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PROTECTIVE EFFECT OF QUERCETIN ON CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

***Sonja Ilić*[1*], *Nenad Stojiljković*[1], *Milica Veljković*[1], *Slavimir Veljković*[1], *Gordana Stojanović*[2]**

[1]*Department of Physiology, University of Niš, Faculty of Medicine, Niš, Serbia*
[2]*Department of Chemistry, University of Niš, Faculty of Natural Sciences and Mathematics, Niš, Serbia*

Abstract. *The aim of this study was to investigate the possible effect of quercetin on cisplatin-induced nephrotoxicity in rats. Experiments were done on thirty-two Wistar rats divided into four groups of 8 animals each. 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) on the fifth day. 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 and functional alterations in the kidneys were performed by histopathological and biochemical analyses. Histological sections of kidney in CIS group revealed mild degenerative changes of proximal tubules and focal apoptosis of tubulocites, while glomeruli had reduced lobular appearance. In CISQ group these changes were ameliorated and less visible. Analysis of biochemical parameters showed significantly higher urea and creatinine serum concentrations in CIS group in comparison with C group and CISQ group ($p<0.001$). The concentrations of potassium and sodium in the CIS group were lower, but not statistically significant in comparison to the C group. Kidney MDA levels were found to be significantly higher in CIS group than those in C group ($p<0.001$), whereas the values for CISQ group were significantly lower than MDA recorded for CIS group ($p<0.001$). The results suggest that quercetin has the nephroprotective action and reduces lipid peroxidation in cisplatin-treated rats.*

Key words: *Cisplatin, quercetin, nephrotoxicity, rats*

Introduction

Cisplatin is an important chemotherapeutic agent useful in the treatment of several cancers: tumors of the testis (including extragonadal germ cell tumors), ovarian cancer, small cell and non-small cell lung cancer, squamous cell carcinomas of the head and neck. Several side effects of cisplatin have been reported, mainly nephrotoxicity and myelosuppression, that limit its clinical use [1–4]. Renal impairment begins several days after the application of cisplatin, as revealed by increases in the serum creatinine and blood urea nitrogen concentrations. The urine output is usually preserved (non-oliguric) and the urine may contain glucose and small amounts of protein, indicative of proximal tubular dysfunction [5]. The exact mechanism of action of cisplatin has not yet been fully understood. Recent studies showed that inflammation, oxidative stress injury, and apoptosis probably explain part of its nephrotoxicity. Toxic effects occur primarily in the proximal tubule, particularly in S3 segment of the tubular epithelial cells; glomeruli and distal tubules are affected subsequently [6, 7]. Substantial evidence indicates that oxidative stress is involved in renal injury secondary to cisplatin administration [8–10]. Reactive oxygen species (ROS) production, depletion of antioxidant systems and stimulation of renal accumulation of lipid peroxidation products have been listed as the main mechanisms associated with cisplatin-induced nephrotoxicity [7, 11]. That is why in recent years many authors investigated the effects of numerous antioxidants on cisplatin-induced nephrotoxicity. Flavonoids are a group of natural antioxidants, which are increasingly used in the prevention and ameliorating of these and similar pathological conditions. Flavonoids affect basic cell function such as growth, differentiation and apoptosis, because of their radical scavenging activity. Quercetin is natural flavonoid present in high concentration in fruits and vegetables like apples, onion, potatoes, broccoli, tea, soybeans, red wine. It has been shown to have very potent antioxidant and cytoprotective effects in preventing endothelial apoptosis caused by oxidants [12, 13].

The aim of this study was to investigate the protective effect of quercetin on cisplatin-induced nephrotoxicity in rats.

Correspondence to: Sonja Ilić
Department of Physiology, University of Niš, Faculty of Medicine
81 Dr Zoran Đinđić Blvd., 18000 Niš, Serbia
Phone: +381 18 4226644 Fax: +381 18 4238770
E-mail: sonjaili@yahoo.com

Material and Methods

Thirty-two Wistar albino rats, weighing 200–250g, were used in this study. The animals were maintained under standard laboratory conditions 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 ad libitum. All experimental procedures were conducted in accord with the principles for the care and use of laboratory animals in research and approved by the local ethics committee.

