

## Beneficial Effects of Hesperidin against Cisplatin-Induced Nephrotoxicity and Oxidative Stress in Rats

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**Abstract:** Cisplatin has been frequently used for treatment of wide variety of tumors. The use of cisplatin is associated with severe cytotoxicity such as nephrotoxicity, hepatotoxicity and spermiotoxicity which radically limits its clinical use. The present study aimed to investigate the possible protective effects of multiple doses of hesperidin against cisplatin-induced nephrotoxicity induced by single i.p injection of cisplatin (7.5 mg/kg). Hesperidin was given to rats at two different doses (100 and 200 mg/kg p.o) for 7 days starting one day before cisplatin injection. Blood samples were collected for determination of serum creatinine and Blood Urea Nitrogen (BUN) levels. Kidneys were used for the determination of Malondialdehyde (MDA), Glutathione (GSH) and total nitrate and nitrite contents. Liver samples were also used for histopathological examination. Results showed that hesperidin significantly reduced cisplatin-induced elevations in serum creatinine and BUN levels. It also significantly reduced kidney MDA and NO content and elevated GSH content. In conclusion, hesperidin greatly protected kidney against cisplatin-induced toxicity in a dose-dependent manner.

**Keywords:** Blood urea nitrogen, cisplatin, creatinine, hesperidin, nephrotoxicity, oxidative stress

### INTRODUCTION

Cisplatin (*cis*-diammine-dichloro-platinum) has been frequently considered as the first choice chemotherapeutic agent for treatment of a wide variety of solid tumors, including ovarian, testicular, bladder, head and neck, esophageal and small cell lung cancers (Weijl *et al.*, 1997; Previati *et al.*, 2006; Shona *et al.*, 2012). About 70-80% of patients respond to platinum treatment. However, such an initial effect is not robust and results in a 5-year patient survival of only 15-20%, as tumors become resistant to therapy (Siddik, 2003). The relapse of the disease and the emergence of resistance in initially responsive tumors occur with 18-24 months (Siddik, 2003; Shi *et al.*, 2007). The dose scale necessary to overcome even a small increase in cellular resistance can lead to severe cytotoxicity in normal cells such as nephrotoxicity, hepatotoxicity and spermiotoxicity (Zicca *et al.*, 2002; Atessahin *et al.*, 2006; Palipoch *et al.*, 2014) which radically limits the clinical usefulness of cisplatin-based therapy.

The mechanism of cisplatin-induced nephrotoxicity has been clearly reported through multiple mechanisms including hypoxia, the generation of free radicals, inflammation and apoptosis with an increase in the pro-apoptotic protein Bax and a decrease in the anti-apoptotic protein Bcl-2 (Tsuruya *et al.*, 2003).

Studies showed that the generation of Reactive Oxygen Species (ROS) such as hydroxyl radical and superoxide anion are involved in the mechanism of cisplatin toxicity (Hussein *et al.*, 2012) which causes an elevation in Lipid Peroxidation (LPO), reduction in levels of protein bound sulfhydryl groups and glutathione (Pratibha *et al.*, 2006; Iraz *et al.*, 2006).

Knowing that cisplatin chemotherapy induces a fall in plasma antioxidant levels, which may reflect a failure of the antioxidant defense mechanism against the oxidative damage induced (Weijl *et al.*, 1998), consequently the aim of the present study is that combination treatment of cisplatin and a naturally occurring antioxidant flavonoid hesperidin may be a useful strategy to protect against cisplatin-induced nephrotoxicity.

Hesperidin, flavonoid found in vegetables and fruits (Justesen *et al.*, 1998), has many useful effects such as antiallergic, antioxidant and anti-inflammatory (Garg *et al.*, 2001). It has also anticarcinogenic effects in different variety of tumors (Lee *et al.*, 2010). Hesperidin was proved to have antioxidant and free radicle scavenging properties (Fraga *et al.*, 1987).

