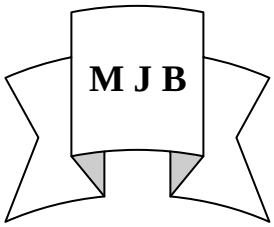
Protection from the Acute Cisplatin-Induced Nephrotoxicity by Simvastatin in Rats

Al-Thamir, S. N. K. Al-Shalah, H. H.* Bairam, A. F. H.†
Dept. of Pharmacology, College of Pharmacy, University of Babylon, Hilla, Iraq.
* Dept. of Clinical Biochemistry, College of Medicine, University of Babylon,Hilla, Iraq.
†Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Kufa, Iraq.



Abstract

Background: The usefulness of cisplatin, a potent anti-tumor, is limited by its ability to induce nephrotoxicity. The cellular changes leading to this toxicity are suggested to be mediated by increased free radicals generation and lipid peroxidation.

Aim of the study: To investigate the protective properties of simvastatin on cisplatin-induced nephrotoxicity in rats using biochemical and histopa- thological approaches.

Material and methods: 35 healthy male Swiss albino rats were used. They were divided into 5 groups (7 animals in each group and all animals supplied with standard food during the experiment): control group (1 ml/kg of normal saline i.p. once daily for 14 days), cisplatin treated group (7.5 mg/kg, i.p. single dose), simvastatin treated group (1 mg/kg, i.p., once daily for 14 days), simvastatin (1 mg/kg) plus cisplatin treated group (i.p., once daily for 7 days before and after cisplatin injection), and simvastatin (2 mg/kg) plus cisplatin treated group (i.p., once daily for 7 days before and after cisplatin injection). Blood samples were collected and used to determine the serum urea, creatinine and total antioxidant status (TAS) levels. kidneys were removed and prepared for histopathological examinations.

Results: In simvastatin plus cisplatin treated groups serum urea and creatinine were significantly lower than those of cisplatin-treated group, While serum TAS was increase. These changes occurred in a dose-dependant manner (simvastatin). Histopathological examinations showed a massive damage in the proximal tubules in cisplatin-treated group. No damage was observed in simvastatin treated groups.

Conclusion: These data show that simvastatin can provide a protective effect against Acute cisplatin-induced nephrotoxicity. This protective effect of simvastatin may be related to the antioxidant status on the kidney.

ألخلاصة

المقدمة: فائدة السزبلاتين كعقار مضاد للسرطان محدودة بسبب تأثيره السمي على الكلية. التغيرات الخلوية المؤدية إلى هذه السمية سببها زيادة في تكوين الجذور الحرة وأكسدة الدهون

الهدف من الدراسة: تقييم التأثير الوقائي للسمفاستاتين على التأثير السمي للسزبلاتين على الكلية في الجرذان باستخدام اختبارات كيميائية حياتية وإجراء الفحص النسيجي.

الحيوانات، المواد وطرق العمل: خمسة وثلاثين ذكرا بالغا من الجرذان البيض السويسرية استخدمت في هذه الدراسة. هذه الحيوانات قسمت بشكل عشوائي إلى خمسة مجاميع (سبعة جرذان في كل مجموعة وجميع الحيوانات أعطيت غذاء طبيعي قياسي طوال التجربة): الحيوانات في المجموعة ألأولى أعطيت ماء ملحي طبيعي ١مل/كغم عن طريق البيريتون مرة واحدة يوميا لمدة أربعة عشر يوم وأُعتبرت مجموعة ألسيطرة ألأولية، الحيوانات في المجموعة الثانية حُقنت بمادة السزبلاتين جرعة واحدة ٧,٥ ملغم/كغم في التجويف ألبطني (البيريتون)، في

حين إن الحيوانات في المجموعة الثالثة حقنت بعقار السمفاستاتين ١ملغ/كغم في التجويف ألبطني (البيريتون) مرة واحدة يوميا لمدة أربعة عشر يوم، الحيوانات في المجموعة الرابعة حقنت بعقار السمفاستاتين (١ملغ/كلغم مرة يوميا عن طريق التجويف ألبطني) لمدة أربعة عشر يوم سبعة أيام قبل وسبعة أيام بعد جرعة السزبلاتين، الحيوانات في المجموعة الخامسة حقنت بعقار السمفاستاتين (٢ملغ/كلغم مرة يوميا عن طريق التجويف ألبطني) لمدة أربعة عشر يوم، سبعة أيام قبل وسبعة أيام بعد جرعة السزبلاتين. عينات الدم جمعت واستخدمت لقياس المؤشرات التالية: مؤشرات وظيفة الكلى وهي يوريا الدم وكرياتينين المصل وفحص مؤشر الأكسدة وهو (تي أي أس) في المصل، كما تم عمل الفحص النسيجي للكلية.

