Received: 15.8.2011 **Accepted: 3.11.2011**

Original Article

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

Fatemeh Eshraghi-Jazi[1], Mehdi Nematbakhsh[2], Hamid Nasri[3], Ardeshir Talebi[4], Maryam Haghighi[1], Zahra Pezeshki[1], Tahereh Safari[1], Farzaneh Ashrafi[5]

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 weigh 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.

J Res Med Sci 2011; 16(11): 1389-1396

Nitric oxide has been well demonstrated to play a pivotal role in endothelial function,[1-5] and there is some evidence that release of NO could be gender related.[6-8] Cisplatin (CP) as an antitumor drug is accompanied by side effects such as nephrotoxcity[9, 10] and endothelial injury.[11-13] Therefore, NO is involved in CP induced nephrotoxicity.[14, 15] 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,[16] but the role of gender is not well known. Women have a lower risk of chronic renal dis-

1- Kidney Basic Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
2- Professor, Kidney Basic Sciences Research Center, and Department of Physiology, School of Medicine, and Kidney diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
3- Professor, Kidney Diseases Research Center, and Department of Internal Medicine, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
4- Associated Professor, Kidney Basic Sciences Research Center, and Department of Clinical Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
6- Assistant Professor, Department of Internal Medicine, School of Medicine, and Kidney Basic Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
Corresponding author: Mehdi Nematbakhsh
E-mail: nematbakhsh@med.mui.ac.ir

ease development than men,[17-22] and female animals are more resistant to ischemic acute renal failure than male animals.[23] NO acts as a strong vasodilator in kidney circulation[24] and the female sex hormones promote NO production.[7] There is a possibility that the preventive role of L-arginine could be gender related,[25] 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 ± 4.1 g) and 15 male (weight: 184.4 ± 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 ± 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 recoded respectively as group 1: 178.1 ± 11.3 g and 175.5 ± 15.2 g, group 2: 158.2 ± 5.8 g and 131.5 ± 5.6 g, group 3: 189.1 ± 9.6 g and 162.9 ± 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.

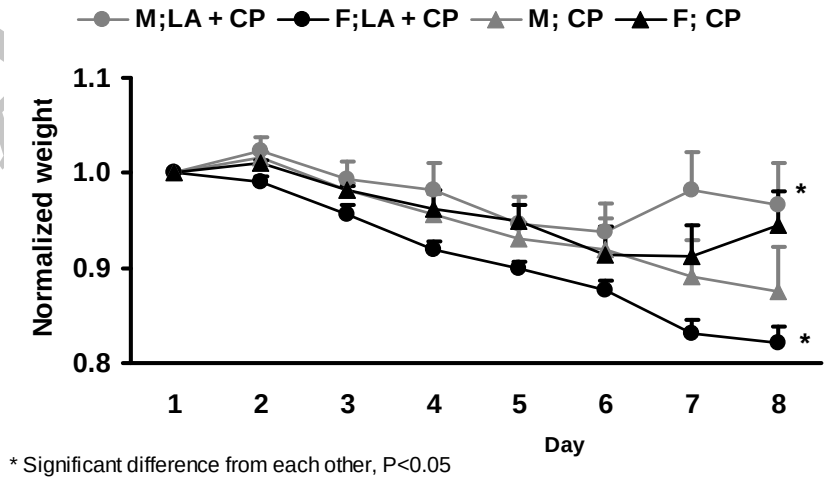
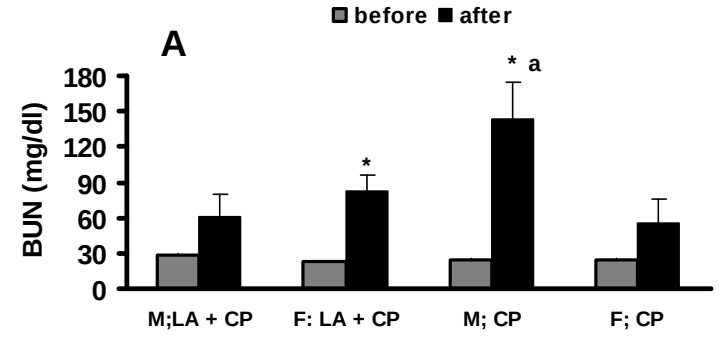
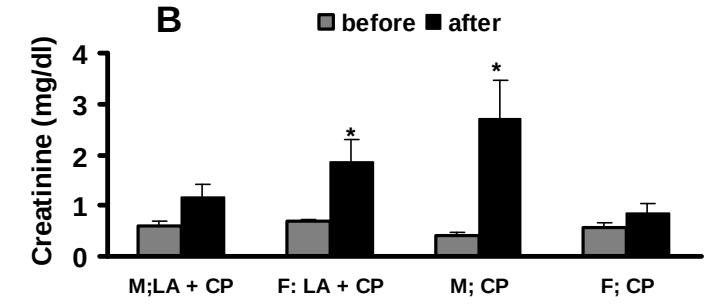


