Original Paper

Protective Activity of *Dendropanax Morbifera* Against Cisplatin-Induced Acute Kidney Injury

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Key Words

Abstract

Background/Aims: Drug-induced acute kidney injury (AKI) has been a severe threat to hospitalized patients, raising the urgent needs to develop strategies to reduce AKI. We investigated the protective activity of *Dendropanax morbifera* (DP), a medicinal plant which has been widely used to treat infectious and pain diseases, on acute kidney injury (AKI) using cisplatin-induced nephropathic models. ***Methods:*** Both *in vitro* renal tubular cells (NRK-52E) and *in vivo* rat models were used to demonstrate the nephroprotective effect of DP. ***Results:*** Methanolic extract from DP significantly reduced cisplatin-induced toxicity in renal tubular cells. Through successive liquid extraction, the extract of DP was separated into n-hexane, $CHCl_3$, EtOAc, n-BuOH, and H_2O fractions. Among these, the $CHCl_3$ fraction (DPCF) was found to be most potent. The protective activity of DPCF was found to be mediated through anti-oxidant, mitochondrial protective, and anti-apoptotic activities. In *in vivo* rat models of AKI, treatment with DPCF significantly reversed the cisplatin-induced increase in blood urea nitrogen and serum creatinine and histopathologic damage, recovered the level of anti-oxidant enzymes, and inhibited renal apoptosis. ***Conclusion:*** We demonstrated that DP extracts decreased cisplatin-induced renal toxicity, indicating its potential to ameliorate drug-associated acute kidney damage.

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Introduction

The kidney is one of the most susceptible target organs for drug-associated toxicity because of its high perfusion rate and high capability for drug uptake and metabolism.

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Various types of drugs can be accumulated in kidney tissue in either the parental form or metabolites, resulting in critical impairment of renal function. This drug-induced acute kidney injury (AKI) is a severe threat to hospitalized patients [1]. There have been intensive efforts to develop strategies to minimize drug-induced AKI. Among several drugs that induce AKI, cisplatin is the most established and has been commonly used in studies aimed to elucidate mechanistic and preventive factors for drug-associated AKI [2, 3].

Cisplatin [cis-dichlorodiammineplatinum (II), CP] is a potent chemotherapeutic drug that is used in the treatment of various solid tumors including testis, breast, lung, and uterine cervix carcinomas [4, 5]. However, the severe adverse effects of CP, including nephrotoxicity, have limited its clinical use in cancer treatment [2, 6]. Approximately one-third of patients undergoing CP treatment have shown clinical nephrotoxicity [2, 3], as indicated by reduced glomerular filtration rate, increased serum creatinine, and dysregulated serum electrolyte levels [3, 7]. The proximal tubular epithelial cells, in which CP is actively accumulated to levels 5-fold higher than that in serum [8], are known to be a major target for CP toxicity [9, 10]. Although the nephrotoxic mechanisms of CP are not completely understood, several studies have reported involvement of the generation of free radicals including reactive oxygen species, disruption of mitochondrial function, depletion of anti-oxidant capacity, and activation of cell death machinery including apoptotic or necrotic pathways [11-13]. To develop novel strategies to prevent and/or reduce drug-induced AKI, diverse pharmacologic and molecular approaches have been investigated using CP-induced AKI [7]; however, the clinical applications of these preventive approaches are still limited [3]. In this context, multi-modal approaches that can modulate the complex mechanisms of AKI may have benefits. Considering their pluripotent activities, natural products and herbal medicine are potential candidates for prevention of CP-induced AKI.

