Luteolin ameliorates cisplatin-induced acute kidney injury in mice by regulation of p53-dependent renal tubular apoptosis

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Abstract

Background. Cisplatin chemotherapy often causes acute kidney injury in cancer patients. The causative mechanisms of cisplatin-induced acute kidney injury include renal inflammation, activation of p53 tumour suppressor protein and tubular apoptosis. Luteolin, a flavone found in medicinal herbs and plants, has been reported to exhibit anti-inflammatory, antioxidant and anticarcinogenic activities. The purpose of this study was to investigate the anti-apoptotic effect of luteolin on cisplatin-induced acute kidney injury and the molecular mechanism.
Methods. C57BL/6 mice were treated with cisplatin (20 mg/kg) with or without treatment with luteolin (50 mg/kg for 3 days). Renal function, histological changes, degree of oxidative stress and tubular apoptosis were examined. The effects of luteolin on cisplatin-induced expression of renal p53, PUMA-α and Bcl-2 family proteins were evaluated.
Results. Treatment of mice with cisplatin resulted in renal damage, showing an increase in blood urea nitrogen and creatinine levels, tubular damage, oxidative stress and apoptosis. Treatment of cisplatin-treated mice with luteolin significantly improved renal dysfunction, reducing tubular cell damage, oxidative stress and apoptosis. Examination of molecules involving apoptosis of the kidney revealed that treatment of cisplatin increased the levels of p53 and its phosphorylation, PUMA-α, Bax and caspase-3 activity that were significantly decreased by treatment with luteolin.
Conclusion. These results indicate that cisplatin induces acute kidney injury by regulation of p53-dependent renal tubular apoptosis and that luteolin ameliorates the cisplatin-mediated nephrotoxicity through down-regulation of p53-dependent apoptotic pathway in the kidney.

Keywords: acute kidney injury; apoptosis; cisplatin; luteolin; tumour suppressor protein p53

Introduction

Platinum-based chemotherapeutic agents are well known to exhibit powerful anticancer activity in the treatment of solid tumours. Among these agents, cisplatin is widely used for chemotherapy against testicular, head and neck, ovarian, cervical and lung cancers [1–3]. However, the use of cisplatin is limited by severe side effects in normal tissues such as kidney, peripheral nerves, gastrointestinal tract and auditory system [3]. Strategy for the prevention of renal dysfunction in cisplatin-based chemotherapy is currently recommended to maintain the urine flow by hydration with saline before and after cisplatin chemotherapy [4,5].

Proposed mechanisms of cisplatin-induced acute kidney injury include renal inflammation, activation of p53 tumour suppression protein and tubular apoptosis [6–8]. Our previous studies have indicated that renal inflammation and oxidative stress contribute to cisplatin-induced acute kidney injury [9,10]. Furthermore, renal tubular apoptosis has been considered as a major mechanism of cisplatin-induced renal tubular injury [3]. In conditions of cellular stress, p53 engages post-translational modification in the apoptosis pathway [11]. Cummings and Schnellmann have demonstrated that p53 is involved in cisplatin-induced renal tubular apoptosis [12]. Therefore, regulation of cisplatin-induced p53 expression by a novel agent may have an important therapeutic target for cisplatin-induced nephrotoxicity.

Luteolin, a flavone found in high concentrations in celery, green pepper and chamomile, has been reported to display anti-inflammatory, antioxidant and anticarcinogenic activities [13–15]. Luteolin exhibits potent anticancer activity against human breast, ovarian and colorectal cancer cell lines *in vitro* and also acts synergistically with cisplatin against cancer via p53 stabilization [16,17]. These observations lead to a hypothesis that modulation of cisplatin-induced p53 expression by luteolin can prevent cisplatin-induced acute kidney injury and that luteolin can act as a potent chemosensitizer in cancer.

Based on the above information, we hypothesized that luteolin protects the kidney from cisplatin-induced injury through regulation of renal oxidative stress and p53-dependent tubular cell apoptosis. Our results showed that luteolin protects the kidney from cisplatin cytotoxicity, reducing oxidative stress and tubular cell apoptosis.

Materials and methods

Animal experiment

Male C57BL/6 mice (7 weeks old, weighing 20–23 g) were purchased from Orient Bio Inc. (Charles River Korea, Seoul, Korea) and maintained under a standard laboratory diet with water *ad libitum*. The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chonbuk National University. The mice were divided into four groups: the control buffer-treated group ($n = 15$), the luteolin group (50 mg/kg; LKT Laboratories, Inc., St Paul, MN; $n = 15$), the cisplatin group (20 mg/kg; Sigma Chemical Co., St Louis, MO; $n = 15$) and the cisplatin plus luteolin group ($n = 15$). The dose of cisplatin and the time of treatment were selected based on our previous work [10]. Luteolin was dissolved in 0.1% dimethylsulfoxide. To determine the optimal concentration of luteolin, we conducted dose-dependent experiments with 5, 50 and 100 mg/kg according to previous reports [18, 19]. A 50 mg/kg dose of luteolin was chosen because it exerted a renal protective effect with no toxicity. Luteolin (50 mg/kg) was administered orally once a day for 3 days, followed by intraperitoneal injection of cisplatin. Maximal renal injury, as assessed by functional and histologic measurements, was observed at 72 h after intraperitoneal injection of 20 mg/kg cisplatin. Kidneys were harvested to evaluate changes in renal injury, degree of tubular apoptosis and protein expression of p53 at 24, 48 and 72 h after cisplatin treatment.

Renal function analysis

On the final experimental day, blood was collected from the mice by intracardiac puncture immediately after anaesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg). The blood urea nitrogen (BUN) and creatinine levels were measured by an enzymatic method using an automatic analyser (Hitachi7180, Tokyo, Japan).

Histologic examinations

The kidneys were fixed in 4% paraformaldehyde and embedded in paraffin. The block was cut into 5 μm sections and stained with periodic acid-Schiff (PAS). Renal tubular injury was assessed as previously described [20]. The magnitude of tubular epithelial cell loss, necrosis, intratubular debris and tubular cast formation was scored at six levels on the basis of the percentage of affected tubules in a high-power field under a light microscope: 0, none; 0.5, <10%; 1, 10–25%; 2, 25–50%; 3, 50–75%; and 4, >75%. The morphometric examinations were performed in a blinded manner by two independent investigators.

