British Journal of Pharmaceutical Research
4(15): 1886-1899, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

The Role of Losartan and Oral Magnesium Sulfate in Cisplatin Induced Nephrotoxicity in Female Rats

Farzaneh Razmjoo[1,2,3], Nepton Soltani[1,2*], Mehdi Nematbakhsh[3,4,5*], Ardeshir Talebi[3,6], Fatemeh Eshraghi-Jazi[3] and Mansooreh Ghayyomi[1,2,3]

[1]Molecular Medicine Research Center, Hormozgan University of Medical Science, Bandar Abbas, Iran.
[2]Department of Physiology, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
[3]Water and Electrolytes Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
[4]Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran.
[5]Isfahan MN Institute of Basic and Applied Sciences Research, Isfahan, Iran.
[6]Department of Clinical Pathology, Isfahan University of Medical Sciences, Isfahan, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MN, NS and AT designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FR, FEJ and MG managed the analyses of the study. Authors FR and NS managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 17th March 2014
Accepted 21st May 2014
Published 1st August 2014

ABSTRACT

Hypomagnesemia is one of the nephrotoxicity signs. In addition, renin-angiotensin system may be involved in pathophysiology of kidney diseases. Therefore, the present study was designed to investigate the possible role of losartan plus oral magnesium sulfate ($MgSO_4$) to reduce CP-induced nephrotoxicity in female rats. The animals were divided into twelve groups: Group 1-6 received saline, $MgSO_4$ (3g/l), $MgSO_4$ (10g/l), losartan, $MgSO_4$ (3g/l) plus losartan, $MgSO_4$ (10g/l) plus losartan, respectively. The animals received $MgSO_4$ via drinking water for 9 days. In addition, losartan (10mg/kg/day; i. p.) was accompanied with

**Corresponding author: Email: solnep2002@yahoo.com, nematbakhsh@med.mui.ac.ir;*

$MgSO_4$ from day 3. Groups 7-12 followed the same regimen of above groups, but CP (2.5mg/kg/day; i. p.) was added to regimen from day 3. At the end of day 9, all animals were sacrificed and the serum levels of blood urea nitrogen (BUN) and creatinine (Cr) were measured. The kidneys were removed rapidly for histopathological study. The Co-administration of losartan and $MgSO_4$ (3g/l) decreased serum Cr and BUN levels in CP treated animals. Also, that was partially attenuated the kidney tissue damage. It was concluded that combination of losartan and $MgSO_4$ (3g/l) may ameliorate kidney function against CP-induced failure.

Keywords: Cisplatin; nephrotoxicity; magnesium sulfate; losartan; female rat.

1. INTRODUCTION

Cisplatin (*cis*-diammine dichloroplatinum, CP) is currently one of the most important cytostatic agents in solid tumors therapy. However, that is limited by side effects such as nephrotoxicity [1-4]. The renin-angiotensin system (RAS) has been reported to have a role in the pathogenesis of kidney diseases [5]. Angiotensin II (AngII) induces cellular hypertrophy in renal tubular cells via the generation of reactive oxygen species (ROS) [5,6]. The potential antioxidant and nephroprotective effects of Ang II receptor 1 (AT1R) inhibitor have been documented in literature [5]. Losartan is an available selective AT1R antagonist and some studies including ours reported that losartan could prevent CP-induced nephrotoxicity in animal model [7,8]. It has also been previously shown that losartan administration provides lower kidney damage in the CP-treated rats than vitamin C therapy [8]. On the other hand, Deegan reported that there would be different responses if losrtan was administered with various manners in nephrotoxicity model [9]. In addition, losartan promotes the CP-induced damage in females, but not male, which may be related to the RAS receptors in the kidneys [10]. CP-induced nephrotoxicity disturbs tubular reabsorption of magnesium (Mg) [11,12] and depletion of Mg enhances nephrotoxicity [13]. Although Mg therapy is associated with gastrointestinal (GI) symptoms resulting in body weight loss [14,15], it is suggested that hypomagnesemia induced by CP is compensated via Mg supplementation [16]. However, in animal experimental model treated with Mg, no protective effect was seen against CP induced nephrotoxicity [15,17]. Usually, decreasing plasma Mg level is observed 2-3 weeks after CP therapy in animal model [18] and Mg depletion in patient treated with CP is considered as serious problem in clinic that may be solved via Mg supplementation [16]. Both Mg and losartan have antioxidant effect [19,20], therefore the combination of both may decrease CP-induced nephrotoxicity. Accordingly, the present study was designed to investigate the possible role of losartan plus oral magnesium sulfate ($MgSo_4$) administration to reduce CP-induced nephrotoxicity in female rats.

2. MATERIALS AND METHODS

2.1 Animals

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee". Eighty six female Wistar rats, weighing 180-250g were selected and maintained at a constant temperature of 22±2ºC with a fixed 12:12-h light-dark cycle. Nutritionally balanced pellets and water were freely available. To obtain a model of CP induced nephrotoxicity, CP

was injected daily instead a single dose. In clinic the CP therapy is not performed by a single dose, and in our recent study we showed that kidney damage could induced by daily inject of CP (15). The animals were divided into twelve groups as following:

Group 1: Sham as negative control (n=6) which treated by saline (placebo, i.p.) for 9 days.
Groups 2 & 3: controls received 3 (named Mg3; n=5) & 10 (named Mg10; n=6) g/l $MgSO_4$ plus saline (placebo, i.p.) for 9 days.
Group 4 (named L: n=5): control received saline (placebo, i.p) for 9 days plus losartan (10 mg/kg/day, i.p.) from the 3th day.
Group 5 (named Mg3+ L: n=5): control received $MgSO_4$ 3 g/l for 9 days plus losartan (10 mg/kg/day, i.p.) from the 3th day.
Group 6 (named Mg10+L, n=5): control received $MgSO_4$ 10 g/l for 9 days plus losartan (10 mg/kg/day, i.p.) from the 3th day.
Groups 7-12 (named in order CP: n=7; Mg3+ CP: n=9; Mg10+ CP: n=9; L+ CP: n=9; Mg3+ L+CP: n=10 and Mg10+ L+ CP: n=10): those received the same regimen of groups 1-6, respectively, but CP (2.5 mg/kg/day; i.p) was added to their regimen from the 3th day.

