



## Effect of Simvastatin on Cisplatin-induced Nephrotoxicity in Male Rats

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### Abstract

Statins have antioxidant and anti-inflammatory effects that are not directly related to their cholesterol-lowering activity. This study aimed to investigate the effect of simvastatin on the extent of tissue damage in cisplatin-induced nephrotoxicity. Simvastatin was orally given to rats in different doses (1, 2 and 4 mg/kg), 1 h prior to cisplatin injection (5 mg/kg, i.p.). All animals were decapitated 5 days after cisplatin administration. Blood urea nitrogen, creatinine, K and Na levels were measured. The kidney samples used for the measurement of malondialdehyde and glutathione levels or were processed for histopathological studies. Simvastatin at 1 mg/kg caused a significant decrease in serum Na and a significant increase in serum K. Simvastatin at 2 mg/kg significantly increased serum Na, and at 4 mg/kg significantly prevented decrease of GSH levels by cisplatin and significantly decreased serum Na levels. The morphological changes induced by cisplatin treatment were prevented only by 4 mg/kg dose of simvastatin. Thus, simvastatin at 4 mg/kg dose is beneficial in cisplatin-induced nephrotoxicity in rats via prevention of lipid peroxidation, inflammation and endothelial function impairment.

**Keywords:** Cisplatin; Nephrotoxicity; Oxidative stress; Simvastatin.

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### 1. Introduction

Statins are the most efficient agents for reducing plasma cholesterol because of their inhibitory effect on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting rate step in its synthesis in the liver and other organs. In

addition, they have pleiotropic non-lipid dependent effects. These effects include anti-inflammatory, antioxidant, antithrombotic and endothelial function improvement effects [1-3].

Cisplatin (cis-diamminedichloroplatinum (II); CDDP) is an antineoplastic drug used in the treatment of many solid-organ cancers, including those of the head, neck, lung, testis, ovary, and breast etc., but nephrotoxicity limits its clinical use. The kidney is not only

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responsible for the excretion of cisplatin but is also the primary site of its accumulation [4-6].

Cisplatin therapy has been demonstrated to induce oxidative stress, principally involving reactive oxygen species (ROS), in renal tubular cells. Oxidative stress is caused by various free-oxygen radicals including superoxide anion, hydrogen peroxide and hydroxyl radical. The interaction of ROS with cellular components may result in damage to DNA, proteins, and lipids. Excessive ROS generation caused by cisplatin may overwhelm the natural antioxidant defenses of the kidney cells and lead to lipid peroxidation and delayed-onset kidney injury [7].

In the present study, we evaluated the extent of tissue damage in cisplatin-induced nephrotoxicity and the protective activity of simvastatin against its toxicity and on the level of oxidative stress.

## 2. Materials and methods

### 2.1. Chemicals

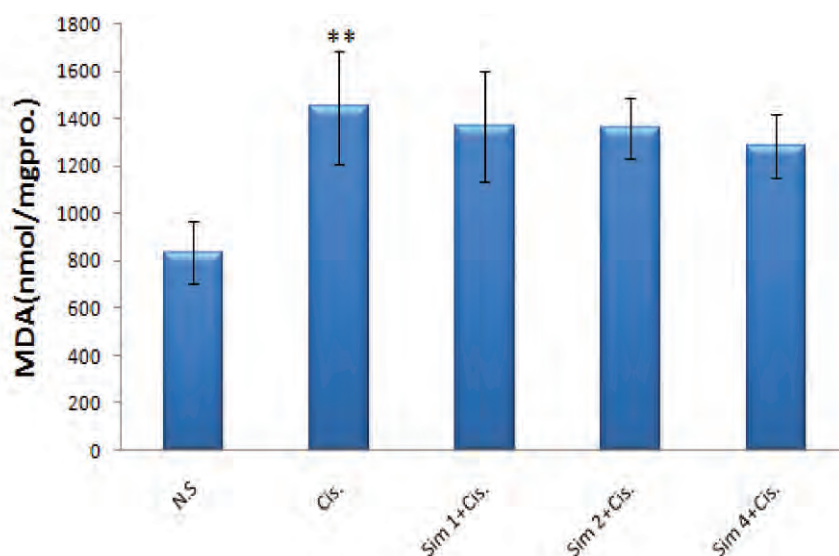
Cisplatin was purchased from Ebewe Pharma (Austria). Simvastatin powder was purchased from Sobhan Company (Iran). It was dissolved DMSO. All other chemicals used in this study were obtained from Merck (Germany).