Dendropanax morbifera (Araliaceae) (DP) is an endemic species found in the southwestern region of South Korea [14]. Although the roots, leaves, seeds, and stems of this plant have long been used in traditional medicine for the treatment of several symptoms including headache, infectious diseases, skin diseases, and dysmenorrhea [15], the biologic activities of DP are not fully understood. Several recent papers reported the beneficial effects of DP extracts or compounds isolated from DP, including anti-complement, anti-atherogenic, anti-cancer, and anti-diabetic activities [16-19], and the anti-oxidant activity of DP was proposed as the main mechanism underlying these biologic benefits. Interestingly, the nephroprotective potential of DP was described in the Bencao Gangmu (Compendium of Materia Medica), the most complete and comprehensive pre-modern textbook on herbal medicine. However, the biologic activity of DP against nephrotoxicity has not been elucidated.

In this study, we evaluated the protective potential of Dendropanax morbifera against CP-induced nephrotoxicity using in vitro renal proximal tubular epithelial cells and in vivo rat models. The mechanisms underlying its protective effects were also investigated. Our study provides new insight into the biologic activity of DP, as well as its therapeutic potential as a novel treatment for drug-associated AKI.

Materials and Methods

Reagents
Cisplatin (CP) was obtained from Sigma-Aldrich (USA) and dissolved in DMEM (Dulbecco's Modified Eagle Medium; Gibco BRL, Canada). Extracts from Dendropanax morbifera Leveille were provided from CL Institute, and dissolved in DMSO (dimethyl sulfoxide, Merck, USA).

Plant material and Preparation of extracts from Dendropanax morbifera
The leaves of Dendropanax morbifera (DP) was purchased at a local herbal market in the southern area of Korea, in September 2012. The plant was authenticated and a voucher specimen has been deposited in the faculty of Herbarium of College of Pharmacy, Hanyang University (No. HYUP-DP-001). The dried leaves
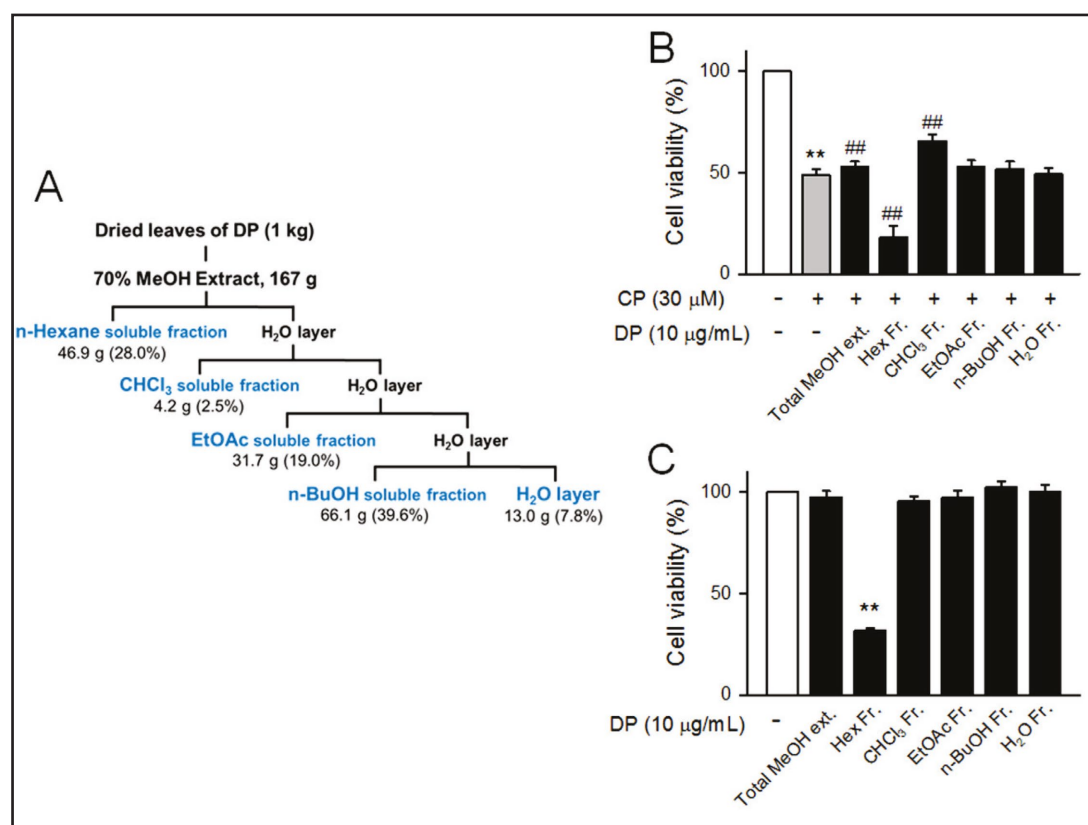
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Fig. 1. Effect of total MeOH extract and sub-fractions from *Dendropanax morbifera* (DP) on cisplatin-induced cytotoxicity in rat renal tubular epithelial cells (NRK-52E). (A) Representative scheme for preparation of each fraction was shown. (B and C) Viability in NRK-52E cells was determined using a MTT assay after treatment with DP extract or sub-fractions (10 μg/mL) with (B) or without (C) cisplatin (CP; 30 μM) for 24 hr. Values are means ± SEM. **p<0.01 vs. control, ##p<0.01 vs. CP alone. B: N=4; C: N=3.