Detection of apoptosis

Apoptosis was assessed by the terminal deoxynucleotidyl transferase-mediated uridine triphosphate (dUTP) nick-end labelling (TUNEL) assay, and the number of apoptotic cells, as defined by chromatin condensation or nuclear fragmentation (apoptotic bodies), was counted as previously described [21]. Apoptosis was detected in the specimen using the Apop-Tag Plus Peroxidase *In Situ* Apoptosis Detection Kit (Millipore Corporation, Billerica, MA) according to the manufacturer's protocol. The number of apoptotic cells in each section was calculated by counting the number of TUNEL-positive apoptotic cells in 10 random, non-overlapping fields per slide at a ×400 magnification.

Western blot analysis

Western blot analysis was performed as described previously [10]. Kidney tissues were homogenized in phosphate-buffered saline (PBS) with a protease inhibitor cocktail (Calbiochem, San Diego, CA), and the protein concentration was quantified by Bradford protein assay. The samples (30 μg protein per lane) were mixed with sample buffer, boiled for 7 min, separated by SDS–polyacrylamide (8–15%) gel electrophoresis and electroblotted onto a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked with 5% non-fat dry milk in Tris-buffered saline with Tween 20 buffer (25 mmol/L Tris, pH 7.5, 150 mmol/L NaCl and 0.1% Tween 20) for 1 h and then incubated overnight at 4°C with rabbit anti-mouse p53 antibody (dilution 1:1000; Cell Signaling Technology, Danvers, MA), rabbit anti-mouse phospho-p53 (Ser 15) (dilution 1:1000; Cell Signaling Technology, Danvers, MA), goat anti-mouse PUMA-α antibody (dilution 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-mouse Bax (dilution 1:1000; Epitomics, Burlingame, CA), rabbit anti-mouse Bcl-2 (dilution 1:500; Cell Signaling Technology, Danvers, MA), rabbit anti-mouse Bcl-xL (dilution 1:1000; Cell Signaling Technology, Danvers, MA) or rabbit anti-mouse caspase-3 (dilution 1:1000; Cell Signaling Technology, Danvers, MA). The blots were washed with PBS and incubated with horseradish peroxidase-conjugated anti-goat and anti-rabbit IgG. Signals were visualized with a chemiluminescent detection kit according to the manufacturer's protocol (Amersham Pharmacia Biotech, London, UK). The membranes were then reprobed with an anti-actin antibody to verify equal loadings of protein in each lane. All signals were analysed by densitometric scanning (LAS-3000; Fuji Film, Tokyo, Japan).

Measurement of renal glutathione level and activity of superoxide dismutase and catalase

Glutathione (GSH) level in kidney tissue was measured by Glutathione Assay Kit II (Calbiochem, Darmstadt, Germany) according to the manufacturer's protocol. Tissue superoxide dismutase (SOD) activity was measured by Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, MI). Catalase activity was determined colorimetrically as described by Sinha [22].

Statistical analysis

Data were expressed as mean ± SD. Multiple comparisons were examined for significant differences using ANOVA, followed by individual comparison with the Tukey post hoc test, with $P < 0.05$ indicating statistical significance.

Results

Luteolin reduces cisplatin-induced renal dysfunction

To investigate effects of luteolin on cisplatin-induced renal injury, we treated mice with cisplatin, luteolin or cisplatin plus luteolin and measured the level of BUN and serum creatinine at 24, 48 and 72 h after cisplatin injection. The levels of BUN and serum creatinine were not significantly changed at 24 and 48 h after cisplatin treatment. However, the levels of BUN (Figure 1A) and serum creatinine (Figure 1B) were significantly increased at 72 h after cisplatin treatment compared to the levels in the control buffer-treated mice. Treatment of cisplatin-treated mice with luteolin significantly decreased the elevated levels of BUN and serum creatinine. Luteolin alone did not show any effect on BUN and creatinine levels.
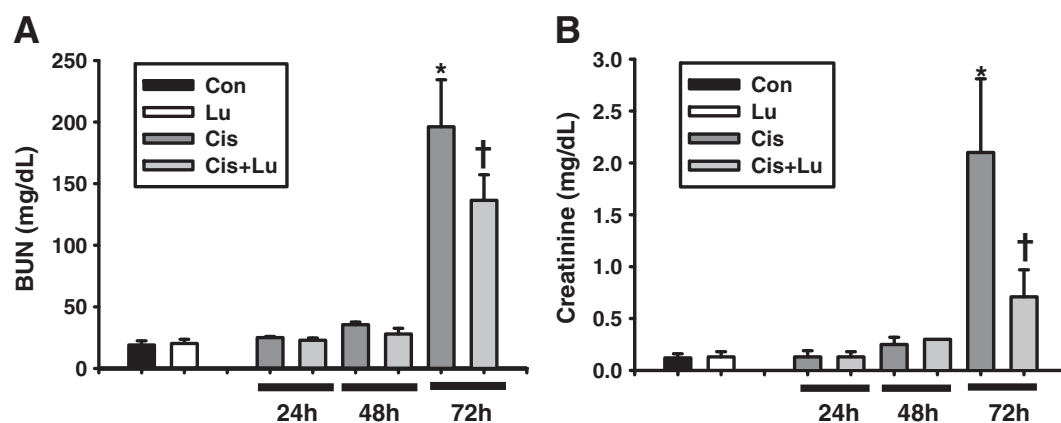
Fig. 1. Effect of luteolin on renal function after cisplatin treatment. Acute kidney injury was induced by cisplatin administration (Cis; 20 mg/kg). Mice were treated with luteolin (50 mg/kg, p.o.) or control buffer (Con) once a day for 3 days, followed by intraperitoneal cisplatin injection. Blood samples were collected 72 h after cisplatin treatment, and BUN (**A**) and creatinine (**B**) levels were measured. Data are expressed as mean ± SD (n = 15 mice per group). *, $P < 0.05$ vs Con or Luteolin; †, $P < 0.05$ vs Cis.

Luteolin ameliorates renal tubular injury after cisplatin treatment

We next evaluated whether luteolin reduces cisplatin-induced renal tubular damage. As presented in Figure 2A, treatment of mice with cisplatin induced tubular cell necrosis, accumulation of PAS-positive material in the tubular lumen, loss of brush border, tubular dilatation and inflammatory cell infiltration at 72 h after cisplatin treatment. Luteolin ameliorated these cisplatin-induced renal tubular damages. When we determined the cisplatin-induced tubular injury score, no significant change in

the tubular injury score was observed at 24 and 48 h after cisplatin treatment compared to the scores of the control buffer- or luteolin-treated groups. However, at 72 h after cisplatin treatment, the tubular injury score was significantly elevated. Luteolin significantly reduced the increased cisplatin-induced renal tubular damage (Figure 2B).

