Canadian Journal of Physiology and Pharmacology

Morphometric analysis of structural renal alterations and beneficial effects of aminoguanidine in acute kidney injury induced by cisplatin in rats

Journal:	<i>Canadian Journal of Physiology and Pharmacology</i>
Manuscript ID	cjpp-2019-0252.R1
Manuscript Type:	Article
Date Submitted by the Author:	10-Jun-2019
Complete List of Authors:	Ilic, Sonja; Faculty of Medicine, University of Nis, Physiology Stojiljkovic, Nenad; Faculty of Medicine, University of Nis, Physiology Sokolovic, Dusan; Department of Biochemistry, University of Nis, Faculty of Medicine, Nis, Serbia Jovanovic, Ivan; Department of Anatomy, University of Nis, Faculty of Medicine, Nis, Serbia, Stojanovic, Nikola; Faculty of Medicine, University of Nis,
Is the invited manuscript for consideration in a Special Issue:	Current Trends in Physiological Sciences Serbia Meeting
Keyword:	Morphometry, cisplatin, antioxidant, nephrotoxicity, oxidative stress

SCHOLARONE™
Manuscripts

Morphometric analysis of structural renal alterations and beneficial effects of aminoguanidine in acute kidney injury induced by cisplatin in rats

Sonja Ilic^{1*}, Nenad Stojiljkovic¹, Dusan Sokolovic², Ivan Jovanovic³, Nikola Stojanovic⁴

¹Department of Physiology, University of Nis, Faculty of Medicine, Bulevar Dr. Zoran Djindjic 81, Nis, Serbia,

²Department of Biochemistry, University of Nis, Faculty of Medicine, Bulevar Dr. Zoran Djindjic 81, Nis, Serbia

³Department of Anatomy, University of Nis, Faculty of Medicine, Bulevar Dr. Zoran Djindjic 81, Nis, Serbia,

⁴University of Nis, Faculty of Medicine, Bulevar Dr. Zoran Djindjic 81, Nis, Serbia,

*Address correspondence to Sonja Ilic,

Department of Physiology, University of Nis, Faculty of Medicine,

Bulevar Dr. Zoran Djindjic 81, Nis, Serbia Phone number: +381184226644 Fax number:

+381-18-42-38-770 E-mail: sonjaili@yahoo.com

Abstract

Since cisplatin-induced nephrotoxicity has very important clinical consequences, purpose of this study was to determine the potential protective effect of aminoguanidine on the acute kidney injury caused by cisplatin. Experiments were done on 40 Wistar rats divided into four groups. CIS group received cisplatin in a single dose of 8 mg/kg, while CISAG group received the same dose of cisplatin and aminoguanidine (100 mg/kg) by intraperitoneal injections. Animals in AG group received only aminoguanidine (100 mg/kg) and those in C group received saline. Quantitative evaluation of structural and functional alterations in the kidneys were performed by analysis of biochemical and parameters of oxidative stress and by histological and morphometric analysis of renal sections. Histological sections of kidney ameliorated structural damages of proximal tubules and glomeruli which were induced by cisplatin. Morphometric analysis revealed statistically significant differences in the area of proximal tubules, size and cellularity of glomeruli between CIS and CISAG group. Glomerular basement membrane thickness was increased in CIS group, while aminoguanidine attenuated these changes in CISAG group of rats. Our results suggest that aminoguanidine acts protectively and repairs structural and functional damages of kidney by engaging the existent antioxidative potential at the level of renal tissue.

Key words: Morphometry, cisplatin, antioxidant, nephrotoxicity, oxidative stress.

INTRODUCTION

The kidney is a common target organ for toxic drugs, such as cyclosporine, gentamicin, cisplatin (Serenio et al. 2015; Veljkovic et al. 2016; Rana et al. 2016). Cisplatin is one of the most potent chemotherapeutic agents widely used for several human cancers (Youssef Nasr and Al Shahat Ibrahim 2015). The major limiting factors of cisplatin administration are the side effects such as ototoxicity, myelosuppression, neurotoxicity, but the primary dose limiting side effect of cisplatin is nephrotoxicity (Sastry and Kellie 2005; Mitazaki et al. 2013).

Cisplatin-induced nephrotoxicity is a consequence of the accumulation of this drug in the epithelial cells from the S3 segment of the proximal tubules (Daugaard 1990). Less frequent are dose-related damages in the glomeruli, distal and collecting tubules (Peres and da Cunha 2013). Typically, renal impairment is characterized by reduced blood flow through the kidneys which causes decreased glomerular filtration rate (GFR) a few days after the first dose of cisplatin. Cisplatin nephrotoxicity is manifested as higher serum creatinine and blood urea concentrations and reduced potassium and magnesium levels (Pabla and Dong 2008). Urine may contain small amounts of glucose and proteins indicating a dysfunction of the proximal tubules (Pabla and Dong 2008).

Mechanisms of cisplatin nephrotoxicity are not fully understood, but several studies suggested that production of reactive oxygen species (ROS), depletion of the antioxidant system and stimulation of renal accumulation of lipid peroxidation products are associated with cisplatin-induced nephrotoxicity (Sanchez-Gonzalez et al. 2011; Silici et al. 2011). Reactive nitrogen species (RNS) may also contribute to cisplatin kidney injury (Chirino et al. 2008; Chirino and Pedraza-Chaverri 2009). Therefore, a number of studies have suggested that several chemical

and natural substances with antioxidant activity may be used for renoprotective effects against cisplatin-induced injuries (Naziroglu et al. 2004; Badary et al. 2005; Trujillo et al. 2016).

