Original Research Article

# MORPHOMETRIC STUDY OF STRUCTURAL KIDNEY DAMAGES CAUSED BY CISPLATIN IN RATS. EFFECTS OF QUERCETIN

S. Ilic[a], N. Stojiljkovic[a]*, S. Veljkovic[a], M. Veljkovic[a], P. Randjelovic[a], D. Sokolovic[b], G. Stojanovic[c]

[a]Department of Physiology, University of Nis, Faculty of Medicine, Bulevar Dr. Zoran Djindjic 81, Nis, Serbia.
[b]Department of Biochemistry, University of Nis, Faculty of Medicine, Bulevar Dr. Zoran Djindjic 81, Nis, Serbia.
[c]Department of Chemistry, University of Nis, Faculty of Natural Sciences and Mathematics, Visegradska 33, Nis, Serbia.

* Corresponding author, Email: nenstojiljkovic@gmail.com, Phone +381184226644.

Received: April 2016. Accepted: November 2016.
Published: December 2016.

## ABSTRACT

Cisplatin is an antioneoplastic drug with serious side effects such as nephrotoxicity. The aim of our morphometric study was to quantify changes of glomeruli, glomerular basal membrane and tubules induced by cisplatin and to examine possible beneficial effect of quercetin on structural damages in this model of nephrotoxicity. Thirty-two male Wistar rats were divided in 4 groups. The CIS group received a single dose of cisplatin (8 mg/kg) intraperitoneally, whereas the CISQ group received quercetin intraperitoneally at a dose of 50 mg/kg for 9 days and a single dose of cisplatin intraperitoneally (8 mg/kg). Animals in the Q group received quercetin (50 mg/kg) and the C group received saline (1 mL/day), both given intraperitoneally for 9 days. Quantitative evaluation of structural damages was performed by histological and morphometric examination of kidneys. Functional alterations in the kidneys were determinated by biochemical and antioxidant parameters. Histological sections of kidney showed that quercetin ameliorated cystic luminal dilatation, cell swelling and degeneration of proximal tubules induced by cisplatin. Quercetin also reduced histopathological changes of glomeruli and glomerular basement membrane. Morphometric analysis revealed statistically significant differences in the area of tubules, size of glomeruli, glomerular basement membrane thickness and cellularity of glomeruli between CIS and CISQ group. Quercetin showed a significant protective effect probably by decreasing level of oxidative stress, which was confirmed through elevating catalase activity and decreasing concentrations of AOPP in kidney tissue in the CISQ group. The results suggest that quercetin has nephroprotective action and ameliorates oxidative stress in cisplatin-treated rats.

**Keywords**: morphometry, cisplatin, nephrotoxicity, quercetin, rats.

## INTRODUCTION

Cisplatin is an antioneoplastic drug commonly used in treatment of various solid tumors such as: germ cell tumors, ovarian cancer, lung cancer, bladder cancer and squamous cell carcinomas of head and neck [1, 2]. Clinical use of cisplatin is limited due to its side effects, especially due to a dose and duration-dependent nephrotoxicity [3]. Cisplatin in the kidneys penetrates the tubular cells and reaches high concentration in the proximal tubules, particularly in S3 segment of the tubular epithelial cells. Glomerular and distal tubules injuries are less frequent [4, 5]. Cisplatin nephrotoxicity is manifested as lower glomerular filtration rate, higher serum creatinine and blood urea concentrations and reduced potassium and magnesium levels [1]. In 20-30% of patients treatment with cisplatin induces acute kidney injury [6]. The exact mechanism of cisplatin-induced nephrotoxicity is complex and incompletely defined. It has been shown that one of pathogenic mechanisms is formation of reactive oxygen species (ROS) and inhibition of the activity of antioxidant enzymes in renal tissue. ROS, such as hydrogen peroxide, hydroxyl radical, singlet oxygen, superoxide anion and peroxyl radical, are

formed inside cells by exposure to several endogenous and exogenous agents and may produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage [7, 8].

It has been shown in many studies that supplementation with antioxidants can ameliorate or prevent cisplatin nephrotoxicity [9, 10, 11]. Quercetin is natural polyphenolic flavonoid present in high concentration in fruits and vegetables such as onion, potatoes, broccoli apples, grapes and berries. An important effect of quercetin is the scavenging of oxygen-derived free radicals. Some investigators showed that quercetin is capable to protect and ameliorate nephrotoxicity of cyclosporine, gentamicin and ferric nitrilotriacetate in rats [12, 13, 14].