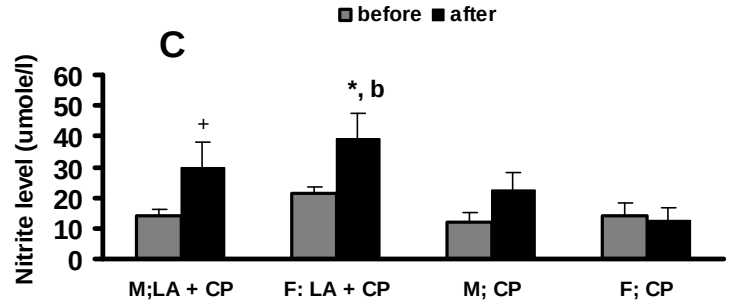
Figure 1. The change of normalized weight in four groups of experiment.
The weight was normalized with respect to animal weight on the first day of experiment. M, F, LA and CP stand for male, female, L-arginine and cisplatin.



Significant difference (*) from before, or (a) from M;LA+CP, P<0.05



*Significant difference from before, P<0.05



Significant difference (*) from before, P<0.05,(+) P<0.1 or (b) from F;CP

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

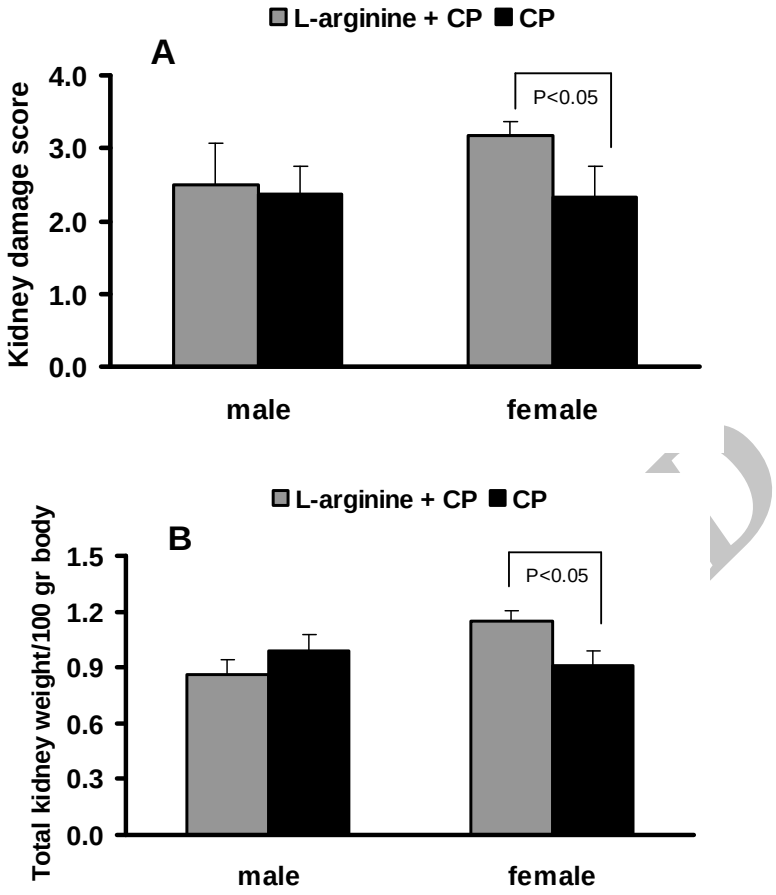


Figure 3. The pathology damage score (A) and total kidney weight/100 g of body weight (B) in male and female animals treated with cisplatin+L-arginine and cisplatin alone. CP stands for cisplatin.