النتائج: في المجاميع المعاملة بالسزبلاتين بالإضافة إلى السمفاستاتين كان مستوى اليوريا و الكرياتتين في المصل بصورة واضحة اقل من مستوياتها في المجموعة المعاملة بالسزبلاتين لوحده, بينما ان مستوى (أي تي أس) في المصل ازداد.هذه التغيرات حدثت بطريقة تعتمد على جرعة السمفاستاتين). الفحص النسيجي بين وجود ضرر كبير في النبيبات الكلوية الملتوية في المجموعة المعاملة بالسزبلاتين لوحده.كما انه ليس هنالك ضرر يلاحظ في المجاميع المعاملة بالسزبلاتين بالإضافة إلى السمفاستاتين.

الاستنتاج: هذه البيانات تبين إن السمفاستاتين يمتلك تأثير وقائي ضد التأثير السمي للسزبلاتين على الكلية وهذا التأثير الوقائي يعزى إلى الفعالية المضادة للأكسدة على الكلية.

Introduction

Cisplatin is an antineoplastic drug, administered to cancer patients as a sterile solution. It is widely used for the treatment of several human malignancies (as standard component of treatment regimens) including head and neck cancers [1], testicular cancer [2], small-cell [3] and non-small cell lung cancer [4], ovarian cancer [5], cervical cancer [6] and bladder cancer [7]. The main dose-limiting side effect of cisplatin is nephrotoxicity [8] Cisplatin nephrotoxicity can present in a number of ways. However, the most serious and one of the more common presentations is the acute renal failure which occurs in 25–35% of patients [9]. Several investigators have hypothesized the oxidative stress mechanism of cisplatin induced nephrotoxicity that is related to depletion of the antioxidant defense system [10] and to inhibitory effect of cisplatin on antioxidant enzymes activities [11]. On the other hand, simvistatin which is a structural analogs of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) can decreased oxidative stress as well as it has a reductase inhibitory effect [12].

Animals, Materials and Methods

Animals: A total of 35 adult male Albino Swiss rats aged 16 - 24 weeks with weight of (170 – 255g), were obtained from Animal Resource Center, the Institute of Embryo Research and Treatment of Infertility, Al-Nahrain University. The animals were apparently healthy and they were housed in individual cages, at temperature controlled environment (25±5°C) with ambient humidity. Lights were maintained on a 12h light/dark cycle. The rats received standard chow diet with water (*ad libitum*). Rats in the study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals USA 1996 [13].

Study design:

After 4 weeks acclimatization period, the animals were randomly separated into 5 groups (7 rats in each group):

i. Normal saline (N.S) treated group: all rats of this group received normal saline (1 ml/kg, i.p., once daily) for 14 days.

ii. Cisplatin treated group: all rats of this group received cisplatin (7.5 mg/kg i.p.) single dose.

iii. Simvastatin treated group: all rats of this group were given simvastatin (1 mg/kg, i.p., once daily) for 14 days.

iv. Simvastatin treated group (1 mg/kg) plus cisplatin: all rats of this group were given Simvastatin (1 mg/kg, i.p.) once daily for 7 days before and 7 days after cisplatin injection.

v. Simvastatin treated group (2 mg/kg) plus cisplatin: all rats of this group were given simvastatin (2 mg/kg, i.p.) once daily for 7 days before and 7 days after cisplatin injection.

Materials and Methods

Cisplatin 50mg/dl vial (Ebewe pharma, Austria), Simvastatin 10 mg tablets (Actavis, Barnstable, United Kingdom). All other chemicals were of the highest available analytical grade. After 24hr from the last injection of all treatments, the rats were anesthetized with phenobarbital (50 mg/kg s.c.). Blood samples (3mls) were obtained from each rat by cardiac puncture using disposable syringe. Sera were obtained via centrifugation at 3000 rpm for 10 minutes and preserved at -20 °C until determination of parameters of serum urea, creatinine and TAS by commercially available colorimetric kits. Rats abdomen was opened through a midline incision and the kidneys were quickly removed and fixed in 10% formalin for histopathological examination.

