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Research Article

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).

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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±2ºC 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 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 hepatptoxicity (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 (NOx) 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% formolsaline 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

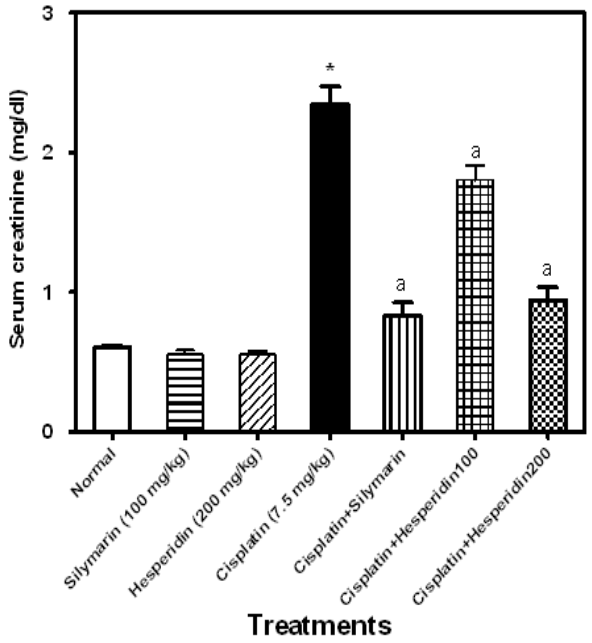

Fig. 1: Effect of hesperidin treatment on serum creatinine level in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean ± SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: significantly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

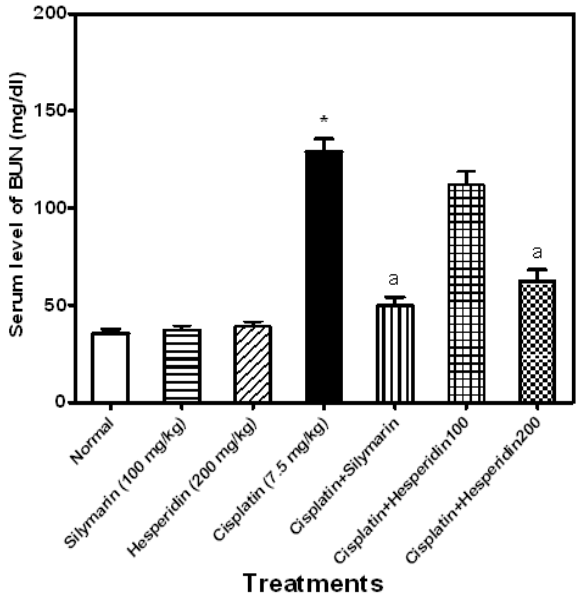

Fig. 2: Effect of hesperidin treatment on serum BUN level in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean ± SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: Significantly different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

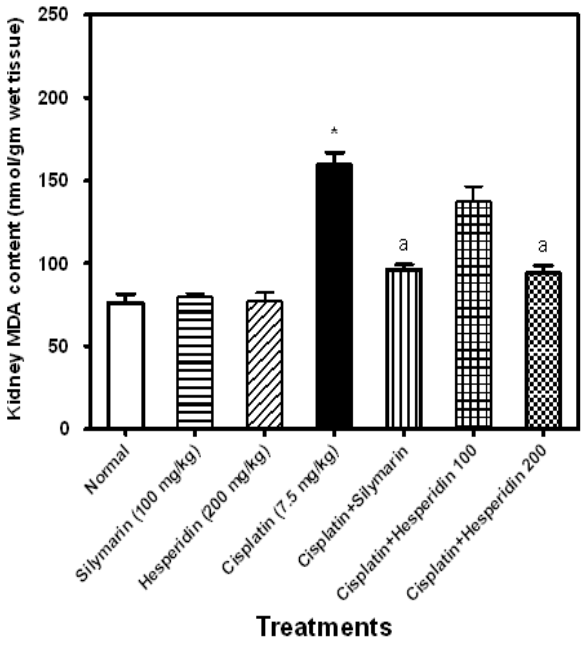

Fig. 3: Effect of hesperidin treatment on kidney MDA content in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean ± SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: Significantly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

was observed in renal MDA content in silymarin or hesperidin control groups (Fig.3).

