

*Research Article***Nephroprotective Role of Naringin Against Cisplatin-Induced Nephrotoxicity****Abd Elmonem A.Taha****, **M. M. A. Khalifa*** and **Mohamed I. Abdel-Salam***** Department of Pharmacology and Toxicology, Faculty of Pharmacy,
Minia University, Egypt**Department of Pharmacology and Toxicology, Faculty of Pharmacy,
Al-Azhar University, Assiut, Egypt**Abstract**

Nephrotoxicity remains one of the most dangerous effect relevant to cisplatin use in chemotherapy. Rat injection with cisplatin in a single dose of 7 mg/kg intraperitoneally resulted in a significant increase in serum level of urea and creatinine. Also, cisplatin caused marked increase in renal content of malondialdehyde (MDA), while depletion in reduced glutathione (GSH). In addition, cisplatin administration notably increased kidney/body weight ratio, renal contents of nitric oxide (NO), tumor necrosis factor alpha(TNF- α) and cyclooxygenase-2 (COX2) protein level as well as renal myeloperoxidase (MPO) activity. Histopathological examination confirmed the biochemical and molecular results and revealed several pathological alteration in the renal tissues following cisplatin. Oral pretreatment of rats with naringin (Nar) (80 mg/kg) for 14 days before and 7 days after cisplatin injection significantly reduced the pathological level of serum urea and creatinine and restored oxidative stress parameters. In the same manner, the inflammatory and apoptotic markers as well as kidney/body weight ratio show great improvement following the treatment. The histopathological examination confirms fit with the biochemical and molecular results. In conclusion, Nar showed a great protective effect against cisplatin-induced nephrotoxicity in rats via its antioxidant, anti-inflammatory and apoptotic roles.

Keywords: Nephrotoxicity, cisplatin, tumor necrosis**Introduction**

Kidney is the main organ that responsible for drug excretion. Approximately 90% of drugs are excreted by the kidneys (Naughton 2008, Pabla and Dong 2008, Kim and Moon 2012). Kidney dysfunction is common side effects for several chemotherapeutic aggets such as cisplatin. This side effect restricts the use of cisplatin against human malignancies and remains the most problem that limited the beneficial roles of this chemotherapeutic agent (Perazella and Moeckel 2010). Cisplatin (CP) is a key chemotherapeutic drug used in the treatment of many solid tumors and hematological malignancies (Yao, Panichpisal et al., 2007). Unfortunately, CP affects healthy and malignant cells; as a result, it accumulates in healthy tissues, leading to severe clinical toxicities in different body organs, including kidneys as

the kidney is the main route of excretion of CP [(Ali and Al Moundhri 2006). Tubular cell injury occurs in one-third of CP-treated patients and manifests by notable increase in serum creatinine and urea concentration as well as imbalanced electrolytes. Although, intense efforts have been devoted over the past decades to find less toxic but equally effective alternatives, CP continues to be the most effective and widely prescribed among platinum chemotherapeutic agents (Ferguson, Vaidya etal) It was postulated that CP-induced nephrotoxicity was mediated via excess generation of reactive oxygen species (ROS), which induces lipid peroxidation and oxidative damage in the renal cells leading to increase renal capillary permeability and tubular atrophy (Davis, Nick et al., 2001, Miller, Tadagavadi et al., 2010).

As Excessive production of ROS causes depletion of endogenous antioxidants defense mechanism and activation of signaling cascade that mediated for immune respons, consequently pathological inflammatory condition will be developed. This inflammation is characterized by overproduction and activation of pro-inflammatory cytokines such as TNF- α , NO and inflammatory cell infiltration. Additionally, CP induces apoptosis of renal tubular cells through activation of both intrinsic and extrinsic mitochondrial pathways as well as its direct tubular toxicity (Cummings and Schnellmann 2002, Domitrović, Cvijanović et al., 2013) . Several strategies and many trials have been done to attenuate CP side effects but no promising results have been obtained. Thus, there is a need for identifying safer sources capable of protecting the renal cells from oxidative injury, inflammatory responses, apoptotic action, which are occurred following cisplatin.