It should be noted that the animals received $MgSo_4$ via drinking water. Body weight of all animals was recorded daily using a digital weighing scale. At the end of day 9, all animals were anesthetized by injection of ketamine HCl (75mg/kg, i.p.), and blood samples were taken and finally rats were sacrificed. The kidneys were removed and weighed rapidly.

2.2 Biochemical Assay

The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were determined using quantitative diagnostic kits (Pars Azmoon, Tehran, Iran).

2.3 Histopathological Procedures

The left kidney was fixed in 10% neutral formalin solution and embedded in paraffin for histopathological staining. The staining was applied through H&E staining to examine the tubular damage. The CP induced kidney damage was scored blindly by an expert pathologist. Presence of tubular atrophy, cast, debris, and necrotic material in the tubular lumen and lymphocytes in interstitial tissue were considered to score the induced damage from 1 to 4 while zero score was assigned to normal tissue.

2.4 Drugs

The following drugs were used: CP was purchased from EBWE Pharma Ges. m. b. H (Unterach, Austria), $MgSO_4$ was obtained from Sigma (St. Louis, MO, USA), and ketamine HCl was obtained from Rotex medica (Trittau, Germany) and losartan was obtained from Daroo Pakhsh drug company (Tehran, Iran).

2.5 Statistical Analysis

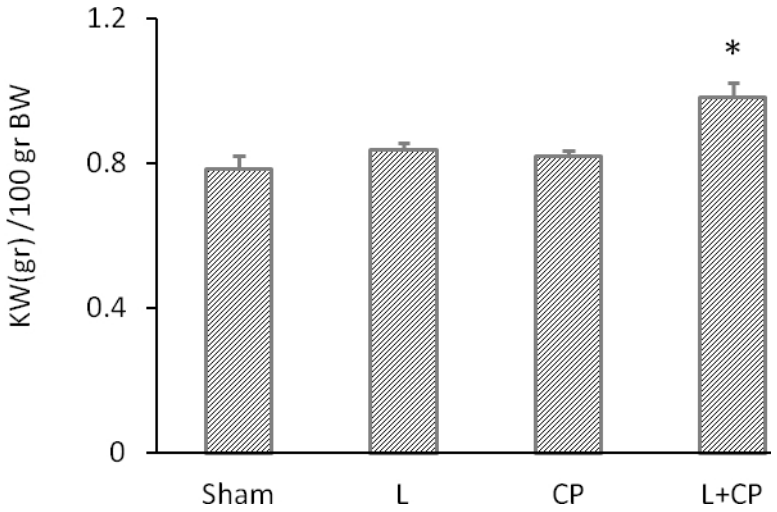
Data were expressed as mean ± S.E.M. Differences among groups were evaluated by one-way ANOVA with the Tukey post-hoc test. The Mann-Whitney test was used to compare the

score of pathology between groups. P<0.05 was selected for acceptance of statistical significant.