In our earlier investigation [15], we began research of acute renal injury caused by cisplatin and protective effects of quercetin. In this study, we have continued research by determining the level of oxidative changes of proteins and quantification of alterations in the kidney induced by cisplatin and quercetin by morphometric analysis, which has not been done in this experimental model up to date. In accordance with this, the aim of our morphometric study was to quantify changes of glomeruli, glomerular basal membrane (GBM) and tubules induced by cisplatin and to examine possible beneficial effect of quercetin on structural damages in this model of nephrotoxicity in rats.

## MATERIALS AND METHODS

Experiments were performed on 32 male Wistar rats, weighing 200–250 g. The animals were housed inside a well-ventilated room with controlled temperature (20 ± 2°C) and humidity (60%) with regular light cycle (12 light/12 dark). The animals were acclimatized for 1 week before the study and had free access to standard laboratory food and water. All experimental procedures were conducted in accord with the principles for the care and use of laboratory animals in research 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 consisting of 8 animals. The control group of rats (C group) was administered with 1 ml saline solution (0.9%) intraperitoneally for 9 days. The cisplatin group (CIS group) received a single dose of cisplatin (Pfizer PTY. Limited, Bentley, Australia) intraperitoneally on the fifth day of experiment at a dose of 8 mg/kg. The quercetin group (Q group) was used as a positive control group and received quercetin (Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in physiological saline solution, intraperitoneally, at a dose of 50 mg/kg for 9 days and the cisplatin-quercetin group (CISQ group) received quercetin intraperitoneally at a dose of 50 mg/kg for 9 days and cisplatin intraperitoneally on the fifth day of treatment at a dose of 8 mg/kg.

Ten days after the beginning of the experiment, all animals were anaesthetized with ketamine (Ketamidor 10%, Richter Pharma AG, Wels, Austria) given intraperitoneally (80 mg/kg) and then sacrificed. Blood samples for biochemical analysis were taken from the aorta (2 mL), and the kidney was subsequently removed and separated into two parts for biochemical analysis and light microscopic examination.

### Biochemical analysis

After finishing the experiment, blood samples taken from the aorta were analyzed for markers of renal impairment. Urea and creatinine concentrations in serum were measured 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 [15]. Kidney tissue specimens were stained with Periodic Acid Schiff (PAS) 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).

**Morphometric analysis**

After rat kidneys were histologically processed and stained, digital images were taken under the objective lens magnification 10x and 40x, using a digital camera connected to a light microscope Leica DMR (Leica Microsystems AG, Wetzlar, Germany). Morphometric analysis was done with computerized image analysis system ImageJ. Spatial calibration, by object micrometer was performed before each analysis. The following morphometric parameters were measured: area of proximal and distal tubules ($\mu m^2$), the nuclear-cytoplasmic (N/C) ratio of the epithelial cells of proximal and distal tubules, glomerular basement membrane (GBM) thickness (μm), glomerular area ($\mu m^2$), cellularity (cells/$\mu m^2$), perimeter (μm), Feret's diameter (μm) and circularity. The area was the number of pixels from the object. The nuclear-cytoplasmic (N/C) ratio was calculated as a ratio of the area of the nucleus of a cell and area of the cytoplasm of that cell. The GBM thickness was estimated as a mean distance after manual tracing of two lines along both sides of the basement membrane. Cellularity was the number of cells in the $\mu m^2$ of area. The perimeter was the length of the outline of each object. Feret's diameter was the longest distance between any two points along the selection boundary. Circularity of each object was determined by the following formula: $(4\pi * \text{area}) / (\text{perimeter}^2)$, with a value of 1.0 indicating a perfect circle. In each animal at least 50 glomeruli proximal and distal tubules were measured.

**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. To determine the parameters of oxidative stress the concentration of proteins was measured according to *Lowry's* method using bovine serum as standard [16].

**Determination of catalase activity**

Tissue catalase (CAT) activity was determined by spectrophotometric method described by *Goth* [17], 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).

**Determination of protein oxidation**

Levels of advanced oxidation protein products (AOPP) were measured by spectrophotometric method [18]. 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 μmol/mg of protein.

**Statistical Analysis**

The values of parameters obtained were expressed as the mean ± SD. Statistically 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). The $p<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

The main finding of our study was that quercetin showed protective effects on structural damages of kidneys induced by cisplatin. We induced acute renal injury by administration of single dose (8mg/kg) of cisplatin. Nephrotoxicity was confirmed by significant increased serum concentrations of urea and creatinine in the CIS group compared to the C group ($p<0.001$) (Fig. 1) and by pathohistological examination of renal sections in CIS group of rats.