of DP (1 kg) were extracted with 3 L of 70% MeOH for 2 weeks at room temperature. The extract solution of DP was vaporized under reduced pressure to yield approximately 167 g of crude total methanolic extract (yield 16.7%). For fractionation, the methanolic extract of DP was partitioned successively with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol (Fig. 1A). Each extract was vaporized to dryness under reduced pressure and stored at 4°C. The concentrations of DP extracts used in *in vitro* and *in vivo* studies were determined based on the literatures on bioactivity of extracts from natural products and our pilot studies.

Rat renal tubular cell culture

NRK-52E cells (rat renal tubular epithelial cells) were obtained from ATCC (VA, USA) and grown in DMEM supplemented with 5% heat-inactivated FBS (fetal bovine serum, Gibco BRL, USA) and 100 units/mL penicillin and 100 μg/mL streptomycin (Welgene, Korea). Cells were maintained in an incubator (95% air, 5% CO_2) at 37°C. Experiments were performed with NRK-52E cells at 80% confluence.

Cell viability

Cell viability was measured by MTT assay (Sigma-Aldrich, USA). Cells were seeded in 96-well plates at a density of 5×10^3 cells/well and incubated at 37°C for 24 h. After treatment with various concentrations of CP and/or DP extracts for 24 h, MTT solution was added (final concentration 5 mg/mL) and the cells were incubated at 37°C for 2 h. The supernatants were removed and 100 μL DMSO was added to each well to extract the formazan. The plate was mixed on plate shaker and the absorbance at 570 nm was recorded. Cell viability was calculated as a percentage compared to control cells.

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Intracellular reactive oxygen species (ROS) and mitochondrial membrane potential

Generation of ROS (reactive oxygen species) was examined using CM-H$_2$DCFDA (5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester, Invitrogen, UK) [20]. Cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated at 37°C for 24 h. After treatment with 30 μM CP and/or 10 μg/mL DP extract for 24 h, DCF (final 10 μM) was added to each well and the plates were incubated at 37°C for 20 min in the dark. Images were obtained by fluorescence microscopy (Nikon Eclipse Ti). The fluorescence signal score was calculated using a region of interest (ROI) statistics program for fluorescence microscopy.

Mitochondrial membrane potential was measured using JC-1 (Invitrogen, UK). After treatment with CP and/or DP as described above, JC-1 (final 5 μM) was added to each well and the plates were incubated for 30 min at 37°C in the dark. Images were captured by fluorescence microscopy. Red fluorescence corresponds to the aggregate form of JC-1 at normal membrane potential and green fluorescence indicates the monomeric form at disrupted lower membrane potential [21].

Caspase-3 activation

Activation of caspase-3, an enzyme responsible for apoptotic cell death, was examined by immunoblot analysis of cleaved active caspase-3 and enzymatic assay.