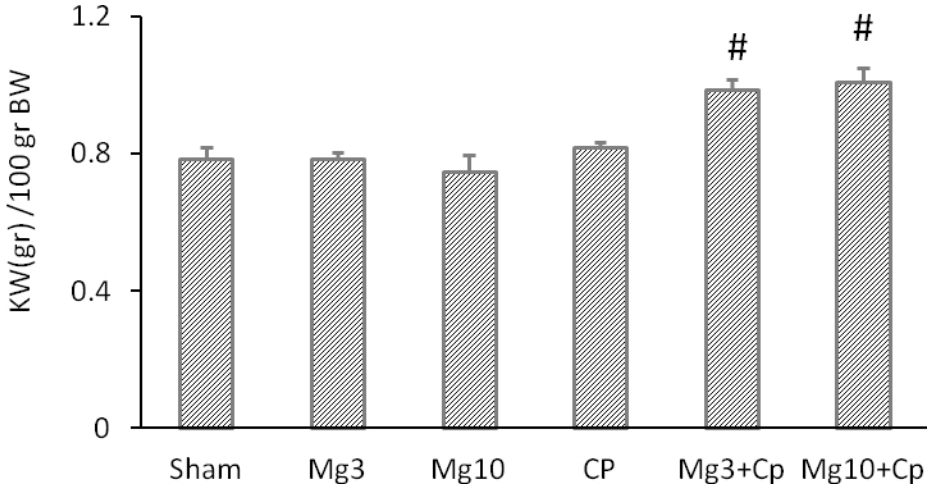
3. RESULTS

3.1 Changes in Kidney Weight

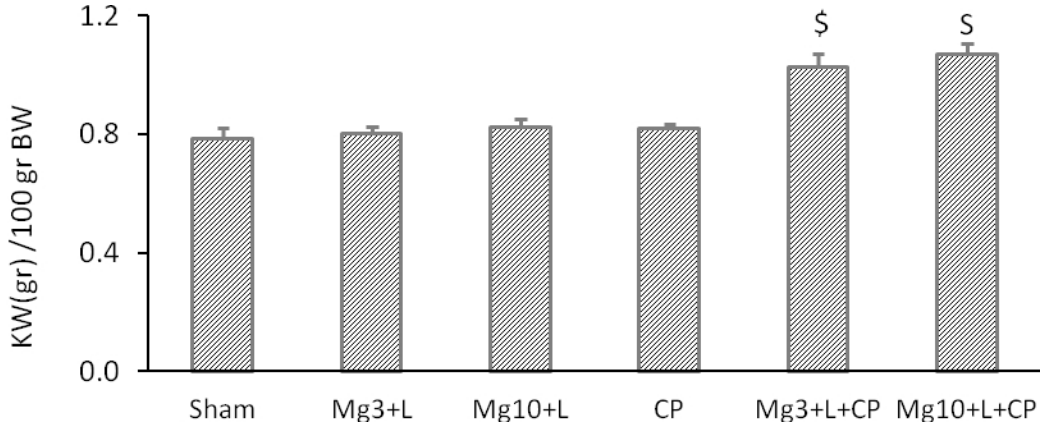
Kidney weight (gr)/100gr of body weight ratio (KW/100gr BW) in all groups have shown in Fig. 1. This ratio didn't change in CP alone treated animals in compared with sham group. However, treatment with losartan, Mg3, Mg10, Mg3+L and Mg10+L in CP treated rats significantly increased KW/100gr BW ratio in compared with other groups (P<0.05).



A



B



C

Fig. 1(A), (B) and (C): The comparison of kidney weight (gr)/100 gr of body weight ratio (KW/100gr BW) in sham, cisplatin (CP), losartan (L), the combination of losartan and CP (L+CP) , Mg-treated with 3 g/l MgSO4 adding to the drinking water (Mg3), Mg-treated with 10 g/l MgSO4 adding to the drinking water (Mg10), the combination of 3 g/l MgSO4 and losartan (Mg3+ L), the combination of 10g/l MgSO4 and losartan (Mg10+ L), the combination of 3g/l MgSO4 and CP (Mg3+CP), the combination of 10g/l MgSO4 and CP (Mg10+CP), the combination of 3g/l MgSO4 and losartan and CP (Mg3+L+ CP), the combination of 10g/l MgSO4 and losartan and CP (Mg10+L+ CP) groups (data are expressed as mean± SEM)

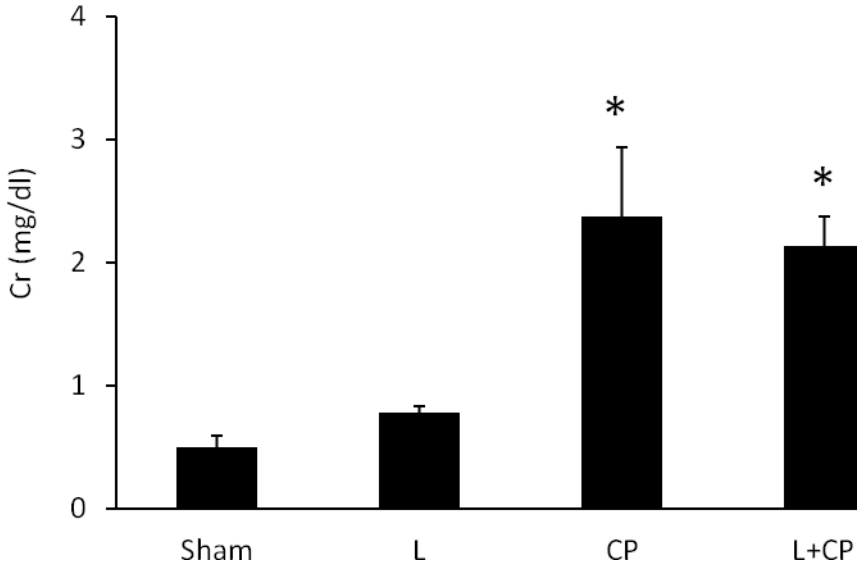
**L+CP vs sham, L and CP (p<0.05)*
 # Mg3+CP and Mg10+CP vs sham, Mg3, Mg10 and CP (p<0.05)
$ Mg3+L+CP and Mg10+L+CP vs sham, CP, Mg3+L and Mg10+L (p<0.05)

3.2 Changes in Serum Cr and BUN

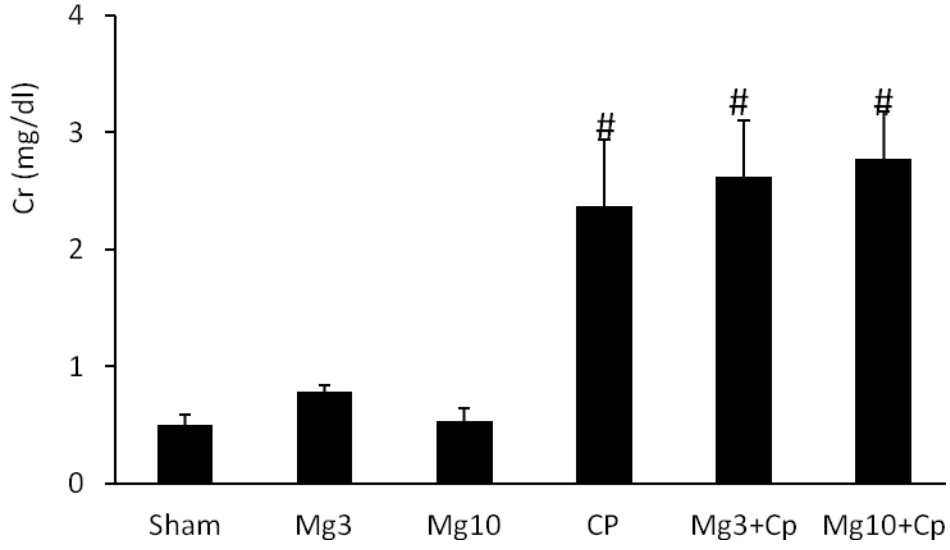
Administration of CP increased serum Cr and BUN levels in all groups (P<0.05). However, the administration of both 3 and 10 g/l $MgSO_4$ alone did not decrease these parameters in CP treated groups. Our results also showed that administration of losartan alone didn't have any protective effect on kidney against CP, but co-administration of losartan and 3 g/l $MgSO_4$ decreased serum Cr (insignificantly) and BUN (significantly, P<0.05) levels in CP treated animals (Figs. 2 and 3).