### 2.2. Animals

The protocol of this study was approved by animal Ethical Committee of Shiraz University of Medical Sciences. Experiments were carried out in adult male Sprague-Dawley rats, with weights ranging from 150-250 g. They were obtained from the nest animals of the Laboratory Animals Research Center of Shiraz University of Medical Sciences, Shiraz, Iran. Rats were maintained under standard conditions of temperature  $23\pm 2$  °C with regular 12 h light: 12 h dark cycle, and were allowed free access to conventional laboratory food and water for 7 days.

### 2.3. Experimental design

Firstly, in our laboratory, pilot study tests were carried out to find suitable toxic dose of cisplatin for renal toxicity. Toxic dose of cisplatin for kidney damage was obtained to be 5 mg/kg according to the serum parameters (potassium, sodium, urea and creatinine). Then, the rats were randomly divided into five groups with five animals in each as follows: Group I (normal control) received 0.9% NaCl; group II (Experimental control) received DMSO; Group III was treated with a single dose of cisplatin (5 mg/kg) intraperi-



**Figure 1.** Effect of simvastatin treatment on cisplatin -induced changes in MDA contents in kidney tissue of male rats.;\*\*: Significantly different from NS group at  $p<0.01$ .

toneally. Rats in Groups IV, V and VI were treated with 1, 2 or 4 mg/kg oral doses of simvastatin, respectively, 1 h before cisplatin injection. Finally on the 5th day, animals were decapitated to collect blood for various biochemical evaluations and kidney for histological studies. Blood samples were allowed to clot and serum was separated by centrifugation at room temperature.

Kidney samples were immediately removed, rinsed in saline (0.9%). One of the kidneys was placed in 10% formalin for histopathological studies and was sent to the pathobiology laboratory of Namazi hospital, Shiraz, Iran. The other kidney was used for checking the amounts GSH, malondialdehyde (MDA) and protein. Two pieces of the latter kidney (weight 200 and 500 mg) were isolated. The piece of 200 mg was used for evaluation of GSH levels and the piece of 500 mg was used for evaluation of MDA and protein levels.

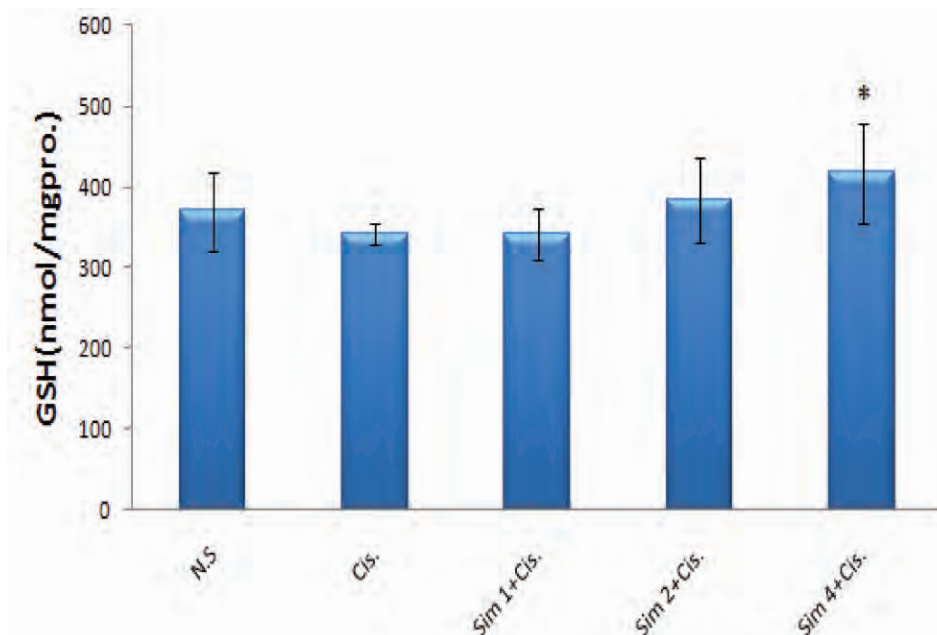
#### 2.4. Renal function tests

Serum urea and creatinine concentrations were measured by modified rate Jaffe's kinetic method and BUN method. Serum

concentrations of  $\text{Na}^+$  and  $\text{K}^+$  ions were determined by flame emission spectrophotometry [8-10].

