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Potential Protective Effect Of Quercetin Against Cisplatin-Induced Acute Nephrotoxicity In Male Rats

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ABSTRACT

Cisplatin-induced nephrotoxicity is a significant dose limiting side effect. We investigated the possible nephroprotective effect of quercetin before and after inducing acute renal damage using cisplatin in male rats. Cisplatin administration resulted in renal damage represented by the elevated renal function parameters, elevated oxidative stress and inflammatory markers, and diminished production of the antioxidant marker. Treatment with quercetin showed reduced levels of renal function parameters, improved antioxidant status, and reduced levels of MDA and TNF-α. This study revealed a potential role of quercetin in protection against cisplatin-induced nephrotoxicity.

Keywords: Cisplatin, quercetin, nephrotoxicity, oxidative stress, lipid peroxidation, GSH, MDA, TNF-α.

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INTRODUCTION

Cisplatin is a well-known antineoplastic agent that is widely used for the treatment of several types of solid tumors (Addie et al. 2015; Chaudhari et al. 2012; Cullen et al. 2007; Ilson 2008; Ismaili, Amzerin, and Flechon 2011). It belongs to chelating anticancers that induce their anticancer effect mainly by the formation of cisplatin-DNA cross links causing cytotoxic lesions in tumors (Dasari and Tchounwou 2014). However, its use is associated with a dose limiting side effect which is nephrotoxicity. Kidney damage could be acute, which requires an intensive care to restore the renal function. Otherwise, it could subsequently be converted to chronic kidney damage, which requires more advanced intervention such as dialysis or renal transplantation (DiPiro et al. 2014).

Many studies have shown that cisplatin propagates oxidative stress (Ajith et al., 2007; Ajith *et al.*, 2009). In addition, it may participate in lipid peroxidation (Ognjanović et al. 2012). Moreover, it induces DNA damage (Cohen and Lippard 2001). Hence, the use of antioxidants has shown an ameliorating effect for cisplatin-induced renal toxicity in various animal species (Barberino et al. 2017; Somani et al. 2000). The mechanism by which antioxidants exert their protective effect against cisplatin nephrotoxicity is not fully understood.

It has been concluded that quercetin has cardiac and hepatic protection features in addition to its beneficial effect in patients with osteoarthritis and atherosclerosis (Mohammad D Al-Rekabi et al. 2014; Mohammed Dakhil Al-Rekabi 2014). Herbs, vegetables, fruits, and beverages are the main sources of flavonoids (Shahidi and Zhong 2010). Recently, interest in flavonoids has been dramatically increased due to their wide range of beneficial effects (Russo et al. 2012). Quercetin acts as a free radical scavenger, TNF-α inhibitor, and anticarcinogenic (Jan et al. 2010). Quercetin intake has shown lower incidence of cardiovascular diseases (Patel et al. 2018). In the present study, quercetin was studied for its protective effect against cisplatin-induced renal damage in male rats.

MATERIALS AND METHODS

Materials

Quercetin capsule (500 mg) was from Slovay/USA. The powder was dissolved in 5 ml of warm water and given orally by gastric savage. Cisplatin 100 ml vial containing 50 mg was from Ebewe pharma/Austria. Creatinine and urea assay kits were from Roche, Germany, rat GSH and MDA kit were from Northwest/USA and rat TNF kit was from Ray bio/USA.

Animals

Thirty adult Albino Swiss male rats (20 - 25 weeks old) with weight of (200 – 300 g) were obtained from the college of Veterinary Medicine / University of Baghdad. All animals were healthy and they were kept in cages, at temperature (25±5˚C) with ambient humidity. A cycle of 12 hour light/dark was maintained. They were fed a standard chow diet and water. The Guide for the Care and Use of Laboratory Animals USA, (1996) was followed in dealing with animals in this study.

Experimental design

After adaptation, the animals were randomly separated into 5 groups (5 rats in each group):

Control group: Animals in this group were injected with normal saline (5 ml) intraperitoneal (i.p).

Cisplatin treated group: Animals in this group were injected with cisplatin 10.5 mg/kg i.p. as a single dose.

Quercetin protection group: Animals in this group were given quercetin (100 mg/kg body weight) orally by gastric savage 8 hours before and 24 hours after cisplatin injection (Ahmed 2010).

Quercetin only treated group: Animals in this group were given quercetin 100 mg orally 8 hours before and 24 hours after injection with normal saline 5 ml i.p.

Preparation of samples

48 hours after cisplatin injection, about 3 ml of blood were obtained from each rat by cardiac puncture and the separated serum was used for the estimation of creatinine and urea. After decapitation, kidneys were rapidly removed and washed with cold isotonic saline. Each kidney was divided into two portions. One part was homogenized in phosphate buffer saline using an electronic homogenizer. Then, 10 % w/v homogenate was prepared and centrifuged (3000 rpm for 10 minutes at 4˚C). The total protein concentration was measured in the supernatant using Lowry assay to be used for the

estimation of renal tissue MDA, GSH and TNF-α. The other part of the kidney was fixed with 10% formalin, embedded in paraffin to prepare 4 µm thickness sections, and stained with hematoxylin and eosin (H and E) stain for histopathological examinations with a light microscope.