For immunoblotting, cells were seeded in 6-well plates at a density of 1×10^5 cells/well and incubated at 37°C for 24 h. After treatment with 30 μM CP and/or 10 μg/mL DP extracts for 24 h, the cells were lysed using RIPA buffer (Thermo Fisher Scientific, USA) containing a proteinase inhibitor cocktail (Thermo Scientific, USA) and harvested by centrifugation. The protein concentrations in the supernatant were determined by bicinchoninic acid assay (Thermo Scientific). Protein extracts were mixed with Laemmli sample buffer and heated at 95°C for 3 min. Samples were separated by SDS-PAGE using 12% gels and transferred to polyvinylidene difluoride membranes (Bio-Rad, USA). After blocking, the membrane was incubated with primary antibody targeting caspase-3 (Cell Signaling Technology, USA) or α-actinin overnight at 4°C. After washing, the membrane was incubated with secondary antibody and protein bands were detected by the SuperSignal® West Pico Chemiluminescent Substrate (Thermo Scientific, USA).

Activity of caspase-3 was examined using a caspase-3 assay kit (Cayman Chemical, USA). Cells were treated with CP and/or DP as described above, and lysed according to the protocol. Activity of caspase-3 was detected using a fluorescent substrate. Fluorescent intensity (λ_{ex}= 485 nm; λ_{em}=535 nm) was recorded and expressed as a percentage of the absorbance from the control cells.

DNA fragmentation

Cells were treated with 30 μM CP and/or 10 μg/mL DP extracts for 24 h, detached by trypsinization, and fixed with 70% EtOH at -20°C for 24 h. Samples were washed with PBS containing 1% FBS, and then incubated with Ribonuclease A (10 mg/mL; USB Co, USA) for 30 min at 37°C in the dark. Propidium iodide (PI, 2 mg/mL; Sigma, USA) was added and DNA fragmentation was analyzed by flow cytometry using a Guava® easyCyte™ 8 Flow Cytometer (Millipore, USA).

Animal treatment

All animal protocols were approved by the Institutional Animal Care and Use Committee at Hanyang University (IACUC 2012-105). Sixteen male Sprague Dawley rats (8 weeks old, body weight 200±10 g) were used (KOATECH, Korea). The rats were randomly divided into four groups and housed under standard laboratory conditions (12 h light: 12 h dark cycle at 25±2°C). Prior to initial treatment, all animals were fasted overnight although water was provided *ad libitum*. Body weight and consumption of food and water were measured on a daily basis. CP (6 mg/kg) was intraperitoneally (i.p.) injected into animals to induce nephrotoxicity. Dried extracts from DP were dissolved in DMSO and administered to animals by i.p. injection (1% DMSO in saline). DP was administered once at 24 hr before CP injection, and once a day for 5 consecutive days after CP injection.

The rats in the four groups were treated as follows; Group 1 (vehicle control): 1% DMSO in saline; Group 2 (CP alone): single administration of CP (6 mg/kg); Group 3 (DP 25 + CP): CP (6 mg/kg) with 25 mg/kg DP; Group 4 (DP 25 alone): 25 mg/kg DP.

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Rats were sacrificed on the seventh day after CP injection and blood and kidney samples were isolated to examine nephrotoxicity. Blood was collected and centrifuged for 15 min at 12,000 rpm to obtain serum. The levels of blood urea nitrogen (BUN) and creatinine were measured in Neodin VetLab (Seoul, Korea). The kidneys were weighed and subjected to histologic observation and biochemical assays.

Histopathologic examination

Kidneys were fixed in 4% formalin solution. After dehydration in graded alcohol, formalin-fixed kidneys were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) for evaluation by light microscopy. Histologic changes induced by acute tubular necrosis (ATN score) were evaluated in the outer stripe of the outer medulla and the cortex and quantified by counting the percentage of tubules that displayed cell necrosis, cast formation, loss of brush border, and tubule dilatation as follows: 0=none, 1= <10%, 2= 11-25%, 3= 26-45%, 4=46-75%, and 5= >76% [22]. At least 10 fields (×200) were evaluated for each slide.