Aminoguanidine (AG) is an analogue of L-arginine, known as a specific inhibitor of inducible nitric oxide synthase (iNOS), a potent antioxidant and a free radical scavenger (Parthasarathy et al. 2014). AG has many other activities such as inhibition of diamine oxides and preventing advanced glycosylation (Jedidi et al. 2003). Previous studies have reported that AG ameliorates nephrotoxicity of gentamicin, amikacin and acetaminophen (Parlakpınar et al. 2004; Polat et al. 2006; Abdel-Zaher et al. 2007).

According to our knowledge, there are only a few studies about the effects of AG on the acute renal injury caused by cisplatin (Mansour et al. 2002; Atasayar et al. 2009), but there are no data about effects of AG on levels of oxidative modified proteins in cisplatin nephrotoxicity and effects of AG on glomerular basement membrane (GBM) changes in this model of nephrotoxicity, which were the main subjects of our study. In accordance to this, the purpose of our experimental research was to determine the possible protective effect of AG on the acute kidney injury caused by cisplatin in rats, as well as to quantify the changes of the kidney structures by histological and morphometric analysis of the Jones methenamine silver stained renal sections, which were not described in similar studies.

MATERIALS AND METHODS

Forty adult male Wistar rats, weighing 250–300g were used in the present study. The animals were housed inside a well-ventilated room with room temperature at $20 \pm 2^{\circ}\text{C}$ and regular light cycle (12 light/12 dark). All experimental procedures were conducted in accordance with the

principles for the Guide for the Care and Use of Laboratory Animals (Eight Edition, 2011) and had been approved by the Animal Ethics Board of the Medical Faculty in Nis. It was documented under number 01-2625-9.

Experimental protocol

The animals were randomly divided into four groups, each containing 10 animals. The control group of rats (C group) received saline 1 ml/day intraperitoneally for 9 days. The cisplatin group (CIS group) received cisplatin (Pfizer PTY. Limited, Bentley, Australia) intraperitoneally in a single dose of 8 mg/kg on the fifth day of experiment. The aminoguanidine group (AG group) was used as a positive control group and received aminoguanidine (Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in physiological saline solution, intraperitoneally, in a dose of 100 mg/kg/24h for 9 days. The cisplatin- aminoguanidine group (CISAG group) of rats received aminoguanidine intraperitoneally in a dose of 100 mg/kg/24h for 9 days and cisplatin intraperitoneally on the fifth day of treatment in a dose of 8 mg/kg.

Ten days after the beginning of the experiment all animals were anaesthetized using 80 mg/kg ketamine (Ketamidol 10%, Richter Pharma AG, Wels, Austria) and sacrificed. Immediately, blood samples were taken from the aorta and the kidneys were subsequently removed.

Biochemical analysis

Biochemical analysis of blood included determination of concentrations of urea, creatinine, sodium and potassium using an automatic biochemical analyzer (A25 Biosystems, Barcelona, Spain) in the laboratory of the Department of Nephrology and Dialysis Clinical Center Niš.

Histological analysis

Preparation of kidney tissue for histological analysis was described in detail in our previous work (Ilic et al. 2014). Kidney tissue specimens were stained with hematoxylin-eosin (HE) and Jones methenamine silver according to conventional staining protocols. The histological sections were examined with a light microscope Leica DMR (Leica Microsystems AG, Wetzlar, Germany).

Homogenate Preparation

The kidney tissue was cut in small pieces and homogenized in ice cold water, using homogenizer (IKA® Works do Brasil Ltda, Rio de Janeiro, Brasil). The homogenates (10% w/v) were centrifuged at 1500×g for 10 min at 4°C. The resulting supernatant was separated and used for the biochemical analysis.

Determination of protein oxidation

To determine the concentration of advanced oxidation protein products (AOPP) the concentration of proteins was measured according to Lowry's method (1951) using bovine serum as standard. Levels of AOPP were measured by spectrophotometric method (Witko- Sarsat et al. 1996). This method is based on the reaction of AOPP with potassium iodide in an acidic medium. The color intensity was recorded immediately at 340 nm. The values were expressed in $\mu\text{mol}/\text{mg}$ of protein.

Determination of catalase activity

The activity of renal antioxidant enzyme catalase (CAT) was determined by spectrophotometric method described by Goth (1991), which is based on the ability of catalase to dissolve the substrate (H_2O_2), whereby enzymatic reaction is stopped by the addition of ammonium

molybdate. Yellow complex of molybdate and H_2O_2 was measured at 405 nm. Enzyme activity was expressed in catalytic units per gram of protein (kU/g).

Estimation of lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde (MDA). The intensity of LPO in kidney tissue was spectrophotometrically measured based on the thiobarbituric (TBA) response products (Ohkawa et al. 1979). Homogenate absorption was measured at 532 nm. The malondialdehyde/lipid peroxidation end-product concentration was expressed in mg/protein using the molecular extinction coefficient of MDA ($1.56 \times 10^{-5} \text{ mol cm}^{-1}$).