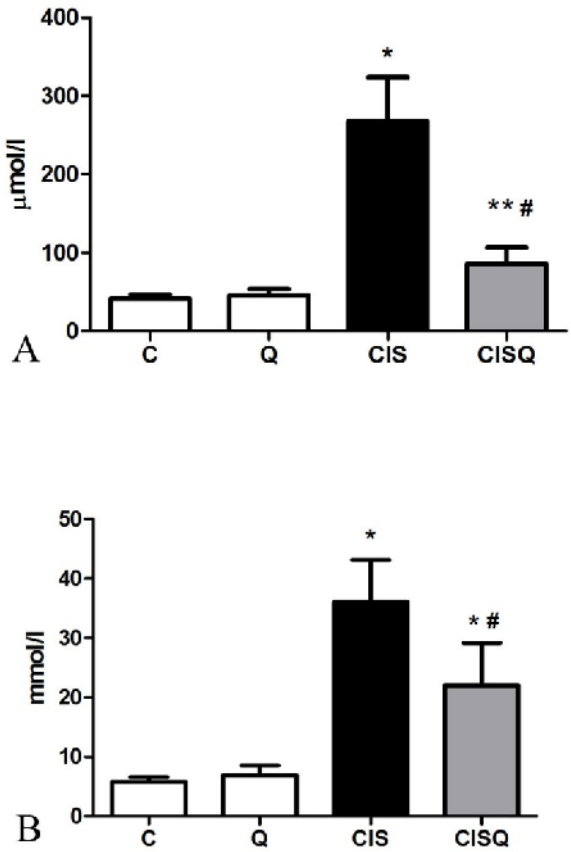


**Fig. 1.** The results of biochemical analysis in control and experimental groups of rats. Serum concentrations of: (A) creatinine (B) urea. Data are presented as a mean ± SD. #p<0.001 versus CIS group, *p<0.001 versus C group, **p<0.05 versus C group.

Histopathological findings of kidney tissues from both vehicle (Figs. 2A and 3A) and quercetin-treated rats (Figs. 2B and 3B) showed intact histo-morphology with normal glomerular and tubular structure. Kidney sections from cisplatin treated rats (CIS group) revealed extensive morphological damage in the form of widespread tubular cell swelling and degeneration, cystic luminal dilatation with accumulation of homogenous eosinophilic casts in the lumen of the tubules (Fig. 2C). Distal tubules were histologically almost unaltered. Tubular damages were similar to those found in ischemia/reperfusion injury in rats [19].

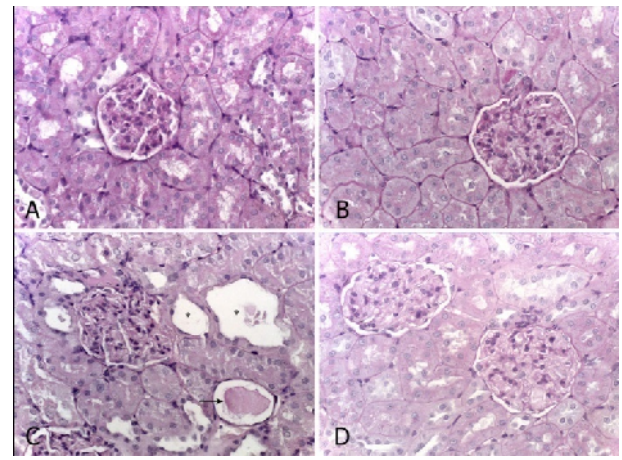


**Fig. 2**. Light photomicrographs of PAS stained sections of rats renal cortex showing: normal histology of kidney tissue and regular and even PAS staining in control (A) and quercetin-treated (B) animals; (C) Rats treated with cisplatin showing reduction of the staining properties and degenerated and dilated tubules (*) with hyaline casts (arrow) in kidney cortex. (D) Section from rat treated with cisplatin and quercetin reveal almost complete prevention of histopathological alterations. Periodic acid-Schiff (PAS) staining, original magnification, 400×

The glomeruli in CIS group displayed generalized congestion with reduction in Bowman's space (Fig. 3C). Animals in CIS group, in comparison to those in other groups, had enlarged glomeruli and irregularly thickened glomerular basement membrane with neutrophil cell infiltration (Fig. 3). These findings are similar with earlier reports where histological changes were also consistent with laboratory findings [20, 21]. Our results confirmed previous suggestions that cisplatin primarly damages proximal tubules, due to its accumulation in this part of nephron and conversion into nephrotoxic metabolites. According to our knowledge, this is the very first morphometric analysis of rats' kidneys in this

experimental model. Measured parameters of proximal and distal tubules in each group are shown in Table 1.