Statistical analysis of data: Statistical analyses were performed using SPSS version 18 (GrraphPad, ISI software, Philadelphia, PA, USA, 1993) computer program. Data are expressed as means ± standard deviation (M±SD). Multiple comparisons were done using one way ANOVA Bonferroni test. The ($p<0.05$) level of probability was chosen as a criterion for the lowest level of significance.

Results

Effects of cisplatin and simvastatin treatment on serum urea and creatinine concentrations :

According to Figures 1 and 2, serum urea and creatinine levels were significantly ($p<0.01$) higher in cisplatin-treated group than the controls. Simvastatin produced a significant ($p<0.01$) and dose dependent reduction in the urea and creatinine concentrations when compared with the cisplatin treated group.

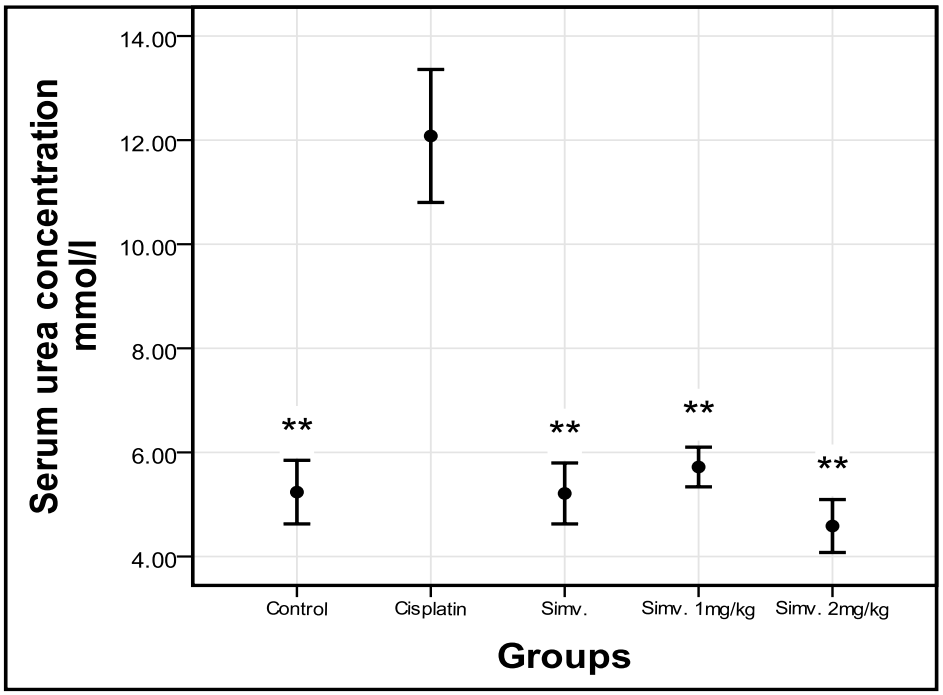


Figure 1. Concentration of serum urea in different treated groups. Data was shown as mean ± S.D. ** = p<0.01 as compared with cisplatin-treated group (n = 7). Simv. = simvastatin.