Luteolin decreases cisplatin-induced renal oxidative stress

Luteolin exhibits potent antioxidative activity [23], and oxidative stress is one of the proposed mechanisms of
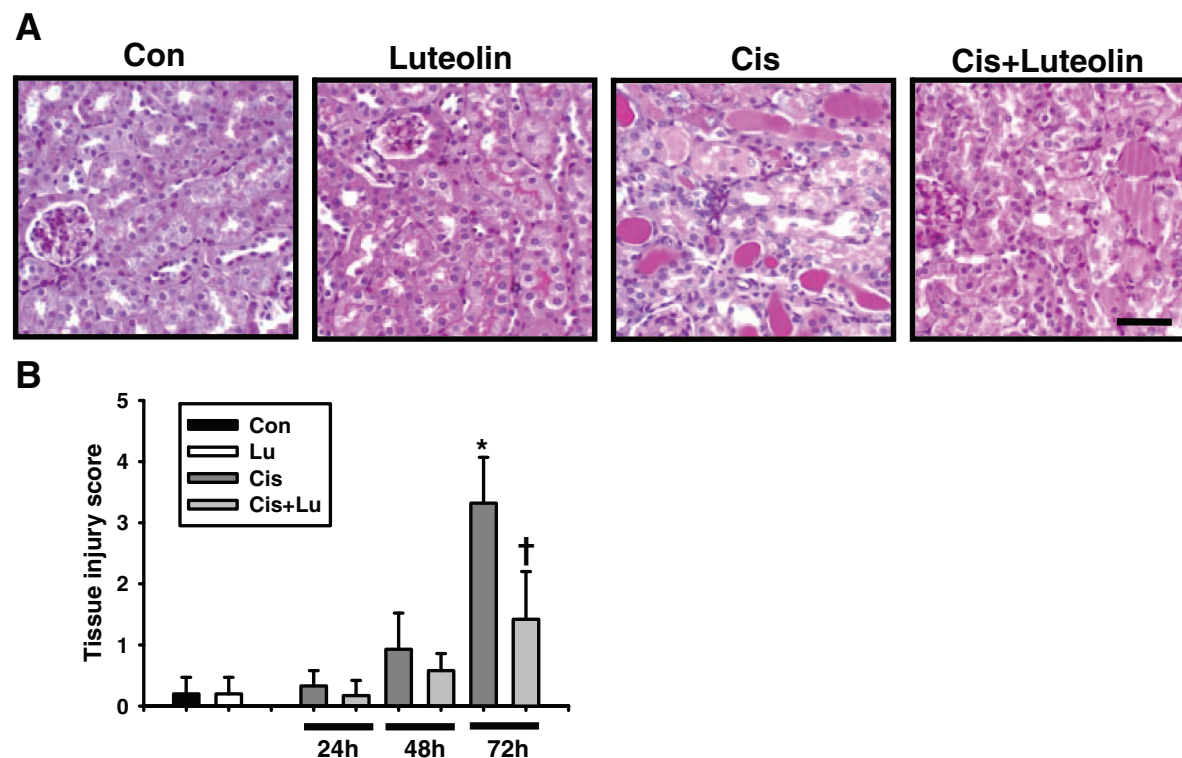

Fig. 2. Effect of luteolin on cisplatin-induced renal tubular damage. (**A**) Histologic sections of kidney at 72 h after treatment with control buffer (Con), luteolin, cisplatin (Cis) and cisplatin plus luteolin (Cis+Luteolin). Bar = 50 μm. (**B**) Semiquantitative scoring of tubular injury was concomitant with histologic analysis (n = 15 for each experimental group). Data are expressed as mean ± SD. *, $P < 0.05$ vs Con or Luteolin; †, $P < 0.05$ vs Cis.
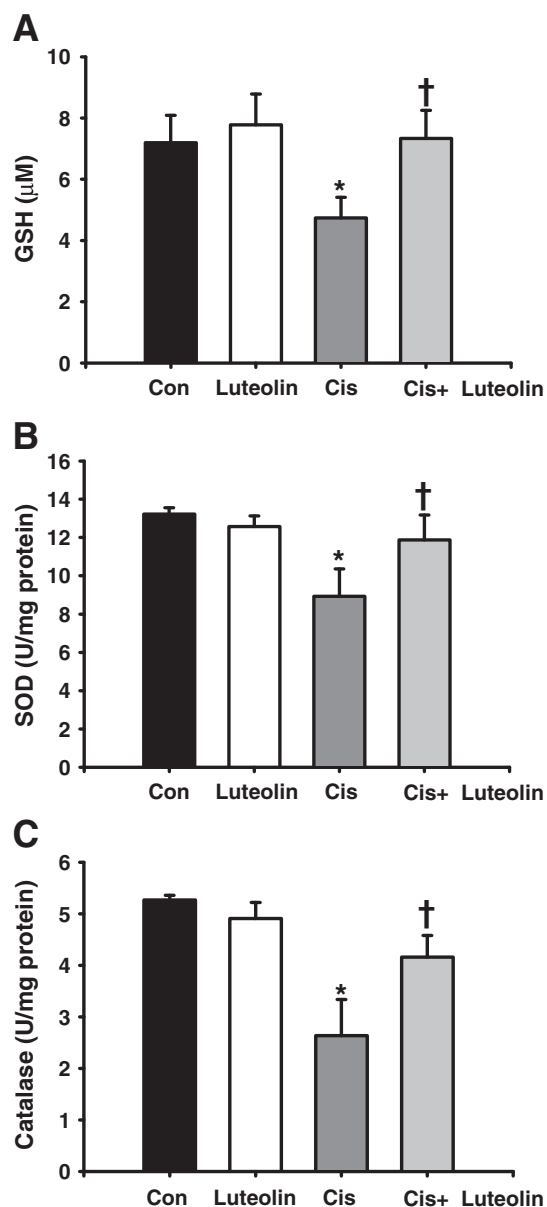
Fig. 3. Effect of luteolin on GSH content, SOD and catalase activities in cisplatin-induced acute kidney injury. Tissue GSH content (**A**), SOD (**B**) and catalase (**C**) activities were measured in the whole kidney lysate. Control buffer treatment (Con), luteolin treatment alone, cisplatin treatment (Cis), cisplatin and luteolin treatment (Cis+Luteolin). Data are expressed as mean ± SD (n = 5–6 mice per each group). *, P < 0.05 vs Con or Luteolin; †, P <0.05 vs Cis.