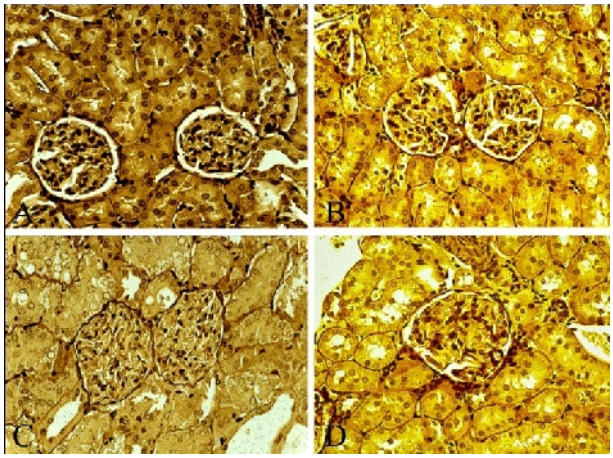


**Fig. 3.** Light photomicrographs of Jones methenamine silver stained kidney sections showing thin glomerular basement membrane in C (A) and Q (B) group of rats; (C) Animals in CIS group had enlarged glomeruli and irregularly thickened glomerular basement membrane; (D) Treatment with quercetin ameliorated changes induced by cisplatin in kidneys and glomerular basement membrane was thinner in this (CISQ) group of rats. Jones methenamine silver, original magnification, 400×

Area of proximal tubules was statistically significant increased in the group of animals treated with cisplatin in comparison to control group ($p<0.001$). In the CISQ group area of proximal tubules was significantly decreased compared to the CIS group of animals ($p<0.001$) (Table 1). The value of nuclear-cytoplasmic ratio in the epithelial cells of proximal tubules in CIS group of animals was statistically significant smaller compared to the control and CISQ group ($p<0.001$) (Table 1). Area of distal tubules and nuclear-cytoplasmic ratio in the epithelial cells of distal tubules showed no statistically significant difference between control and experimental group of animals (Table 1).

In addition to proximal tubules tubular injuries there are glomerular effects that are much less well documented. When cisplatin is administred to experimental animals, there is marked fall in renal blood flow and glomerular filtration rate with rise in renal vascular resitance, which is induced by structural changes in glomerular structures and without a direct relationship with tubular damages [22]. Due to this assumption, main goal of our study was to quantify changes of glomeral structures, especially GBM thickness in cisplatin induced nephrotoxicity and to examine possible protective effects of quercetin on these damages. Morphometric analysis of glomeruli revealed that cisplatin treated animals had larger glomeruli. This was confirmed with significantly larger values of glomerular area, perimeter and Feret´s diameter compared to control group ($p<0.001$) (Table 1). Analysis of glomerular circularity did not show statistically significant differences in the control group in relation to the experimental group of animals (Table 1). The mean glomerular membrane thickness was significantly greater in CIS group compared to C group ($p<0.001$) and CISQ group of rats ($p<0.001$) (Table 1). *Stojiljkovic* et al. [23] published that in gentamicin-induced nephrotoxicity glomerular basement membrane was irregularly thickened. Our study showed similar changes indicating that cisplatin and gentamicin caused same damages of glomerular basement membrane. Taken all together our findings showed that there are significant structural changes of glomeruli and glomerular basement membrane in animals treated with cisplatin. Changes of GBM cause altered glomerular filtration and functional impairment of kidneys. Glomerular hemodynamics, glomerular contraction, filtration surface area and ultrafiltration coefficient depend of mesangial cells functions. We also have found that number of cells in glomerular area (cellularity) is decreased, which indicates that there is significant drug-induced apoptotic reaction in renal glomerular tissue (Table 1). *Kohn* et al. [24] have suggested that mitochondrial malfunction may play a significant role in the glomerular cells injury.