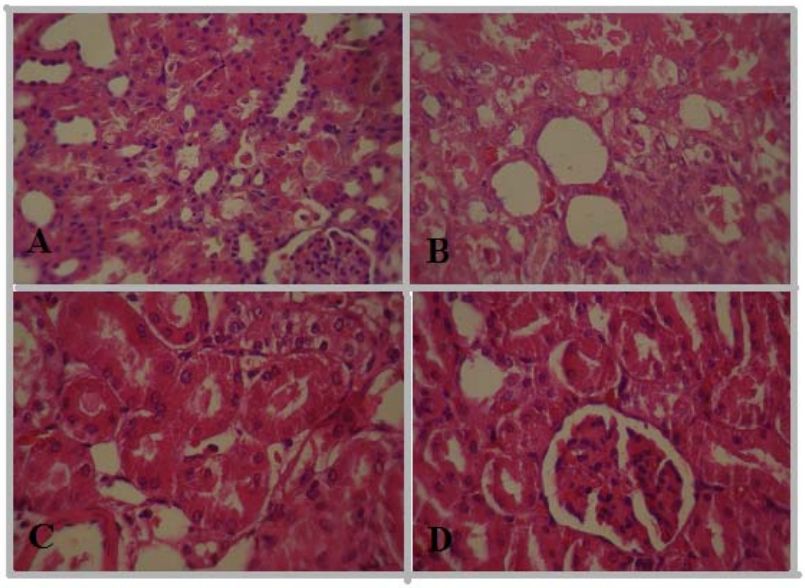


Figure 4. The pathology images (magnification: 400X) of kidney tissue in four groups of experiment. A: group 1 (male; L-arginine + cisplatin), B: group 2 (female; L-arginine + cisplatin), C: group 3 (male; cisplatin), D: group 4 (female; cisplatin). More tissue damage is shown in B.

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,[16,34] but such observation was not documented in female. Our results for BUN and Cr in male rats was similar to others findings,[16,34] 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.[35] In addition, it is possible that L-arginine antagonizes the hemodynamic effects of CP on renal function.[36] 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.[37-39] Estrogen as a sex hormone induces production of NO[40] and increases activity of NO synthase enzyme. It also causes more release of NO in female than male.[8, 41] The result from other studies showed that blockade of NO pathways during CP chemotherapy may reduce the CP side effects[42] and NO enhances cisplatin cytotoxicity.[43] 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.[44] On the other hand, it is mentioned that production of NO is gender-related[40] 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.

Acknowledgment
The research was supported by Isfahan University of Medical Sciences (grant no. 290200)

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.

References
1. Clapp BR, Hirschfield GM, Storry C, Gallimore JR, Stidwill RP, Singer M, et al. Inflammation and endothelial function: direct vascular effects of human C-reactive protein on nitric oxide bioavailability. Circulation 2005; 111(12): 1530-6.
2. Linke A, Erbs S, Hambrecht R. Flow-mediated vasodilation partially reflects nitric oxide-mediated endothelial function. J Appl Physiol 2005; 99(4): 1622.
3. John S, Schneider MP, Delles C, Jacobi J, Schmieder RE. Lipid-independent effects of statins on endothelial function and bioavailability of nitric oxide in hypercholesterolemic patients. Am Heart J 2005; 149(3): 473.
4. Alexander MY, Brosnan MJ, Hamilton CA, Downie P, Devlin AM, Dowell F, et al. Gene transfer of endothelial nitric oxide synthase improves nitric oxide-dependent endothelial function in a hypertensive rat model. Cardiovasc Res 1999; 43(3): 798-807.
5. Antoniades C, Shirodaria C, Crabtree M, Rinze R, Alp N, Cunnington C, et al. Altered plasma versus vascular biopterins in human atherosclerosis reveal relationships between endothelial nitric oxide synthase coupling, endothelial function, and inflammation. Circulation 2007; 116(24): 2851-9.