3.3 Changes in Kidney Histopathology Score

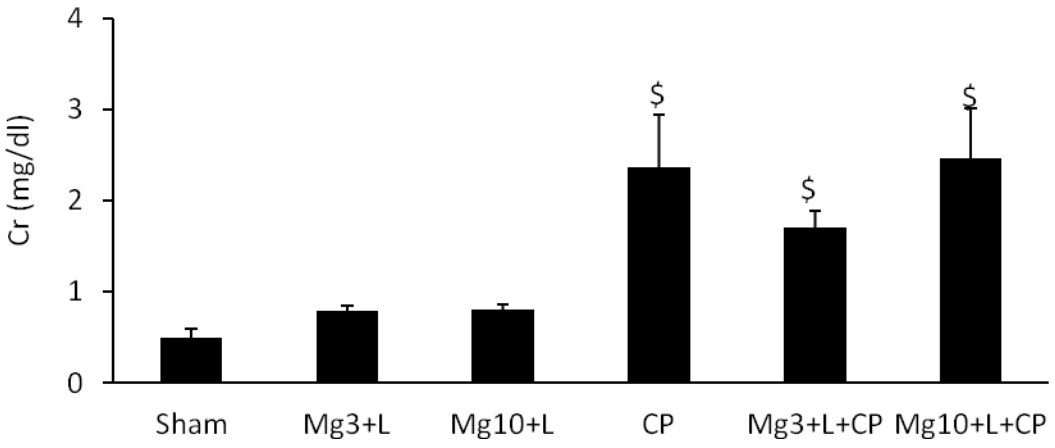
These data were presented in Fig. 4. Significantly, higher kidney damage was detected in all CP-treated groups when compared with other groups (P<0.05), although either the combination of losartan and 3g/l $MgSO_4$ or losartan and 10g/l $MgSO_4$ attenuated kidney tissue damage induced by CP insignificantly. The images of kidney tissue in all groups were illustrated in Fig. 5.


4
3
2
1
0
Cr (mg/dl)
*
*
Sham
L
CP
L+CP

A


4
3
2
1
0
Cr (mg/dl)
#
#
#
Sham
Mg3
Mg10
CP
Mg3+Cp
Mg10+Cp

B

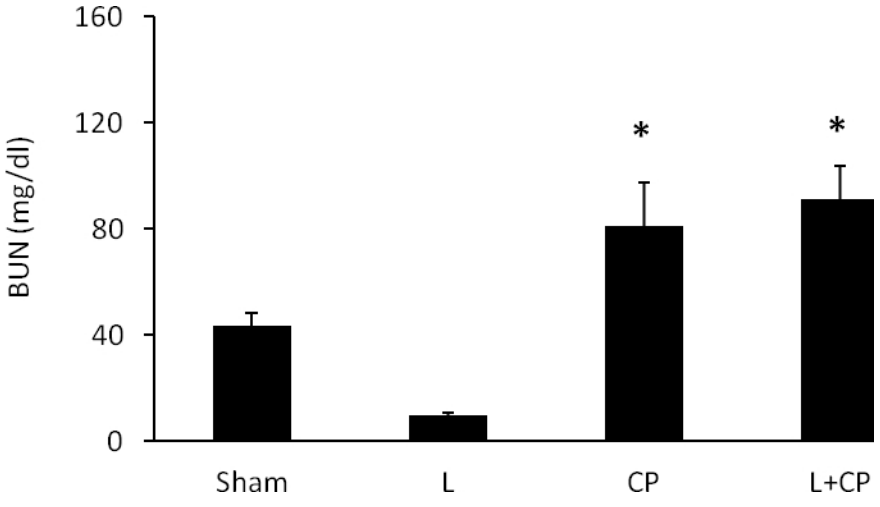


C

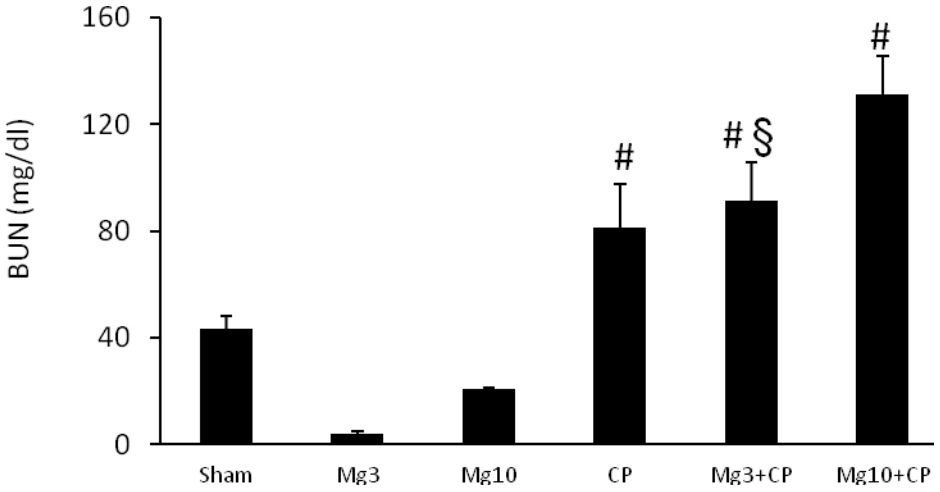
Fig. 2(A), (B) and (C) The comparison of serum creatinine level (Cr) in sham, cisplatin (CP), losartan (L), the combination of losartan and CP (L+CP) , Mg-treated with 3 g/l MgSO4 adding to the drinking water (Mg3), Mg-treated with 10 g/l $MgSO_4$ adding to the drinking water (Mg10), the combination of 3 g/l MgSO4 and losartan (Mg3+ L), the combination of 10g/l $MgSO_4$ and losartan (Mg10+ L), the combination of 3g/l $MgSO_4$and CP (Mg3+CP), the combination of 10g/l $MgSO_4$ and CP (Mg10+CP), the combination of 3g/l $MgSO_4$ and losartan and CP (Mg3+L+ CP), the combination of 10g/l $MgSO_4$ and losartan and CP (Mg10+L+ CP) groups (data are expressed as mean± SEM)