#### 2.5. Measurement of tissue MDA and GSH levels

The most widely used index of lipid peroxidation is MDA formation, often assayed with the thiobarbituric acid (TBA) assay [11]. In our study, approximately 500 mg of the kidney tissue was cut and washed with saline. Then, 4.5 ml of sucrose solution (0.25 M) was added to it and homogenized with a homogenizer. After homogenization, it was centrifuged at 1000 rpm for 10 min and at 2000 rpm for 30 min., respectively. Then, 0.2 ml of the upper suspension, 0.2 ml of SDS (8.1%), 1.5 ml of TBA (0.8%), 1.5 ml of acetic acid (20%, pH 3.5) and 0.3 ml of distilled water were mixed and put in a boiling water for about 60 min. After cooling, 0.5 ml of TCA (50%) was added and centrifuged at 4000 rpm for about 10 min. Finally, the absorbance of MDA was measured by UV spectrophotometer at 535 nm [12]. GSH was determined by spectrophotometric method which is a modification of Ellman procedure [13].



**Figure 2.** Effect of simvastatin treatment on cisplatin -induced changes in GSH contents of kidney tissue of male rats. \*: Significantly different from cisplatin group at  $p < 0.05$

### 2.6. Histopathological examinations

Kidneys from all groups were fixed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin. Fine sections were obtained, mounted on glass slides and counter-stained with hematoxylin and eosin for light microscopic analyses. The slides were coded and were examined by a histopathologist who was blinded to the treatment groups. The renal injury was based on focal chronic pyelonephritis, sever congestion, focal coagulation necrosis, and intratubular RBC.

### 2.7. Statistical analysis

All data are presented as mean±SD. Statistical analysis was done by independent t-test or One-way Analysis of Variance (ANOVA) followed by Dunnet post test. All calculations were done with computer and SPSS (version 16) software. The statistical significance of difference was taken as  $p < 0.05$ .

## 3. Results

### 3.1. Effect of simvastatin on cisplatin-induced lipid peroxidation

As shown in Figure 1, cisplatin injection in a dose of 5 mg/kg caused a significant

increase in MDA content of kidney tissue, when compared with NS group. But simvastatin at 1, 2 and 4 mg/kg did not produce a significant change in MDA content of kidney tissue when compared with cisplatin group.

### 3.2. GSH

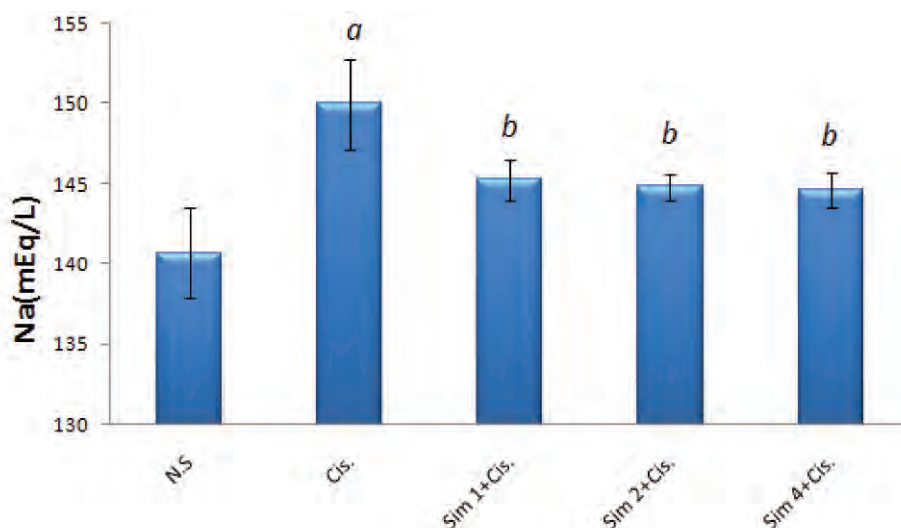
As shown in figure 2, cisplatin injection in dose of 5 mg/kg did not decrease GSH content of kidney tissue significantly when compared with NS group. Simvastatin at 4mg/kg caused a significant increase in GSH content of kidney tissue when compared with cisplatin group.