6. Palacios J, Marusic ET, Lopez NC, Gonzalez M, Michea L. Estradiol-induced expression of N(+)-K(+)-ATPase catalytic isoforms in rat arteries: gender differences in activity mediated by nitric oxide donors. Am J Physiol Heart Circ Physiol 2004; 286(5): H1793-H1800.
7. Calderone V, Baragatti B, Breschi MC, Nieri P, Martinotti E. Hormonal influence on the release of endothelial nitric oxide: gender-related dimorphic sensitivity of rat aorta for noradrenaline. J Pharm Pharmacol 2002; 54(4): 523-8.
8. Kauser K, Rubanyi GM. Gender difference in bioassayable endothelium-derived nitric oxide from isolated rat aortae. Am J Physiol 1994; 267(6 Pt 2): H2311-H2317.
9. Santos NA, Catao CS, Martins NM, Curti C, Bianchi ML, Santos AC. Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch Toxicol 2007; 81(7): 495-504.
10. Shord SS, Thompson DM, Krempl GA, Hanigan MH. Effect of concurrent medications on cisplatin-induced nephrotoxicity in patients with head and neck cancer. Anticancer Drugs 2006; 17(2): 207-15.
11. Eguchi R, Fujimori Y, Ohta T, Kunimasa K, Nakano T. Calpain is involved in cisplatin-induced endothelial injury in an in vitro three-dimensional blood vessel model. Int J Oncol 2010; 37(5): 1289-96.
12. Kohn S, Fradis M, Podoshin L, Ben-David J, Zidan J, Robinson E. Endothelial injury of capillaries in the stria vascularis of guinea pigs treated with cisplatin and gentamicin. Ultrastruct Pathol 1997; 21(3): 289-99.
13. Ito H, Okafuji T, Suzuki T. Vitamin E prevents endothelial injury associated with cisplatin injection into the superior mesenteric artery of rats. Heart Vessels 1995; 10(4): 178-84.
14. Gulec M, Iraz M, Yilmaz HR, Ozyurt H, Temel I. The effects of ginkgo biloba extract on tissue adenosine deaminase, xanthine oxidase, myeloperoxidase, malondialdehyde, and nitric oxide in cisplatin-induced nephrotoxicity. Toxicol Ind Health 2006; 22(3): 125-30.
15. Saad SY, Najjar TA, Daba MH, Al-Rikabi AC. Inhibition of nitric oxide synthase aggravates cisplatin-induced nephrotoxicity: effect of 2-amino-4-methylpyridine. Chemotherapy 2002; 48(6): 309-15.
16. Saleh S, El-Demerdash E. Protective effects of L-arginine against cisplatin-induced renal oxidative stress and toxicity: role of nitric oxide. Basic Clin Pharmacol Toxicol 2005; 97(2): 91-7.
17. Guan Y. Nuclear receptors link gender dimorphism of renal disease progression. Kidney Int 2006; 70(11): 1889-90.
18. Kang DH, Yu ES, Yoon KI, Johnson R. The impact of gender on progression of renal disease: potential role of estrogen-mediated vascular endothelial growth factor regulation and vascular protection. Am J Pathol 2004; 164(2): 679-88.
19. Silbiger SR, Neugarten J. The role of gender in the progression of renal disease. Adv Ren Replace Ther 2003; 10(1): 3-14.
20. Neugarten J. Gender and the progression of renal disease. J Am Soc Nephrol 2002; 13(11): 2807-9.
21. Seliger SL, Davis C, Stehman-Breen C. Gender and the progression of renal disease. Curr Opin Nephrol Hypertens 2001; 10(2): 219-25.
22. Neugarten J, Acharya A, Silbiger SR. Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. J Am Soc Nephrol 2000; 11(2): 319-29.
23. Wei Q, Wang MH, Dong Z. Differential gender differences in ischemic and nephrotoxic acute renal failure. Am J Nephrol 2005; 25(5): 491-9.
24. Bergstrom G, Rudenstam J, Creutz J, Gothberg G, Karlstrom G. Renal and haemodynamic effects of nitric oxide blockade in a Wistar assay rat during high pressure cross-circulation of an isolated denervated kidney. Acta Physiol Scand 1995; 154(2): 241-52.
25. Wideman L, Weltman JY, Patrie JT, Bowers CY, Shah N, Story S, et al. Synergy of L-arginine and growth hormone (GH)-releasing peptide-2 on GH release: influence of gender. Am J Physiol Regul Integr Comp Physiol 2000; 279(4): R1455-R1466.
26. Ohno T, Kato S, Wakatsuki M, Noda SE, Murakami C, Nakamura M, et al. Incidence and temporal pattern of anorexia, diarrhea, weight loss, and leukopenia in patients with cervical cancer treated with concurrent radiation therapy and weekly cisplatin: comparison with radiation therapy alone. Gynecol Oncol 2006; 103(1): 94-9.
27. Endo Y, Kanbayashi H. Modified rice bran beneficial for weight loss of mice as a major and acute adverse effect of Cisplatin. Pharmacol Toxicol 2003; 92(6): 300-3.
28. Miya T, Goya T, Yanagida O, Nogami H, Koshiishi Y, Sasaki Y. The influence of relative body weight on toxicity of combination chemotherapy with cisplatin and etoposide. Cancer Chemother Pharmacol 1998; 42(5): 386-90.
29. Ammer U, Natochin Y, David C, Rumrich G, Ullrich KJ. Cisplatin nephrotoxicity: site of functional disturbance and correlation to loss of body weight. Ren Physiol Biochem 1993; 16(3): 131-45.
30. Appenroth D, Frob S, Kersten L, Splinter FK, Winnefeld K. Protective effects of vitamin E and C on cisplatin nephrotoxicity in developing rats. Arch Toxicol 1997; 71(11): 677-83.
31. Kennedy JA, Kirk SJ, McCrory DC, Halliday MI, Barclay GR, Rowlands BJ. Modulation of immune function and weight loss by L-arginine in obstructive jaundice in the rat. Br J Surg 1994; 81(8): 1199-201.

