

*Original Article***The protective role of endogenous nitric oxide donor (L-arginine) in cisplatin-induced nephrotoxicity: Gender related differences in rat model**

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**Abstract**

**BACKGROUND:** Cisplatin (CP) as a potential drug for solid tumors produces nephrotoxicity and disturbs endothelial function. CP induced nephrotoxicity may be gender related. Nitric oxide plays a pivotal role in endothelial function and L-arginine as endogenous NO donor promotes endothelial function. The role of L-arginine in CP induced nephrotoxicity model and its gender related was investigated in this study.

**METHODS:** Thirty three Wistar rats were randomly assigned to four groups. The groups 1 (male, n = 6) and 2 (female, n = 11) received a single dose of L-arginine (300 mg/kg, ip), and the day after, they received a single dose of CP (7 mg/kg). The group 3 (male, n = 9) and 4 (female, n = 7) were assigned to the same regimen except for saline instead of L-arginine. All animals were sacrificed one week after CP administration. The levels of blood urea nitrogen (BUN), creatinine and nitrite were measured. The kidneys were also removed for pathological investigations.

**RESULTS:** Five animals died. All CP treated animals lost weight. The normalized weight loss was significantly different between male and female in CP+L-arginine treated animals ( $p < 0.05$ ). BUN and creatinine were increased significantly in male treated with CP and in female treated with CP+L-arginine ( $p < 0.05$ ). L-arginine reduced BUN in male (not in female) when compared with control groups ( $p < 0.05$ ). The level of nitrite was increased significantly in L-arginine treated animals. Kidney tissue damage score and normalized kidney weight were greater in females treated with CP+L-arginine than female received CP alone ( $p < 0.05$ ).

**CONCLUSIONS:** L-arginine may protect against CP induced nephrotoxicity in male, but it promotes the induced damage in female. The exact mechanism need to be defined.

**KEYWORDS:** Gender, L-arginine, Cisplatin, Nephrotoxicity, Rat.

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Nitric oxide has been well demonstrated to play a pivotal role in endothelial function,<sup>1-5</sup> and there is some evidence that release of NO could be gender related.<sup>6-8</sup> Cisplatin (CP) as an antitumor drug is accompanied by side effects such as nephrotoxicity<sup>9, 10</sup> and endothelial injury.<sup>11-13</sup> Therefore, NO is

involved in CP induced nephrotoxicity.<sup>14, 15</sup> L-arginine is the main precursor of NO in vascular endothelium, and it was reported that L-arginine administration has preventive role to protect the kidney against CP nephrotoxicity,<sup>16</sup> but the role of gender is not well known. Women have a lower risk of chronic renal dis-

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ease development than men,<sup>17-22</sup> and female animals are more resistant to ischemic acute renal failure than male animals.<sup>23</sup> NO acts as a strong vasodilator in kidney circulation<sup>24</sup> and the female sex hormones promote NO production.<sup>7</sup> There is a possibility that the preventive role of L-arginine could be gender related,<sup>25</sup> particularly in CP induced nephrotoxicity. Accordingly, this study was designed to investigate the protective role of L-arginine against CP induced nephrotoxicity in male and female rat models.

## Methods

### Animals

Eighteen adult female (weight:  $162.0 \pm 4.1$  g) and 15 male (weight:  $184.4 \pm 7.2$  g) Wistar rats (Animal Centre, Ahvaz University of Medical Sciences, Ahvaz, Iran) were used for this research. The rats were housed at a temperature of 23–25°C. Rats had free access to water and rat chow. The rats were acclimatized to this diet for at least 1 week prior to experiment. The experimental procedures were approved in advance by the Isfahan University Medical Sciences Ethics Committee.