Inducible nitric oxide synthase (iNOS) activity determination

Kidney tissue homogenate levels of inducible nitric oxide synthase (iNOS) were determined using CUSABIO ELISA kit (CSB-E08325r) following protocol given by the manufacturer. Enzyme activity was calculated from the standard curve and the results are presented as IU/mg of tissue proteins.

Morphometric analysis

After histological processing of tissue, images of each section were taken using a light microscope Leica DMR (Leica Microsystems AG, Wetzlar, Germany). Morphometric analysis was done with computerized image analysis system ImageJ. Kidney sections were stained with hematoxylin and eosin and Jones methenamine silver and their images were captured by a digital camera under the objective lens magnification $10\times$ and $40\times$. Spatial calibration, by object micrometer was performed before each analysis. The following morphometric parameters were measured: area of proximal and distal tubules (μm^2), the nuclear-cytoplasmic (N/C) ratio of the

epithelial cells of proximal and distal tubules, GBM thickness (μm), glomerular area (μm^2), glomerular cellularity (cells/ μm^2), perimeter (μm), Feret's diameter (μm) and circularity. In each animal at least 50 glomeruli, proximal and distal tubules were measured.

Statistical Analysis

Results were expressed as the mean $\pm$ standard deviation (SD). Statistical significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison (Graphpad Prism version 5.03, San Diego, CA, USA). Differences were considered to be significant when $p < 0.05$.

RESULTS

Biochemical analysis

Analysis of biochemical parameters showed a significant increase of blood urea and serum creatinine concentrations in the CIS group when compared to C group ($p < 0.001$). Serum creatinine and blood urea concentrations in the CISAG group were significantly decreased when compared to the CIS group ($p < 0.001$) (Table 1). The concentrations of sodium ($p < 0.001$) and potassium ($p < 0.05$) in the serum were significantly decreased in the CIS group than concentrations of sodium and potassium in the C group. The concentrations of sodium ($p < 0.001$) and potassium ($p < 0.05$) in the CISAG group were significantly decreased when compared to CIS group of rats (Table 1).

Histological analysis

Histopathological findings of kidney tissues from C group (Figures 1A and 2A) showed a normal histological appearance of glomerular and tubular structures. Histological analysis in CIS group

of rats showed a large number of proximal tubules with focal apoptosis and tubular cell swelling, degeneration of tubular cells with cytoplasm vacuolization, luminal dilatation of proximal tubules, peritubular inflammatory cell infiltration and extensive hyperemia (Figure 1C). Distal tubules were almost unaltered (Figure 1C). The glomeruli in CIS group displayed generalized congestion with reduced lobular appearance with periglomerular infiltration (Figure 1C). Animals in CIS group, in comparison to those in other groups, had enlarged glomeruli with reduction in Bowman's space and irregularly thickened glomerular basement membrane (Figure 2). These cisplatin-induced pathological lesions were remarkably reduced by the application of AG where signs of glomerular congestion, leucocyte infiltration or tubular dilatation were not observed (Figure 1D). Glomerular basement membrane in CISAG group was thickened only in some segments of the glomeruli (Figure 2D). Furthermore, there were not any kidney tissue alterations in AG group rats when compared to the control (Figures 1B and 2B).

Determination of Protein Oxidation

Cisplatin administration to rats significantly increased the AOPP levels in kidney tissue compared to C group ($p < 0.001$). Administration of AG with cisplatin significantly decreased concentration of renal AOPP in CISAG group compared to the CIS group ($p < 0.001$) (Table 2).

Catalase activity

The catalase activity in the kidney tissue was statistically significant reduced in the CIS group in comparison to C group ($p < 0.001$). In the CISAG group the catalase activity was increased when compared to the CIS group ($p < 0.001$) (Table 2).

Estimation of lipid peroxidation

Cisplatin administration to rats significantly increased the MDA levels in kidney tissue compared to C group ($p<0.001$). Administration of aminoguanidine in the CISAG group reduced lipid peroxidation, as evidenced by significantly decreased level of MDA than those in the CIS group ($p<0.001$) (Table 2).

Inducible nitric oxide synthase (iNOS) activity determination

The iNOS activity in the kidney tissue was statistically significant increased in the CIS group in comparison to C group ($p<0.001$). In the CISAG group the iNOS activity was reduced when compared to the CIS group of animals ($p<0.01$) (Table 2).

Morphometric analysis

The glomerular morphometric parameters of animals from the experimental and control groups are presented in Table 3. Statistically significant differences were found between the experimental group of animals treated with cisplatin and control groups in glomerular size (glomerular area, perimeter and Feret's diameter) ($p<0.001$). In CISAG group, glomerular area, perimeter and Feret's diameter were statistically significant decreased compared to the CIS group of rats ($p<0.001$). The cellularity was significantly lower in CIS treated group compared to control and CISAG group of animals ($p<0.001$) (Table 2). Analysis of glomerular circularity did not show statistically significant differences between experimental and control groups of rats (Table 3).

The mean glomerular membrane thickness was significantly higher in CIS group compared to C group ($p < 0.001$). In the CISAG group membrane thickness was decreased when compared to the CIS group ($p < 0.001$) (Figure 3).