### 3.3. Serum Na

As shown in figure 3, cisplatin injection in a dose of 5 mg/kg caused a significant increase in serum Na when compared with NS group but simvastatin at 1, 2 and 4mg/kg produced a significant decrease in serum Na when compared with cisplatin group.

### 3.4. Serum K

As shown in figure 4, cisplatin injection in dose of 5 mg/kg did not change serum K value significantly when compared with NS group. Simvastatin at 1mg /kg increased



**Figure 3.** Effect of simvastatin treatment on cisplatin -induced changes in serum Na of male rats.; a: Significantly different from NS group at  $p < 0.05$ . ; b: Significantly different from cisplatin group at  $p < 0.05$ .

serum K value significantly when compared with cisplatin group, but at 2 and 4 mg/kg did not produced a significant change in serum K value.

### 3.5. Serum Urea

Figure 5 shows that cisplatin injection in dose of 5 mg/kg increased serum urea significantly when compared with NS group. But simvastatin at 1, 2 and 4 mg/kg did not produce a significant change in serum urea when compared with cisplatin group.

### 3.6. Serum Creatinine

As shown in figure 6, cisplatin injection in dose of 5 mg/kg increased serum creatinine significantly when compared with NS group. But simvastatin at 1, 2 and 4 mg/kg did not decrease serum creatinine significantly when compared with cisplatin group.

### 3.7. Histopathological results

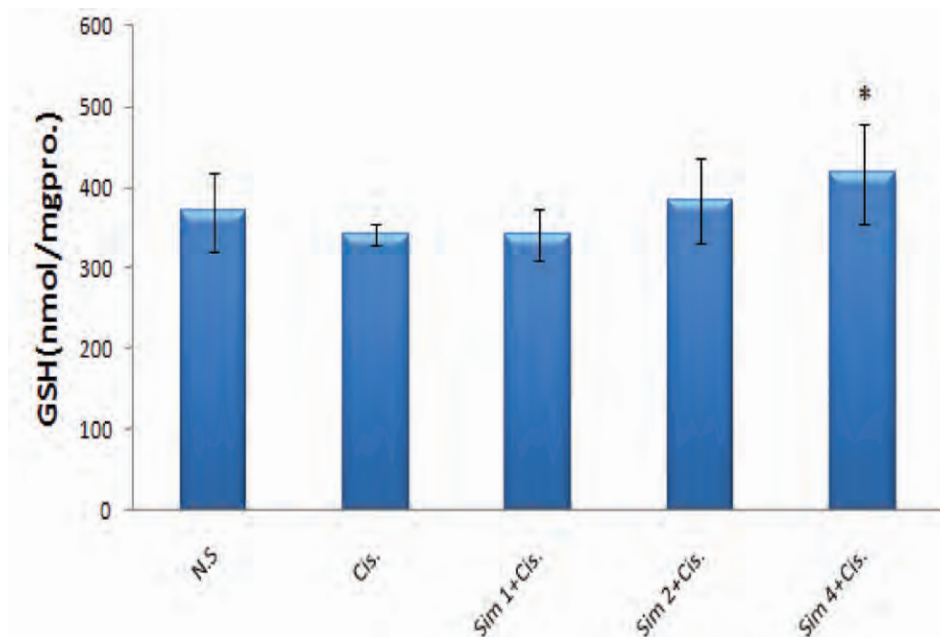
Sections from NS group showed normal histological structure of kidney tissue (Figure 7). In renal sections from the rats receiving cisplatin, simvastatin 1 mg/kg<sup>+</sup> cisplatin and simvastatin 2 mg/kg<sup>+</sup> cisplatin there were

focal chronic pyelonephritis, sever congestion, focal coagulation necrosis and intratubular RBC were observed (Figure 8). The severity of these symptoms was dose-dependent. The histopathological changes induced by cisplatin administration were completely prevented by pretreatment of simvastatin at dose 4 mg/kg (Figure 7).