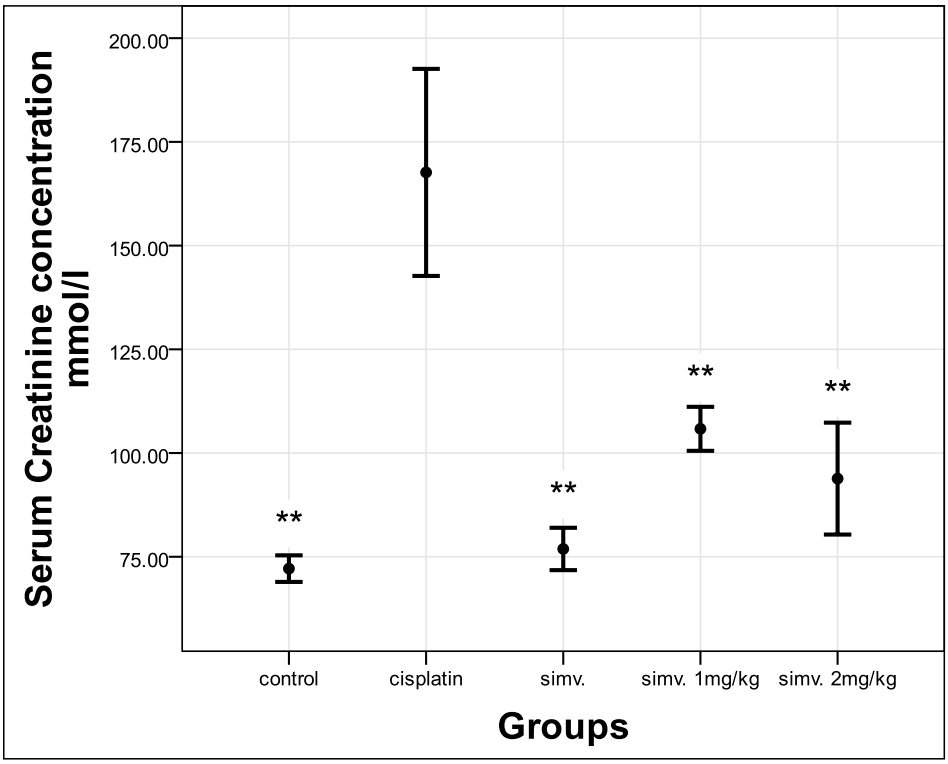


Figure 2 Concentration of serum creatinine in different treated groups. Data was shown as mean ± S.D. ** = p<0.01 as compared with cisplatin- treated group (n = 7).

Effects of cisplatin and simvastatin treatment on serum TAS :

According to Figures 3, serum TAS were significantly ($p<0.01$) lower in cisplatin-treated group than the controls. Simvastatin produced a significant ($p<0.01$) and dose dependent elevation in the serum TAS when compared with the cisplatin treated group.

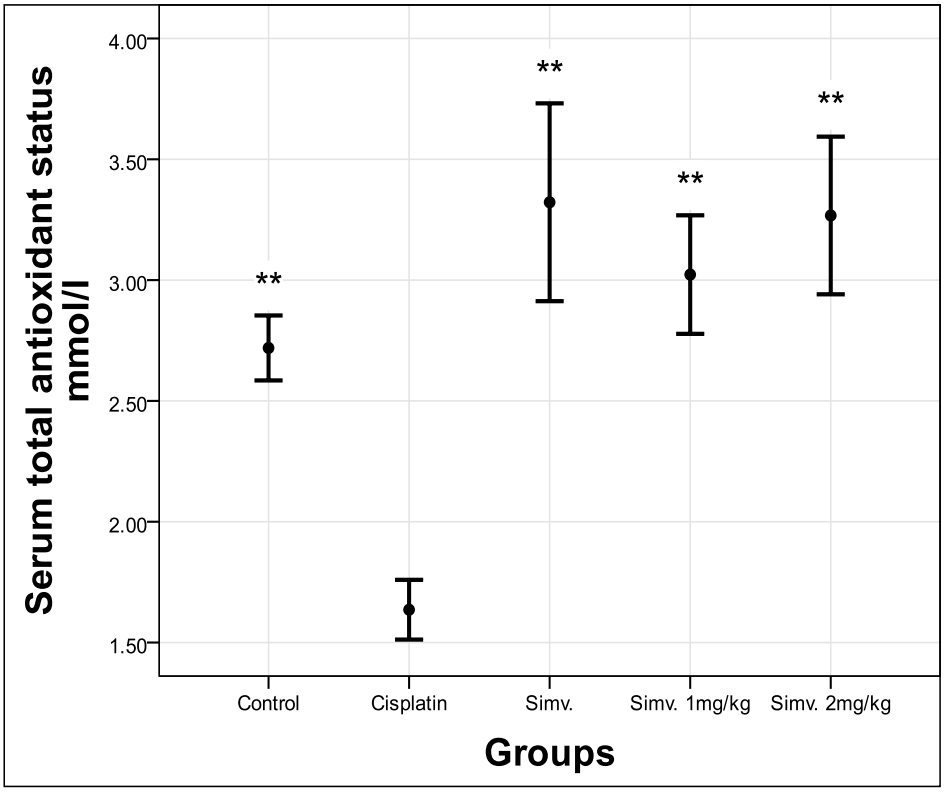


Figure 3 Concentration of serum total antioxidant status in different treated groups. Data was shown as mean ± S.D. ** = $p<0.01$ as compared with cisplatin- treated group (n = 7).