Naringin (Nar) ,flavanone glycoside, is isolated from the grape and citrus fruit species (Kim, Song et al., 2009, Adil, Visnagri et al., 2014).]. Nar has been reported to possess beneficial role in several pathological conditions including cancer, obesity, apoptotic condition and atherosclerosis. The anti-inflammatory and antioxidant roles of Nar represent the corner stone in role of Nar against these pathological conditions (Choe, Kim et al., 2001, Rajadurai and Prince 2009, Chtourou, Aouey et al., 2015). Therefore, the current study was done to investigate the possible nephroprotective effect of Nar against CP-induced nephrotoxicity in rats.

Materials and Methods

Drugs and Chemicals

Cisplatin was obtained from MYLAN United Pharmaceuticals Co., Egypt. Nar was purchased from Sigma–Aldrich (Seeize, Germany).Elman’s reagent, thio-barbituric acid, reduced glutathione (GSH), 1, 1, 3, 3-tetraethoxypropane, N-(1-Naphthyl) ethylenediamine dihydrochloride and trichloroacetic acid were purchased from Sigma–Aldrich (St. Louis, MO). All

other chemicals were obtained from local sources with highest analytical grade.

Animals

Male Swiss albino rats weighing 200–230 g were housed in the animal house of the Faculty of Medicine, Assiut University. The animals were kept one week to adapt with the environmental conditions. Rats were fed a standard diet and allowed freely accessed to water. Experiment was carried out according to the accepted guidelines for animal care (Council 1996).

Experimental Design

Fourty male adult Swiss albino rats were allocated into five groups (eight rats each); two rats from each group were used for histopathological and immunohistological examination.

Group 1: Rats were received saline and served as controls.

Group 2: Animals were injected with CP in a single dose of 7 mg/kg, i.p. (Atessahin, Yilmaz et al., 2005).

Group 3: Animals were treated with Nar in a daily dose of 80 mg/kg, p.o. for 14 consecutive days. This is followed by CP injection and Nar treatment for another 7 consecutive days (Nie, Wu et al., 2012).

At The end of the experiment, rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) by IP Injection, blood samples were withdrawn by a direct cardiac puncture. Sera were collected in non-heparinized tubes after blood centrifugation for 20 min at 4000×g, then stored at –20 °C for biochemical assay.

The kidneys were dissected out, washed with ice-cold isotonic saline (0.9%) and divided into three portions. the first portion was kept in 10% neutral buffered formalin solution for immunohistochemistry and histopathological examinations. The second portion was homogenized (20%) with (Cole-Parmer instrument company, USA) in cold phosphate buffered saline (PBS). Tissue homogenates were centrifuged at 3000 rpm for 15 min at 4°C prior to collection of supernatant, which was stored at – 70°C for biochemical evaluations. The

third portion was frozen in liquid nitrogen and kept at -70°C for molecular analysis.

Methods

Assessment of kidney function testes

Serum level of urea and creatinine were estimated colorimetrically according to methods of Fawcett and Scott (Fawcett and Scott 1960), Bartles et al, (Kammeraat 1978), respectively.

Evaluation of oxidative stress markers

Estimation of renal contents of GSH was done using the method described by Ellman (Ellman 1959), while renal contents of MDA were determined according to the method depicted by Uchiyama and Mihara (Uchiyama and Mihara 1978).

Estimation of inflammatory markers.

Renal content of TNF- α was determined by immunohistochemical analysis, while COX-II protein level was determined Using ELISA kit according to the manufacturing instruction (Wkea med supplies Corp, Changchun Jilin, China).

Nitric oxide was measured according to the method described by Montgomery and Dymock (1961), while the activity of myeloperoxidase (MPO) enzyme was assessed using a method depicted by Bradley et al., (1982).

Histopathological examination

Kidney samples were buffered in standard 10% formalin prior to staining with haematoxylin and eosin. Qualitative examination of histologic samples was undertaken by histopathologist blind to specimen group allocation.

