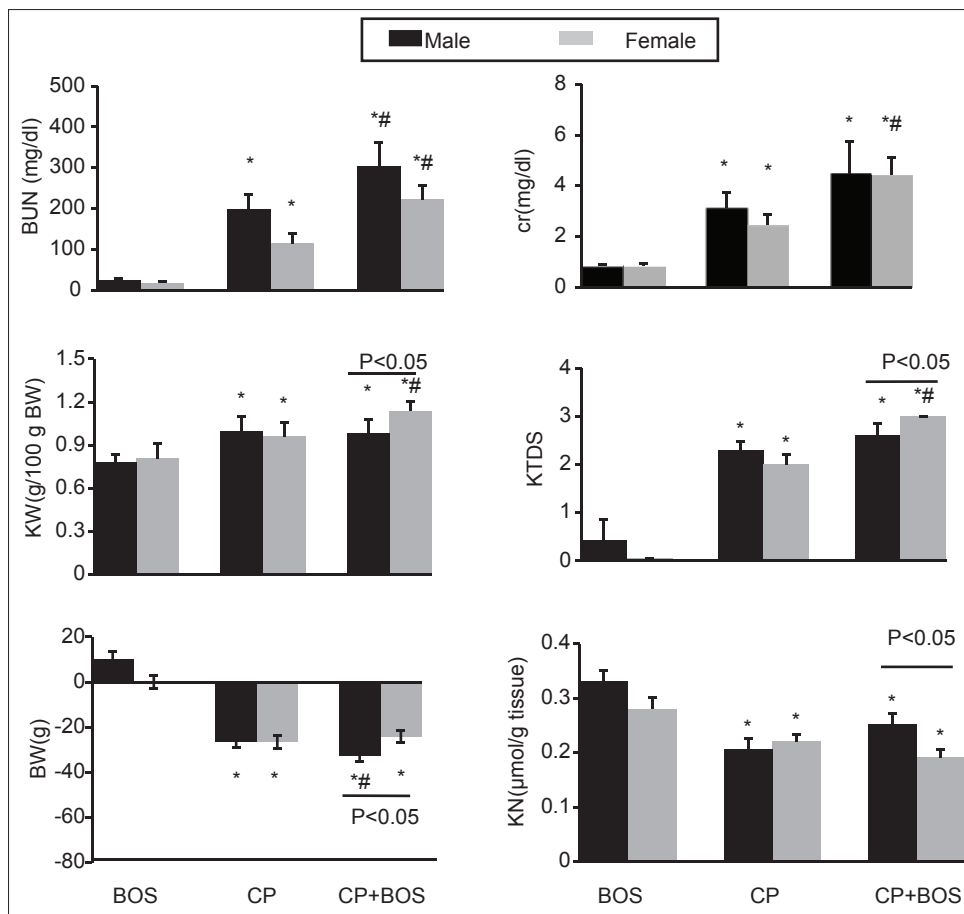
The male groups were not significantly different with regard to the testis weight; however, the uterus weight in CP alone and CP plus BOS-treated groups decreased significantly ( $P < 0.05$ ) [Table 1].

### Effect of BOS on bodyweight

Bodyweight decreased significantly in CP alone and CP plus BOS-treated groups compared with the negative control in both genders ( $P < 0.05$ ). However, this body weight loss was higher in male animals ( $P < 0.05$ ) [Figure 1].

### DISCUSSION

This study is intended to investigate the role of BOS on nephrotoxicity induced by CP in male and female rats. BOS did not show nephroprotective effect against CP, and kidney injury was greater in female than in male rats. CP alone increased serum levels of BUN and Cr, KTDS, and KW; and decreased bodyweight and kidney nitrite level in both males and females, which indicates induced nephrotoxicity. These findings are in agreement with other studies.<sup>[24,25,30-34]</sup> CP nephrotoxicity is related to apoptosis,<sup>[35]</sup> oxidative stress,<sup>[36]</sup> and accumulation of CP in the proximal tubule.<sup>[37]</sup> Increasing KW by CP may be related to

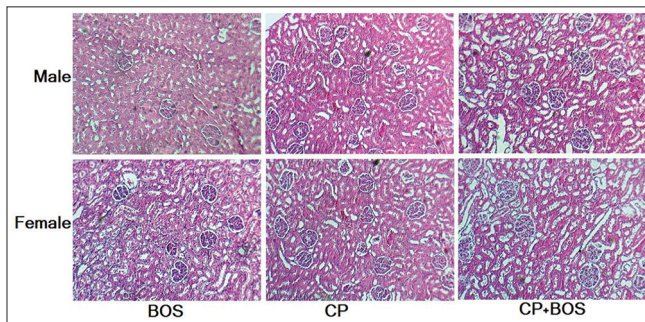


**Figure 1:** Comparison of the sham (treated with bosentan [BOS] alone), positive control (treated with cisplatin [CP] alone), and case (treated with BOS plus CP) groups in each gender with regard to the measured BUN, creatinine, kidney weight, bodyweight change, kidney tissue damage score, and kidney nitrite. Data are reported as mean  $\pm$  standard error of mean \*indicates significant difference from the sham group and #indicates significant difference from the positive control group, and –indicates significant difference between the case groups in the two genders. BUN: Blood urea nitrogen, Cr: Creatinine, KW: Kidney weight/100 g bodyweight, BW: Bodyweight change, KTDS: Kidney tissue damage score, KN: Kidney nitrite, BOS: Bosentan, and CP: Cisplatin