## Discussion

Results from our study revealed significant increases in serum creatinine and BUN levels (markers of impaired glomerular function) with severe tubular necrosis following treatment with a single dose of 5 mg/kg of cisplatin. This was also associated with marked increase in renal MDA and a significant Na elevation with mild K decrease, suggesting an acute renal failure.

The electrolyte disturbances may be due to a specific membrane or transport system abnormality. potassium decrease may be secondary to hypomagnesaemia [14-15]. Elevation of serum creatinine and BUN levels indicates glomerular damage consequence of ROS and it already reported by other studies [16] [10, 17-20]. Cisplatin-induced



**Figure 4.** Effect of simvastatin treatment on cisplatin -induced changes in serum K of male rats.; \*\*: Significantly different from cisplatin group at  $p < 0.01$ .

nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney tissues. The drug causes generation of reactive oxygen species and inhibits the activity of antioxidant enzymes in renal tissue.[2]. The biological effect of ROS is controlled by a wide spectrum of enzymatic and non enzymatic defense mechanisms such as SOD, CAT and GSH [21]. The balance between oxidants and antioxidants is crucial for the maintenance of the biological integrity of the tissues [7].

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular and organelle components, reduced the most mitochondrial survival and lipid fluidity [22]. Lipid peroxidation that assessed by measuring MDA in cisplatin group significantly decreased and GSH was decreased non-significantly compared with the control rats. These results are similar to findings from other studies [2, 10, 17, 20, 23]. Increased lipid peroxidation and a concomitant decrease in tissue glutathione content which are consequence of ROS, suggest that the cisplatin at 5 mg/kg dose led

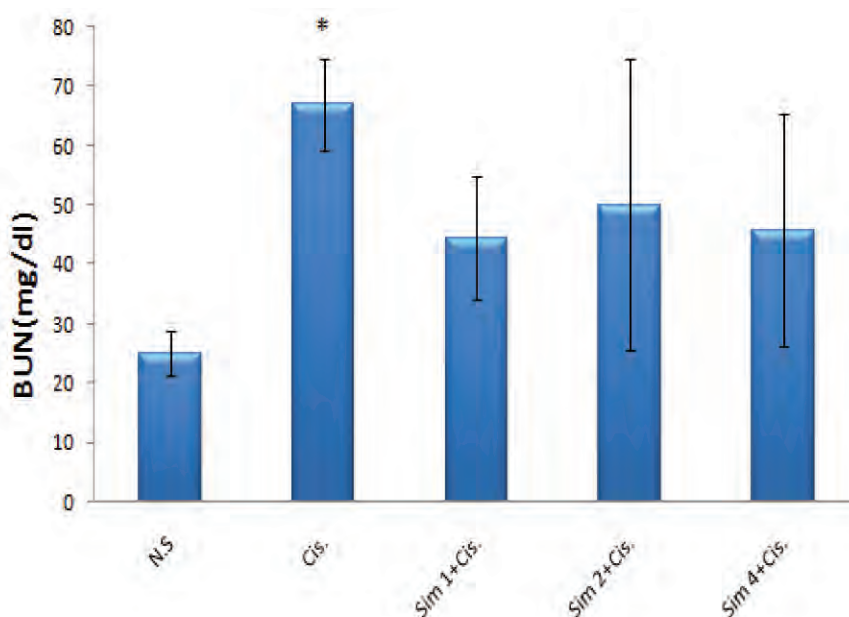
to nephrotoxicity in this study.

Some data from cell culture studies support the notion that GSH depletion is a mechanism of cisplatin toxicity [24], but others do not [25]. In addition, while some reports show GSH to be depleted in cisplatin nephrotoxicity, others show GSH levels to be elevated or normal under this condition. According to Meyer and Madias [26] these observations are not necessarily contradictory; they may represent different phases of the toxic response to cisplatin.

Both our study and others [27] indicated that the nephrotoxicity of cisplatin is associated with depletion in renal glutathione content. This may be due to the interaction of cisplatin with the enzymes which contain sulphhydryl groups.

It was reported that the expression and/or activity of several antioxidant defense proteins, like catalase, superoxide dismutase and hemeoxygenase-1 (HO-1) are upregulated by statins either in animals or in cultured cells [28].