### MATERIAL and METHODS

**Animals:** Adult male Wistar rats (120-150 g) obtained from the National Research Centre (Giza, Egypt) were

used in the current study. They were housed 10 per cage and maintained at 25±2°C with free access to water and food, under a 12/12 h light-dark cycle. All procedures in this study were carried out according to guidelines of Ethics Committee of Faculty of Pharmacy, Cairo University.

**Drugs and chemicals:** Cisplatin was purchased from Sigma-Aldrich (USA), silymarin and hesperidin was purchased from Sigma-Aldrich (USA). Thiobarbituric acid, Ellman's reagent, vanadium trichloride, N-(1-Naphtyl) ethylenediamine dihydrochloride and sulfanilamide were purchased from Sigma-Aldrich (USA). Blood Urea Nitrogen Kit and Creatinine Kit were purchased from (Diamond Laboratory Reagents).

**Experimental design:** Rats were divided into 7 groups, each of 6-8 rats. It was proved that intra- peritoneal injection of cisplatin at a dose of 7.5 mg/kg causes induction of hepatotoxicity (Mansour *et al.*, 2006):

**Group 1 :** Received vehicle (2% Tween 80, p.o.) and acts as negative control.

**Group 2 :** Received silymarin (100 mg/kg/day, p.o.) and acts as silymarin control.

**Group 3 :** Received hesperidin (200 mg/kg/day, p.o.) and acts as hesperidin control.

**Group 4 :** Received vehicle (2% Tween 80, p.o.) and acts as cisplatin control.

**Group 5 :** Received silymarin (100 mg/kg/day, p.o.).

**Group 6 :** Received hesperidin (100 mg/kg/day, p.o.).

**Group 7 :** Received hesperidin (200 mg/kg/day, p.o.).

Groups 1-3 received a single i.p. saline on second day. Groups 4-7 received a single dose of cisplatin (7.5 mg/kg i.p) on second day 1 hour after vehicle or drug administration.

At the end of the experiment, rats were fasted overnight, blood was collected and serum was separated by centrifugation at 3000 r.p.m. and used for biochemical estimations including:

- Serum creatinine (Cr) and Blood Urea Nitrogen (BUN) using colorimetric assay kits (Diamond Diagnostics, Egypt)

For tissue biomarkers and histopathological estimations; animals were sacrificed then kidneys were dissected out, washed with cold normal saline and dried between two filter papers.

A portion of each kidney tissue was homogenized and the homogenates were centrifuged at 1000×g for 15 min and the obtained supernatants were used for the estimation of tissue biomarkers namely, reduced Glutathione (GSH) according to the method described by Beutler *et al.* (1963) and Arab *et al.* (2014) and expressed as mg%, Thiobarbituric acid Reactive Substances (TBARS) content, estimated as Malondialdehyde (MDA) according to the method

described by Mihara and Uchiyama (1978) and Mehany *et al.* (2013) and expressed as nmol/ml and total nitrate/nitrite (NO<sub>x</sub>) content according to the method described by Miranda *et al.* (2001).

**Histopathological examination:** Another portion of each kidney tissue was preserved in formalin solution in saline for a time sufficient for hardening of tissue to be sectioned for histopathological examination. Autopsy samples were taken from the liver and kidney of rats in different groups and fixed in 10% formalsaline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidgemicrotome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain for routine examination then examination was done through the light electric microscope (Bancroft *et al.*, 1996).

**Statistical analysis:** The values of the measured parameters were presented as mean ± SEM. Comparisons between different treatments were carried out using one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparisons test. Differences were considered statistically significant at p<0.05.

## RESULTS

**Effect of hesperidin on cisplatin-induced elevations in serum Cr and BUN levels:** Cisplatin significantly increased serum Cr and BUN levels when compared to normal control group. Oral administration of silymarin 100 mg/kg and hesperidin 200 mg/kg significantly decreased cisplatin-induced increase in serum Cr and BUN levels when compared to cisplatin control group. Administration of hesperidin 100 mg/kg significantly reduced elevated serum Cr but did not significantly affect elevated BUN level. No significant difference was observed in serum Cr and BUN levels between normal control and silymarin or hesperidin control groups (Fig.1 and 2).