cisplatin-induced acute kidney injury [3]. Therefore, we evaluated whether luteolin can modulate cisplatin-induced renal oxidative stress by measuring GSH level and activity of enzymes involved in antioxidation. The renal GSH level was significantly decreased at 72 h after cisplatin treatment compared to levels in the control group (Figure 3A). Administration of luteolin significantly increased the cisplatin-induced depletion of GSH level. Renal SOD activity was significantly decreased at 72 h after cisplatin treatment compared to the activity in the control group, and luteolin attenuated the cisplatin-induced decrease of SOD activity (Figure 3B). Catalase activity was also decreased at 72 h

after cisplatin treatment compared to the activity in the control group. Luteolin effectively blocked the cisplatin-induced decrease of catalase activity (Figure 3C). Luteolin alone did not affect the renal GSH level and antioxidant enzyme system.

Luteolin decreases cisplatin-induced renal tubular apoptosis

Renal tubular apoptosis has been suggested as a mechanism of cisplatin-induced acute kidney injury [3]. As shown in Figure 4A and B, there were no significant changes in renal tubular apoptosis at 24 h after cisplatin or luteolin treatment. The number of TUNEL-positive cells was significantly increased at 48 h after cisplatin treatment and then further increased at 72 h compared to the number of the control buffer-treated group. Luteolin administration significantly decreased the number of cisplatin-induced TUNEL-positive cells. Luteolin alone did not induce significant tubular cell apoptosis.

Luteolin decreases cisplatin-induced p53 activation and PUMA-α protein expression

Cisplatin-induced nephrotoxicity involves up-regulation of p53 tumour suppressor protein in the kidney tissue following cisplatin injection [8]. Western blot analyses showed that p53 protein expression was significantly increased at 72 h after treatment with cisplatin compared to the level of the control buffer-treated group and that luteolin treatment significantly decreased the cisplatin-induced increase of p53 expression. As parallel with p53 protein expression, phosphorylation of p53 (Ser15) was significantly increased at 72 h after treatment with cisplatin, and luteolin significantly decreased the cisplatin-induced increase of p53 phosphorylation (Figure 5A). Luteolin alone did not show any effect on the p53 protein expression and its phosphorylation. In addition, we evaluate time-dependent changes in p53 phosphorylation after cisplatin or cisplatin plus luteolin treatment. The phosphorylation of p53 was significantly increased at 48 and 72 h after cisplatin treatment, and luteolin significantly decreased the cisplatin-induced increase of p53 (Supplementary Figure 1).

A p53-responsive pro-apoptotic factor, PUMA-α is induced under cellular stress conditions such as cisplatin-induced nephrotoxicity [8]. Therefore, we also evaluated the effect of luteolin on cisplatin-induced PUMA-α expression. As presented in Figure 4B, cisplatin treatment increased significantly PUMA-α protein expression compared to the control buffer-treated group. Treatment of cisplatin-treated mice with luteolin effectively decreased the rise of PUMA-α expression. Luteolin alone did not induce PUMA-α protein expression.

Luteolin modulates Bcl-2 family proteins and caspase-3 expressions following cisplatin treatment

We further investigated the molecular basis of a protective effect of luteolin against cisplatin-induced tubular cell apoptosis. Treatment of mice with cisplatin increased pro-apoptotic Bax expression by approximately 7.1-fold over
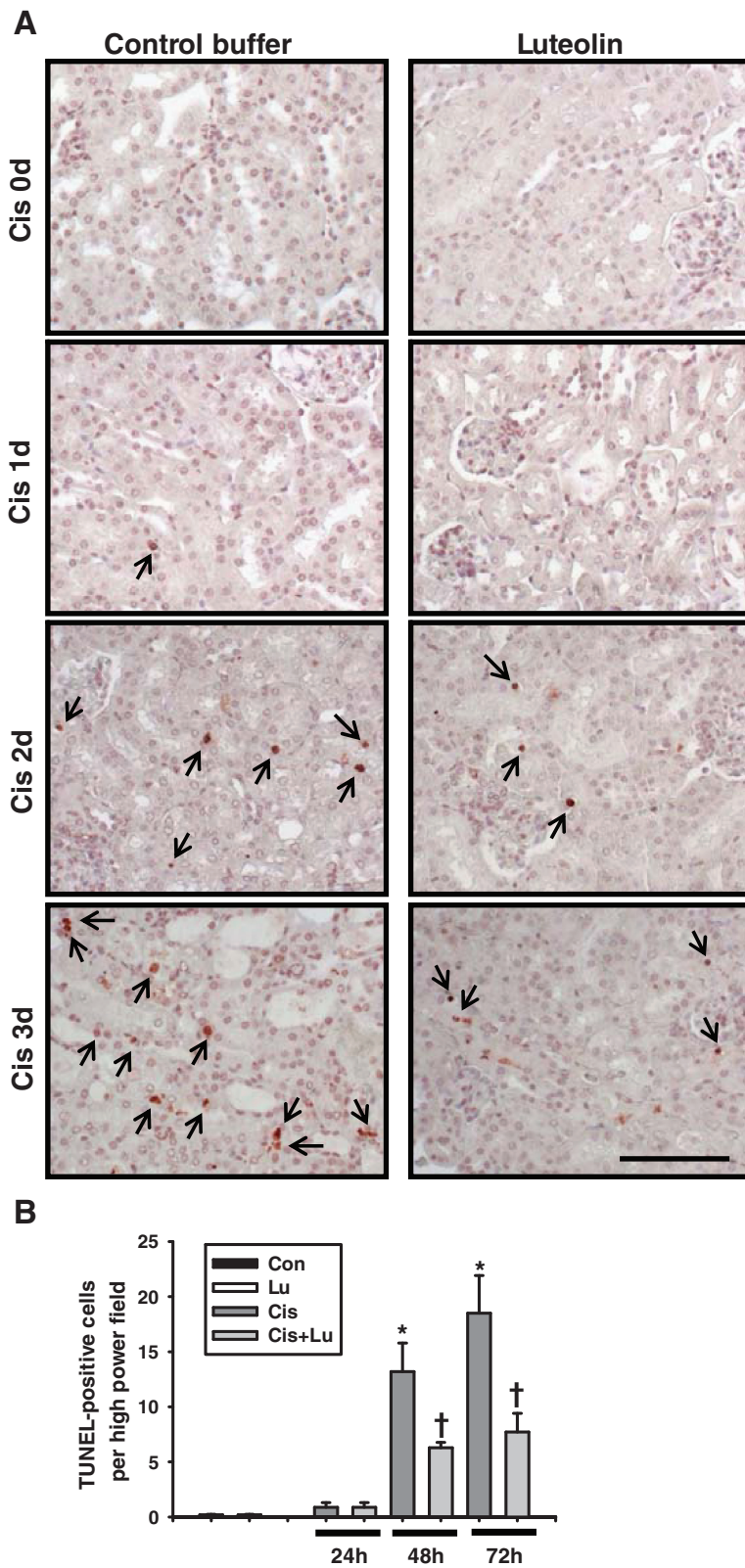
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B