Immunohistochemical analysis

Tissue samples were flushed and fixed at 10% neutral buffered formalin for 72 hrs. Samples were trimmed and processed by dehydration in alcohols, clearing in xylene, synthetic wax infiltration and blocking out into Paraplast tissue embedding media. 3-5 μ sections were cut by rotatory microtome. The sections were stained with Harris Hematoxylin and Eosin. Tumor necrosis factor-alpha immunohistochemical staining by using rabbit polyclonal antibody RB-

9034-R7 From Thermo scientific Co. Expression Area percentage of TNF- α were obtained from (ex. 10 random fields) according to by using a full HD microscopic camera attached to the Leica application suite for immunoeexpression analysis (Leica Biosystems- Germany. (Banchroft, J. et al.,1996).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5.0 software (GraphPad, San Diego, Ca., USA). Data were presented as mean \pm SE. Multiple comparisons were achieved using one way ANOVA with Tukey-Kramer test as multiple comparison post ANOVA test. $P < 0.05$ was chosen to indicate statistical significance.

Result

Effect of Nar on kidney functions test

Injection of CP resulted in a significant increase in serum urea (280%) and creatinine (323%) compared to control group. In contrast, oral administration of Nar significantly reduced the elevated levels of urea and creatinine in serum by 52% and 61%, respectively in comparison with a murine model. At the end of the experiment the kidney-body weight ratio were significantly increased in rats challenged with CP respected to control rats. Treatment with Nar significantly reduced kidney-body weight ratio regarded to CP rats.

Effect of Nar on oxidative stress biomarkers .

Administration of CP was associated with marked elevation of renal content of MDA (116%), while significant reduction in renal contents of GSH (66%) compared to control group. On the other hand, treatment of rats with Nar resulted in a notable correction of oxidative stress markers.

Nar effects on inflammatory biomarkers.

CP injection was showed a significant increase in kidney contents of TNF- α , COX-2 and NO as well as MPO activity in related to control animals. Nar treatment remarkably ameliorated renal contents of TNF- α , COX-2 and NO as well as MPO activity in related to a murine challenged with CP.

Results

Table (1): Effect of treatment of Nar on serum urea, creatinine, final body weight and kidney–body weight ratio in CP-treated rats:

Parameters Groups	Urea (mg/dl)	Creatinine (mg/dl)	Kidney–Body Weight ratio(1000×)
Control saline	30.50±2.95	0.92 ± 0.077	5.6 ± 0.29
CP	114 ± 5.71 ^a	3.9 ± 0.14 ^a	13.85±0.0 ^a
CP + Nar	72.75±5.93 ^{a,b}	1.81 ± 0.102 ^{a,b}	9.45±0.3 ^{a,b}

Data are expressed as mean ± SEM of eight rats per group.

^a Significantly different from the control saline group.

^b Significantly different from the CP-treated group.

ANOVA followed by the Tukey–Kramer test for multiple comparison at $p \leq 0.05$

Table (2): Effect of Treatment of Nar on Kidney Contents of MDA and GSH in CP-Treated Rats:

Parameters Group	MDA (nmol/g Tissue)	GSH (µmol/ g tissue)
Control Saline	20.75±1.54	8.97±0.319
CP	44.5±2.84 ^a	3.025±0.213 ^a
CP + Nar	28.75±2.016 ^b	7.47±0.311 ^{a,b}

Data are expressed as mean ± SEM of six rats per group.

^a Significantly different from the control saline group.

^b Significantly different from the CP-treated group.

Table (3): Effect of treatment with Nar on inflammatory biomarkers in CP-treated rats:

Parameters Group	COX-2 (pg/ml)	MPO (U/g tissue)	NO (µmol/g tissue)	TNF-α Area%
Control Saline	45±2.95	10.98±0.833	2.37±0.228	0.750±.064
CP	132.4±3.77 ^a	37.8±1.93 ^a	6.57±0.268 ^a	41.5±1.93 ^a
CP + Nar	84.75±2.64 ^{a,b}	22±2 ^{ab}	3.87±0.149 ^b	22±2 ^{a,b}

Data are expressed as mean ± SEM of six rats per group.