### Experimental protocol

Wistar rats were randomly assigned to four groups. The groups 1 (male,  $n = 6$ ) and 2 (female,  $n = 11$ ) received a single dose of L-arginine (300 mg/kg, ip) and the day after, they received a single dose of CP (7 mg/kg). The group 3 (male,  $n = 9$ ) and 4 (female,  $n = 7$ ) were assigned to the same regimen except for saline instead of L-arginine. All animals were sacrificed one week after CP administration. The levels of blood urea nitrogen (BUN), creatinine (Cr), and nitrite were measured. CP [cis-Diammineplatinum (II) dichloride, code P4394] was purchased from Sigma (Germany). Blood samples were obtained from each animal before and 7 days after CP administration. The animals' body weight was recorded daily. At the end of the experiment, the kidney was removed and weighted rapidly for histopathological investigations.

### Measurement

The levels of serum Cr and BUN were determined using quantitative diagnostic kits (Pars Azmoon, Iran). The serum level of nitrite (stable NO metabolite) was measured using a colorimetric assay kit (Promega Corporation, USA) that involves the Griess reaction.

### Histopathological Procedures

The removed kidney was fixed in 10% formalin solution, embedded in paraffin for histopathological staining. The hematoxylin and eosin stain was applied to examine the tubular damage. Presence of acute tubular injury such as tubular dilation and simplification, tubular cells swelling and necrosis, tubular casts and intra luminal cell debris with inflammatory cells infiltration were considered. Based on the intensity of tubular lesions as mentioned above, we scored from 1 to 4, while the score of zero was assigned to the normal tissue without damage.

### Statistical Analysis

Data are expressed as mean  $\pm$  SEM. To compare the weight change between the groups, repeated measured analysis was applied. Paired and unpaired t-tests also were applied to compare kidney weight and the serum levels of BUN, Cr and nitrite within and between the groups. Due to the qualitative nature of scoring, Mann-Whitney or Kruskal-Wallis tests were applied to compare the pathology damage score between the groups. Values of  $p < 0.05$  were considered statistically significant.

## Results

From a total of 33 animals, 5 rats were expired during the experiment (Table 1). Therefore 28 animals were remained for final investigations.

### Effect of CP on body weight

Before and after the experiment, the weight of animals in each group was recorded respectively as group 1:  $178.1 \pm 11.3$  g and  $175.5 \pm 15.2$  g, group 2:  $158.2 \pm 5.8$  g and  $131.5 \pm 5.6$  g, group 3:  $189.1 \pm 9.6$  g and  $162.9 \pm 9.6$  g, group 4:

**Table 1.** The mortality rate of animals in each group

Group	N	Day								n
		1	2*	3	4	5	6	7	8	
# 1 : Male treated with L-arginine and CP	6	-	-	-	-	-	-	-	-	6
# 2 : Female treated with L-arginine and CP	11	-	-	-	-	-	-	1	1	9
# 3 : Male treated with CP	9	-	-	-	-	-	1	-	-	8
# 4 : Female treated with CP	7	-	-	-	-	-	-	2	-	5

N: total number of animals, n: number of experimental animals, CP: cisplatin.

\* CP was administrated on day 2.

169.0 ± 3.6 g and 154.7 ± 8.1 g. All animals lost weight. The animals weight were normalized versus of first day of experiment, and the normalized weight loss was significantly different between male and female in L-arginine treated animals ( $p < 0.05$ ) (Figure 1).

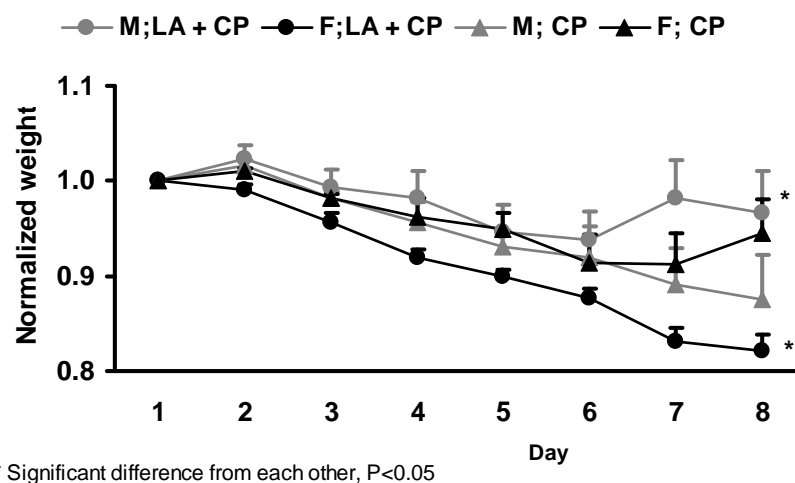
#### Effect of CP on serum BUN, Cr and nitrite levels