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

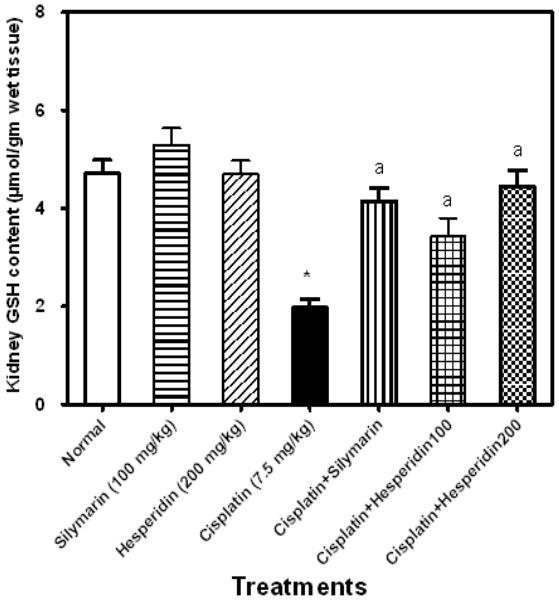

Fig. 4: Effect of hesperidin treatment on kidney GSH content in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean ± SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: significantly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

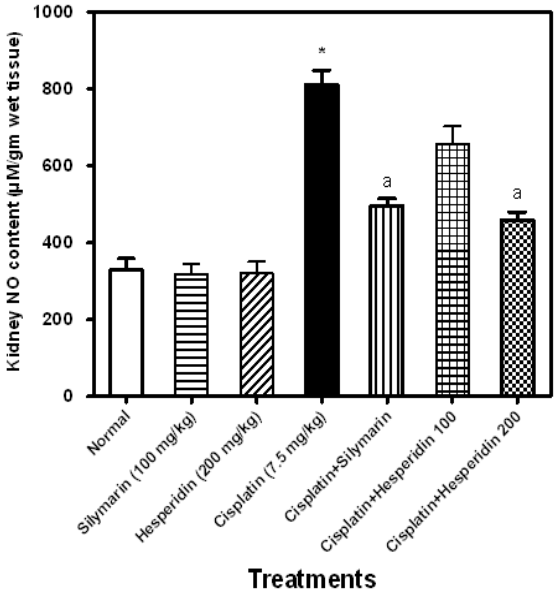

Fig. 5: Effect of hesperidin treatment on kidney NO content in cisplatin administered rats; N = 6-8 rats per group. Each value represents the mean ± SE of the mean; Statistics were carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: significantly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

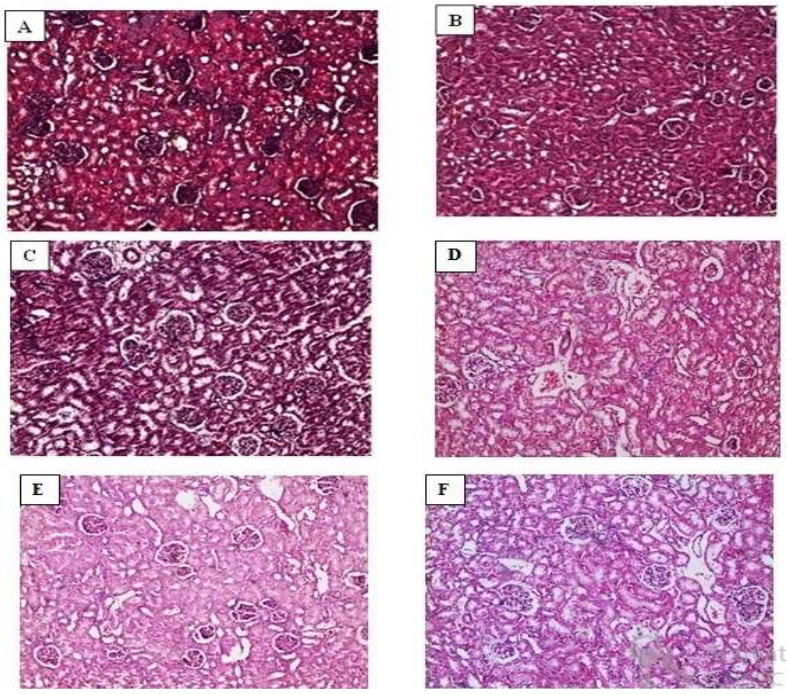

Fig. 6: Photo micrograph of rat kidney tissues H&E staining; (A): Normal control group; (B): Silymarin control group and (C): Hesperidin control group, sections of kidney showing normal histological structure of the glomeruli and tubules at the cortex and the tubules at the corticomedullary; (D): Cisplatin 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 peroxyl 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 peroxidatin. 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 α-tocopherol, β-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.