^a Significantly different from the control saline group.

^b Significantly different from the CP-treated group.

Table (4): Effect of Treatment of Nar on Histopathological Findings of Kidney Tissues of Cisplatin -Treated Rats:

Histopathological Findings \ Groups	Normal Saline	CP	CP + Nar
Vacuolation of renal tubular epithelium and glomerular tufts	-	+++	+
Congestion of glomerular tufts	-	++	+
Cystic dilatation of renal tubules	-	+++	-
Necrosis of renal tubular epithelium	-	++	+
Cellular cast in the lumen of renal tubules	-	++	-
Chronic interstitial nephritis	--	+++	-

(-)normal (+)milde (++)moderate (++++) sever

figure 2: Effect of Treatment of Nar on Histopathological Findings of Kidney Tissues of CP-Treated Rats:

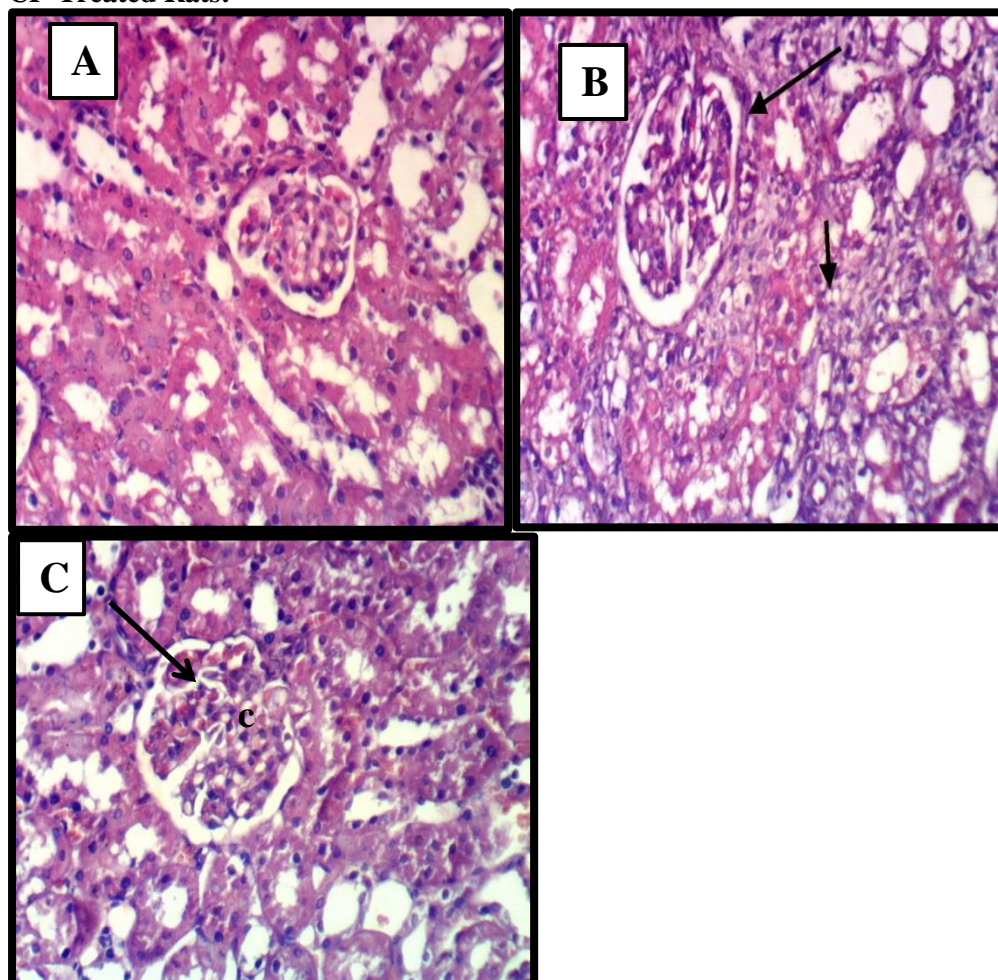
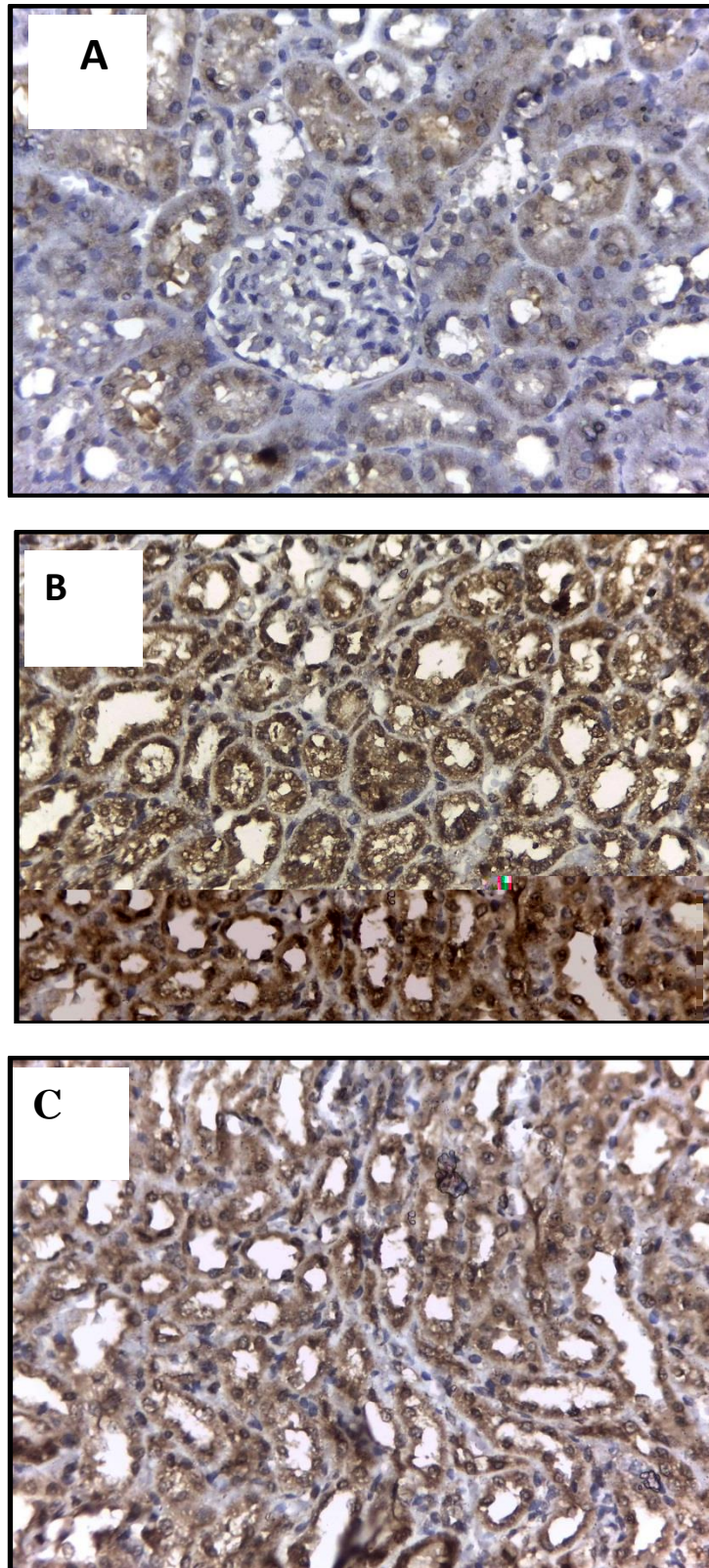


Figure 2;(A) control saline: shows that normal histological structure of renal parenchyma, (B) CP-treated group: shows that marked vacuolation (v) and necrosis (n) of renal tubular epithelium as well as periglomerular fibroblast proliferation ,(C) Nar + CP-treated group: shows that slight congestion (c) of glomerular tuft.