Renal activity of superoxide dismutase and caspase-3

Kidney tissue was washed with cold phosphate buffered saline (PBS) to remove blood and homogenized in sucrose buffer (0.25 M sucrose, 10 mM Tris, and 1 mM EDTA, pH 7.4). Kidney homogenates were centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was used for determination of total superoxide dismutase (SOD) activities using a SOD determination kit (Sigma-Aldrich Chemicals GmbH, Bushs, Switzerland). The absorbance at 450 nm was recorded and SOD activity was calculated using the manufacturer's protocol.

Statistical analysis

The means and standard errors of means (SEM) were calculated for all treatment groups. Data were subjected to one-way ANOVA (analysis of variance) test and t-test to determine the statistical significance. For all analyses, a *p* value < 0.05 was considered significant.

Results

In vitro protective effect of DP against CP-induced toxicity in renal tubular epithelial cells

To examine the protective potential of *Dendropanax morbifera* (DP) on cisplatin (CP)-induced nephrotoxicity, we used tubular epithelial cells which are the most susceptible kidney cell type to xenobiotic-induced toxicity. Treatment with CP (30 μM) for 24 hr significantly decreased viability of rat kidney tubular epithelial cells (NRK-52E) (Fig. 1B). To evaluate the protective effect of DP, NRK-52E cells were treated with various DP extracts (10 μg/mL; total methanol extract and successive sub-fractions using n-hexane, $CHCl_3$, EtOAc, n-BuOH, and H_2O). Among these DP extracts, total MeOH extract and the $CHCl_3$ sub-fraction significantly protected cells against CP-induced cytotoxicity (Fig. 1B). In contrast, the viability of tubular cells exposed to CP with the DP hexane fraction was significantly decreased, possibly because of a cytotoxic effect of the DP hexane fraction (Fig. 1C). The total MeOH extract or other sub-fractions did not induce any cytotoxicity (Fig. 1C).

Next, we focused on the protective activity of the $CHCl_3$ sub-fraction from DP (DP chloroform fraction; DP CF), which showed the most potent protection among all sub-fractions. DP CF (0 to 10 μg/mL) significantly reversed CP-induced renal tubular cytotoxicity in a dose-dependent manner (Fig. 2A). Morphologic examination also revealed the protective effect of DP CF against CP-induced tubular cell damage (Fig. 2A).