Measured parameters of proximal and distal tubules in each group are shown in Table 4. Area of proximal tubules was statistically significant increased in the group of animals treated with cisplatin in comparison to control groups ($p < 0.001$). In the CISAG group area of proximal tubules was significantly decreased compared to the CIS group. The nuclear- cytoplasmic ratio in the epithelial cells of proximal tubules was significant decreased in the CIS group of animals when compared to the C group ($p < 0.001$). In the CISAG group nuclear-cytoplasmic ratio in the epithelial cells of proximal tubules was significantly increased compared to the CIS group of animals. Additionally, morphometric parameters measured in distal tubules did not significantly differ between the groups (Table 4).

DISCUSSION

The main finding of this study was that aminoguanidine exerted protective potential against functional and structural damaging effects of cisplatin. Despite many reports, the exact mechanisms of cisplatin-induced nephrotoxicity still remain unclear because the pathophysiological mechanism is complex and involves a number of interconnected factors such as accumulation of cisplatin in the proximal tubules, conversion into nephrotoxins, DNA damage, mitochondrial dysfunction, oxidative and nitrosative stress, inflammatory response and activation of apoptotic pathways (Peres and da Chuncha 2013). Oxidative and nitrosative stress are probably among the major factors of cell damaging.

In our experimental study morphological changes were detected by histological and morphometric analysis of renal structures. In group of rats treated with cisplatin, apoptosis, degeneration and tubular cell swelling, and luminal dilatation of proximal tubules were found. The glomeruli in CIS group displayed generalized congestion with inflammatory cell infiltration. Biochemical analyses of blood in rats treated with cisplatin indicated acute renal injury and results correlate with histological findings. Levels of blood urea and serum creatinine in CIS group were statistically significantly increased, while the values of serum sodium and potassium were decreased in comparison to the control group. This observation is in accordance with findings from similar studies (Ashrafi et al. 2012; Palipoch and Punsawad 2013). Treatment with AG resulted in a decrease in blood urea and serum creatinine levels and attenuated morphological changes in kidneys most likely as a result of cytoprotective effect by inhibition of ROS and peroxynitrite. Analysis of kidney sections stained with Jones methenamine silver showed that animals in CIS group, in comparison to those in other groups, had enlarged glomeruli with reduction in Bowman's space and irregularly thickened glomerular basement membrane. These cisplatin-induced pathological lesions were remarkably reduced by the application of AG where signs of tubular and glomerular damages and leucocyte infiltration were considerably less marked compared to the group of animals treated by cisplatin only.

In available data, there are no reports about effects of AG on GBM changes in cisplatin induced nephrotoxicity. In our investigation, we have done morphometric analysis of kidney sections to quantify changes on GBM for the first time in this model of nephrotoxicity. We found that mean glomerular membrane thickness was significantly higher in rats treated with cisplatin. Our findings are in accordance with earlier suggestions that both vascular (glomerular) and tubular targets are involved in cisplatin-induced nephrotoxicity (Peres and da Chuncha 2013). Kohn et

al. (2002) showed that cisplatin may damage glomerular capillaries including their endothelial cells; basement membrane, podocytes, mesangial cells and parietal cells of Bowman capsule in Guinea pigs. Our study has contributed in determining and quantifying changes of GBM in cisplatin-induced nephrotoxicity in rats. Also, very important was our measurement of GBM in CISAG group of rats, where we found that administration of AG attenuated changes of GBM induced by cisplatin.

In the present study we have done morphometric analysis of glomeruli and tubules, which also wasn't used in earlier investigations of cisplatin-induced nephrotoxicity. The obtained results indicate strong correlation between histological and morphometric alterations in cisplatin-induced acute renal injury. Morphometric analysis of tubules showed that area and nuclear-cytoplasmic ratio were of rats treated with cisplatin. It is known that cisplatin primarily affects proximal tubules due its accumulation in this part of nephron (Yao et al. 2007). The glomerular morphometric analysis revealed that the glomeruli of the rats treated with cisplatin were significantly larger than in the control group. Similar findings we reported in our previous research in gentamicin-induced nephrotoxicity (Stojiljkovic et al. 2012). An undetermined part of the nephrotoxicity of cisplatin is due to vascular damage and vasoconstriction, which are derived mainly from the endothelial dysfunction and abnormal vascular autoregulation. Cisplatin-induced glomerular injury is characterized by a marked fall in GFR and renal blood flow, which is associated with decrease of ultrafiltration coefficient (K_f) and increased renal vascular resistance, indicating preglomerular vasoconstriction (Rodriguez-Barbero et al. 2000). Pathophysiological mechanisms that regulate K_f depend on the activity of intraglomerular mesangial cells contraction (Sanchez-Gonzalez et al. 2011). Thus, the contractile state of the glomeruli seems to be the major determinant of glomerular hemodynamic regulation.

We showed that glomerular cellularity was significantly lower in CIS group compared to control and CISAG group of animals. The explanation of this may be that cisplatin at the dose of 8 mg/kg predominantly induces apoptosis versus proliferation of mesangial cells, considering that cisplatin simultaneously induces proliferation and apoptosis of these cells (Rodriguez-Barbero et al. 2000; Ju et al. 2014). Opposed to this, in CISAG group morphometric analysis of tubules and glomeruli showed that area of proximal tubules, glomerular area, perimeter and Feret's diameter were statistically significant decreased compared to the group of rats treated only with cisplatin, which indicates a significant protective effect of AG on kidneys.