Fig. 4. Effect of luteolin on cisplatin-induced tubular apoptosis. (**A**) Kidneys from mice treated with control buffer, luteolin, cisplatin and cisplatin plus luteolin were stained using the TUNEL assay. Bar = 100 μm. (**B**) Number of TUNEL-positive cells per ×400 magnification. Data are expressed as mean ± SD (n = 15 mice per each group). *, P < 0.05 vs Con or Luteolin; †, P < 0.05 vs Cis.
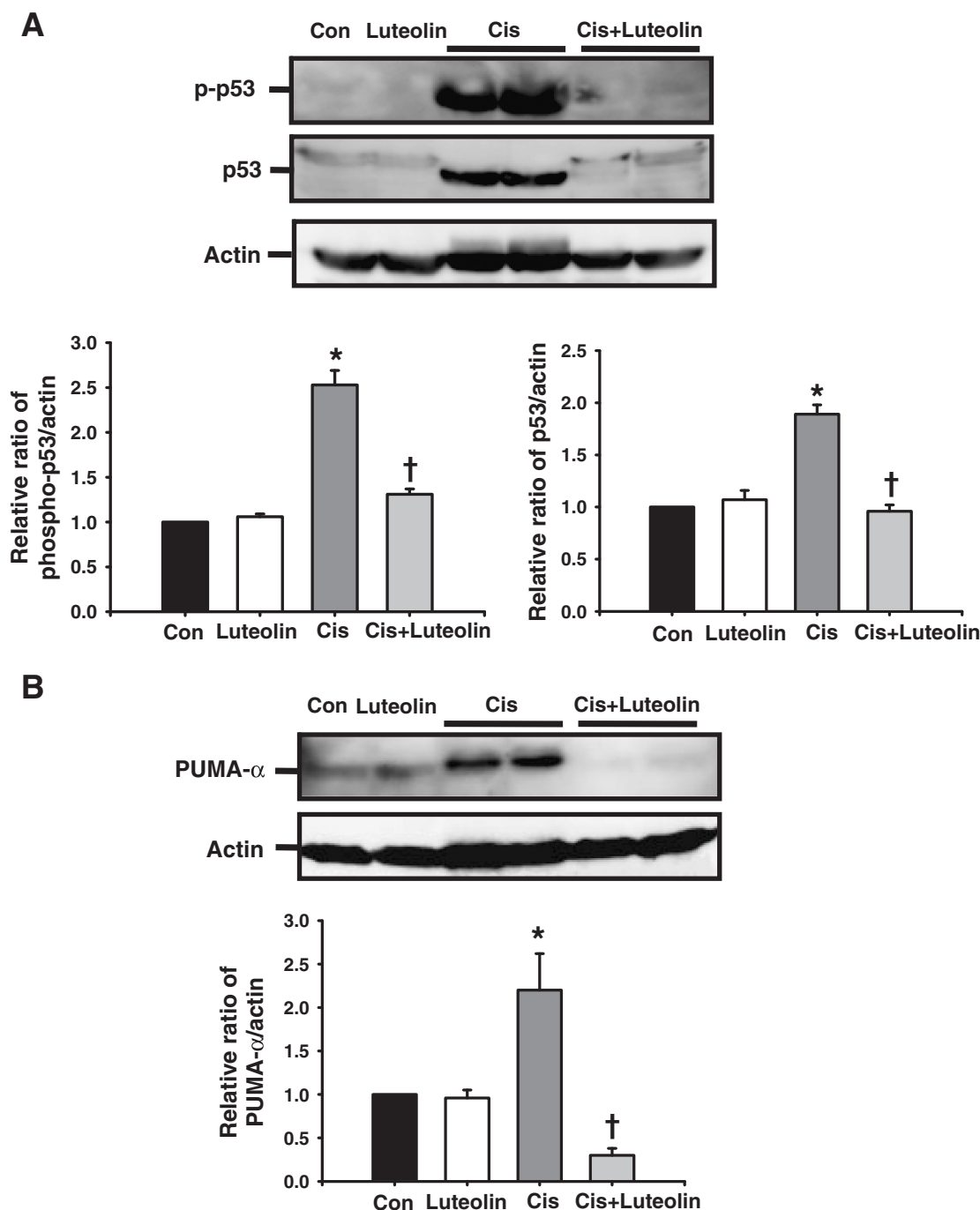
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Fig. 5. Effect of luteolin on cisplatin-induced p53 and PUMA-α protein expressions. Kidneys from mice treated with control buffer (Con), luteolin, cisplatin (Cis) and cisplatin plus luteolin (Cis+Luteolin) were evaluated for phospho-p53 (Ser15) and p53 (**A**) and PUMA-α protein expressions (**B**) by western blot analysis. Densitometric analyses are presented as the relative ratio of each protein to actin. The relative ratio measured in the kidneys from control mice is arbitrarily presented as 1. Data are expressed as mean ± SD of three independent experiments. *, $P < 0.05$ vs Con or Luteolin; †, $P < 0.05$ vs Cis.

that of the control buffer-treated group (Figure 6A and B). Treatment of cisplatin-treated mice with luteolin significantly inhibited the increase of Bax expression. We also evaluated the expression of anti-apoptotic protein, Bcl-2 and Bcl-xL after cisplatin treatment. The Bcl-2 protein expression after treatment with cisplatin was not significantly changed. However, luteolin significantly increased Bcl-2

expression by approximately 1.85-fold over that of the control buffer-treated group. Expression of Bcl-xL protein was unchanged after cisplatin administration with or without luteolin (Figure 6A and B).