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REFERENCES

Abdelmeguid, N.E., H.N. Chmaisse and N.S. Abou Zeinab, 2010a. Silymarin ameliorates cisplatin-induced hepatotoxicity in rats: Histopathological and ultrastructural studies. Pak. J. Biol. Sci., 13(10): 463-479.

Abdelmeguid, N.E., H.N. Chmaisse and N.A. Zeinab, 2010b. Protective effect of silymarin on cisplatin-induced nephrotoxicity in rats. Pakist. J. Nutr., 9(7): 624-636.

Ahmad, S.T., W. Arjumand, S. Nafees, A. Seth, N. Ali, S. Rashid and S. Sultana, 2012. Hesperidin alleviates acetaminophen induced toxicity in Wistar rats by abrogation of oxidative stress, apoptosis and inflammation. Toxicol. Lett., 208(2): 149-161.

Arab, H.H., S.A. Salama, A.H. Eid, H.A. Omar, S.A. Arafa el and I.A. Maghrabi, 2014. Camel's milk ameliorates TNBS-induced colitis in rats via downregulation of inflammatory cytokines and oxidative stress. Food Chem. Toxicol., 69: 294-302.

Atessahin, A., I. Karahan, G. Turk, S. Gur, S. Yilmaz and A.O. Ceribasi, 2006. Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Reprod. Toxicol., 21(1): 42-47.

Bancroft, J.D., A. Stevens and D.R. Turner, 1996.Theory and Practice of Histological Techniques.4th Edn., Churchill Livingstone,New York, London, San Francisco, Tokyo.

Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Cayir, K., A Karadeniz, A. Yildirim, Y. Kalkan, A. Karakoc, M. Keles and S.B. Tekin, 2009. Protective effect of L-carnitine against cisplatin-induced liver and kidney oxidant injury in rats. Cent. Eur.J. Med., 4(2): 184-191.

Chirino, Y., R.Hernández-Pando, J. Pedraza-Chaverrí 2004. Peroxynitrite decomposition catalyst ameliorates renal damage and protein nitration in cisplatin-induced nephrotoxicity in rats. BMC Pharmacol., 4(1): 20.

Cohen, S.M. and S.J. Lippard, 2001. Cisplatin: From DNA damage to cancer chemotherapy. Prog. Nucl. Acid Res. Mol. Biol., 67: 93-130.

Comelli, M.C., U. Mengs, C. Schneider and M. Prosdocimi, 2007. Toward the definition of the mechanism of action of silymarin: Activities related to cellular protection from toxic damage induced by chemotherapy. Integr. Cancer Ther., 6(2): 120-129.

Cotelle, N., J.L. Bernier, J.P. Catteau, J. Pommery, J.C. Wallet and E.M. Gaydou, 1996. Antioxidant properties of hydroxy-flavones. Free Radic. Biol Med., 20(1): 35-43.

Dashti-Khavidaki, S., F. Shahbazi, H. Khalili and M. Lessan-Pezeshki, 2012. Potential renoprotective effects of silymarin against nephrotoxic drugs: A review of literature. J. Pharm. Pharm. Sci., 15(1): 112-123.

Fraga, C.G., V.S. Martino, G.E. Ferraro, J.D. Coussio and A. Boveris, 1987. Flavonoids as antioxidants evaluated by< i> in vitro</i> and< i> in situ</i> liver chemiluminescence. Biochem.Pharmacol., 36(5): 717-720.

Gaedeke, J., L.M. Fels, C. Bokemeyer, U. Mengs, H. Stolte and H. Lentzen, 1996. Cisplatin nephrotoxicity and protection by silibinin. Nephrol. Dial. Transplant., 11(1): 55-62.

Garg, A., S. Garg, L.J. Zaneveld and A.K. Singla, 2001. Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. Phytother. Res., 15(8): 655-669.

Goldstein, R.S.and G.H. Mayor, 1983. Minireview. The nephrotoxicity of cisplatin. Life Sci., 32(7): 685-690.