Kidney histology:

All parts of kidney showed normal appearance in the control group (Figure 4 A). On the other hand, rats treated with cisplatin showed tubular degeneration which varied according to kidney zone. Coagulative necrosis was observed in the tubular lining epithelium at the cortex and corticomedullary junction, the protein casts are seen in the lumen of proximal tubules and loss of the epithelial brush-border (Figure 4 B1 and B2). Coagulative necrosis is an irreversible grade of necrotic cell death and tubular cells could not regenerate after removal of toxic insult. Treatment with Simvastatin alone didn't show any significant histopathological changes compared to the control group (Figure 4 C). The treatment with simvastatin 1 mg/kg and 2 mg/kg for one week before and after cisplatin injection showed nephroprotective and antinecrotic effect of the histopathological changes induced by cisplatin in a dose dependant manner (Figures 4 D and E) respectively.

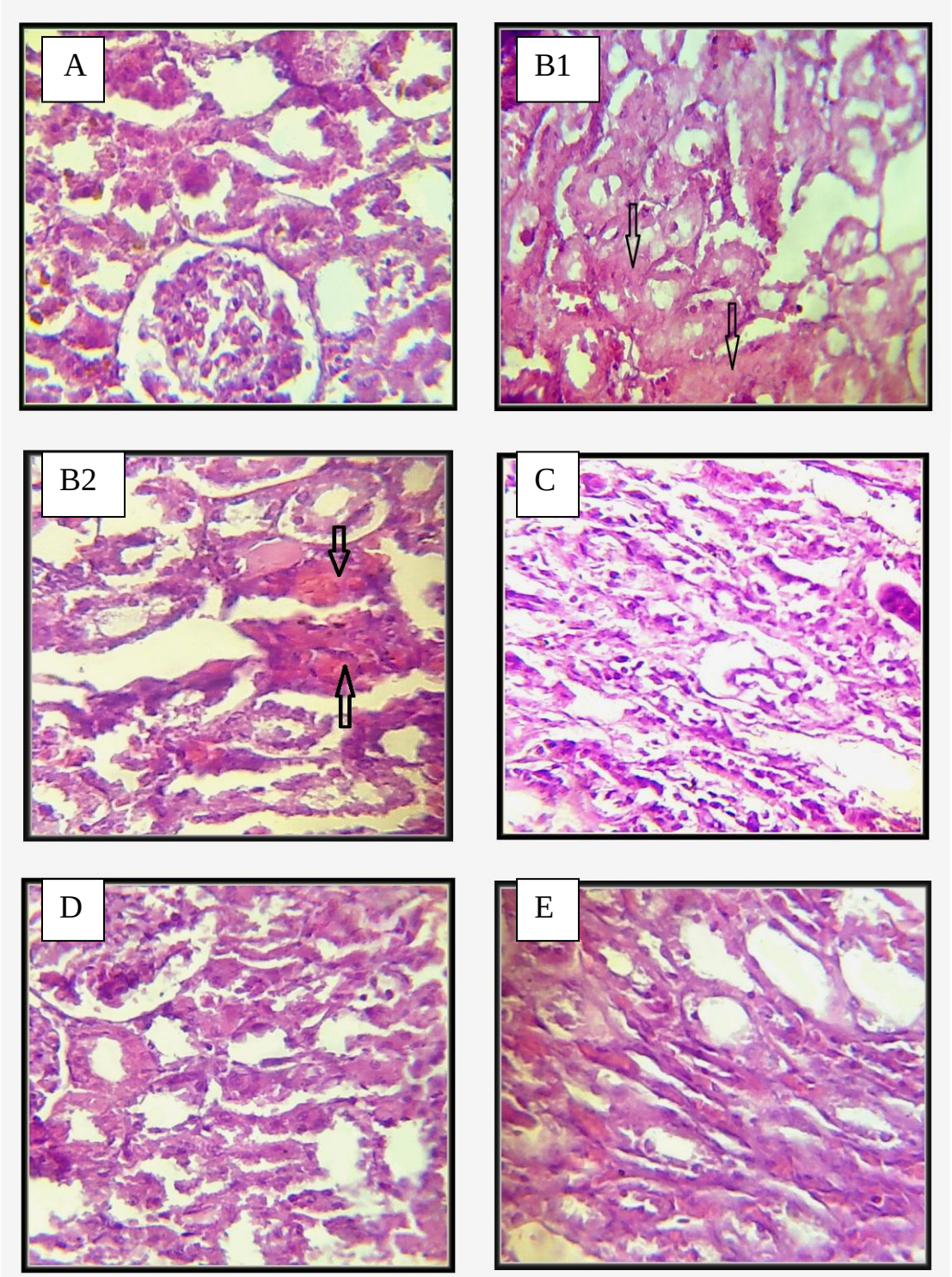


Figure 4 Light microscopy of renal tissue stained with hematoxylin and eosin stain in different treated groups (high magnification power, × 400); (A) Control group, renal tubules show normal appearance. (B1) Cisplatin treated group, Proximal tubules show coagulative necrosis in the cortico-medulary junction (arrow). (B2) Cisplatin treated group, the tubular epithelial cell casts are in the lumen of the proximal tubules (arrow). (C) Simvastatin treated group (simvastatin alone), show no histopathological changes compared with the control group. (D) Simvastatin (1mg/kg, i.p.) plus cisplatin treated group, almost all renal proximal tubules are intact. (E) Simvastatin (2mg/kg, i.p.) plus cisplatin treated group, all of the renal proximal tubules in the cortex are intact and show no histopathological changes compared with the control group.

Discussion

Cisplatin is one of the most effective anticancer agents. It is used to treat solid tumors such as testicular, bladder, ovarian, breast and lung cancers [14]. The present study indicated that simvastatin dramatically protected the cisplatin-induced *in vivo* nephrotoxicity in rat.