**Table 1.** Morphometric analysis of proximal and distal tubules and glomeruli in experimental and control group of rats

| Variable | C group | Q group | CIS group | CISQ group |
|---|---|---|---|---|
| **Glomerular area** ($\mu m^2$) | 7635.63 ± 1278.06 | 7674.63 ± 1112.56 | 11598.20±2293.85*** | 8035.95 ± 933.74**# |
| **Cellularity** (cells/$\mu m^2$) | 0.0072 ± 0.0012 | 0.0070 ± 0.0011 | 0.0052 ± 0.0007*** | 0.0067 ± 0.0009***# |
| **Perimeter** ($\mu m$) | 319.04 ± 27.03 | 319.82 ± 22.53 | 395.73 ± 36.99*** | 326.98 ± 20.90**# |
| **Feret´s diameter** ($\mu m$) | 111.42 ± 11.21 | 111.70 ± 10.13 | 140.65 ± 14.19*** | 114.63 ± 10.21**# |
| **Circularity** | 0.9317 ± 0.0274 | 0.9319 ± 0.0270 | 0.9368 ± 0.0274 | 0.9318 ± 0.0283 |
| **GBM thickness** ($\mu m$) | 0.5581±0.0258 | 0.5820 ± 0.0432 | 0.8561 ± 0.0384*** | 0.6363 ± 0.0391*# |
| **Area of proximal tubules ($\mu m^2$)** | 1310 ± 97.30 | 1348 ± 101.0 | 2279 ± 158.4*** | 1505 ± 39.20**# |
| **N/C of proximal tubules** | 0.7105 ± 0.0408 | 0.7216 ± 0.0278 | 0.4165 ± 0.0671*** | 0.6297 ± 0.0519*# |
| **Area of distal tubules ($\mu m^2$)** | 1139 ± 76.50 | 1160 ± 87.83 | 1241 ± 76.78 | 1220 ± 74.72 |
| **N/C of distal tubules** | 0.8895 ± 0.0799 | 0.8872 ± 0.0706 | 0.7971 ± 0.0504 | 0.8234 ± 0.0365 |

Data are presented as a mean ± SD. *p<0.05 vs C, **p<0.01 vs C, ***p<0.001 vs C, #p< 0.001 vs CIS group.

The flavonoids are a family of phenolic compounds that have biochemical and pharmalogical activities like antibacterial, antiviral, antiinflammatory, antiallergic, antithrombotic, antimutagenic, antineoplastic, as well as neuroprotective properties [25]. Flavonoids affect basic cell function such as growth, differentiation and apoptosis, because of their radical scavenging activity [26]. Quercetin has been reported as a renoprotective agent in cisplatin-induced nephrotoxicity in vitro [27] and in vivo [28, 29]. In addition, *Sanchez-Gonzalez* et al. [20] showed that quercetin may diminish inflammation in kidneys without affecting the anticancer efficacy of cisplatin.

In our study, administration of quercetin, 4 days before and 4 days after single dose of cisplatin, produced a significant protection against nephrotoxicity induced by cisplatin. Amelioration of nephrotoxicity was evidenced by significant reductions in serum urea and creatinine concentrations compared to the group treated only with cisplatin (Fig. 1). Protective effect of quercetin was similar to those published by *Behling* et al. [28] who showed that quercetin treatment was able to restore plasma creatinine to control levels in cisplatin-treated animals. On the other hand, administration of quercetin was demonstrated by the normalization of histopathological alterations. There was focal degeneration of tubular cells without glomerular congestion, leucocyte infiltration or tubular dilatation (Fig. 2D). Glomerular basement membrane in cisplatin-quercetin group of animals was thinner than that in CIS group of rats (Fig. 3D).

Our morphometric analysis revealed that the glomeruli of rats treated with cisplatin and quercetin together were significantly smaller than in the group of rats treated only

with cisplatin which was confirmed with significant differences between CIS and CISQ groups in area, perimeter and Feret´s diameter ($p<0.001$) (Table 1). Damages of proximal tubules were also significantly attenuated by quercetin adminsitration in CISQ group of rats (Table 1). Taken together, these data suggest that quercetin exerts an ameliorative effect on kidneys structural damages caused by cisplatin administration.