To identify the underlying mechanism of the protective activity of DP, we investigated the anti-oxidant effect of DP CF on CP-induced intracellular ROS generation using DCF fluorescence. Cells were co-treated with 10 μg/mL DP extract and 30 μM CP for 24 h. CP alone increased ROS generation in NRK-52E cells, but this was significantly reduced by DP CF (Fig. 2B). Mitochondrial damage resulting from ROS generation is a major mechanism underlying CP-induced nephrotoxicity. We therefore examined whether DP CF reduced
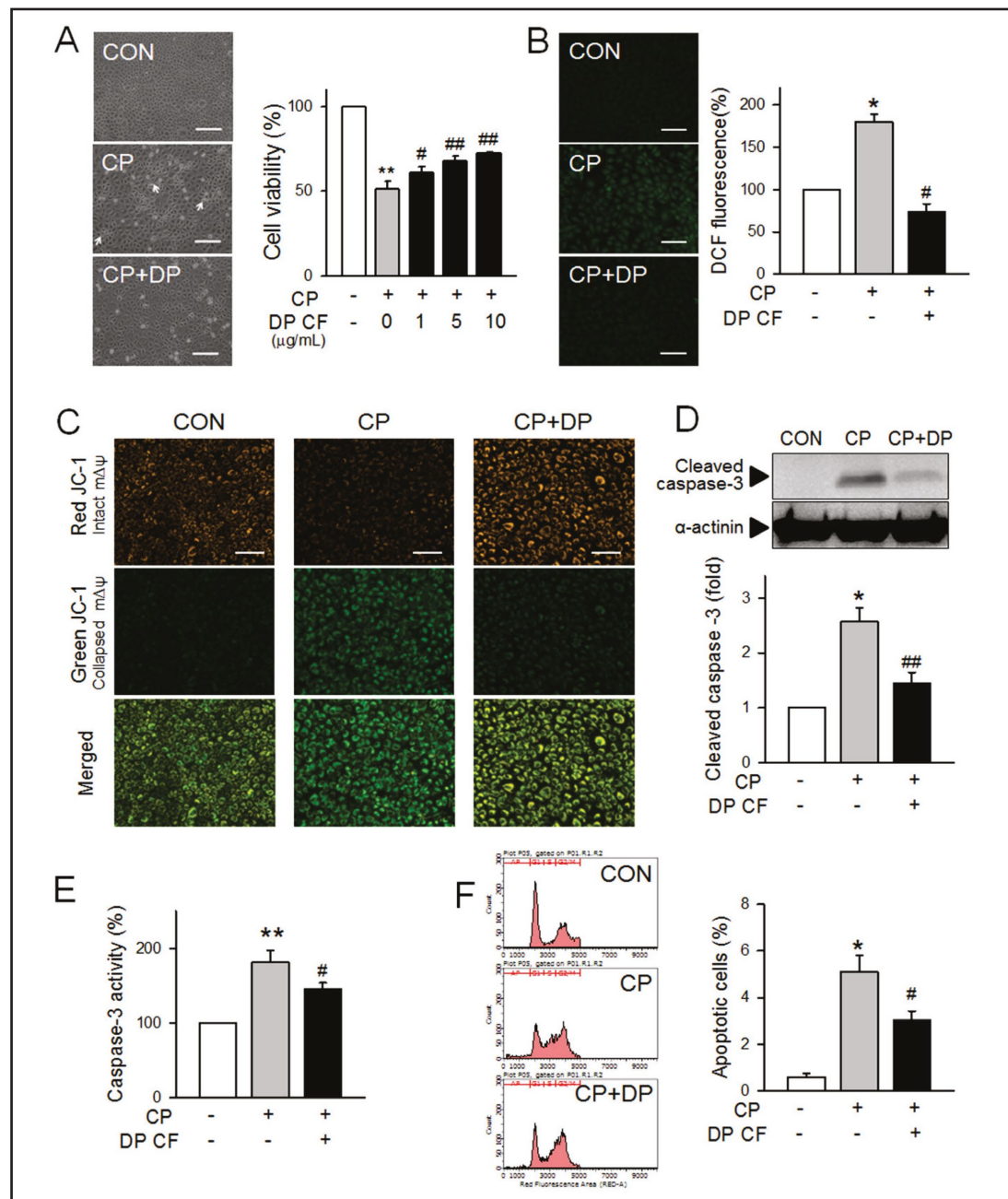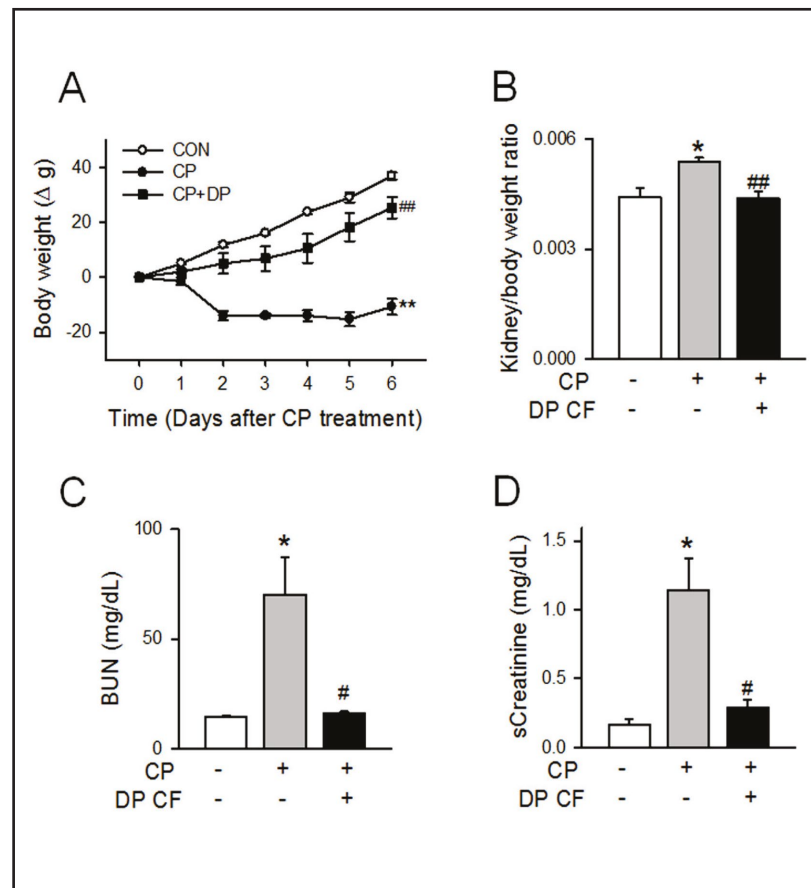
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Fig. 2. Protective effect of the $CHCl_3$ fraction of DP (DP CF) on CP-induced cellular damage in NRK-52E cells. NRK-52E cells were treated with CP (30 μM) and DP CF (0, 1, 5, or 10 μg/mL for A; 10 μg/ml for B to F) for 24 hr. (A) Morphologic changes in cells were observed by microscopy (left panel) and cell viability was examined by MTT assay (right panel). (B) ROS generation was measured by DCF fluorescence and representative photographs are shown (left panel). The DCF fluorescence signal score was obtained using a ROI statistics program for fluorescence microscopy (right panel). (C) Mitochondria membrane potential was measured using JC-1. Representative images of cells obtained by fluorescence microscopy are presented. (D) The level of caspase-3 cleavage (17-19 kDa) was detected by western blotting. Band densities were calculated using ImageJ program. (E) The activities of caspase-3 were examined by fluorescence assay. (F) Apoptotic DNA fragmentation was detected using flow cytometric analysis after staining the DNA with propidium iodide (PI). Scale Bar; 100 μm. Values are means ± SEM. *$p<0.05$, **$p<0.01$ vs. control, #$p<0.05$, ##$p<0.01$ vs. CP alone. A, B, C, D, and F: N=3; E: N=4.