In addition, the expression of active caspase-3 was also increased after treatment with cisplatin compared to the control buffer-treated group. Luteolin decreased the cis-
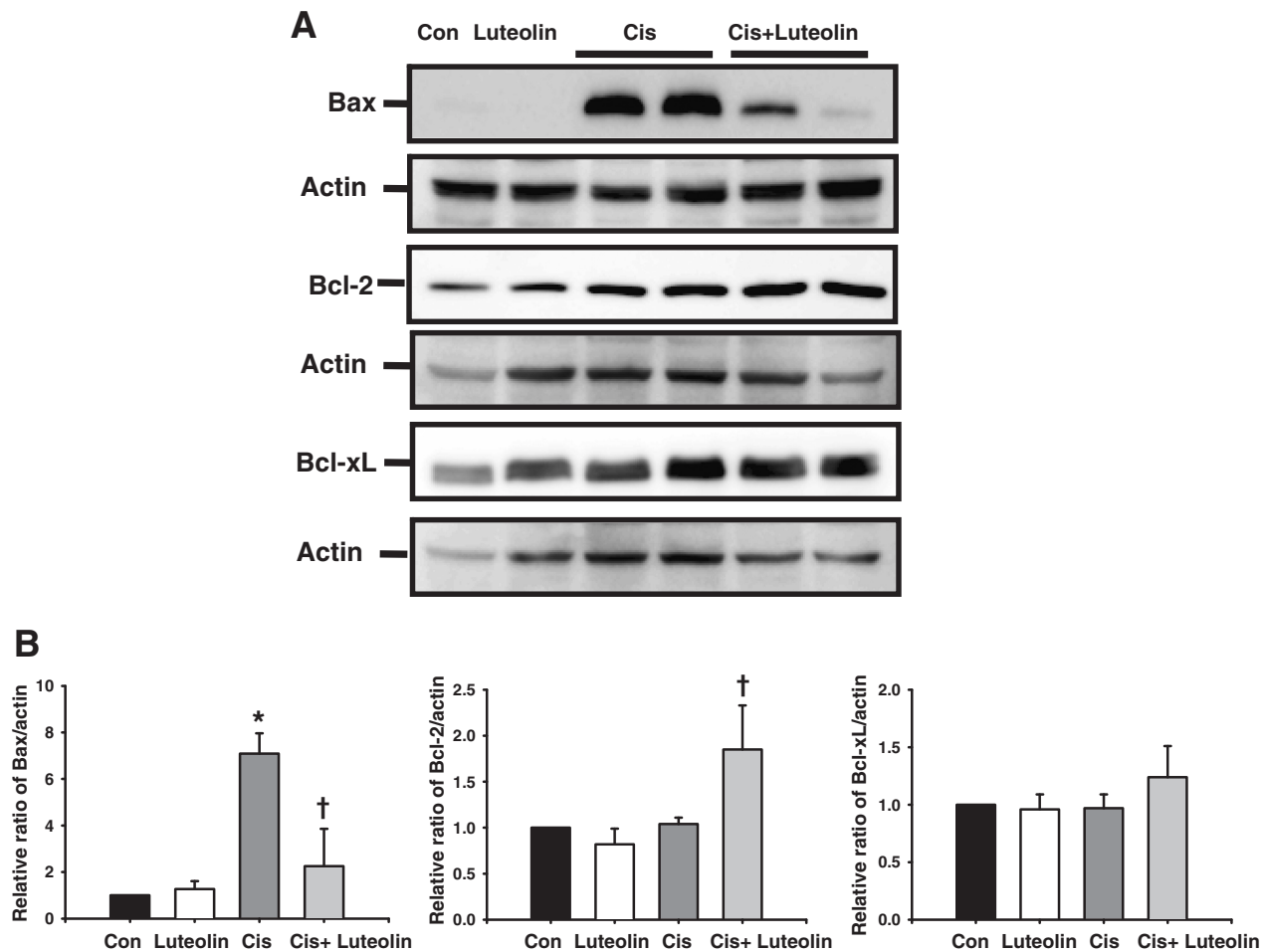
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Fig. 6. Effect of luteolin on cisplatin-induced Bcl-2 family protein expression. (**A**) Kidneys from mice treated with control buffer (Con), luteolin, cisplatin (Cis) and cisplatin plus luteolin (Cis+Luteolin) were evaluated for Bax, Bcl-2 and Bcl-xL protein expressions by western blot analysis. (**B**) Densitometric analyses for Bax, Bcl-2 and Bcl-xL are presented as the relative ratio of each protein to actin. The relative ratio measured in the kidneys from control mice is arbitrarily presented as 1. Data are expressed as mean ± SD of three independent experiments. *, $P < 0.05$ vs Con or Luteolin; †, $P < 0.05$ vs Cis.

platin-induced increase of active caspase-3 expression. Luteolin alone did not induce expression of active caspase-3 (Figure 7).

Discussion

A pitfall of cisplatin chemotherapy is an induction of tubulointerstitial inflammation and tubular cell apoptosis, which lead to acute kidney injury. Thus, finding of a novel pharmacologic agent with potent protective effect of cisplatin-induced nephrotoxicity is an enduring challenge. In this study, we have examined effects of a multifunctional natural compound, luteolin, on cisplatin-induced acute kidney injury. The results revealed that treatment of cisplatin-treated mice with luteolin reduces cisplatin-induced renal injury, oxidative stress and renal tubular cell apoptosis. Molecular basis for the protective effects of luteolin appears to be a reduction of cisplatin-induced increase in oxidative stress, expression of p53, PUMA-α and Bcl-2 family proteins, including reduction of caspase-3 activity.

A number of studies have shown that flavonoids have the ability to regulate a variety of enzyme systems involved in cell division, proliferation, detoxification, inflammation and immune response [13,24,25]. Among these flavonoids, luteolin is a flavone that is found in medicinal plants as well as in vegetables and has antioxidant, anticancer, anti-inflammatory and anti-allergic effects [23,26,27]. In terms of anticancer activities, luteolin sensitizes not only TNF-α-induced apoptosis but also cisplatin-induced apoptosis in human cancer cell lines [14,17]. Since cisplatin is used as a chemotherapeutic agent and luteolin displays anticancer and anti-inflammatory activities, we have observed that luteolin may exert a dual effect on sensitization of cisplatin chemotherapy and prevention of cisplatin-induced acute kidney injury.