**L+CP and CP vs sham and L (p<0.05) # Mg3 +CP and Mg10+CP and CP vs sham, Mg3, Mg10 (p<0.05)*

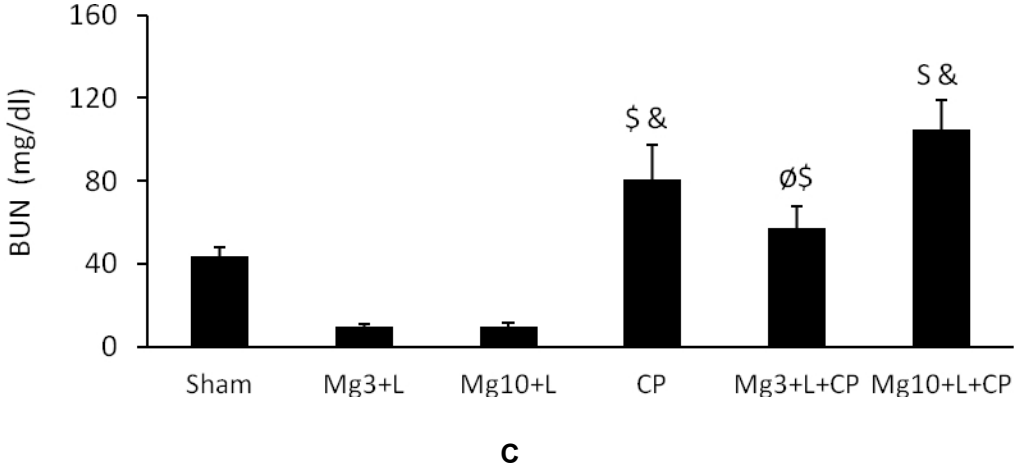
$ Mg3+ L+CP and Mg10+ L+CP and CP vs sham, Mg3+L and Mg10+L (p<0.05)