Experimental protocol

After a quarantine period of 7 days, 32 rats were randomly divided into four groups, each consisting of 8 animals. The control group of rats (C group) received 1 ml saline solution per day intraperitoneally for 9 days. The cisplatin group (CIS group) received a single dose of cisplatin (Pfizer Pty Ltd, Bentley, Australia) intraperitoneally on the fifth day of the experiment at a dose of 8 mg/kg. The quercetin group (Q group) was used as a positive control and received quercetin (Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in physiological saline solution, intraperitoneally, at a dose of 50mg/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 using 80 mg/kg ketamine (Ketamidor 10%, Richter Pharma AG, Wels, Austria) and 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.

Histological analysis

Paraformaldehyde-fixed kidney tissues were dehydrated in ascending graded series of alcohol and embedded in paraffin. Kidney tissue specimens were cut into slices of 5 μm thickness using a HistoRange microtome (model: LKB 2218, LKB-Produkter AB, Bromma, Sweden) followed by staining with hematoxylin and eosin (HE) according to conventional staining protocols. The histological sections were examined with a light microscope Leica DMR (Leica Microsystems AG, Wetzlar, Germany).

Biochemical analysis

After finishing the experiment, blood samples taken from the aorta were analyzed for markers of renal impairment. Urea, creatinine, sodium and potassium 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.

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 [14]. 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 (1.56×10^{-5} mol cm^{-1}).

Statistical Analysis

All data were expressed as the mean ± SD. Statistical comparison between different groups were done 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). $P< 0.05$ were considered to be statistically significant.

Results

Histological analysis

In the CIS group of animals a large number of proximal tubules showed degeneration of tubular architecture, with numerous vacuoles in cytoplasm of tubular cells and focal apoptosis of tubulocytes. The distal tubules were with normal histological appearance. Glomeruli had reduced lobular appearance with hyperemia (Fig. 1). In the CISQ group these changes were less pronounced with focal degenerative changes in proximal tubules, while glomeruli were not affected (Fig. 2). Renal sections from the C and Q groups showed no histological changes (Figs. 3 and 4).

Biochemical analysis

In the CIS group, when compared to the C group, analysis of biochemical parameters showed a significant increase of urea and creatinine serum concentrations ($p <0.001$). The concentration of potassium and sodium in the CIS group were lower, but not statistically significant in comparison to the C group. In the CISQ group, creatinine concentrations were significantly elevated compared to the first group ($p <0.05$), but also these values were significantly decreased compared to the CIS group ($p<0.001$). Levels of urea were significantly elevated in CISQ group in comparison with the C group ($p<0.001$) and significantly lower when compared to the CIS group of animals ($p<0.001$). The concentrations of potassium and sodium in the CISQ group were not significantly different compared to the other groups (Table 1).

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 quercetin in the CISQ group reduced lipid peroxidation, as evidenced by significantly decreased level of MDA than those in the CIS group ($p<0.001$) (Fig. 5).

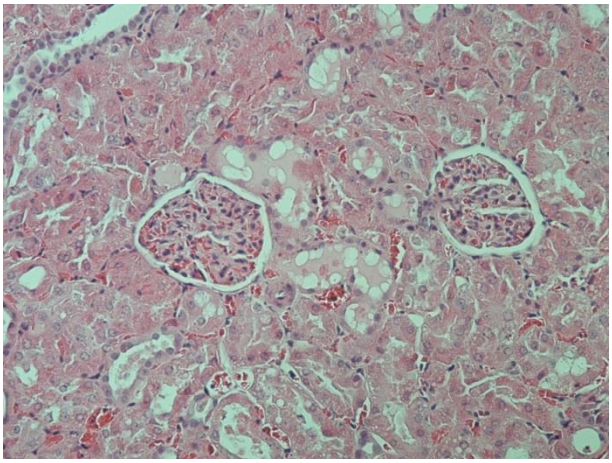


Fig. 1. Histopathological view of renal sections of CIS group of rats (HE × 200)