**Effect of hesperidin on renal lipid peroxides content:** Cisplatin significantly elevated renal MDA content as compared to normal control group. Silymarin 100 mg/kg p.o and hesperidin 200 mg/kg p.o significantly decreased cisplatin-induced elevation in renal MDA content when compared to cisplatin control group. Hesperidin 100 mg/kg p.o did not significantly affect elevated renal MDA content. No significant difference was observed in renal MDA content in silymarin or hesperidin control groups (Fig.3).

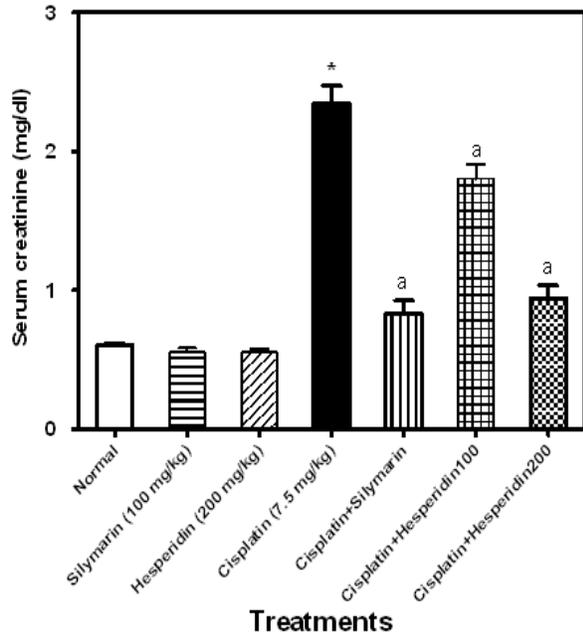


Fig. 1: Effect of hesperidin treatment on serum creatinine level in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean  $\pm$  SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; \*: significantly different from normal control at  $p < 0.05$ ; @: Significantly different from cisplatin control at  $p < 0.05$

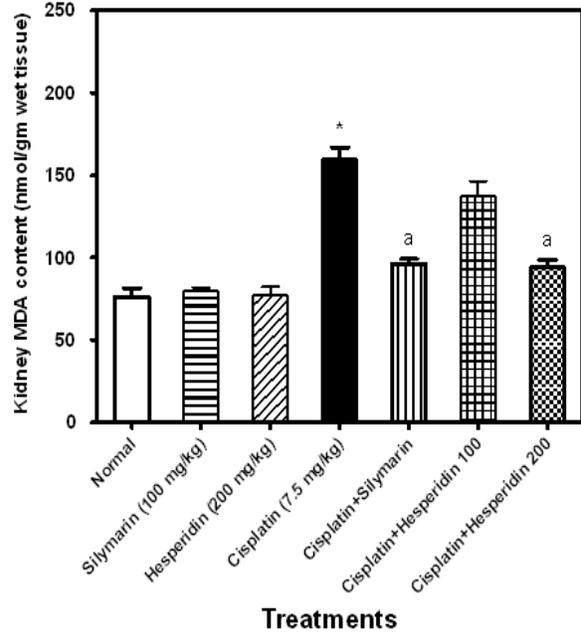


Fig. 3: Effect of hesperidin treatment on kidney MDA content in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean  $\pm$  SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; \*: Significantly different from normal control at  $p < 0.05$ ; @: Significantly different from cisplatin control at  $p < 0.05$