BUN and Cr were increased in all CP treated groups, but it was only statistically significant in male treated with CP and in female treated with CP+L-arginine ( $p < 0.05$ ). L-arginine attenuates the levels of BUN and Cr in male but not in female when compared with control groups (the BUN reduction was significantly different,  $p < 0.05$ ). The nitrite level increased in L-arginine treated animals (male,  $p < 0.1$ ; female,  $p < 0.05$ ), but at the end of the experiment, a significant difference in nitrite level was only detected between females groups ( $p < 0.05$ ) (Figure 2). On the whole, these

findings indicated that L-arginine provides different pattern of effect on BUN, Cr and nitrite levels in CP-induced nephrotoxicity model in male and female rats.

#### Effect of CP on kidney damage

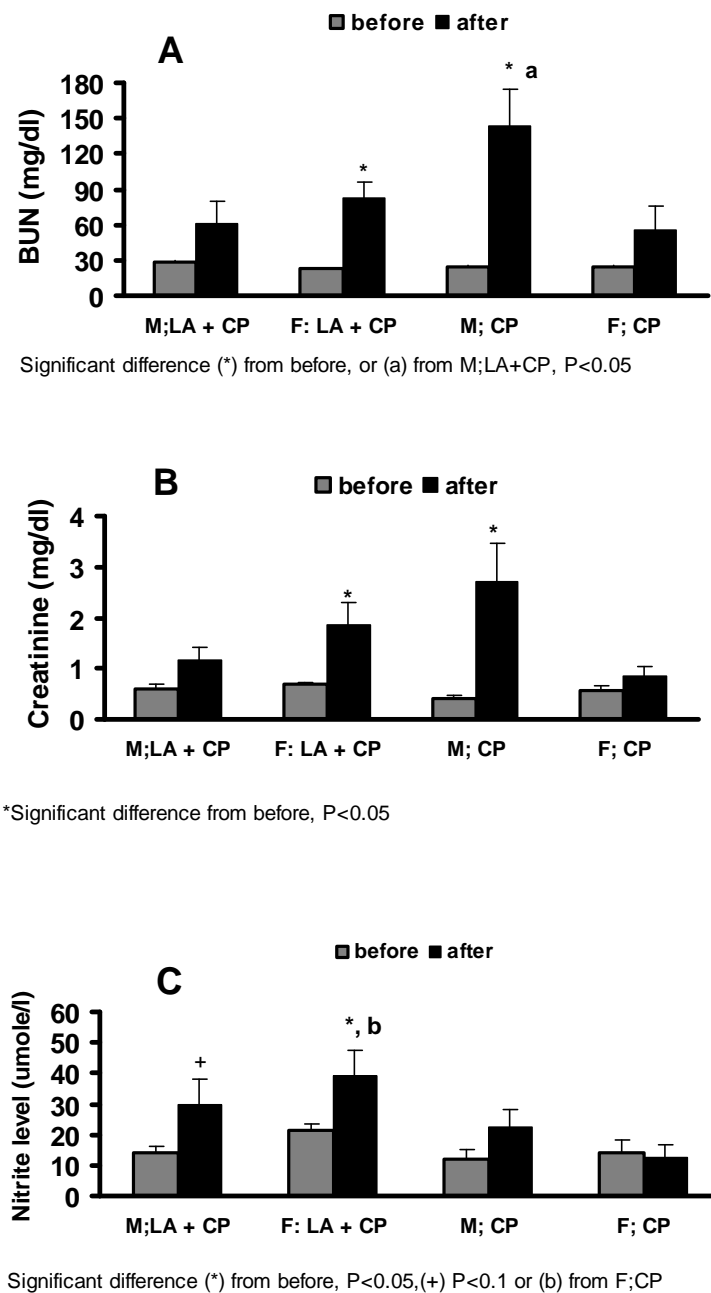
The kidney damage induced by CP was evaluated and scored by two independent pathologists. The scores given by the two pathologists were compared by Wilcoxon test and no statistically significant difference was obtained ( $p = 0.9$ ). The score obtained for each animal and then for each group was considered as the final damage tissue score. This data is demonstrated in figure 3. It indicates that the kidney damage score and normalized kidney weight (kidney weight/100 g body weight) obtained in female treated with L-arginine +CP was significantly greater than in female treated with CP alone ( $p < 0.05$ ) and such difference was not observed in male. The pathology images are also demonstrated in figure 4.