The results of the present study demonstrate that a single dose (4mg/kg) of simvastatin treatment of rats markedly



**Figure 5.** Effect of simvastatin treatment on cisplatin -induced changes in serum urea of male rats; \*: Significantly different from NS group at  $p < 0.05$ .

improves cisplatin-induced kidney dysfunction damage as confirmed by microscopic examination and biochemical assays. Pretreatment with simvastatin at the noted doses, 1h before cisplatin administration showed different effects on renal function and oxidant-antioxidant status of kidney. Simvastatin at 4 mg/kg caused an insignificant decrease in MDA and a significant increase in GSH content of kidney when compared with cisplatin group. Serum Na also significantly decreased in comparison with cisplatin group. Histopathological results showed a normal structure in renal tissue of the rats that received 4mg/kg of simvastatin.

Serum Na significantly decreased at 2mg/kg of simvastatin when compared with cisplatin group. Simvastatin at 1mg/kg caused a significant decrease in serum Na and a significant increase in serum K when compared with cisplatin group. The morphological changes in the kidneys that induced by cisplatin administration was not changed by 1 and 2 mg/kg doses.

Serum creatinine and BUN elevation that induced by cisplatin were prevented non-significantly at the three doses of simvastatin

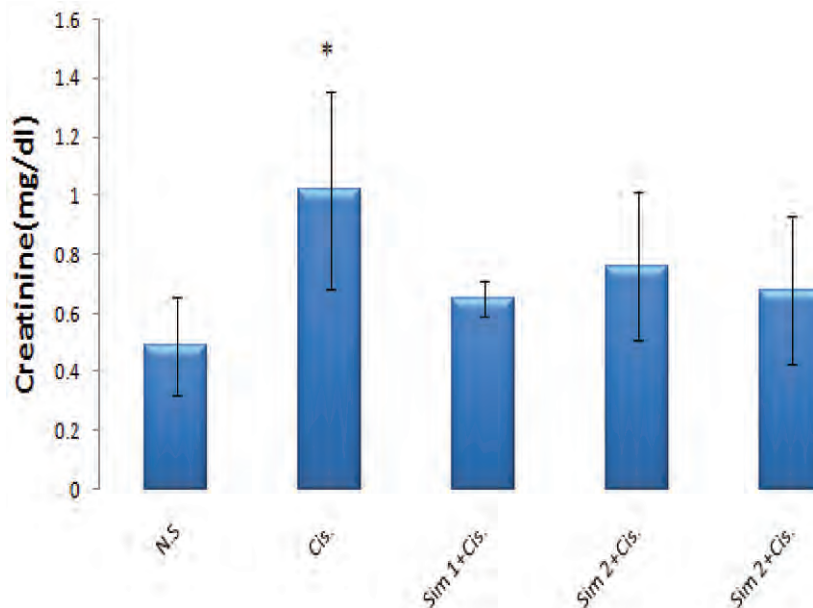
while seri, S., F. Ercan, and N. Gedik shown that simvastatin (1mg/kg) in co-administration with cisplatin reversed the increases in serum creatinine and BUN induced by cisplatin treatment significantly [2].

The decrease of serum creatinine and urea levels, Na decrease and k elevation may be due to improve the glomerular filtration rate [30-31] or may be secondary to decreased ROS [31].

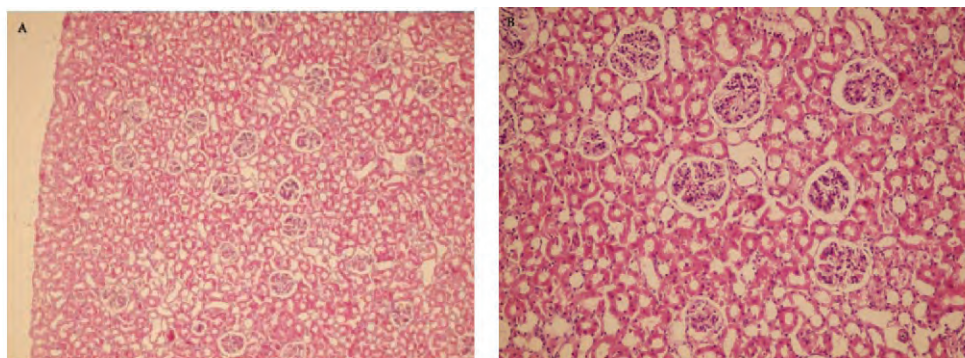
It was observed that the treatment with simvastatin at the three doses, dose dependently, prevented the elevation of renal MDA caused by cisplatin nonsignificantly while seri, S., F. Ercan, and N. Gedik reported that renal MDA significantly decreased. Insignificant decrease of renal MDA at used doses of simvastatin may be due to low dose of simvastatin [17].