Figure 3: Immunohistochemical assay of TNF- α in CP-treated rats:-



A: Normal control group
B: CP- treated group
C: Nar+CP treated group

Discussion

Nephrotoxicity remains the most complicating factor for uses of CP in chemotherapy. The exact molecular mechanism that underlies CP-induced nephrotoxicity is still divisive. However, the excessive generation of free radical in a rate that exceed the endogenous ability to scavenge ROS is involved in the development and prognosis of CP-induced nephrotoxicity. ROS over-production with subsequent oxidative stress lead to tubular damages and activation of inflammatory mediators genes including cytokines and chemokines (Miller, Tadagavadi et al., 2010]. Many researchers have established the benefit role of natural antioxidants against several pathological conditions (Rajadurai and Prince 2009).

Therefore, the present study was designed to evaluate the possible protective effects of Nar against CP-induced nephrotoxicity in rats. CP-induced renal injury was characterized by significant elevations in serum level of creatinine, urea and kidney-body weight ratio (Miller, Tadagavadi et al., 2010) . These abnormalities could be attributed to reduction in glomerular filtration rate, increase in glomerular volume and cellular degenerative changes as well as severe body weight reduction due to vomiting and loss of appetite (Ferguson, Vaidya et al., 2008). Moreover, our data showed that a single dose of CP produces pathological changes in oxidative stress parameters such as significant increase in renal contents of MDA and marked depletion in GSH levels. These results are in consistent with Ali et al and Fouad et al, whom found that CP generates a large amount and different members of ROS including superoxide anions, hydrogen peroxide, and hydroxyl radicals, which induces lipid peroxidation causing an oxidative damage in renal cells and tubular atrophy as well as, depletion of endogenous antioxidants. Additionally, NO reacts with superoxide anion to generate peroxynitrite radical, a potent prooxidant and cytotoxic intermediate that causes protein nitration and tissue injury (Pabla and Dong 2008). These results are consistent with de Oliveira

Mora et al., (2003) and Shimeda et al., (2005) who found that CP generates a large amount of ROS includes superoxide anion, hydrogen peroxide, and hydroxyl radicals which, induces lipid peroxidation causing an oxidative damage in renal cells and tubular atrophy as well as, depletion of endogenous antioxidants system.

Furthermore, our study revealed that CP provoked a significant elevation in renal contents of TNF- α , COX-2, NO and MPO activity which is a marker of neutrophil infiltration. This is attributed to excessive ROS production, with subsequent activation of inflammatory signaling cascade and upregulation of inflammatory cytokines including TNF- α and COX-2 and chemokines. TNF- α upregulates iNOS with subsequent increase of NO production. Cytokines and chemokines provoked leukocyte infiltration at the site of inflammation (Kang, Kim et al., 2009, Kumar, Rajmane et al., 2014) (Ueki, Ueno et al., 2013).

The present study revealed that pretreatment with Nar ameliorated CP-induced alterations in serum levels of creatinine, urea and kidney-body weight ratio. In addition, Nar significantly mitigated the oxidative stress condition as described by decreased renal contents of MDA, and increased GSH levels. These results could be attributed to the potential antioxidant effect of Nar (Chen, Nie et al., 2013).

Also, Nar therapy obviously mitigated CP-induced renal inflammation and apoptosis. These is approved by a significant decrease in the renal contents of TNF- α , COX-2, NO and MPO enzyme activity following Nar treatment. In the same manner, Nar notably decreased caspase-3 and significantly increased in Bcl-2 genes expression. These findings are inconsistent with Kumar, Rajmane et al., 2014., who demonstrated that Nar has anti-inflammatory, anti-apoptotic activities.

The histopathological findings demonstrated that administration of CP-induced

various degenerative changes in kidney cells. In contrast, the pretreatment with Nar obviously mitigated the histopathological changes induced by CP (Benavente-Garcia and Castillo 2008). A similar study was reported by Y Chtourou et al., 2015 who demonstrated the decisive role of Nar in suppressing ROS-mediated activation of NF- κ B signaling pathways.

In conclusion, the present study revealed the nephroprotective effect of Nar against CP-induced might be attributed to their antioxidant, anti-inflammatory, and antiapoptotic effects.

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