The nephroprotective mechanism of AG appears to be through modulation of the oxidant and antioxidant parameters in the renal tissue. We examined antioxidative effects of AG by determining levels of AOPP, MDA, catalase and iNOS activity in the kidneys. MDA is parameter of lipid peroxidation, CAT shows activity of antioxidant system and AOPP is parameter of oxidative modified proteins and it wasn't determined in previous examinations in cisplatin-induced nephrotoxicity. Inducible nitric oxide synthase (iNOS) is one of three key enzymes generating nitric oxide (NO) from the amino acid L-arginine. Our results confirmed that cisplatin increases production of ROS and inhibits antioxidant enzymes. ROS damage the lipid components of the cell membrane by peroxidation and denature proteins and contribute to the DNA damaging and apoptosis (Hanigan and Devarajan 2003). In addition to ROS, nitrosative stress plays a significant role in cisplatin-induced nephrotoxicity. Cellular effects of ROS are increased by the production of large amounts of nitric oxide (NO). NO production, probably as a result of synthesis of iNOS, leads to the continuous formation of peroxynitrite, which contributes to cisplatin-induced nephrotoxicity (Yildirim et al. 2003; Ozen et al. 2004). In our study, we found that increased levels of AOPP and MDA and decreased catalase activity in cisplatin

treated group were attenuated by AG administration. Amelioration of redox status by AG very probably contributes to tubular and glomerular protection. Our results showed that administration of cisplatin increases activity of iNOS. In contrary, application of AG in CISAG group significantly reduced activity of this enzyme. The protective effect of AG in cisplatin-induced renal damage may be achieved by two mechanisms. Firstly, AG inhibits the activity of iNOS and reduces the formation of peroxynitrite (Radi 2004; Parthasarathy et al. 2014). When the production of nitric oxide is decreased by the administration of AG, the source of peroxynitrite is reduced. Thus, less peroxynitrite is produced, the oxidative damage of lipids and proteins is decreased and the cellular antioxidant systems are spared (Reinartz et al. 2008). Secondly, there are many evidences that AG has direct antioxidant potential and prevents the formation of ROS and RNS acting as a powerful scavenger of ROS and peroxynitrite and preventing lipid peroxidation and depletion of cellular antioxidant activity (Di Paola et al. 2004; Abd El- Gawad and El-Sawalhi 2004; Ara et al. 2006).

Our results indicate that AG considerably reduces histological and functional damages of kidney associated with cisplatin treatment. Especially, we showed protective effects of AG on GBM changes caused by cisplatin. As cisplatin is believed to induce oxidative and nitrosative stress which initiates a whole chain of complex biochemical reactions resulting in cell damages, here we showed that, when concomitantly administered, AG acts protectively and repairs structural and functional damages to glomeruli and renal tubules by engaging the existent antioxidative potential at the level of renal tissue and inhibiting lipid peroxidation and protein oxidation.

References

- Abd El-Gawad, H.M., and El-Sawalhi, M.M. 2004. Nitric oxide and oxidative stress in brain and heart of normal rats treated with doxorubicin: role of aminoguanidine. *J. Biochem. Mol. Toxicol.* 18(2): 69-77. DOI: 10.1002/jbt.20013. PMID: 15122648.
- Abdel-Zaher, A.O., Abdel-Rahman, M.M., Hafez, M.M., and Omran, F.M. 2007. Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. *Toxicology.* 234 (1-2): 124-134. DOI: 10.1016/j.tox.2007.02.014. PMID:17391827.
- Ara, C., Karabulut, A.B., Kirimlioglu, H., Yilmaz, M., Kirimliglu, V., and Yilmaz, S. 2006. Protective effect of aminoguanidine against oxidative stress in an experimental peritoneal adhesion model in rats. *Cell. Biochem. Funct.* 24(5): 443-448.
- Ashrafi, F., Nematbakhsh, M., Safari, T., Talebi, A., Nasri, H., Khazaei, M., et al. 2012. A combination of vitamin C and losartan for cisplatin-induced nephrotoxicity in rats. *Iran. J. Kidney. Dis.* 6(5): 361-365. PMID: 22976262.
- Atasayar, S., Güreer-Orhan, H., Orhan, H., Gürel, B., Girgin, G., and Ozgüneş, H. 2009. Preventive effect of aminoguanidine compared to vitamin E and C on cisplatin-induced nephrotoxicity in rats. *Exp. Toxicol. Pathol.* 61(1): 23-32. DOI: 10.1016/j.etp.2008.04.016. PMID: 18684602.
- Badary, O.A., Abdel-Maksoud, S., Ahmed, W.A., and Owieda, G.H. 2005. Naringenin attenuates cisplatin nephrotoxicity in rats. *Life. Sci.* 76(18): 2125-2135. DOI: 10.1016/j.lfs.2004.11.005. PMID: 15826879.

Chirino, Y.I., Trujillo, J., Sánchez-González, D.J., Martínez-Martínez, C.M., Cruz, C., Bobadilla, N.A., et al. 2008. Selective iNOS inhibition reduces renal damage induced by cisplatin. *Toxicol. Lett.* 176(1): 48-57. DOI: 10.1016/j.toxlet.2007.10.006. PMID: 18063323.