Under the experimental condition of our study, simvastatin at 4mg/kg showed good protection on cisplatin-induced nephrotoxicity on adult male Sprague Dawley rat kidneys.

The protective effects of simvastatin may be to prevention the cisplatin-induced renal antioxidant status, inflammation and endothelial function Impairment.



**Figure 6.** Effect of simvastatin treatment on cisplatin -induced changes in serum creatinine of male rats.; \*: Significantly different from control group at  $p < 0.05$ .



**Figure 7.** The normal histological features of NS or simvastatin 4mg/kg + cisplatin isolated kidneys. (A) Magnification: 100 x. (B) Magnification: 250 x.

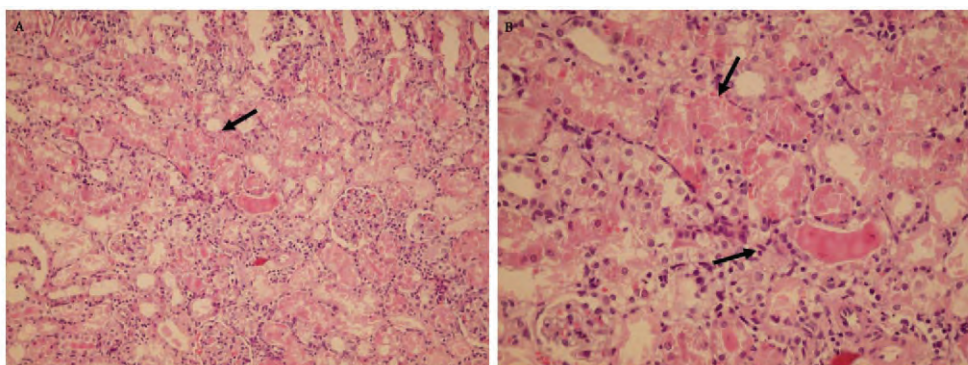
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Conflict of interest: None declared.

### References

- [1] Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Funda Clin Pharm.* 2005;19(1):117-25.
- [2] Iseri S, Ercan F, Gedik N. Simvastatin attenuates cisplatin-induced kidney and liver damage in rats. *Toxicol.* 2007;230(2-3):256-64.
- [3] Epstein M, Campese V. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors on renal function. *Am J kidney dis.* 2005;45(1):2-14.
- [4] Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. *Toxins.* 2010;2(11):2490-518.
- [5] Casarett L, Klaassen C, Doull J, editors. Casarett and Doull's toxicology: the basic science of poisons. New York: McGraw-Hill; 2008.
- [6] Ali B, Al Moundhri M. 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.
- [7] Yi A, Xin H, Yan W, Zhou X. Amelioration of cisplatin-induced nephrotoxicity by pravastatin in mice. *Exp Toxicol Pathol.* 2011;63:215-9.
- [8] Owen JA, Iggo B, Scandert F, Stewart C. The determination of creatinine in plasma or serum, and in urine; a critical examination. *Biochem J.* 1954 58(3):426.
- [9] Hosten A, editor. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd ed. Boston Butterworths; 1990.
- [10] Ekor M, Emerole G, Farombi E. Phenolic extract of soybean (*Glycine max*) attenuates cisplatin-induced nephrotoxicity in rats. *Food Chem Toxicol.* 2010;48(4):1005-12.
- [11] Zwart d, Loeckie L, Meerman JH, Commandeur JN, Vermeulen NP. Biomarkers of free radical damage : Applications in experimental animals and in humans. *Free Radical Biol Medicine.* 1999;26 (1-2):202-26.
- [12] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid



**Figure 8.** Representative examples of acute renal necrosis in kidney tissue. (A) Magnification: 100 x. (B) Magnification: 250 x.