The important mechanisms of cisplatin-induced nephrotoxicity are oxidative stress and p53 tumour suppressor protein activation [8]. It has also been reported that oxidative stress may link to cisplatin-induced p53 activation [28]. PUMA-α has an important role in cisplatin-induced tubular cell apoptosis [7]. In the present study, treatment of mice with cisplatin resulted in the decrease of GSH level
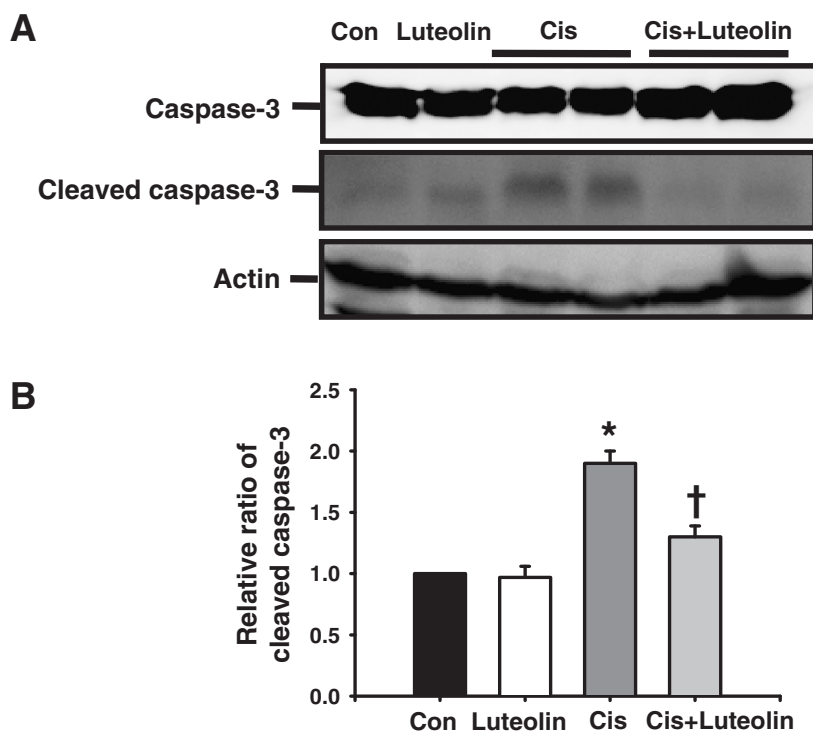
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Fig. 7. Effect of luteolin on cisplatin-induced caspase-3 expression. (**A**) Kidneys from mice treated with control buffer (Con), luteolin, cisplatin (Cis) and cisplatin plus luteolin (Cis+Luteolin) were evaluated for caspase-3 expression by western blot analysis. (**B**) Densitometric analyses are presented as the relative ratio of each protein to actin. The relative ratio measured in the kidneys from control mice is arbitrarily presented as 1. Data are expressed as mean ± SD of three independent experiments. *, $P < 0.05$ vs Con or Luteolin; †, $P < 0.05$ vs Cis.

and the activity of SOD and catalase while expression of p53 and its phosphorylation were increased. Treatment of cisplatin-treated mice with luteolin attenuated the cisplatin-induced decrease of GSH level and SOD and catalase activities. Treatment of cisplatin-treated mice with luteolin also reduced the cisplatin-induced increase of p53 protein expression and its phosphorylation. Interestingly, the phosphorylation of p53 was observed at 48 h after cisplatin treatment. Consistent with this observation, renal tubular apoptosis was significantly increased at 48 h after cisplatin treatment despite of normal renal function and minimal histologic changes. The protective action of luteolin may start at an early stage of cisplatin-induced nephrotoxicity. Therefore, these findings suggest that early therapeutic intervention for the prevention of cisplatin-induced nephrotoxicity has an important clinical relevance. Supporting the observations that p53 expression and its phosphorylation were increased, cisplatin-mediated increase of a p53-responsive pro-apoptotic factor, PUMA-α, was also increased and inhibited by luteolin. Consistent with our findings, Wei *et al.* studied p53 activation in cisplatin-induced nephrotoxicity and demonstrated that modulation of p53 by pifithrin, a pharmacologic inhibitor of p53, effectively ameliorates cisplatin-induced renal injury [29].

In contrast to our results, Shi *et al.* has shown that luteolin increases p53 phosphorylation and stabilization in human cancer cells [17]. To clarify this discrepancy, we compared the effect of luteolin on cell viability of mPT cells (a mouse proximal tubular cell line) and B16f10 cells (a melanoma cell line) treated with cisplatin. The results showed that luteolin decreased the cisplatin-induced death of mPT cells (Supplementary Figure 2). In contrast, luteolin increased the cisplatin-induced death of B16f10 cells. These observations suggest that luteolin acts differently depending on cell type. In fact, it has been suggested that cellular microenvironment is a critical factor in determining the outcome of redox regulation effect of luteolin [30]. Taken together, the dual roles of luteolin in protecting normal cells and helping tumour cell death by therapeutics such as cisplatin could be an advantage of this flavonoid for cancer therapy.

To further understand the inhibition of cisplatin-mediated induction of p53 and PUMA-α by luteolin, modulation of Bcl-2 family protein and caspase-3 activity by luteolin has been evaluated. A study has demonstrated that p53 directly activates the pro-apoptotic Bax and induces transcription-independent apoptosis [31]. Neutralization of anti-apoptotic Bcl-2 protein by PUMA may promote apoptosis [32]. In the present study, examination of the cisplatin-mediated expression and regulation of Bcl-2 family proteins revealed that expression of pro-apoptotic Bax is increased by cisplatin treatment and that this increase is marginally, but significantly, suppressed by luteolin and expression of anti-apoptotic Bcl-2, not Bcl-xL, was significantly increased after cisplatin plus luteolin treatment. In addition, cisplatin-mediated activation of caspase-3 was attenuated by luteolin. These observations indicate that luteolin may exert protective effect on cisplatin-induced renal tubular apoptosis through modulation of the cisplatin-induced expression of Bcl-2 family proteins as well as the expression of p53 and PUMA-α proteins.