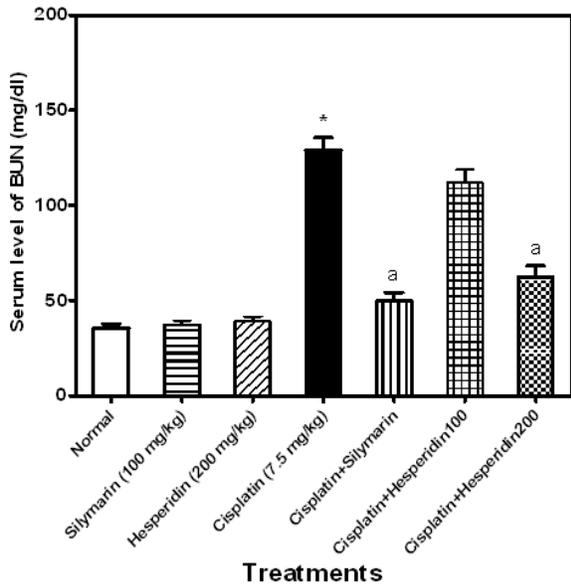


Fig. 2: Effect of hesperidin treatment on serum BUN level in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean  $\pm$  SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; \*: Significantly different from normal control at  $p < 0.05$ ; @: Significantly different from cisplatin control at  $p < 0.05$

**Effect of hesperidin on renal reduced glutathione content:** Cisplatin significantly reduced renal GSH content as compared to normal control group. Oral administration of silymarin 100 mg/kg and hesperidin 100 or 200 mg/kg significantly increased cisplatin-induced reduction in GSH content when compared to cisplatin control group. No significant difference was observed in renal GSH content between normal control and silymarin or hesperidin control groups (Fig. 4).

**Effect of hesperidin on renal nitrate/nitrite (NO) content:** Cisplatin significantly elevated renal NO content in cisplatin control group as compared to normal control group. Silymarin 100 mg/kg p.o and hesperidin 200 mg/kg significantly decreased cisplatin-induced elevation in renal NO content when compared to cisplatin control group. No significant difference was observed in renal NO content between normal control and silymarin or hesperidin control groups (Fig. 5).

**Histopathology:** There was no histopathological alteration and the normal histological structure of the glomeruli and tubules at the cortex and the tubules at the corticomedullary portion were recorded in Fig. 6A. Silymarin 100 mg/kg and hesperidin 200 mg/kg on normal rats showed normal structure of the

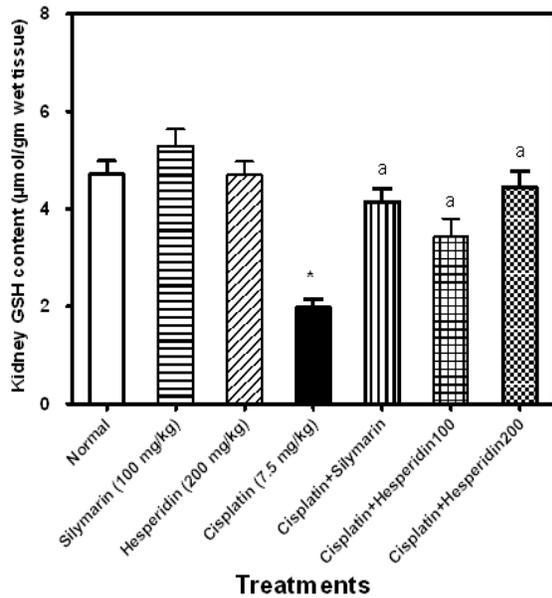


Fig. 4: Effect of hesperidin treatment on kidney GSH content in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean  $\pm$  SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; \*: significantly different from normal control at  $p < 0.05$ ; @: Significantly different from cisplatin control at  $p < 0.05$