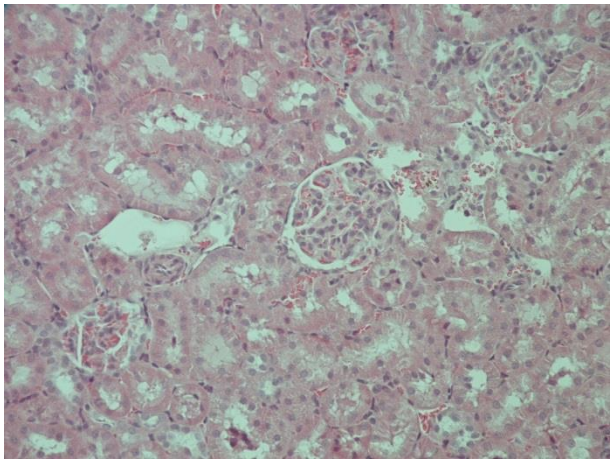


Fig. 3. Histopathological view of renal sections of C group of rats (HE × 200)

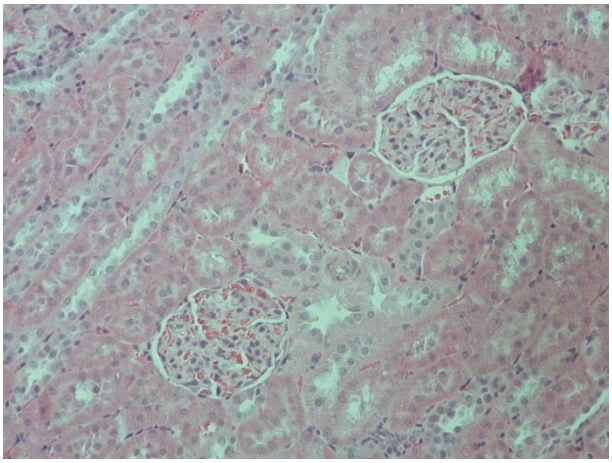


Fig. 2. Histopathological view of renal sections of CISQ group of rats (HE × 200)

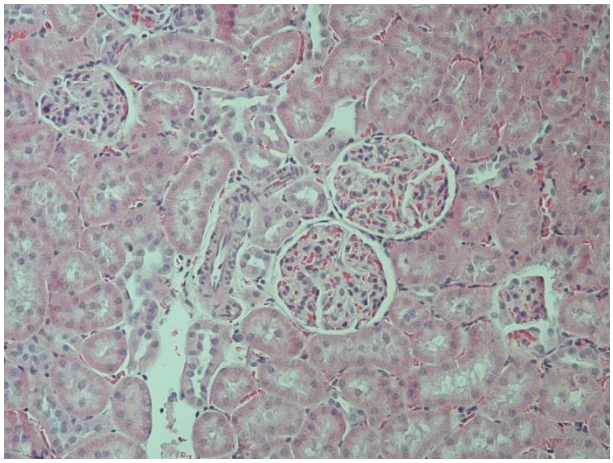
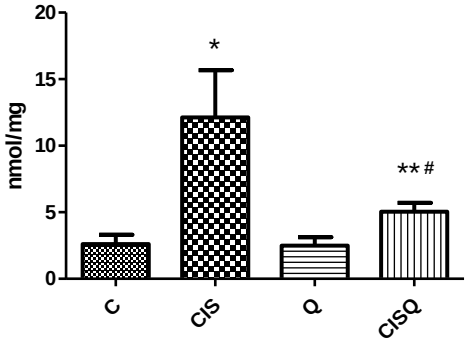


Fig. 4. Histopathological view of renal sections of Q group of rats (HE × 200)

Table 1. Biochemical analysis of rat serum creatinine, urea and electrolytes concentrations.

Serum contretation	C group	CIS group	Q group	CISQ group
Creatinine (µmol/L)	41.36 ± 5.33	268.4 ± 55.39*	46.31 ± 7.665	86.14 ± 20.57**#
Urea (mmol/L)	5.75 ± 0.8142	36.07 ± 7.059*	6.888 ± 1.625	22.03 ± 7.109*#
Sodium (mmol/L)	139.1 ± 2.167	137.5 ± 2.777	140.1 ± 2.8	138.4 ± 2.299
Potassium (mmol/L)	4.95 ± 0.3546	4.375 ± 0.6228	4.988 ± 0.6728	4.771 ± 0.3251