32. Fenster CP, Darley-Usmar VM, Landar AL, Gower BA, Weinsier RL, Hunter GR, et al. Weight loss and race modulate nitric oxide metabolism in overweight women. Free Radic Biol Med 2004; 37(5): 695-702.
33. Maneschi F, Benedetti-Panici P, Scambia G, Salerno MG, D'Agostino G, Mancuso S. Menstrual and hormone patterns in women treated with high-dose cisplatin and bleomycin. Gynecol Oncol 1994; 54(3): 345-8.
34. Li Q, Bowmer CJ, Yates MS. Effect of arginine on cisplatin-induced acute renal failure in the rat. Biochem Pharmacol 1994; 47(12): 2298-301.
35. Cernadas MR, Lopez-Farre A, Riesco A, Gallego MJ, Espinosa G, Digiuni E, et al. Renal and systemic effects of aminoacids administered separately: comparison between L-arginine and non-nitric oxide donor aminoacids. J Pharmacol Exp Ther 1992; 263(3): 1023-9.
36. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43(2): 109-42.
37. Riazi S, Madala-Halagappa VK, Dantas AP, Hu X, Ecelbarger CA. Sex differences in renal nitric oxide synthase, NAD(P)H oxidase, and blood pressure in obese Zucker rats. Gend Med 2007; 4(3): 214-29.
38. Loyer X, Oliviero P, Damy T, Robidel E, Marotte F, Heymes C, et al. Effects of sex differences on constitutive nitric oxide synthase expression and activity in response to pressure overload in rats. Am J Physiol Heart Circ Physiol 2007; 293(5): H2650-H2658.
39. Pontari MA, Ruggieri MR. Sex differences and role of nitric oxide in blood flow of canine urinary bladder. Am J Physiol 1999; 276(2 Pt 2): R407-R413.
40. Hayashi T, Ishikawa T, Yamada K, Kuzuya M, Naito M, Hidaka H, et al. Biphasic effect of estrogen on neuronal constitutive nitric oxide synthase via Ca(2+)-calmodulin dependent mechanism. Biochem Biophys Res Commun 1994; 203(2): 1013-9.
41. Kauser K, Rubanyi GM. Gender difference in endothelial dysfunction in the aorta of spontaneously hypertensive rats. Hypertension 1995; 25(4 Pt 1): 517-23.
42. Srivastava RC, Farookh A, Ahmad N, Misra M, Hasan SK, Husain MM. Evidence for the involvement of nitric oxide in cisplatin-induced toxicity in rats. Biometals 1996; 9(2): 139-42.
43. Wink DA, Cook JA, Christodoulou D, Krishna MC, Pacelli R, Kim S, et al. Nitric oxide and some nitric oxide donor compounds enhance the cytotoxicity of cisplatin. Nitric Oxide 1997; 1(1): 88-94.
44. Jeremy RW, McCarron H, Sullivan D. Effects of dietary L-arginine on atherosclerosis and endothelium-dependent vasodilatation in the hypercholesterolemic rabbit. Response according to treatment duration, anatomic site, and sex. Circulation 1996; 94(3): 498-506.