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Fig. 3. *In vivo* effect of DP CF on CP-induced renal dysfunction in rats. CP (6 mg/kg) and/or DP (25 mg/kg) were administered to rats as described in the Methods section. (A) The change in body weight was monitored throughout the treatment schedule. (B) On the sixth day of CP treatment, the kidney was isolated and weighed. The ratio between kidney and body weight was calculated. (C and D) Renal function was determined by the level of blood urea nitrogen (BUN; C) and serum creatinine (sCreatinine; D). Values are means ± SEM. *$p<0.05$, **$p<0.01$ vs. rats in control group, #$p<0.05$, ##$p<0.01$ vs. rats treated with CP alone. A to D: N=4 rats/group.
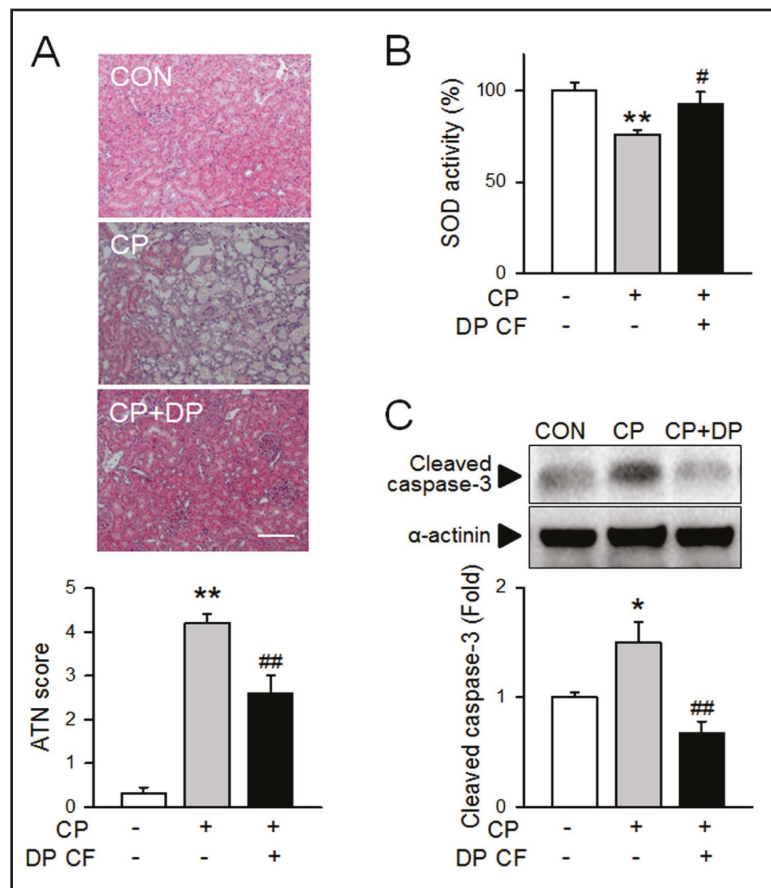