\* Significant difference from each other,  $P < 0.05$

**Figure 1.** The change of normalized weight in four groups of experiment.

The weight was normalized with respect to animal weight on the first day of experiment. M, F, LA and CP stand for male, female, L-arginine and cisplatin.

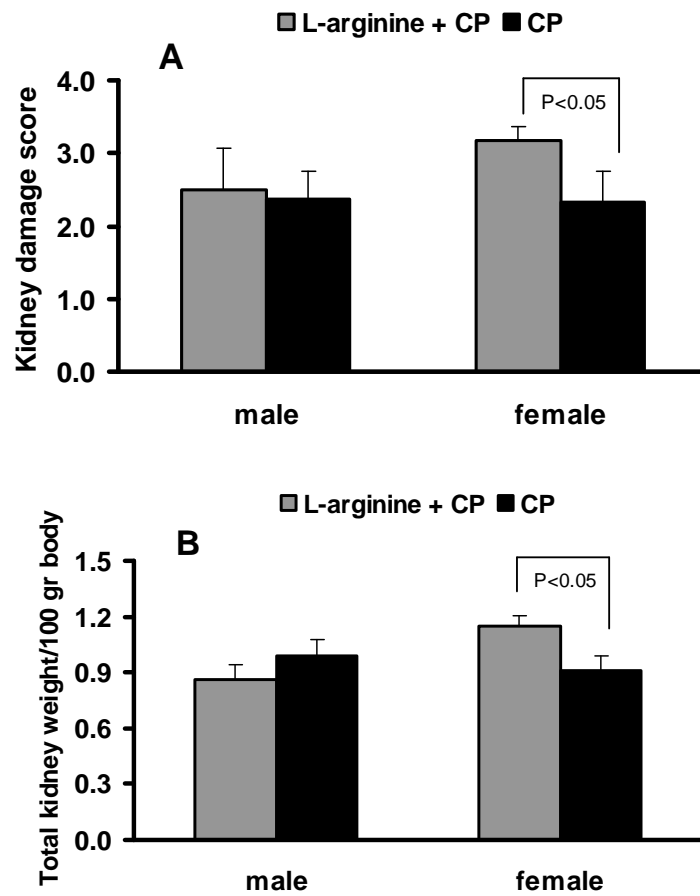


**Figure 2.** Serum level of blood urea nitrogen (BUN) (A), creatinine (B) and nitrite levels (C) in cisplatin treated groups before and after intervention. M, F, LA and CP stand for male, female, L-arginine and cisplatin.