Although exact mechanism of cisplatin-induced nephrotoxicity is incompletely defined, many studies showed that cisplatin has multiple intracellular effects, causing direct cytotoxicity with reactive oxygen species, activating mitogen-activated protein kinases, inducing apoptosis and stimulating inflammation [26]. In the present study, we also examined mechanisms of cisplatin nephrotoxicity, as well as quercetin nephroprotective effects through analysis of parameters of oxidative stress. In our previous study [15], we reported that cisplatin administration lead to elevated levels of malondyaldehyde which is the end-product of lipid peroxidation and also that quercetin inhibits lipid peroxidation and protects a lipid components in cell membranes. Catalase is one of the endogenous antioxidant enzymes that play a pivotal role in the reduction of oxidative modification of lipids and scavenge hydrogen peroxide and thus interrupt the propagation of the lipid peroxidation reaction [26]. In present study, catalase activity in the kidney tissue was statistically significant reduced in the CIS group in comparison to C group ($p<0.001$) (Fig 4 A) after a single dose of cisplatin (8mg/kg) which is in agreement with earlier findings [30, 31]. These observations support the evidence that part of the mechanism of nephrotoxicity in cisplatin-treated rats is related to depletion of antioxidant system [26]. In addition, cellular proteins may also be affected by ROS. It is generally known that proteins are susceptible to damage by ROS in vitro and in vivo, and oxidative modification of proteins may lead to the structural alteration and functional inactivation of many enzyme proteins [32].

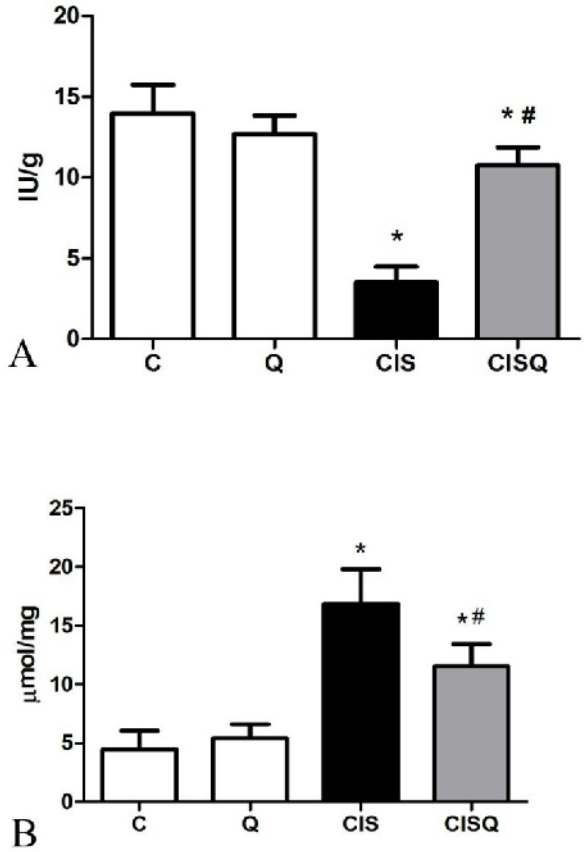


**Fig. 4.** Effect of quercetin administration on (A) catalase activity and (B) AOPP levels in kidneys of rats treated with cisplatin. Results represent mean ± SD. $^{\#}p<0.001$ versus CIS group, $*p<0.001$ versus C group.

Nephrotoxicity was further confirmed by a significant increase of the concentration of AOPP in kidney tissue in CIS group of rats compared to those of control group ($p<0.001$) (Fig. 4 B).

The nephroprotective effect of quercetin seems to be related with its antioxidant activity and also with its capacity to inhibit renal inflammation and tubular cell apoptosis [20]. Quercetin protects membrane lipids, nuclear DNA and proteins from oxidative damage through its strong capacity to inhibit oxidative stress [33]. Many studies confirmed antioxidant activity of quercetin, through its ability to direct inhibit lipid peroxidation [28]. *Ferralli* et al. [34] proposed that

possible antioxidant mechanism of quercetin may be due to its iron-chelating and iron-stabilizing properties. Another possible mechanism by which quercetin act is through interaction with various enzyme systems. In our study the antioxidant properties of quercetin was confirmed by statistically significant increased of catalase activity in rats treated with quercetin and cisplatin in relation to the group of rats treated with cisplatin alone ($p<0.001$) (Fig. 4A), which showed that quercetin can increase the function of the endogenous antioxidants. Our results confirmed previous findings about the beneficial antioxidant effect of quercetin [20]. Administration of quercetin with cisplatin also significantly decreased concentration of renal AOPP in CISQ group compared to the CIS group ($p<0.001$) (Fig. 4B). These results indicate role of quercetin in scavenging free radicals generated by cisplatin and in protecting proteins in cells. This ability of quercetin may contribute to protective effects on structural damages of kidneys which we quantified by morphometry. Furthermore, some effects may be a result of a combination of radical scavenging and an interaction with enzyme functions.