Statistical analysis

Our data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was carried out using one way ANOVA to test the significant difference between groups. Data analysis and plots were generated by GraphPad Prism 7. Those data with p value less than 0.05 were considered statistically significant.

RESULTS

Renal function parameters (blood urea and serum creatinine)

Animals injected with cisplatin showed a significantly higher level of blood urea and serum creatinine ($p < 0.05$) when compared to control. However, treatment with quercetin resulted in significantly lower urea and creatinine levels ($p < 0.05$) when compared to cisplatin group (**Figure 1 A** and **B**, respectively).

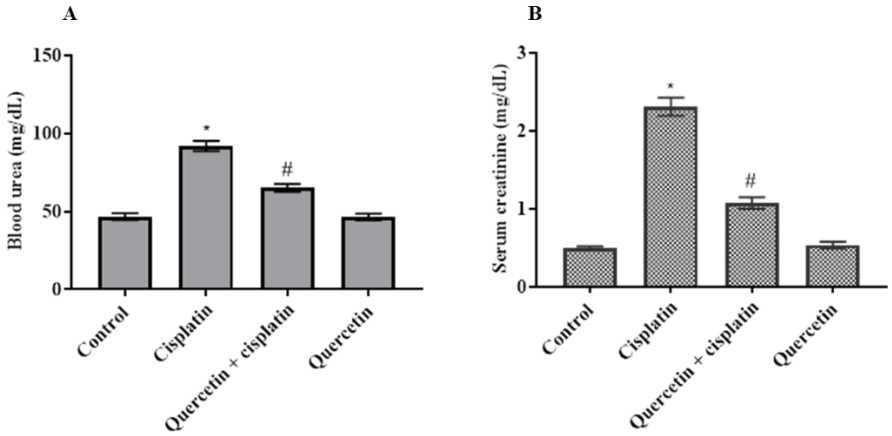

Figure 1: Renal function test in control, cisplatin-treated, quercetin plus cisplatin (protection) and quercetin only groups **A**: blood urea and **B**: serum creatinine (n=5 and data are presented as Mean±SEM). *: p<0.05 when compared to control and #: p<0.05 when compared to cisplatin group.

Renal tissue parameters

Treatment with cisplatin showed a significantly lower level of renal tissue GSH ($p < 0.05$) in comparison with control group. However, protection with quercetin significantly improved the level of GSH ($p < 0.05$) when compared to cisplatin group (**Figure 2 A**).

Cisplatin injection resulted in a significantly higher level of tissue MDA ($p < 0.05$) in comparison with control. Treatment with quercetin showed a significantly lower level of tissue MDA ($p < 0.05$) when compared to cisplatin group (**Figure 2 B**).

The level of renal tissue TNF-α in cisplatin group was significantly higher ($p < 0.05$) than its level in the control group. Treatment with quercetin showed a significantly lower level of TNF-α ($p < 0.05$) when compared to cisplatin only group (**Figure 2 C**).

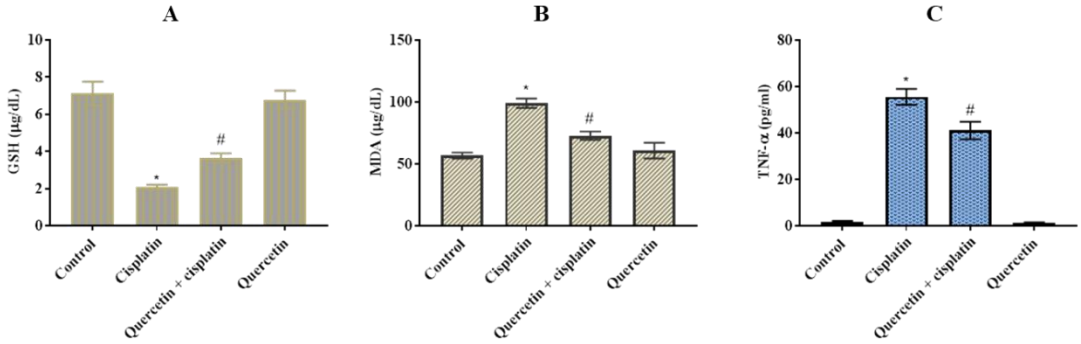

Figure1: Renal tissue parameters in control, cisplatin-treated, quercetin plus cisplatin (protection) and quercetin only groups **A**: GSH **B**: MDA and **C**: TNFα (n=5 and data are presented as Mean±SEM). *: p<0.05 when compared to control and #: p<0.05 when compared to cisplatin group.

Histopathological sections

There was normal appearance for all parts of the kidney the control group (**Figure 3 A**). However, animals treated with cisplatin showed tubular degeneration with variable severity according to kidney zone (**Figure 3 B**). On the other hand, animals treated with quercetin showed significant improvement in the histopathological appearance when compared to the cisplatin group (**Figure 3 C**).