A



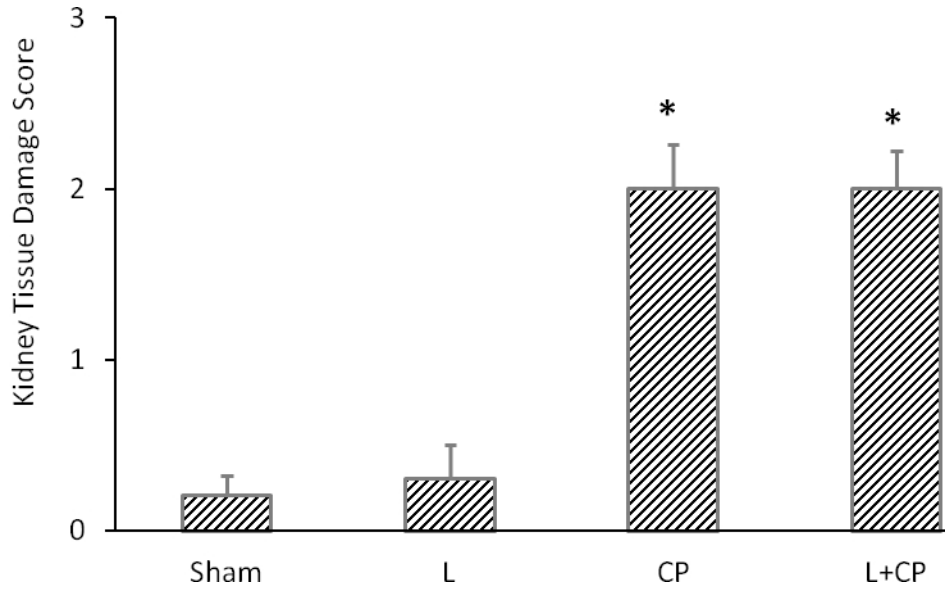
B



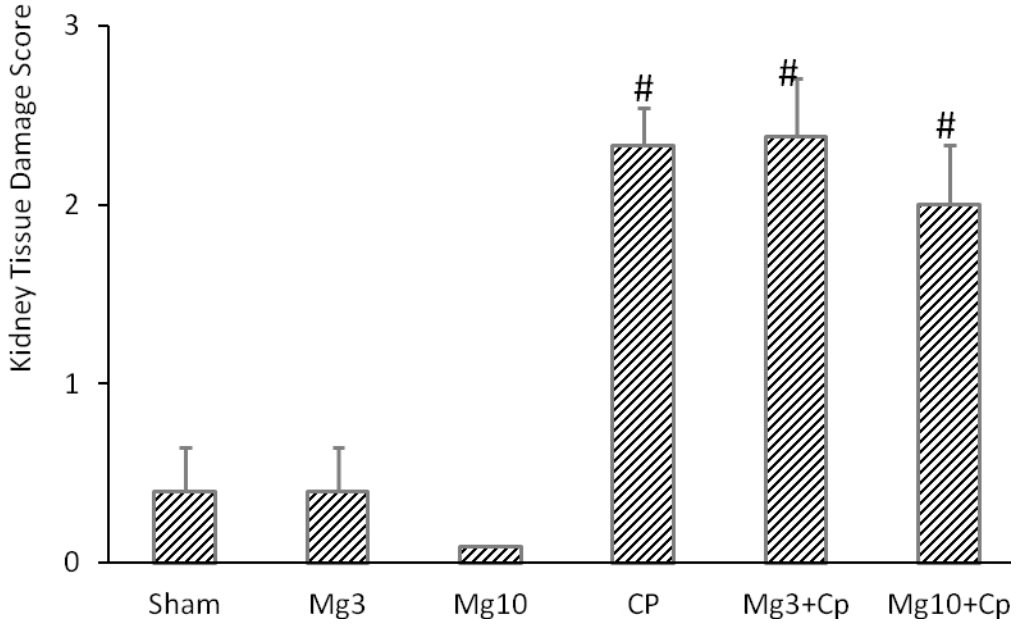
C

Fig. 3(A), (B) and (C) The comparison of serum BUN level in sham, cisplatin (CP), losartan (L), the combination of losartan and CP (L+CP) , Mg-treated with 3 g/l MgSO4 adding to the drinking water (Mg3), Mg-treated with 10 g/l MgSO4 adding to the drinking water (Mg10), the combination of 3g/l MgSO4 and losartan (Mg3+ L), the combination of 10g/l MgSO4 and losartan (Mg10+ L), the combination of 3g/l MgSO4 and CP (Mg3+CP), the combination of 10g/l MgSO4 and CP (Mg10+CP), the combination of 3g/l MgSO4 and losartan and CP (Mg3+L+ CP), the combination of 10g/l MgSO4 and losartan and CP (Mg10+L+ CP) groups (data are expressed as mean± SEM)

**CP and L+CP vs sham and L (p<0.05) # CP and Mg3+CP and Mg10+CP vs sham, Mg3 and Mg10 (p<0.05) $ CP and Mg3+L+ CP and Mg10+L+ CP vs Mg3+L and Mg10+L (p<0.05) & CP and Mg10+L+ CP vs sham (p<0.05)Ø Mg3+L+ CP vs Mg10+L+ CP (p<0.05)§ Mg3+CP vs Mg10+CP (p<0.05)*


Kidney Tissue Damage Score
3
2
1
0
*
*
Sham
L
CP
L+CP

A


Kidney Tissue Damage Score
3
2
1
0
#
#
#
Sham
Mg3
Mg10
CP
Mg3+Cp
Mg10+Cp

B

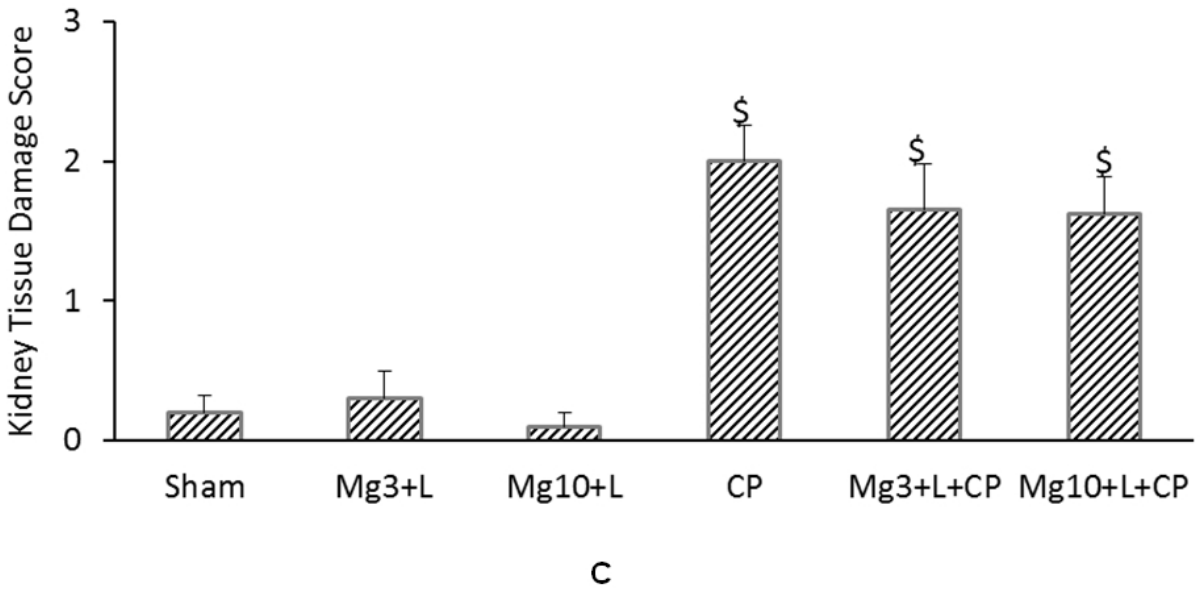


Fig. 4. The comparison of kidney tissue damage score in sham, cisplatin (CP), losartan (L), the combination of losartan and CP (L+CP) , Mg-treated with 3 g/l $MgSO_4$ adding to the drinking water (Mg3), Mg-treated with 10 g/l MgSO4 adding to the drinking water (Mg10), the combination of 3 g/l $MgSO_4$ and losartan (Mg3+ L), the combination of 10g/l $MgSO_4$ and losartan (Mg10+ L), the combination of 3g/l $MgSO_4$ and CP (Mg3+CP), the combination of 10g/l $MgSO_4$ and CP (Mg10+CP), the combination of 3g/l $MgSO_4$ and losartan and CP (Mg3+L+ CP), the combination of 10g/l $MgSO_4$ and losartan and CP (Mg10+L+ CP) groups (data are expressed as mean± SEM)

**L+CP and CP vs sham and L (p<0.05)# Mg3 +CP and Mg10+CP and CP vs sham and Mg3 and Mg10 (p<0.05)$ Mg3+L+ CP and Mg10+L+ CP and CP vs sham, Mg3+L and Mg10+L (p<0.05)*