Chirino, Y.I., and Pedraza-Chaverri, J. 2009. Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. *Exp. Toxicol. Pathol.* 61(3): 223-242. DOI: 10.1016/j.etp.2008.09.003. PMID: 18986801.

Daugaard G. 1990. Cisplatin nephrotoxicity: experimental and clinical studies. *Dan. Med. Bull.* 37(1): 1-12. PMID: 2178884.

Di Paola, R., Marzocco, S., Mazzon, E., Dattola, F., Rotondo, F., Britti, D., et al. 2004. Effect of aminoguanidine in ligature-induced periodontitis in rats. *J. Dent. Res.* 83(4): 343- 348. DOI: 10.1177/154405910408300414. PMID: 15044511.

Goth, L. 1991. A simple method for determination of serum catalase activity and revision reference range. *Clin. Chim. Acta.* 196(2-3): 143-152. PMID: 2029780.

Hanigan, M.H., and Devarajan, P. 2003. Cisplatin nephrotoxicity: molecular mechanisms. *Cancer. Ther.* 1:47-61. PMID: 18185852.

Jedidi, I., Therond, P., Zarev, S., Cosson, C., Couturier, M., Massot, C., et al. 2003. Paradoxal protective effect of aminoguanidine toward low-density lipoprotein oxidation: inhibition of apolipoprotein B fragmentation without preventing its carbonylation. Mechanism of action of aminoguanidine. *Biochemistry.* 42(38): 11356-11365. DOI: 10.1021/bi034539w. PMID: 14503886.

Ju, S.M., Pae, H.O., Kim, W.S., Kang, D.G., Lee, H.S., and Jeon, B.H. 2014. Role of reactive oxygen species in p53 activation during cisplatin-induced apoptosis of rat mesangial cells. *Eur. Rev. Med. Pharmacol. Sci.* 18(8): 1135-1141. PMID: 24817286.

Kohn, S., Fradis, M., Ben-David, J., Zidan, J., and Robinson, E. 2002. Nephrotoxicity of combined treatment with cisplatin and gentamicin in the guinea pig: glomerular injury findings. *Ultrastruct. Pathol.* 26(6): 371-382. DOI: 10.1080/01913120290104683. PMID: 12537762.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193(1): 265-275. PMID: 14907713.

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(2): 123-128. PMID: 12106889.

Mitazaki, S., Hashimoto, M., Matsushashi, Y., Honma, S., Suto, M., Kato, N., et al. 2013. Interleukin-6 modulates oxidative stress produced during the development of cisplatin nephrotoxicity. *Life. Sci.* 92(12): 694-700. DOI: 10.1016/j.lfs.2013.01.026. PMID: 23384965.

Naziroglu, M., Karaoglu, A., and Aksoy, A.O. 2004. Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. *Toxicology.* 195(2-3): 221-230. PMID: 14751677.

Ohkawa, H., Ohishi, N., and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95(2): 351-358. PMID: 36810.

Ozen, S., Akyol, O., Iraz, M., Söğüt, S., Ozuğurlu, F., Ozyurt, H., et al. 2004. Role of caffeic acid phenethyl ester, an active component of propolis, against cisplatin-induced nephrotoxicity in rats. *J. Appl. Toxicol.* 24(1): 27-35. DOI: 10.1002/jat.941. PMID: 14745844.

Pabla, N., and Dong, Z. 2008. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney. Int.* 73(9): 994-1007. DOI: 10.1038/sj.ki.5002786. PMID: 18272962.

Palipoch, S., and Punsawad, C. 2013. Biochemical and histological study of rat liver and kidney injury induced by Cisplatin. *J. Toxicol. Pathol.* 26(3): 293-299. DOI: 10.1293/tox.26.293. PMID: 24155562.

Parlakpınar, H., Koc, M., Polat, A., Vardi, N., Ozer, M.K., Turkoz, Y., et al. 2004. Protective effect of aminoguanidine against nephrotoxicity induced by amikacin in rats. *Urol. Res.* 32(4): 278-282. DOI: 10.1007/s00240-004-0399-5. PMID: 15278326.

Parthasarathy, A., Gopi, V., Devi, K.M.S., Balaji, N., and Vellaichamy, E. 2014. Aminoguanidine inhibits ventricular fibrosis and remodeling process in isoproterenol- induced hypertrophied rat hearts by suppressing ROS and MMPs. *Life. Sci.* 118(1): 15-26. DOI: 10.1016/j.lfs.2014.09.030. PMID: 25445437.

Peres, L.A., and da Cunha, A.D. Jr. 2013. Acute nephrotoxicity of cisplatin: molecular mechanisms. *J. Bras. Nefrol.* 35(4): 332-340. DOI: 10.5935/0101-2800.20130052. PMID: 24402113.

Polat, A., Parlakpınar, H., Tasdemir, S., Colak, C., Vardi, N., Ucar, M., et al. 2006. Protective role of aminoguanidine on gentamicin-induced acute renal failure in rats. *Acta. Histochem.* 108(5): 365-371. DOI: 10.1016/j.acthis.2006.06.005. PMID: 16999986.

Radi, R. 2004. Nitric oxide, oxidants, and protein tyrosine nitration. *Proc. Natl. Acad. Sci. USA*. 101(12): 4003-4008. DOI: 10.1073/pnas.0307446101. PMID: 15020765.