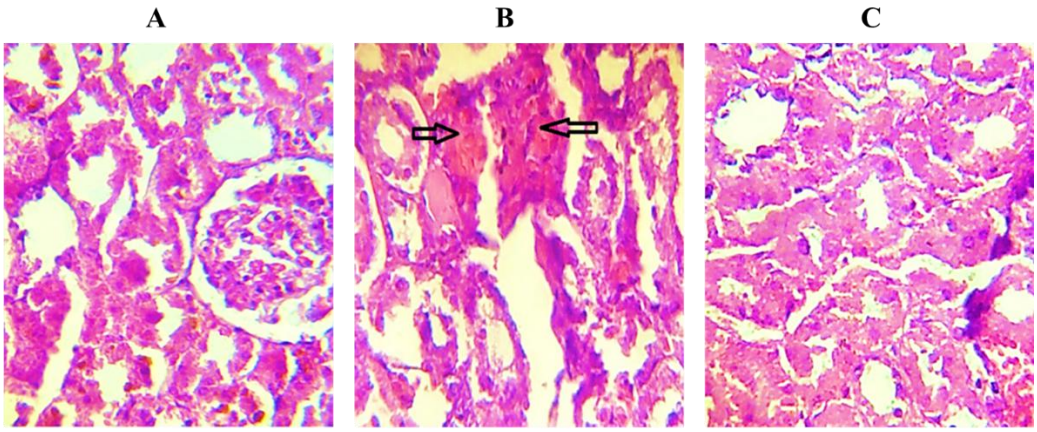

Figure 3: Light microscopy of renal tissue of control (**A**), cisplatin (**B**) and quercetin plus cisplatin treated (**C**) groups stained with H and E stain (high magnification power, × 400). The control shows normal appearance of renal tissues while cisplatin treatment resulted in proximal tubules coagulative necrosis in the cortico-medulary junction (arrows). The tubular epithelial cells casts are in the lumen of the proximal tubule. Quercetin treatment resulted in mild eosinophilic degeneration in the proximal tubules.

DISCUSSION

Cisplatin nephrotoxicity has been reported in several animal models as well as at the clinical level (Yao et al. 2007). High levels of serum urea and creatinine concentrations compared to the control group indicated the presence of acute renal damage. A similar effect was reported by Somani *et al.* (2000) and Badary et al. (2005). Elevated urea and creatinine levels in cisplatin group and decreased levels of urea and creatinine in quercetin protection groups indicate an acute toxic effect of cisplatin and a protective effect of quercetin, respectively.

The renal antioxidant status, including the activity of GSH enzyme is one of the important mechanisms involved in the oxidative homeostatic balance. When GSH depleted, organs become more sensitive to oxidative stress and cell injury (Ahmed and Zaki, 2009). GSH depletion could also be an important factor involved in lipid peroxidation in cisplatin group. Quercetin reversed the depletion of GSH that means it has a potential protective effect to the kidney (Ajith et al., 2007). The cisplatin-induced nephrotoxicity may be attributed to renal accumulation of platinum as well as covalent binding of platinum to renal proteins (Murata et al. 2004).

Superoxide dismutase (SOD) depletion could be responsible for initiation and propagation of lipid peroxidation, a process that may be attributed to inactivation of SOD induced by the loss of copper and zinc, or due to reactive oxygen species (Yilmaz et al. 2005). MDA production is elevated due to lipid peroxidation in cisplatin treated group. Lipid peroxidation and oxygen reactive species were antagonized by treatment with quercetin which resulted in prevention of lipid peroxidation and enhancement of renal SOD, catalase (CAT) and GPx activities since phenolic compounds are powerful antioxidants, inhibitors of lipid peroxidation and ROS scavenging activity (Rodrigues et al., 2013). Free radicals may have effects such as decreased membrane fluidity, disrupted membrane structure, and impaired membrane function (Habon et al. 2001; Haraguchi et al., 1997). *In vitro* and *in vivo* studies have revealed that flavonoids are strong radical scavengers. In addition, their antioxidant activity and hepatoprotective effect have been confirmed (Al-Rasheed et al. 2016; Mohammad D Al-Rekabi et al. 2014).

TNF-α activates the initiator caspases (1, 8, and 9) which in turn activate caspase 3, an important executioner caspase in renal tubule apoptosis (Gao et al. 2007; Tsuruya et al. 2003). Apoptosis is a well known mode of cell death. Cisplatin can also induce apoptosis even in the absence of Fas. However, whether this process is involved in cisplatin nephrotoxicity is not fully investigated (Dimanche-Boitrel et al. 2005). Interestingly, Ramesh and Reeves (2002) reported a protective effect of TNF-α-neutralizing antibody from cisplatin nephrotoxicity.

In conclusion, quercetin ameliorates the renal damage induced by cisplatin in rats. The protection showed by using quercetin may be partially attributed to the prevention of cisplatin-induced inhibition of renal antioxidant system. It could also be related to inhibited lysosomal membrane damage. Future work may include the assessment of a range of doses of quercetin, using different members of flavonoids or assessment of any potential protective effect for the brain.

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