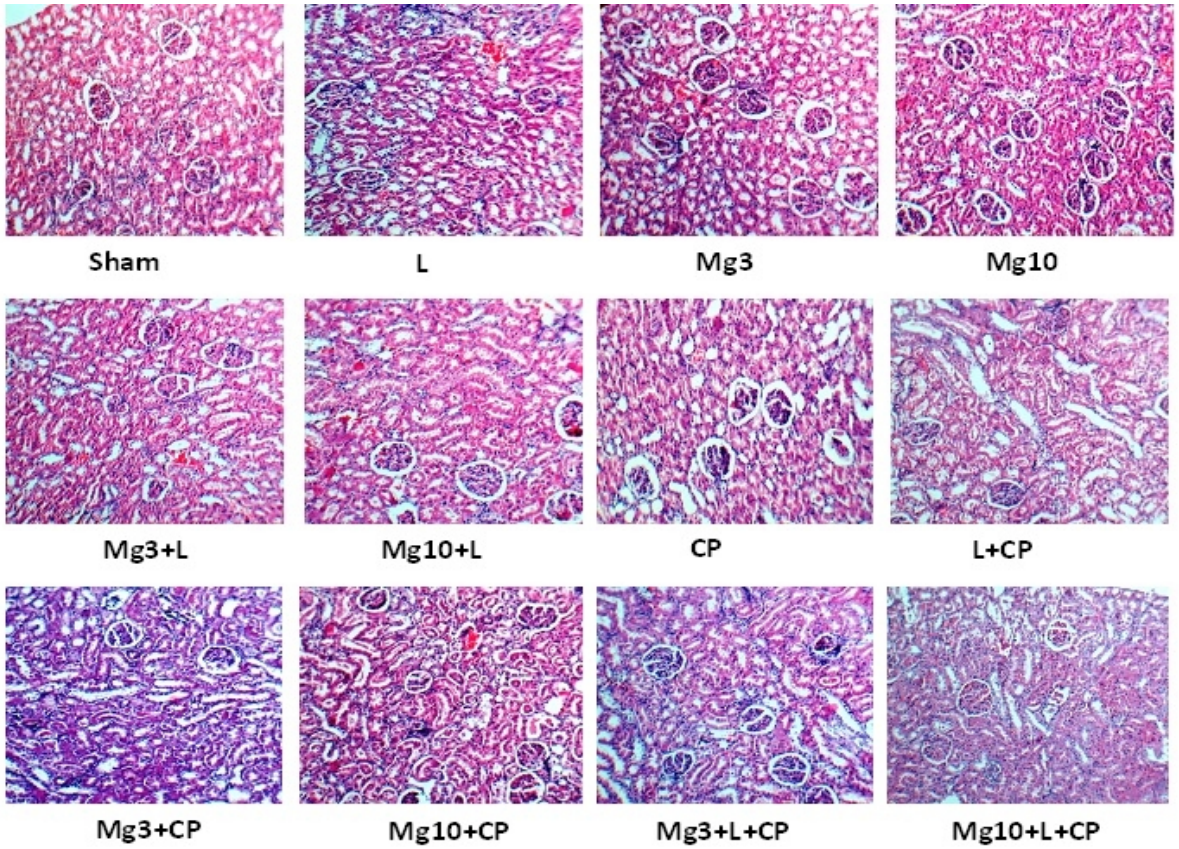


Fig. 5. The images (magnification100) of the kidney tissue. Sham, losartan(L), Mg-treated with 3 g/l of $MgSO_4$ adding to the drinking water (Mg3), Mg-treated with 10 g/l of $MgSO_4$ adding to the drinking water (Mg10), the combination of 3 g/l $MgSO_4$ and losartan (Mg3+L), the combination of 10g/l of $MgSO_4$+ losartan (Mg10+ L),cisplatin(CP),the combination of losartan and CP (L+CP), the combination of 3g/l $MgSO_4$ and CP (Mg3+CP), the combination of 10g/l $MgSO_4$ and CP (Mg10+CP), the combination of 3g/l $MgSO_4$ and losartan and CP (Mg3+L+ CP), the combination of 10g/l $MgSO_4$ and losartan and CP (Mg10+L+ CP) groups

4. DISCUSSION

In the present study, we attempted to examine whether the combination of losartan and oral administration of $MgSO_4$ could decrease CP-induced nephrotoxicity in female. Our previous studies have reported that inhibition of AT1R by losartan has protective effect against CP-induced nephrotoxicity in male, but not in female [8,10,21]. It seems that this difference is probably related to RAS, which acts differently in male and female kidneys [22,23]. Results of present study showed that administration of losartan alone could not ameliorate nephrotoxicity induced by CP in female. These findings were in agreement with our previous reports [10,21].

It is demonstrated that plasma and intracellular of Mg concentrations decrease after CP therapy [11,12,16]. Human organic cation transporter 2 (OCT2) is responsible for uptake of organic cations across the basolateral membrane in kidneys [24]. Moreover, OCT2 is Mg-dependent and hypomagnesemia causes up regulation of OCT2, which may increase the accumulation of CP in the kidney [13]. The low dose of $MgSO_4$ administration (20mg/kg) via intraperitoneal injection had a harmful effect on CP-induced nephrotoxicity [17]. So it was assumed that continually dose of $MgSO_4$ alone may reduce CP-induced nephrotoxicity. Contrary to our expectations, the present study showed that oral administration of both low and high doses of $MgSO_4$ alone couldn't ameliorate CP-induced failure. Therefore in the next step, we tried to use the combination of losartan and different concentrations of $MgSO_4$ to test its effect on CP-induced nephrotoxicity. According to our findings, administration of combination of losartan and $MgSO_4$ increased KW/100 gr BW ratio. In our previous studies KW was correlated with kidney tissue damage score (KTDS) [10,17,21], however in this study, KW in Mg3+L+CP and Mg10+L+CP groups increased while KTDS decreased. It seems that Mg plays an important role here to decrease the KTDS. Mg itself affects the gastrointestinal (GI) system, and Mg therapy is associated with GI disturbance symptoms [14,15,17]. Similarly, weight loss in our model may be related to both CP and $MgSO_4$ (data were not shown). Our results also showed that administration of losartan plus low dose of $MgSO_4$ accompanied with CP decreased serum Cr and BUN levels in comparison with CP alone treated group. Finally and in overall, we can conclude that the combination of losartan and low dose of MgSO4 may ameliorate nephrotoxicity induced by CP. However according our results, we cannot suggest our medications to the physicians who are involved in human cancers chemotherapy. But it seems that the physicians should be changed their protocols for cancers chemotherapy and use of losartan or Mg supplementations in female very limit.