#p< 0.001 vs. CIS, *p< 0.001 vs. C, **p<0.05 vs. C



# p<0.001 vs. C, ** p<0.05 vs. C, # p<0.001 vs. CIS

Fig. 5. Values of kidney malondialdehyde in rats treated with cisplatin

Discussion

The anticancer drug cisplatin is a very effective compound in the treatment of several cancers. Its clinical use, however, is associated with severe side effects. Main side effect which limits its use in treatment of cancers is nephrotoxicity [1, 3, 15]. Cisplatin in the kidneys penetrates the tubular cells and reaches high concentration in the proximal tubules (S3 segment), the sites most dramatically affected [7]. Glomerular injury is less frequent. Tubular damage manifests through impaired reabsorption which is characterized by reduced glomerular filtration rate, increased serum creatinine and blood urea concentrations, hypokalemia. In 20–30% of patients treatment with

cisplatin induces acute kidney injury [5, 16]. The mechanism underlying cisplatin nephrotoxicity is incompletely defined. The pathophysiological mechanism of cisplatin-induced tubular damage is complex and involves a number of interconnected factors such as accumulation of cisplatin mediated by membrane transportation, conversion into nephrotoxins, DNA damage, mitochondrial dysfunction oxidative stress, inflammatory response, activation of signal transducers and intracellular messengers and activation of apoptotic pathways [7]. In our study, quantitative evaluation of cisplatin-induced structural alterations and degree of functional alterations in the kidneys were performed by histopathological and biochemical analyses in order to determine potential beneficial effects of quercetin on cisplatin-induced nephrotoxicity. Histopathological analyses in CIS group showed easy to moderate disturbed organization of the epithelium with mild degree of degeneration and abundant cytoplasm in the proximal tubules. There were also a large number of vacuoles in the cytoplasm of tubulocytes, as well as apoptosis of certain tubules. Distal tubules were not significantly altered. Glomeruli had changed reduced lobular appearance and pronounced hyperemia. Palipoch et al. showed that the nephrotoxicity of cisplatin is dose-dependent; administration of different doses of cisplatin caused various degrees of renal impairment. At the dose of 10 mg/kg cisplatin caused presence of proteinaceous casts in the tubular lumen. Higher doses caused mild to moderate tubular necrosis, especially in the proximal tubules, in the same study. In our experiments, rats treated with quercetin revealed an almost complete prevention of histopathological alterations. There were focal degenerative changes in proximal tubules, without vacuoles and apoptosis, while glomeruli were not affected. These findings are in agreement with earlier reports [18–20] where histological changes were also consistent with laboratory findings. We found that a single dose of cisplatin caused significant increase of urea and creatinine serum concentrations ($p < 0.001$) compared with control group. The concentrations of potassium and sodium in the CIS group were lower, but not statistically significant in comparison to the C group. The toxic effects of cisplatin in our study were similar to those shown by Badary et al., who caused nephrotoxicity by cisplatin administration (7 mg/kg) as evidenced by significantly increased levels of urea and creatinine levels compared with control group. We showed that administration of quercetin (50 mg/kg), 4 days before and 4 days after single dose of cisplatin had beneficial effect on kidney treated with cisplatin. This protection was evidenced by significantly reduced levels of urea and creatinine in CISQ group in relation to the group of animals that received only cisplatin. These findings are in accordance with the results described previously [18, 19, 22]. Shimeda et al. [23] and Koyner et al. [24] suggested that underlying mechanism in cisplatin-induced nephrotoxicity is oxidative stress through elevation of ROS and reduction of the antioxidant defense system. Cisplatin generates ROS such as superoxide anion and hydroxyl radicals and stimulates renal lipid peroxidation [25–27]. It has been shown in various studies that cisplatin administrations are associated with increased formation of free radicals, and with heavy oxidative stress [26, 28, 29]. As a marker of oxidative stress and lipid peroxidation we evaluated MDA in kidneys. Levels of MDA were significantly increased in rats treated with cisplatin when compared to the control group probably due to impairment of antioxidant system. However, lipid peroxidation was significantly reduced in the animals that received cisplatin and quercetin compared with CIS group. Quercetin significantly attenuated the increase of MDA levels in renal tissue probably because of its capacity to scavenge oxygen free radicals in the kidney tubular cells of rats. Many studies suggested that quercetin has a broad range of pharmacological activities, such as anticancer, antioxidant and anti-inflammatory [30–32]. In addition to its protective effects, quercetin alone was found to be safe and did not induce any histopathological or biochemical changes in the kidneys.

Conclusion

The findings in our study clearly showed that quercetin ameliorated oxidative and histological damage caused by cisplatin. These results may indicate that quercetin is beneficial as a protective agent in cisplatin-induced nephrotoxicity.

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