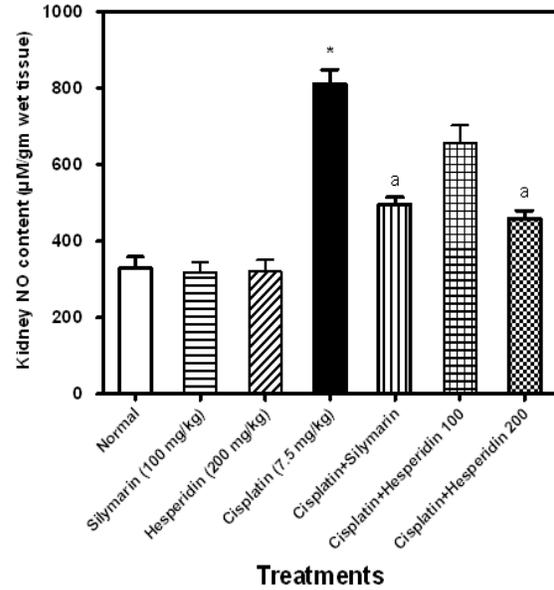


Fig. 5: Effect of hesperidin treatment on kidney NO content in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean  $\pm$  SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; \*: significantly different from normal control at  $p < 0.05$ ; @: Significantly different from cisplatin control at  $p < 0.05$

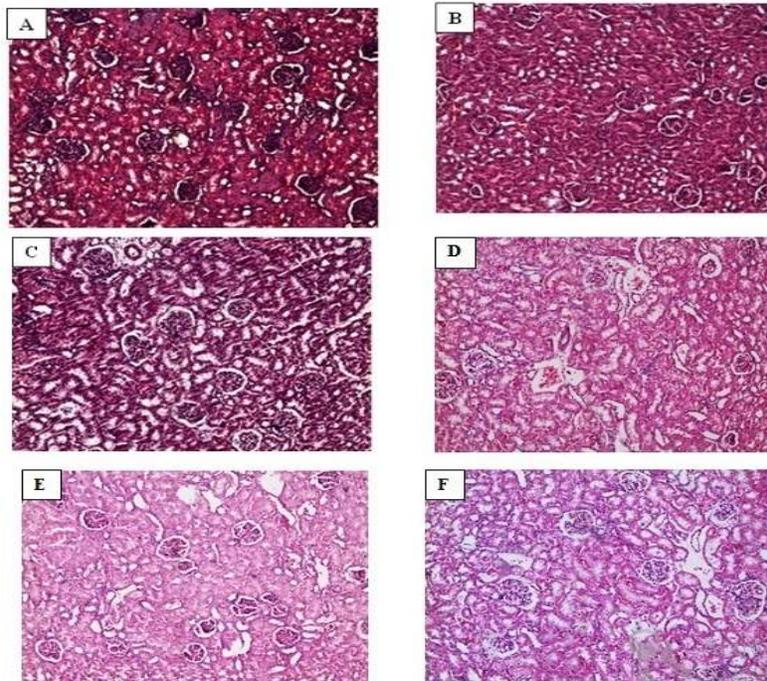


Fig. 6: Photo micrograph of rat kidney tissues H&E staining; (A): Normal control group; (B): Silymarin control group and (C): Hesperidin control group, sections of kidney showing normal histological structure of the glomeruli and tubules at the cortex and the tubules at the corticomedullary; (D): Cisplatin control group, showing Coagulation necrosis was noticed in most of the tubules associated with congestion in the glomerular tuft and cystic dilatation as well as cast formation in the tubules of both cortical and corticomedullary portions; (E): Silymarin + Cisplatin and (F): Hesperidin (200 mg/kg) + Cisplatin, showing nearly normal structure of the kidney with no histopathological alterations observed

kidney; (Fig. 6B and 6C). Cisplatin control group showed coagulation necrosis in most of the tubules associated with congestion in the glomerular tuft and cystic dilatation as well as cast formation in the tubules of both cortical and corticomedullary portions (Fig. 6 D). Silymarin 100 mg/kg and hesperidin 200 mg/kg on cisplatin administered rats showed nearly normal structure of the kidney with no histopathological alterations observed (Fig. 6 E and 6F).