Rana, M.A., Khan, R.A., Nasiruddin, M., and Khan, A.A. 2016. Amelioration of cisplatin induced nephrotoxicity by ethanolic extract of *Bauhinia purpurea*: An in vivo study in rats. *Saudi. J. Kidney. Dis. Transpl.* 27(1): 41-48. DOI: 10.4103/1319-2442.174068. PMID: 26787565.

Reinartz, M., Ding, Z., Flögel, U., Gödecke, A., and Schrader, J. 2008. Nitrosative stress leads to protein glutathiolation, increased s-nitrosation, and up-regulation of peroxiredoxins in the heart. *J. Biol. Chem.* 283(25): 17440-17449. DOI: 10.1074/jbc.M800126200. PMID: 18426799.

Rodriguez-Barbero A, L'Azou B, Cambar J, and López-Novoa JM. 2000. Potential use of isolated glomeruli and cultured mesangial cells as in vitro models to assess nephrotoxicity. *Cell. Biol. Toxicol.* 16(3): 145-153. PMID: 11032358.

Sanchez-Gonzalez, P.D., Lopez-Hernandez, F.J., Lopez-Novoa, J.M., and Morales, A.I. 2011. An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. *Crit. Rev. Toxicol.* 41(10): 803-821. DOI: 10.3109/10408444.2011.602662. PMID: 21838551.

Sastry, J., and Kellie, S.J. 2005. Severe neurotoxicity, ototoxicity and nephrotoxicity following high-dose cisplatin and amifostine. *Pediatr. Hematol. Oncol.* 22(5): 441-445. DOI: 10.1080/08880010590964381. PMID: 16020136.

Sereno, J., Vala, H., Nunes, S., Rocha-Pereira, P., Carvalho, E., Alves, R., et al. 2015. Cyclosporine A-induced nephrotoxicity is ameliorated by dose reduction and conversion to sirolimus in the rat. *J. Physiol. Pharmacol.* 66(2): 285-299. PMID: 25903959.

Silici, S., Ekmekcioglu, O., Kanbur, M., and Deniz, K. 2011. The protective effect of royal jelly against cisplatin-induced renal oxidative stress in rats. *World. J. Urol.* 29(1):127-132. DOI: 10.1007/s00345-010-0543-5. PMID: 20369241.

Stojiljkovic N, Stoilkovic M, Mihailovic D, Randjelovic P, Ilic S, Gocmanac-Ignjatovic M, et al. 2012. Beneficial effects of calcium oral coadministration in gentamicin-induced nephrotoxicity in rats. *Ren. Fail.* 34(5): 622-627. DOI: 10.3109/0886022X.2012.664809. PMID: 22417102.

Trujillo, J., Molina-Jijón, E., Medina Campos, O.N., Rodriguez-Munoz, R., Reyes, J.L., Loredó, M.L., et al. 2016. Curcumin prevents cisplatin-induced decrease in the tight and adherens junctions: relation to oxidative stress. *Food. Funct.* 7(1): 279-293. DOI: 10.1039/c5fo00624d. PMID: 26467482.

Veljkovic, M., Pavlovic, D.R., Stojiljkovic, N., Ilic, S., Petrovic, A., Jovanovic, I., et al. 2016. Morphological and morphometric study of protective effect of green tea in gentamicin-induced nephrotoxicity in rats. *Life. Sci.* 147:85-91. DOI: 10.1016/j.lfs.2016.01.035. PMID: 26812477.

Witko-Sarsat, V., Friedlander, M., Capeillère-Blandin, C., Nguyen-Khoa, T., Nguyen, A.T., Zingraff, J., et al. 1996. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney. Int.* 49(5): 1304-1313. PMID: 8731095.

Yao, X., Panichpisal, K., Kurtzman, N., and Nugent, K. 2007. Cisplatin nephrotoxicity: A review. *Am. J. Med. Sci.* 334: 115-124. DOI: 10.1097/MAJ.0b013e31812dfe1e. PMID: 17700201.

Yildirim, Z., Sogut, S., Odaci, E., Iraz, M., Ozyurt, H., Kotuk, M., et al. 2003. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. *Pharmacol. Res.* 47(2):149-156. PMID: 12543063.

Youssef Nasr, A., and Al Shahat Ibrahim, A. 2015. Aged garlic extract ameliorates the oxidative stress, histomorphological, and ultrastructural changes of cisplatin-induced nephrotoxicity in adult male rats. *Microsc. Res. Tech.* 78 (6): 452-461. DOI: 10.1002/jemt.22494. PMID: 25810378.

Draft

Table 1. Biochemical analysis of serum levels of electrolytes, blood urea, and creatinine in experimental and control groups of rats.