Hakova, H., E. Misurova and K. Kropacova, 1996. The effect of silymarin on concentration and total content of nucleic acids in tissues of continuously irradiated rats. Vet. Med. (Praha), 41(4): 113-119.

Haller, C. and I. Hizoh, 2004. The cytotoxicity of iodinated radiocontrast agents on renal cells in vitro. Invest. Radiol., 39(3): 149-154.

Hussein, A., A.A. Ahmed, S.A. Shouman and S. Sharawy, 2012. Ameliorating effect of DL-alpha-lipoic acid against cisplatin-induced nephrotoxicity and cardiotoxicity in experimental animals. Drug Discov. Ther., 6(3): 147-156.

Iraz, M., E. Ozerol, M. Gulec, S. Tasdemir, N. Idiz, E. Fadillioglu, M. Naziroglu and O. Akyol, 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.

Jean, T. and M.C. Bodinier, 1994. Mediators involved in inflammation: effects of Daflon 500 mg on their release. Angiology, 45(6 Pt 2): 554-559.

Justesen, U., P. Knuthsen and T. Leth, 1998. Quantitative analysis of flavonols, flavones and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. J. Chromatogr. A, 799(1-2): 101-110.

Karadeniz, A., N. Simsek, E. Karakus, S. Yildirim, A. Kara, I. Can, F. Kisa, H. Emre and M. Turkeli, 2011. Royal jelly modulates oxidative stress and apoptosis in liver and kidneys of rats treated with cisplatin. Oxid. Med. Cell Longev., 2011: 981793.

Karimi, G., M. Ramezani and Z. Tahoonian, 2005. Cisplatin nephrotoxicity and protection by milk thistle extract in rats. Evid.-Based Compl. Alt., 2(3): 383-386.

Karimi, G., M. Vahabzadeh, P. Lari, M. Rashedinia and M. Moshiri, 2011. "Silymarin":A promising pharmacological agent for treatment of diseases. Iran J. Basic Med. Sci., 14(4): 308-317.

Kaur, G., N. Tirkey and K. Chopra, 2006. Beneficial effect of hesperidin on lipopolysaccharide-induced hepatotoxicity. Toxicology, 226(2-3): 152-160.

Kawai, Y., T. Nakao, N. Kunimura, Y. Kohda and M. Gemba, 2006. Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. J. Pharmacol. Sci., 100(1): 65-72.

Lee, K.H., M.H. Yeh, S.T. Kao, C.M. Hung, C.J. Liu, Y.Y. Huang and C.C. Yeh, 2010. The inhibitory effect of hesperidin on tumor cell invasiveness occurs via suppression of activator protein 1 and nuclear factor-kappaB in human hepatocellular carcinoma cells. Toxicol. Lett., 194(1-2): 42-49.

Loguercio, C. and A. Federico, 2003. Oxidative stress in viral and alcoholic hepatitis. Free Radic. Biol. Med., 34(1): 1-10.

Mansour, H.H., H.F. Hafez and N.M. Fahmy, 2006. Silymarin modulates Cisplatin-induced oxidative stress and hepatotoxicity in rats. J. Biochem. Mol. Biol., 39(6): 656-661.

Mehany, H.A., A.M. Abo-Youssef, L.A. Ahmed, E.S.A. Arafa and H.A.A. El-Latif, 2013. Protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. Beni-Suef Univ. J. Basic Appl. Sci., 2(2): 96-102.

Mihara, M. and M. Uchiyama, 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 86(1): 271-278.

Miranda, K.M., M.G. Espey and D.A. Wink, 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nato Sci. S A Lif. Sci., 5(1): 62-71.

Miyake, Y., K. Yamamoto, N. Tsujihara and T. Osawa, 1998. Protective effects of lemon flavonoids on oxidative stress in diabetic rats. Lipids, 33(7): 689-695.

Morel, I., G. Lescoat, P. Cogrel, O. Sergent, N. Pasdeloup, P. Brissot, P. Cillard and J. Cillard, 1993. Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem. Pharmacol., 45(1): 13-19.

Naqshbandi, A., W. Khan, S. Rizwan and F. Khan, 2012. Studies on the protective effect of flaxseed oil on cisplatin-induced hepatotoxicity. Hum. Exp. Toxicol., 31(4): 364-375.

Pabla, N. and Z. Dong, 2008. Cisplatin nephrotoxicity: Mechanisms and renoprotective strategies. Kidney Int., 73(9): 994-1007.