In the present study, a single dose of cisplatin (7.5 mg/kg, i.p.) induced nephrotoxicity as revealed from the increase in serum urea, creatinine levels and decrease serum TAS as well as by histopathological findings. These changes occurred acutely (24 hours) after cisplatin injection into rats. These results are consistent with those experimentally reported by [15,16].

The main mechanism of cisplatin induced nephrotoxicity is oxidative stress through lipid peroxidation and free radical generation in the tubular cells causing tubular cell necrosis as reported by [17]. The proposed mechanisms by which cisplatin causes oxidative stress are:

a. Depletion of the antioxidant defense system [10].

b. Inhibitory effect of cisplatin on antioxidant enzymes activities as catalase (CAT), glutathione peroxidase (GSH-Px) and super oxide dismutase (SOD), [11].

The increase in free radical generation and the decrease in antioxidant defense system results in an increase in serum and renal lipid peroxidation that lead to decrease the level of TAS production in serum and renal tissue. The results of this study confirm the nephro-protective activity of simvastatin, When the rats received simvastatin (1mg and 2mg/kg for 14 days), 7 days before and 7 days after cisplatin injection, the serum urea, creatinine concentrations are significantly lower than that in cisplatin administered group, while the serum TAS is more than that in cisplatin administered group. These results are in agreement with [18, 19].

Conclusions

Simvastatin is able to protect the kidneys against cisplatin-induced acute renal failure. But, before a conclusive statement on potential

usefulness of simvastatin as adjunct to the cisplatin therapy, there is a need for further studies including human trials.

References

1-Planting, A., Catimel, G., de Mulder, P., de Graeff, A., Hoppener, F., Verweij, J. Oster, W. and Vermorken, J. (1999): Randomized study of a short course of weekly cisplatin with or without amifostine in advanced head and neck cancer. Ann. Oncol.: 10: 693–700.

2-Loehrer, P. J., Gonin, R., Nichols, C. R., Weathers, T. and Einhorn, L. H. (1998): Vinblastine plus ifosphamide plus cisplatin as initial salvage therapy in recurrent germ cell tumor. J. Clin. Oncol.: 16: 2500-2504.

3-Noda, K., Nishiwaki, Y., Kawahara, M., Negoro, S., Sugiura, T., Yokoyama, A. and Saijo, N. (2002): Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N. Engl. J. Med.: 346: 85–91.