	C	AG	CIS	CISAG
CREATININE ($\mu\text{mol/L}$)	39.14 ± 2.92	$42.59 \pm 4.92^\#$	$193.4 \pm 24.25^*$	$97.98 \pm 9.17^{*\#}$
UREA (mmol/L)	5.86 ± 0.84	$6.31 \pm 1.04^\#$	$37.52 \pm 4.60^*$	$15.43 \pm 0.72^{*\#}$
SODIUM (mmol/L)	139.1 ± 2.16	$139.4 \pm 0.91^\#$	$133.3 \pm 4.02^*$	$139.5 \pm 2.39^\#$
POTASSIUM (mmol/L)	5.02 ± 0.52	$5.01 \pm 0.54^{##}$	$4.27 \pm 0.52^{**}$	$4.92 \pm 0.12^{##}$

Data are presented as the mean $\pm$ SD. * $p < 0.001$ versus C group; ** $p < 0.05$ versus C group; $^\#p < 0.001$ versus CIS group; $^{##}p < 0.05$ versus CIS group

Draft

Table 2. Levels of AOPP, CAT, MDA and iNOS in kidney tissue in experimental and control groups of rats.

	C	AG	CIS	CISAG
AOPP ($\mu\text{mol/mg}$)	6.09 ± 0.44	$5.79 \pm 0.68^{\#}$	$17.48 \pm 2.26^{***}$	$8.88 \pm 1.59^{***\#}$
CAT (kU/g)	13.45 ± 1.73	$13.58 \pm 2.09^{\#}$	$3.46 \pm 0.75^{***}$	$10.82 \pm 1.83^{***\#}$
MDA (nmol/mg)	2.60 ± 0.70	$2.50 \pm 0.48^{\#}$	$12.13 \pm 3.54^{***}$	$5.25 \pm 1.01^{*\#}$
iNOS (IU/mg)	0.26 ± 0.005	$0.28 \pm 0.013^{\#\#}$	$0.38 \pm 0.04^{***}$	$0.29 \pm 0.03^{\#\#}$

Data are presented as the mean $\pm$ SD. * $p < 0.05$ versus C group, ** $p < 0.01$ versus C group,

*** $p < 0.001$ versus C group, # $p < 0.001$ versus CIS group, ## $p < 0.01$ versus CIS group

Draft

Table 3. Morphometric characteristics of glomeruli in experimental and the control groups of rats.

	C	AG	CIS	CISAG
AREA (μm^2)	7719 $\pm$ 301.81	7627 $\pm$ 397.42 [#]	11830 $\pm$ 487.44 [*]	8283 $\pm$ 319.03 ^{***#}
CELLULARITY (cells/ μm^2)	0.00755 $\pm$ 0.00060	0.00741 $\pm$ 0.00047 [#]	0.00533 $\pm$ 0.00031 [*]	0.00674 $\pm$ 0.00024 ^{***#}
PERIMETER (μm)	311.9 $\pm$ 2.86	316.8 $\pm$ 4.83 [#]	384.1 $\pm$ 19.25 [*]	330.1 $\pm$ 4.79 ^{**#}
FERET'S DIAMETER (μm)	110.9 $\pm$ 8.49	111.1 $\pm$ 2.81 [#]	137.4 $\pm$ 1.22 [*]	119.1 $\pm$ 2.68 ^{***#}
CIRCULARITY	0.9313 $\pm$ 0.00638	0.9372 $\pm$ 0.00630	0.9368 $\pm$ 0.00473	0.9346 $\pm$ 0.00442

Data are presented as the mean $\pm$ SD. ^{*}p<0.001 versus C group, ^{**}p<0.01 versus C group,

^{***}p<0.05 versus C group, [#]p<0.001 versus CIS group

Table 4. Morphometric characteristics of proximal and distal tubules in experimental and the control groups of rats.

	C	AG	CIS	CISAG
AREA (μm^2) (proximal tubules)	1244 $\pm$ 144.44	1271 $\pm$ 102.93 [#]	2279 $\pm$ 158.42***	1553 $\pm$ 131.75*** [#]
AREA (μm^2) (distal tubules)	998.8 $\pm$ 45.73	992.3 $\pm$ 67.65	1108 $\pm$ 84.89	1034 $\pm$ 86.70
N/C RATIO (proximal tubules)	0.7120 $\pm$ 0.04071	0.7090 $\pm$ 0.04484 [#]	0.3742 $\pm$ 0.03513***	0.6164 $\pm$ 0.09151** [#]
N/C RATIO (distal tubules)	0.9287 $\pm$ 0.07535	0.9186 $\pm$ 0.07187	0.8304 $\pm$ 0.09409	0.8654 $\pm$ 0.07377

Data are presented as the mean $\pm$ SD. *p<0.05 versus C group, **p<0.01 versus C group,

***p<0.001 versus C group, #p<0.001 versus CIS group

Draft

Figure legends:

Figure 1. Histopathology of renal sections of experimental and control groups of rats: (A) Vehicle-treated group (control); (B) Aminoguanidine-treated rats; (C) Renal section of animals treated with cisplatin; (D) Renal section of animals treated with cisplatin and aminoguanidine. Hematoxylin and eosin (HE) staining, original magnification, 200×

Figure 2. Light photomicrographs of Jones methenamine silver stained kidney sections of: (A) control group of rats; (B) AG group of rats; (C) CIS group of rats; (D) CISAG group of rats. Jones methenamine silver, original magnification, 400×

Figure 3. Glomerular membrane thickness in experimental and the control groups of rats. Data are presented as the mean $\pm$ SD. * $p < 0.001$ versus C group, # $p < 0.001$ versus CIS group

216x162mm (300 x 300 DPI)

209x155mm (300 x 300 DPI)

104x67mm (300 x 300 DPI)