## CONCLUSIONS

Our results strongly support the concept that cisplatin causes structural alterations of glomeruli and GBM, as well as alterations of proximal tubules in rats. In addition, results of our study indicate that administration of quercetin in a dose 50 mg/kg afforded significant protection against histological and functional glomerular and tubular impairment induced by cisplatin probably by acting as a potent scavenger of ROS. These findings indicate that quercetin has a potent shielding effect against the neprotoxiciy of cisplatin and might be clinically useful.

## DECLARATION OF INTEREST

The authors declare no conflicts of interest.

## REFERENCES

[1] Pabla N., Dong Z. (2008) "Cisplatin nephrotoxicity: mechanisms and renoprotective strategies" *Kidney Int.,* 73(9):994-1007.

[2] Rjiba-Touati K., Ayed-Boussema I., Bouaziz C., Belarbia A., Azzabi A., Achour A., Hassen W., Bacha H. (2012) "Protective effect of erythropoietin against cisplatin-induced nephrotoxicity in rats: antigenotoxic and antiapoptotic effect" *Drug Chem Toxicol.* 35(1):89-95.

[3] Zamani Z., Nematbakhsh M., Eshraghi-Jazi F., Talebi A., Jilanchi S., Navidi M., Shirdavani S., Ashrafi F. (2016) "Effect of enalapril in cisplatin-induced nephrotoxicity in rats; gender-related difference" *Adv Biomed Res.* doi: 10.4103/2277-9175.175253.

[4] Chirino Y.I., Pedraza-Chaverri J. (2009) "Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity" *Exp Toxicol Pathol.* 61(3):223-242.

[5] Peres L.A., da Cunha Jr. A.D. (2013) "Acute nephrotoxicity of cisplatin: molecular mechanisms" *J Bras Nefrol.* 35(4):332-340.

[6] Miller R.P., Tadagavadi R.K., Ramesh G., Reeves W.B. (2010) "Mechanisms of cisplatin nephrotoxicity" *Toxins (Basel).* 2(11):2490-2518.

[7] Yao X., Panichpisal K., Kurtzman N., Nugent K. (2007) "Cisplatin nephrotoxicity: a review" *Am J Med Sci.* 334(2):115-124.

[8] Oh G.S., Kim H.J., Shen A., Lee S.B., Yang S.H., Shim H., Cho E.Y., Kwon K.B., Kwak T.H., So H.S. (2016) "New Therapeutic Concept of NAD Redox Balance for Cisplatin Nephrotoxicity" *Biomed Res Int.* 2016:4048390.

[9] Iraz M., Ozerol E., Gulec M., Tasdemir S., Idiz N., Fadillioglu E., Naziroglu M., Akyol O. (2006) "Protective effect of caffeic acid phenethyl ester

(CAPE) administration on cisplatin-induced oxidative damage to liver in rat" *Cell Biochem Funct.* 24(4):357-361.

[10] Aksoy A, Karaoglu A, Akpolat N, Naziroglu M, Ozturk T, Karagoz ZK. (2015) "Protective Role of Selenium and High Dose Vitamin E against Cisplatin - Induced Nephrotoxicty in Rats" *Asian Pac J Cancer Prev.* 16(16):6877-6882.

[11] Malik S., Bhatia J., Suchal K., Gamad N., Dinda A.K., Gupta Y.K., Arya DS. (2015) "Nobiletin ameliorates cisplatin-induced acute kidney injury due to its anti-oxidant, anti-inflammatory and anti-apoptotic effects" *Exp Toxicol Pathol.* 67(7-8):427-433.

[12] Satyanarayana P.S., Singh D., Chopra K. (2001) "Quercetin, a bioflavonoid, protects against oxidative stress-related renal dysfunction by cyclosporine in rats" *Methods Find Exp Clin Pharmacol.* 23(4):175-181.

[13] Singh D., Chander V., Chopra K. (2004) "Quercetin, a bioflavonoid, attenuates ferric nitrilotriacetate-induced oxidative renal injury in rats" *Drug Chem Toxicol.* 27(2):145-156.

[14] Abdel-Raheem I.T., Abdel-Ghany A.A., Mohamed G.A. (2009) "Protective effect of quercetin against gentamicin-induced nephrotoxicity in rats" *Biol Pharm Bull.* 32(1):61-67.

[15] Ilić S., Stojiljković N., Veljković M., Veljković S., Stojanovic G. (2014) "Protective effect of quercetin on cisplatin-induced nephrotoxicity in rats" *FU Med Biol.* 16(2):71-75.