4-Gatzemeier, U., Von Pawel, J., Ten Velde, G., Mattson, K., DeMarinis, F., Harper, P., Salvati, F., Robinet, G., Lucenti, A., Bogarerts, J. and Gallant, G. (2000): Phase III comparative study of high-dose cisplatin versus a combination of paclitaxel and cisplatin in patients with advanced non-

smallcell lung cancer. J. Clin. Oncol.: 18: 3390 - 3399.

5-Hoskins, P., Eisenhauer, E., Vergote, I., Dubuc-Lissoir, J., Fisher, B., Grimshaw, R., Oza, A., Plante, M., Stuart, G. and Vermorken, J. (2000): Phase II feasibility study of sequential couplets of Cisplatin/Topotecan followed by paciltaxel/cisplatin as primary treatment of advanced epithelial ovarian cancer: A National Cancer Institute of Canada Clinical Trials Group study. J. Clin. Oncol.: 18: 4038-4044.

6-Rose, P., Bundy, B., Watkins, E., Thigpen, J., Deppe, G., Maiman, M., Clarke-Pearson, D. and Insalaco, S. (1999): Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N. Engl. J. Med.: 340: 1144-1153.

7-Coppin, C., Gospodarowicz, M., James, K., Tannock, I., Zee, B., Carson, J., Peter, J. and Sullivan, D. (1996): Improved local control of invasive bladder cancer by concurrent cisplatin and preoperative or definitive radiation. J. Clin. Oncol.: 14: 2901-2907.

8-Sastry, J. and Kellie, S. J. (2005): Severe neurotoxicity, ototoxicity and nephrotoxicity following high-dose cisplatin and amifostine. Pediatr. Hematol. Oncol.: 22: 441-445.

9-Lee, K. W., Jeong, J. Y., Lim, B. J., Chang, Y.K., Lee, S. J., Na, K. R., Shin, Y. T. and Choi, D. E. (2009): Sildenafil attenuates renal injury in an experimental model of rat cisplatin-induced nephrotoxicity. Toxicology: 257: 137-143.

10-Silva, C. R., Greggi Antunes, L. M. and Bianchi, M. L. (2001): Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. Pharmacol. Res.: 43: 561-566.

11-Yildirim, Z., Sogut, S., Odaci, E., Iraz, M., Ozyurt, H., Kotuk, M. and Akyol ,O. (2003): Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. Pharmacol. Res.: 47:149-156.

12-Malloy, M. J. and Kane, J. P. (2007): Agents Used in Hyperlipidemia : In ; Basic and clinical Pharmacology, Katzung, B. G. (ed.), tenth edition, McGraw-Hill: p. 796.

13-Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C. 1996.

14-Giaccone, G. (2000): Clinical perspectives on platinum resistance. Drugs: 4: 9-17.

15-Mansour, M. A., Mostafa, A. M., Nagi, M. N., Khattab, M. M. and Al-Shabanah, O. A. (2002): Protective effect of aminoguanidine against nephrotoxicity induced by cisplatin in normal rats. Comp. Biochem. Physiol. C Toxicol. Pharmacol.: 132: 123-128.

16-Mora, L. O., Antunes, L. M., Francescato, H. D. and Bianchi, M. L. (2003): The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. Pharmacol. Res.: 47: 517-522.

17-Maliakel, D. M., Kagiya, T. V. and Nair, C. K. K. (2008): Prevention of cisplatin-induced nephrotoxicity by glycosides of ascorbic acid and [alpha]-tocopherol. Exp. Toxicol. Pathol.: 60: 521-527.

18-Abd Elbaky, N. A., Hammad, L. N. and Atia, T. A. (2006): Protective Effect of Simvastatin against adriamycin-induced nepherotoxicity in rats; Biochemical and Histological Study. Egyp. J. Hosp. Med.: 25: 725 - 739.

19-Puttananjaiah, M. H., Dhale, M. A., Gaonkar, V. and Keni, S. (2011): Statins: 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors demonstrate anti-atherosclerotic character due to their antioxidant capacity, Appl. Biochem. Biotechnol.: 163(2): 215-222.

20-Hong, M. E., Hong, J. C., Stepkowski, S. and Kahan, B. D. (2005): Correlation between cyclosporine-induced nephrotoxicity in reduced nephron mass and expression of kidney injury molecule-1 and aquaporin-2 gene. Transplant. Proc.: 37(10): 4254 -4258.