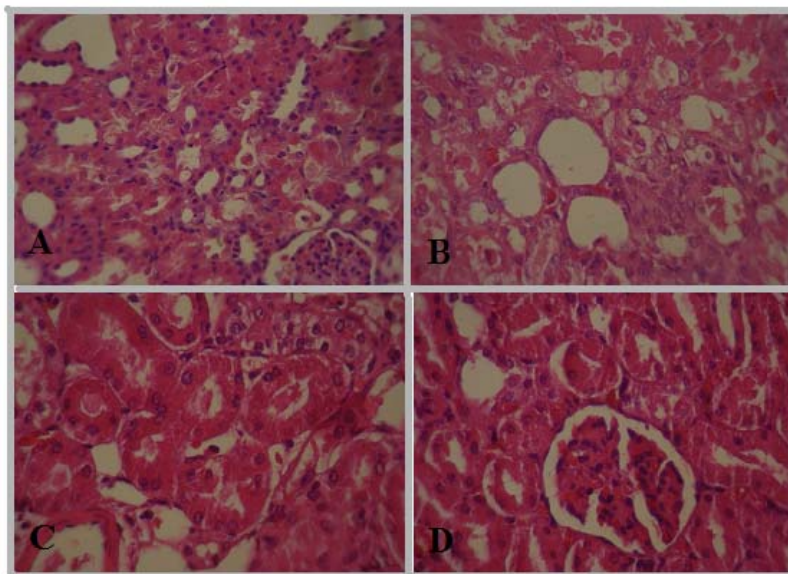
## Discussion

The main objective of this study was to determine the gender related difference in protective role of L-arginine in a model of nephrotoxicity. The induced weight loss by CP was significantly different in male and female treated with L-arginine. CP reduces weight which is

related to gastrointestinal disturbances.<sup>26-30</sup> In line with our results, saleh et al. showed that L-arginine ameliorates weight loss induced by CP in male rats.<sup>16</sup> L-arginine may diminish the weight loss in male rat<sup>31</sup> and NO can be modulated by weight loss in women.<sup>32</sup> CP also has interaction with women hormonal system,<sup>33</sup>



**Figure 3.** The pathology damage score (A) and total kidney weight/100 g of body weight (B) in male and female animals treated with cisplatin+L-arginine and cisplatin alone. CP stands for cisplatin.



**Figure 4.** The pathology images (magnification: 400X) of kidney tissue in four groups of experiment. A: group 1 (male; L-arginine + cisplatin), B: group 2 (female; L-arginine + cisplatin), C: group 3 (male; cisplatin), D: group 4 (female; cisplatin). More tissue damage is shown in B.



and therefore it seems that endogenous NO has not protective role to diminish the CP induced weight loss in female.

It is reported that L-arginine reduces the serum level of BUN and Cr in male treated with CP,<sup>16,34</sup> but such observation was not documented in female. Our results for BUN and Cr in male rats was similar to others findings,<sup>16,34</sup> but different results were obtained in female rats. It is reported that L-arginine increases glomerular filtration rate and renal plasma flow via NO as mediator.<sup>35</sup> In addition, it is possible that L-arginine antagonizes the hemodynamic effects of CP on renal function.<sup>36</sup> Our study showed that, L-arginine did not reduce the levels of BUN and Cr induced by CP in female. This difference may be related to sex differences of NO synthase expression or the role of NO on renal system.<sup>37-39</sup> Estrogen as a sex hormone induces production of NO<sup>40</sup> and increases activity of NO synthase enzyme. It also causes more release of NO in female than

male.<sup>8, 41</sup> The result from other studies showed that blockade of NO pathways during CP chemotherapy may reduce the CP side effects<sup>42</sup> and NO enhances cisplatin cytotoxicity.<sup>43</sup> It seems that L-arginine has some interaction with sex hormones. For example, dietary L-arginine indicates less atherosclerosis in male but not in females.<sup>44</sup> On the other hand, it is mentioned that production of NO is gender-related<sup>40</sup> and L-arginine is precursor of NO, therefore it is possible that additional amounts of NO in female sex increase nephrotoxicity induced CP via rising serum levels of BUN and Cr. It is concluded that L-arginine improves nephrotoxicity induced CP in male but not in female which is related to gender and sex hormones. Our pathological investigation also confirmed this conclusion.

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### Conflict of Interests

Authors have no conflict of interests.

### Authors' Contributions

FEJ conducted experimental procedures and assisted in data analysis; MN planned and conducted the experimental procedures and data analysis, wrote and finalized it. HN and AT conducted pathological diagnosis; MH, ZP and TS assisted experimental procedures. FA assisted in planning and consulted in final results. All authors read and approved the final draft of the paper.

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