- reaction. *Anal biochem.* 1979;95(2):351-8.
- [13] Sedlak J, Lindsay R. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal biochem.* 1968;25(1):192.
- [14] Sweetman S, Martindale W. The complete drug reference 35th ed. Martindale W, editor. London: *Pharmaceutical Press*; 2005.
- [15] Launay-Vacher V, Rey J-B, Isnard-Bagnis C, Deray G, Daouphars M. Prevention of cisplatin nephrotoxicity: state of the art and recommendations from the European Society of Clinical Pharmacy Special Interest Group on Cancer Care. *Cancer Chemother Pharmacol.* 2008;61:903-9.
- [16] Hughes A, Stricklett P, Padilla E, Kohan D. Effect of reactive oxygen species on endothelin-1 production by human mesangial cells. *Kidney Int.* 1996;49(1):181-9.
- [17] Lovari J, Mesic M, Macan M, Koprivanac M, Kelava M, Vlasta B. Measurement of malondialdehyde (MDA) level in rat plasma after simvastatin treatment using two different analytical methods. *Perid Biol* 2008;110(1):63-7.
- [18] Francescato HsDC, Costa RS, Camargo SMR, Zanetti MA, Lavrador MA, Bianchi MdLP. Effect of oral selenium administration on cisplatin-induced nephrotoxicity in rats. *Pharmacol Res.* 2001;43(1):77-82.
- [19] Saad A, Youssef M, El-Shennawy L. Cisplatin induced damage in kidney genomic DNA and nephrotoxicity in male rats: The protective effect of grape seed proanthocyanidin extract. *Food Chem Toxicol.* 2009;47(7):1499-506.
- [20] Sueishi K, Mishima K, Makino K, Itoh Y, Tsuruya K, Hirakata H, et al. Protection by a radical scavenger edaravone against cisplatin-induced nephrotoxicity in rats. *Eu J Pharmacol.* 2002;451(2):203-8.
- [21] Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant sci.* 2002;7(9):405-10.
- [22] Gutteridge J. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem.* 1995;41(12):1819.
- [23] Bagchi D, Garg A, Krohn R, Bagchi M, Bagchi D, Balmoori J, et al. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *Gen Pharmacol.* 1998;30(5):771-6.
- [24] Suzuki C, Cherian M. The interactions of cis-diamminedichloroplatinum with metallothionein and glutathione in rat liver and kidney. *Toxicol.* 1990;64(2):113-27.
- [25] Montine T, Borch R. Role of endogenous sulfur-containing nucleophiles in an in vitro model of cis-diamminedichloroplatinum (II)-induced nephrotoxicity. *Biochem Pharmacol.* 1990;39(11):1751-7.
- [26] Meyer K, Madias N. Cisplatin nephrotoxicity. *Miner electrol metabol.* 1994;20(4):201.
- [27] Greggi Antunes L, Joana D'arc C, Bianchi MdLP. Protective effects of vitamin C against Cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: A dose- dependent study. *Pharmacol Res.* 2000;41(4):405-11.
- [28] Schupp N, Schmid U, Heidland A, Stopper H. Rosuvastatin protects against oxidative stress and DNA damage in vitro via upregulation of glutathione synthesis. *Atherosclerosis.* 2008;199(2):278-87.
- [29] Athyros V, Mikhailidis D, Papageorgiou A, Symeonidis A, Pehlivanidis A, Bouloukos V, et al. The effect of statins versus untreated dyslipidaemia on renal function in patients with coronary heart disease. A subgroup analysis of the Greek atorvastatin and coronary heart disease evaluation (GREACE) study. *J Clin Path.* 2004;57(7):728.
- [30] Shepherd J, Kastelein JJP, Bittner V, Deedwania P, Breazna A, Dobson S, et al. Effect of intensive lipid lowering with atorvastatin on renal function in patients with coronary heart disease: the Treating to New Targets (TNT) study. *Clin J Am Soc Neph.* 2007;2(6):1131.
- [31] Zhou M, Schuman I, Jaimes E, Raij L. Renoprotection by statins is linked to a decrease in renal oxidative stress, TGF- $\beta$ , and fibronectin with concomitant increase in nitric oxide bioavailability. *Am J Physiol Renal Physiol.* 2008;295(1):F53.