## DISCUSSION

Results of the present study states that cisplatin significantly produced nephrotoxicity in rats. Nephrotoxicity was proved by significant elevations in serum levels of creatinine and BUN as compared to normal control group. Cisplatin administration to rats showed histopathological changes in kidney including coagulation necrosis was noticed in most of the tubules associated with congestion in the glomerular tuft and cystic dilatation as well as cast formation in the tubules of both cortical and corticomedullary portions. These findings are similar to those obtained by previous investigators (Prabhu *et al.*, 2013; Sahu *et al.*, 2013).

Abdelmeguid *et al.* (2010b) showed that cisplatin (5 mg/kg, i.p.), in rats caused significant deterioration of renal corpuscle structure and increased tubular necrosis after 2 weeks. Also Chirino *et al.* (2004) showed that cisplatin treatment induced mesangial cells contraction.

Cisplatin treatment produced several responses including dysfunction of mitochondria, DNA damage, membrane peroxidation and protein synthesis inhibition (Cohen and Lippard, 2001; Sadowitz *et al.*, 2002; Naqshbandi *et al.*, 2012).

Cisplatin stimulates ROS production by damaged mitochondria, which elevates free radical production and reduce antioxidant production (Kawai *et al.*, 2006; Silici *et al.*, 2011).

It was proved that cisplatin exerts its anticancer effect through induction of free radicles such as peroxy radicals, superoxide radicles, hydroxy radicals and singlet oxygen, which cause kidney damage at the same time (Karadeniz *et al.*, 2011). These free radicals attack the highly unsaturated fatty acids of the cell membrane inducing lipid peroxidation which is considered the key process in several pathological events (Weijl *et al.*, 1997; Schinella *et al.*, 2002).

This action of cisplatin is confirmed in the present study by significant increase in lipid peroxidation, measured as MDA level, as compared to normal control rats. Injection of cisplatin caused an increase in kidney lipid peroxides content and a reduction in the antioxidant enzymes activities which protect against tissues lipid peroxidation (Cayir *et al.*, 2009; Goldstein and Mayor, 1983). Several cellular pathways have been suggested to contribute to induction of oxidative stress

and lipid peroxidation. Safirstein (1999) explained that the main targets of cisplatin in kidney are proximal straight and distal convoluted tubules, where it accumulates and encourages cellular damage by multiple mechanisms such as oxidative stress, DNA damage and apoptosis (Karadeniz *et al.*, 2011).

Moreover, the decreased activities of enzymatic antioxidants caused reduction in scavenging of ROS which in turn leads to oxidative stress (Palipoch *et al.*, 2014). This was shown in the present results by the significant reduction in tissue levels of GSH and similar results were obtained by previous studies (Mansour *et al.*, 2006; Sahu *et al.*, 2013).

The present work reveals that cisplatin significantly increased kidney levels of NO. Excessive production of NO causes vasodilatation and hypotension leading to organ hypoperfusion, edema and organ dysfunction. NO can interact with ROS to form peroxynitrite, which is a powerful oxidant and cytotoxic agent and may play an important role in the cellular damage associated with the overproduction of NO (Ahmad *et al.*, 2012). Another possible explanation of cisplatin toxicity is that cisplatin induces a cascade of inflammatory reactions, that play an important pathogenic role in cisplatin-induced tissues injury (Pabla and Dong, 2008; Hussein *et al.*, 2012).

Oral administration of silymarin to cisplatin treated rats significantly reduced the elevated serum levels of creatinine and BUN. It also improved the oxidative stress parameters by significantly decreasing the elevated levels of kidney MDA, NO and significantly increased kidney levels of GSH as compared to cisplatin control groups.

This action of silymarin is attributed to its antioxidant effect and its free radical scavenging activity, consequently protecting membrane permeability (Hakova *et al.*, 1996; Mansour *et al.*, 2006).