In respect to the clinical effectiveness of luteolin for prevention of cisplatin-induced acute kidney injury, an oral preparation of luteolin would be convenient. Despite flavonoids are known to have a poor bioavailability after oral intake, Shimoi *et al.* demonstrated that luteolin can be detected in rodents and human serum after oral treatment using high-performance liquid chromatographic analysis [33]. The oral LD_{50} value of luteolin in mice was reported to be over 2500 mg/kg [23]. Therefore, we adopted an oral dose of 50 mg/kg luteolin in this study, which is relatively safe and can achieve the maximal protective effect after cisplatin administration.

In conclusion, our present study has demonstrated that luteolin treatment ameliorates cisplatin-induced acute kidney injury in mice by regulation of p53-dependent renal tubular cell apoptosis. Therefore, administration of luteolin may be useful in patients who are at risk of renal dysfunction after cisplatin-based chemotherapy.

Supplementary data

Supplementary data is available online at http://ndt. oxfordjournals.org.

References

1. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* 2005; 4: 307–320
2. Cepeda V, Fuertes MA, Castilla J *et al*. Biochemical mechanisms of cisplatin cytotoxicity. *Anticancer Agents Med Chem* 2007; 7: 3–18
3. Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney Int* 2008; 73: 994–1007
4. Cornelison TL, Reed E. Nephrotoxicity and hydration management for cisplatin, carboplatin, and ormaplatin. *Gynecol Oncol* 1993; 50: 147–158
5. Launay-Vacher V, Rey JB, Isnard-Bagnis C *et al*. Prevention of cisplatin nephrotoxicity: state of the art and recommendations from the European Society of Clinical Pharmacy Special Interest Group on Cancer Care. *Cancer Chemother Pharmacol* 2008; 61: 903–909
6. Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest* 2002; 110: 835–842
7. Jiang M, Wei Q, Wang J *et al*. Regulation of PUMA-alpha by p53 in cisplatin-induced renal cell apoptosis. *Oncogene* 2006; 25: 4056–4066
8. Jiang M, Dong Z. Regulation and pathological role of p53 in cisplatin nephrotoxicity. *J Pharmacol Exp Ther* 2008; 327: 300–307
9. Sung MJ, Kim DH, Jung YJ *et al*. Genistein protects the kidney from cisplatin-induced injury. *Kidney Int* 2008; 74: 1538–1547
10. Kang KP, Kim DH, Jung YJ *et al*. Alpha-lipoic acid attenuates cisplatin-induced acute kidney injury in mice by suppressing renal inflammation. *Nephrol Dial Transplant* 2009; 24: 3012–3020
11. Chipuk JE, Green DR. Dissecting p53-dependent apoptosis. *Cell Death Differ* 2006; 13: 994–1002
12. Cummings BS, Schnellmann RG. Cisplatin-induced renal cell apoptosis: caspase 3-dependent and -independent pathways. *J Pharmacol Exp Ther* 2002; 302: 8–17
13. Choi JS, Choi YJ, Park SH *et al*. Flavones mitigate tumor necrosis factor-alpha-induced adhesion molecule upregulation in cultured human endothelial cells: role of nuclear factor-kappa B. *J Nutr* 2004; 134: 1013–1019
14. Shi RX, Ong CN, Shen HM. Luteolin sensitizes tumor necrosis factor-alpha-induced apoptosis in human tumor cells. *Oncogene* 2004; 23: 7712–7721
15. Ju W, Wang X, Shi H *et al*. A critical role of luteolin-induced reactive oxygen species in blockage of tumor necrosis factor-activated nuclear factor-kappaB pathway and sensitization of apoptosis in lung cancer cells. *Mol Pharmacol* 2007; 71: 1381–1388
16. Chiang CT, Way TD, Lin JK. Sensitizing HER2-overexpressing cancer cells to luteolin-induced apoptosis through suppressing p21 (WAF1/CIP1) expression with rapamycin. *Mol Cancer Ther* 2007; 6: 2127–2138
17. Shi R, Huang Q, Zhu X *et al*. Luteolin sensitizes the anticancer effect of cisplatin via c-Jun NH2-terminal kinase-mediated p53 phosphorylation and stabilization. *Mol Cancer Ther* 2007; 6: 1338–1347
18. Hendriks JJ, Alblas J, van der Pol SM *et al*. Flavonoids influence monocytic GTPase activity and are protective in experimental allergic encephalitis. *J Exp Med* 2004; 200: 1667–1672
19. Ziyan L, Yongmei Z, Nan Z *et al*. Evaluation of the anti-inflammatory activity of luteolin in experimental animal models. *Planta Med* 2007; 73: 221–226
20. Liu M, Chien CC, Burne-Taney M *et al*. A pathophysiologic role for T lymphocytes in murine acute cisplatin nephrotoxicity. *J Am Soc Nephrol* 2006; 17: 765–774
21. Lee S, Moon SO, Kim W *et al*. Protective role of L-2-oxothiazolidine-4-carboxylic acid in cisplatin-induced renal injury. *Nephrol Dial Transplant* 2006; 21: 2085–2095
22. Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972; 47: 389–394
23. Seelinger G, Merfort I, Schempp CM. Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin. *Planta Med* 2008; 74: 1667–1677
24. Di Carlo G, Mascolo N, Izzo AA *et al*. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci* 1999; 65: 337–353
25. Hollman PC, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem Toxicol* 1999; 37: 937–942
26. Fotsis T, Pepper MS, Aktas E *et al*. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. *Cancer Res* 1997; 57: 2916–2921
27. Ueda H, Yamazaki C, Yamazaki M. Luteolin as an anti-inflammatory and anti-allergic constituent of Perilla frutescens. *Biol Pharm Bull* 2002; 25: 1197–1202
28. Jiang M, Wei Q, Pabla N *et al*. Effects of hydroxyl radical scavenging on cisplatin-induced p53 activation, tubular cell apoptosis and nephrotoxicity. *Biochem Pharmacol* 2007; 73: 1499–1510
29. Wei Q, Dong G, Yang T *et al*. Activation and involvement of p53 in cisplatin-induced nephrotoxicity. *Am J Physiol Renal Physiol* 2007; 293: F1282–F1291
30. Lin Y, Shi R, Wang X *et al*. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Curr Cancer Drug Targets* 2008; 8: 634–646
31. Chipuk JE, Kuwana T, Bouchier-Hayes L *et al*. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* 2004; 303: 1010–1014
32. Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? *Trends Cell Biol* 2008; 18: 157–164
33. Shimoi K, Okada H, Furugori M *et al*. Intestinal absorption of luteolin and luteolin 7-O-beta-glucoside in rats and humans. *FEBS Lett* 1998; 438: 220–224