Palipoch, S., C. Punsawad, P. Koomhin and P. Suwannalert, 2014. Hepatoprotective effect of curcumin and alpha-tocopherol against cisplatin-induced oxidative stress. BMC Complem. Altern. M., 14(1): 111.

Prabhu, V.V., N. Kannan and C. Guruvayoorappan, 2013. 1,2-Diazole prevents cisplatin-induced nephrotoxicity in experimental rats. Pharmacol. Rep., 65(4): 980-990.

Pratibha, R., R. Sameer, P.V. Rataboli, D.A.Bhiwgade and C.Y. Dhume, 2006. Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. Eur. J. Pharmacol., 532(3): 290-293.

Previati, M., I. Lanzoni, E. Corbacella, S. Magosso, V. Guaran, A. Martini and S. Capitani, 2006. Cisplatin-induced apoptosis in human promyelocytic leukemia cells. Int. J. Mol. Med., 18(3): 511-516.

Sadowitz, P.D., B.A. Hubbard, J.C. Dabrowiak, J. Goodisman, K.A. Tacka, M.K. Aktas, M.J. Cunningham, R.L. Dubowy and A.K. Souid, 2002. Kinetics of cisplatin binding to cellular DNA and modulations by thiol-blocking agents and thiol drugs. Drug Metab. Dispos., 30(2): 183-190.

Safirstein, R.L., 1999. Lessons learned from ischemic and cisplatin-induced nephrotoxicity in animals. Ren. Fail, 21(3-4): 359-364.

Sahu, B.D., M. Kuncha, G.J. Sindhura and R. Sistla, 2013. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. Phytomedicine, 20(5): 453-460.

Saller, R., J. Melzer, J. Reichling, R. Brignoli and R. Meier, 2007. An updated systematic review of the pharmacology of silymarin. Forsch. Komplementmed., 14(2): 70-80.

Schinella, G., H. Tournier, J. Prieto, P.M. De Buschiazzo and J. R±os, 2002. Antioxidant activity of anti-inflammatory plant extracts. Life Sci.,70(9): 1023-1033.

Shi, R., Q. Huang, X. Zhu, Y.B. Ong, B. Zhao, J. Lu, C.N. Ong and H.M. Shen, 2007. Luteolin sensitizes the anticancer effect of cisplatin via c-Jun NH2-terminal kinase-mediated p53 phosphorylation and stabilization. Mol. Cancer Ther., 6(4): 1338-1347.

Shona, S., A.W. Essawy, S.M. Zaki andT.I. Abdel-Galil,2012. Effect of cisplatinum on the liver of the adult albino rat and the possible protective role of vitamin E (Histological and ultrastructural study). Anat. Physiol., 2(3): 1-5.

Siddik, Z.H., 2003. Cisplatin: Mode of cytotoxic action and molecular basis of resistance. Oncogene, 22(47): 7265-7279.

Silici, S., O. Ekmekcioglu, M. Kanbur and K. Deniz, 2011. The protective effect of royal jelly against cisplatin-induced renal oxidative stress in rats. World J. Urol., 29(1): 127-132.

Tirkey, N., S. Pilkhwal, A. Kuhad and K. Chopra, 2005. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC Pharmacol., 5: 2.

Tsuruya, K., T. Ninomiya, M. Tokumoto, M. Hirakawa, K. Masutani, M. Taniguchi, K. Fukuda, H. Kanai, K. Kishihara, H. Hirakata and M. Iida, 2003. Direct involvement of the receptor-mediated apoptotic pathways in cisplatin-induced renal tubular cell death. Kidney Int., 63(1): 72-82.

Weijl, N.I., F.J. Cleton and S. Osanto, 1997. Free radicals and antioxidants in chemotherapy-induced toxicity. Cancer Treat. Rev., 23(4): 209-240.

Weijl, N.I., G.D. Hopman, A. Wipkink-Bakker, E.G. Lentjes, H.M. Berger, F.J. Cleton and S. Osanto, 1998. Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. Ann. Oncol., 9(12): 1331-1337.

Zicca, A., S. Cafaggi, M.A. Mariggio, M.O. Vannozzi, M. Ottone, V. Bocchini, G. Caviglioli and M. Viale, 2002. Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats. Eur. J. Pharmacol., 442(3): 265-272.