**Table 1: Serum and kidney levels of nitrite and malondialdehyde (MDA) and testis weight and uterus weight in six experimental groups**

group	BOSM	BOSF	CPM	CPF	CP+BOSM	CP+BOSF
Nitrit ( $\mu\text{mol/lit}$ )	10.86 $\pm$ 1.86	11.23 $\pm$ 1.18	29.17 $\pm$ 11.83	22.57 $\pm$ 9.00	14.34 $\pm$ 2.34	17.41 $\pm$ 5.24
kidney nitrite ( $\mu\text{mol/grtissue}$ )	0.33 $\pm$ 0.01	0.27 $\pm$ 0.02	0.20 $\pm$ 0.01*	0.22 $\pm$ 0.01*	0.25 $\pm$ 0.01*	0.19 $\pm$ 0.02*
Serum MDA ( $\mu\text{mol/lit}$ )	16.58 $\pm$ 1.62	20.93 $\pm$ 2.37	19.85 $\pm$ 2.32	16.47 $\pm$ 2.16	20.04 $\pm$ 1.79	18.83 $\pm$ 1.50
kidney MDA ( $\text{nmol/grtissue}$ )	10.19 $\pm$ 1.48	11.09 $\pm$ 0.92	11.58 $\pm$ 0.88	12.35 $\pm$ 1.07*	12.69 $\pm$ 0.74	11.15 $\pm$ 0.31
Uterus weight ( $\text{gr}/100\text{grBW}$ )		0.05 $\pm$ 0.00		0.03 $\pm$ 0.00*		0.03 $\pm$ 0.00*
Testis weight ( $\text{gr}/100\text{grBW}$ )	1.18 $\pm$ 0.03		1.26 $\pm$ 0.06		1.25 $\pm$ 0.05	

\*The data are reported as mean $\pm$ SEM. Significant difference from the sham group ( $P < 0.05$ ). SEM: Standard error of mean, BW: Bodyweight change, CP: Cisplatin, CPM: CP in male, CPF: CP in female, BOSM: Bosentan in male BOSF: Bosentan in female

**Figure 2:** Sample image from kidney tissue of all experimental groups

kidney injury and changes in GFR<sup>[38]</sup> and consequently aggregation of water and salt in the renal tissue. Helmy *et al.* reported that co-administration of BOS and CP did not decrease the serum levels of BUN and Cr in male rats treated with CP<sup>[39]</sup> that is in agreement with our results. In the present study, administration of BOS accompanied with CP failed to decrease KW and KTDS in both genders. Some other studies illustrated that BOS could not protect these factors in renal injury. BOS itself may increase ET-1 level in blood,<sup>[40,41]</sup> and this may promote CP effects. Furthermore, our results showed that BOS exacerbated CP-induced nephrotoxicity in females. It was reported that administration of compounds such as L-arginine, erythropoietin, and losartan aggravate CP-induced injury in females.<sup>[24,25,42]</sup> It seems that female sex hormone plays an important role in nephrotoxicity induced by CP. Estrogen itself promote CP-induced nephrotoxicity in ovariectomized female rats.<sup>[43]</sup> Furthermore, the presence of estrogen reversed renoprotective effects of Vitamins C and E and losartan against CP-induced nephrotoxicity in ovariectomized female rats.<sup>[25]</sup> Probably in our study presence of gonad hormones influenced BOS effect on CP-induced nephrotoxicity. Furthermore, ET<sub>B</sub> receptor expression in males is higher than that in female,<sup>[44]</sup> and accordingly the vasodilatory aspect of ET<sub>B</sub> receptor.<sup>[45]</sup> It seems that decrement of injury in males is due to this gender difference of ET<sub>B</sub> receptor. The results of this study also showed that CP decreased kidney nitrite level. Nitric oxide (NO) is synthesized in the vasculature by three isoforms of NO synthases (NOS); namely, neuronal NOS,

endothelial NOS (eNOS), and inducible NOS.<sup>[46]</sup> Administration of CP decreases expression of eNOS in the outer medulla of the kidney.<sup>[47]</sup> In addition, the plasma level of ET-1 was increased by CP,<sup>[11]</sup> and ET-1 impairs the production of NO through inhibition of eNOS synthase.<sup>[48]</sup> Therefore, we may conclude that nitrite renal level was decreased in both genders probably by elevating ET-1 and reducing eNOS following CP administration. CP decreased uterus weight probably via apoptosis and necrosis.<sup>[49,50]</sup> We observed that CP decreased bodyweight in both males and females, which is in agreement with the findings of previous studies.<sup>[9,25,51,52]</sup> Body weight loss is induced by CP due to gastrointestinal problems such as diarrhea and appetite loss.<sup>[51]</sup> BOS could not recover body weight loss in both genders; however, the damage was greater in male compared with female rats. It was reported that treatment with BOS could not ameliorate body weight loss in diabetic rats.<sup>[53]</sup> Accordingly, the patients received BOS exhibited the same clinical problems including skin ulcers, fatigue, upper respiratory tract infection, cough, arthralgia, peripheral edema, diarrhea, bronchitis, sinusitis, and anemia.<sup>[54]</sup> Therefore, it is possible that side effects of BOS such as gastrointestinal problems; especially diarrhea affect the body weight loss in our study. ET-1 also mediated water and sodium excretion through ET<sub>B</sub>-receptors.<sup>[55]</sup> Karet *et al.* reported that ET<sub>B</sub> receptor is available in the kidney.<sup>[44]</sup> Since BOS has a 20-30 folds higher potency for the ET<sub>A</sub>-receptor than for the ET<sub>B</sub>-receptor,<sup>[40]</sup> the greater weight loss in males is probably due to the BOS action on ET<sub>B</sub> receptors that results in increased diuresis. In summary, BOS could not protect renal tissue against nephrotoxicity in males and females; however, this damage was greater in female rats.

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