5. CONCLUSION

According to our findings, we suggest the physicians who are involved in human cancers chemotherapy to change their protocols for cancers chemotherapy and use of losartan or Mg supplementations in female very limit.

ACKNOWLEDGMENT

This research was supported by Hormozgan University of Medical Sciences.

CONFLICT OF INTERESTS

The authors have not a direct financial relation with the commercial identities mentioned in this article. So, the authors have no conflict of interests.

REFERENCES

1. Boulikas T, Vougiouka M. Cisplatin and platinum drugs at the molecular level (Review). Oncology reports. 2003;10(6):1663-1682.
2. Yao X, et al. Cisplatin nephrotoxicity: A review. Am J Med Sci. 2007;334(2):115-24.
3. Ali BH, Al Moundhri MS, Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: A review of some recent research. Food Chem Toxicol. 2006:44(8):1173-83.
4. Daugaard G, Abildgaard U. Cisplatin nephrotoxicity. A review. Cancer Chemother Pharmacol. 1989:25(1):1-9.
5. Kobori H, et al. The intrarenal renin-angiotensin system: From physiology to the pathobiology of hypertension and kidney disease. Pharmacological Reviews. 2007;59(3):251-287.
6. Wolf G, et al. Angiotensin II induces p27Kip1 expression in renal tubules in vivo: Role of reactive oxygen species. Journal of molecular medicine. 2001;79(7):382-389.
7. Saleh S, et al. Protective effects of the angiotensin II receptor blocker losartan on cisplatin-induced kidney injury. Chemotherapy. 2009:55(6):399-406.
8. Ashrafi F, et al. A combination of vitamin C and losartan for cisplatin induced nephrotoxicity in rats. Iranian journal of kidney diseases. 2012;6:5.
9. Deegan PM, et al. The role of the renin-angiotensin system in cisplatin nephrotoxicity. Renal failure. 1995;17(6):665-674.
10. Haghighi M, et al. The role of angiotensin II receptor 1 (AT1) blockade in cisplatin-induced nephrotoxicity in rats: Gender-related differences. Ren Fail. 2012;34(8):1046-51.
11. Martin M, et al. Intravenous and oral magnesium supplementations in the prophylaxis of cisplatin-induced hypomagnesemia. Results of a controlled trial. Am J Clin Oncol. 1992;15(4):348-51.
12. Lajer H, et al. Magnesium and potassium homeostasis during cisplatin treatment. Cancer Chemother Pharmacol. 2005;55(3):231-6.
13. Yokoo K, et al. Enhanced renal accumulation of cisplatin via renal organic cation transporter deteriorates acute kidney injury in hypomagnesemic rats. Clin Exp Nephrol. 2009;13(6):578-84.
14. Ikarashi N, et al. Effects of magnesium sulphate administration on aquaporin 3 in rat gastrointestinal tract. Biol Pharm Bull. 2011;34(2):238-42.
15. Soltani N, et al. Effect of oral administration of magnesium on cisplatin-induced nephrotoxicity in normal and streptozocin-induced diabetic rats. Nephrourol Mon, 2013;5(4):884-90.
16. Hodgkinson E, Neville-Webbe HL, Coleman RE. Magnesium depletion in patients receiving cisplatin-based chemotherapy. Clin Oncol (R Coll Radiol). 2006;18(9):710-8.
17. Ashrafi F, et al. The role of magnesium supplementation in cisplatin-induced nephrotoxicity in a Rat model: no nephroprotectant effect. Int J Prev Med. 2012;3(9):637-43.
18. Mavichak V, et al. Studies on the pathogenesis of cisplatin-induced hypomagnesemia in rats. Kidney Int. 1985;28(6):914-21.
19. Yao EH, et al. Losartan improves the impaired function of endothelial progenitor cells in hypertension via an antioxidant effect. Hypertens Res. 2007;30(11):1119-28.
20. Afanas'ev IB, et al. Study of antioxidant properties of metal aspartates. Analyst. 1995;120(3):859-62.

21. Nematbakhsh M, et al. Vitamin E, Vitamin C, or losartan is not nephroprotectant against cisplatin-induced nephrotoxicity in presence of estrogen in ovariectomized rat model. International journal of nephrology. 2012;2012:284896. doi: 10.1155/2012/284896.
22. Sampson AK, et al. Enhanced angiotensin II type 2 receptor mechanisms mediate decreases in arterial pressure attributable to chronic low-dose angiotensin II in female rats. Hypertension. 2008;52(4):666-671.
23. Safari T, Nematbakhsh M. Angiotensin 1-7 receptor and angiotensin ii receptor 2 blockades prevent the increased serum and kidney nitric oxide levels in response to angiotensin II administration: gender-related difference. Int J Prev Med. 2013.4(3):311-5.
24. Kolb RJ, Ghazi AM, Barfuss DW. Inhibition of basolateral transport and cellular accumulation of cDDP and N-acetyl- L-cysteine-cDDP by TEA and PAH in the renal proximal tubule. Cancer Chemother Pharmacol. 2003;51(2):132-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=607&id=14&aid=5607