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

[17] Goth L. (1991) "A simple method for determination of serum catalase activity and revision reference range" *Clin Chim Acta.* 196(2-3):143-151.

[18] Witko-Sarsat V., Friedlander M., Capeillere-Blandin C., Nguyen-Khoa T., Nguyen A.T., Zingraff J., Jungers P., Descamps-Latscha B. (1996) "Advanced oxidation protein products as a novel marker of oxidative stress in uremia" *Kidney Int.* 49(5):1304-1313.

[19] González Núñeza L., Rodríguez Salgueirob S., Fernández Iglesiasa A., Santos Santosc E., Gutiérrez Albaa N. E., López Marína L., Galán Garciac L., Chávez Muñoza Y., Calunga Fernández J. L. (2010) "Effects of ozone oxidative postconditioning on renal tubular epithelial cells against ischemia/reperfusion injury in rats" *Acta Microscopica.* 19(3):264-270.

[20] Sanchez-Gonzalez P.D., Lopez-Hernandez F.J., Perez-Barriocanal F., Morales A.I., Lopez-Novoa J.M. (2011) Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. *Nephrol Dial Transplant.* 26(11):3484-3495.

[21] Palipoch S., Punsawad C. (2013) "Biochemical and histological study of rat liver and kidney injury induced by Cisplatin" *J Toxicol Pathol.* 26(3):293-299.

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

[23] Stojiljkovic N., Stoiljkovic M., Randjelovic P., Veljkovic S., Mihailovic D. (2012) "Cytoprotective effect of vitamin C against gentamicin-induced acute kidney injury in rats" *Exp Toxicol Pathol.* 64(1-2):69-74.

[24] Kohn S., Fradis M., Ben-David J., Zidan J., Robinson E. (2002) "Nephrotoxicity of combined treatment with cisplatin and gentamicin in the guinea pig: glomerular injury findings" *Ultrastruct Pathol.* 26(6):371-382.

[25] Uivarosi V., Barbuceanu S.F., Aldea V., Arama C.C., Badea M., Olar R., Marinescu D. (2010) "Synthesis, spectral and thermal studies of new rutin vanadyl complexes" *Molecules.* 15(3):1578-1589.

[26] Arjumand W., Seth A., Sultana S. (2011) "Rutin attenuates cisplatin induced renal inflammation and apoptosis by reducing NFκB, TNF-α and caspase-3 expression in Wistar rats" *Food Chem Toxicol.* 49(9):2013-2021.

[27] Kuhlmann M.K., Horsch E., Burkhardt G., Wagner M., Kohler H. (1998) "Reduction of cisplatin toxicity in cultured renal tubular cells by the bioflavonoid quercetin" *Arch Toxicol.* 72(8):536-540.

[28] Behling E.B., Sendao M.C., Francescato H.D., Antunes L.M., Costa R.S., Bianchi Mde L. (2006) "Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys" *Pharmacol Rep.* 58(4):526-532.

[29] Aldemir M., Okulu E., Kösemehmetoğlu K., Ener K., Topal F., Evirgen O., Gürleyik E., Avcı A. (2014) "Evaluation of the protective effect of quercetin against cisplatin-induced renal and testis tissue damage and sperm parameters in rats" *Andrologia.* 46(10):1089-1097.

[30] Abdel Moneim A.E., Othman M.S., Aref A.M. (2014) "Azadirachta indica attenuates cisplatin-induced nephrotoxicity and oxidative stress" *Biomed Res Int.* 2014:647131.

[31] Nasr A.Y., Saleh H.A. (2014) "Aged garlic extract protects against oxidative stress and renal changes in cisplatin-treated adult male rats" *Cancer Cell Int.* 14(1):92.

[32] Sitte N., Merker K., Von Zglinicki T., Davies K.J., Grune T. (2000) "Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part II—aging of nondividing cells" *FASEB J.* 14(15):2503-2510.

[33] Morales A.I., Vicente-Sanchez C., Sandoval J.M., Egido J., Mayoral P., Arévalo M.A., Fernández-Tagarro M., López-Novoa J.M., Pérez-Barriocanal F. (2006) "Protective effect of quercetin on experimental chronic cadmium nephrotoxicity in rats is based on its antioxidant properties " *Food Chem Toxicol.* 44(12):2092-2100.

[34] Ferrali M., Signorini C., Caciotti B., Sugherini .L, Ciccoli L., Giachetti D., Comporti M. (1997) "Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity" *FEBS Lett.* 416(2):123-129.