The nephroprotective properties of silymarin are due to free radical scavenging and elevating the cellular content of glutathione that lead to inhibition of the lipid peroxidation and stabilizing plasma membrane (Karimi *et al.*, 2011). In addition, silymarin stimulates ribosomal protein synthesis by means of stimulating RNA polymerase I and so counteracting the reduction in macromolecule synthesis in the tissues (Gaedeke *et al.*, 1996; Saller *et al.*, 2007; Dashti-Khavidaki *et al.*, 2012). Moreover, silymarin maintains the effective levels of  $\alpha$ -tocopherol,  $\beta$ -carotene and ascorbate, resulting in an increased cell viability and preserved functionality in several animal models and cell lines (Comelli *et al.*, 2007). Several studies revealed that silymarin reduces the nephrotoxic effects of cisplatin without decreasing its anti-tumour activity. The renoprotective effects of silymarin were more apparent when it was administered before cisplatin (Dashti-Khavidaki *et al.*, 2012).

In addition, silymarin greatly improved the histopathological findings observed with cisplatin control as silymarin showed nearly normal histopathological structure of the kidney. In agreement with our results, Karimi *et al.*, (2005) and Abdelmeguid *et al.*, (2010b) showed that silymarin post-treatment attenuated glomerular atrophy that revealed minimal erythrocytes leakage and slightly dilated urinary space. Also previous studies showed that pretreatment with silymarin 2 h before cisplatin significantly decreased the pathological changes induced by cisplatin and appeared highly protective (Abdelmeguid *et al.*, 2010a)

Administration of high dose of hesperidin 200 mg/kg significantly decreased elevated serum levels of creatinine and BUN. On the other hand administration of low dose of hesperidin 100 mg/kg only significantly decreased creatinine but failed to produce any significant decrease in BUN. It is obvious that there is dose-dependent increase in response of hesperidin as by increasing the dose the response becomes better in all measured parameters.

Furthermore, hesperidin 200 mg/kg significantly decreased cisplatin-induced elevation in kidney MDA and NO while increased cisplatin-induced reduction in kidney GSH. Hesperidin 100 mg/kg failed to decrease elevated levels of kidney MDA and NO while significantly increased kidney GSH. Also these results of hesperidin indicate that there is a dose-dependent increase in response. The histopathological findings showed that hesperidin greatly protected kidneys against cisplatin-induced damage.

Studies demonstrated that hesperidin enhanced the antioxidant defense mechanisms (Garg *et al.*, 2001; Loguercio and Federico, 2003). Hesperidin improved GSH levels in kidneys of diabetic rats and decreased DNA fragmentation, in the urine of diabetic rats (Miyake *et al.*, 1998). Hesperidin in combination with diosmin inhibited the reactive oxygen radicals production in Zymosan-stimulated human polymorphonuclear neutrophils (Jean and Bodinier, 1994). Consequently, in-vivo and in-vitro studies have been shown that hesperidin reduced oxidative stress (Tirkey *et al.*, 2005).

In addition studies proved that flavonoids are hydrogen-donors and free-radical scavengers and so they act as potential chainbreaking antioxidants to inhibit the low density lipoprotein oxidation (Cotelle *et al.*, 1996; Kaur *et al.*, 2006). Furthermore, other studies have indicated that flavonoids can chelate transition metal-ions so can inhibit free radical formation and the propagation of free-radical reactions (Morel *et al.*, 1993; Haller and Hizoh, 2004).

In conclusion, the present study proved that oxidative stress plays an important role in cisplatin-induced nephrotoxicity. Hesperidin, decreased cisplatin-induced kidney damage, suppressed cisplatin-induced generation of ROS, lipid peroxidation and

oxidative stress. All these obtained results indicate that hesperidin provide protective effects on cisplatin-induced nephrotoxicity and it may find use as an adjunct in the cancer chemotherapy in which cisplatin is used as first drug.

#### ACKNOWLEDGMENT

This study was supported by a research grant from the Scientific Research Development Center, Beni-Suef University, Beni-Suef, Egypt.

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