damage to the mitochondria membrane potential that is induced by CP. In the JC-1 assay, red fluorescence corresponds to the aggregate form of JC-1 at normal membrane potential whereas green fluorescence indicates the monomeric form present at a disrupted lower membrane potential [21]. Green fluorescence was significantly increased in NRK-52E cells after exposure to CP, suggesting that the mitochondrial membrane potential was impaired by CP. Notably, co-treatment with DP CF recovered the normal membrane potential, resulting in enhanced red fluorescence (Fig. 2C).

Increased ROS generation and disruption of the mitochondrial membrane potential during CP-induced nephrotoxicity induce apoptosis in renal tubular cells. We examined the protective effect of DP on CP-induced apoptosis by analyzing activation of pro-apoptotic caspase-3 and DNA fragmentation. Treatment with CP increased both the level of cleaved caspase-3 (active form) and its enzymatic activity, whereas addition of DP CF significantly reduced the activation of caspase-3 (Fig. 2D and 2E). Consistent with these findings, CP increased the percentage of apoptotic cells with abnormally fragmented DNA whereas co-treatment with DP CF and CP significantly decreased this apoptotic population, demonstrating the inhibitory effect of DP CF against CP-induced renal tubular apoptosis.

In vivo protection of CP-induced renal impairment by DP

To examine the *in vivo* relevance of the nephroprotective activity of DP, we evaluated the effect of DP CF on CP-induced kidney damage in rats. CP (6 mg/kg; dissolved in saline) and DP CF (25 mg/kg; dissolved in DMSO) were administered to rats by i.p. injection (final

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Fig. 4. Changes in kidney tissues after treatment with CP and/or DP CF. CP (6 mg/kg) and/or DP (25 mg/kg) were administered to rats as described in the Methods section. On the sixth day of CP treatment, the kidney was isolated. (A) Histologic changes in renal tissues were observed using H&E staining. Scale Bar; 200 μm. (B) Renal SOD activity in homogenized kidney samples was measured using a SOD determination kit. (C) The level of caspase-3 cleavage (17-19 kDa) was detected western blotting. Values are means ± SEM. *$p < 0.05$, **$p < 0.01$ vs. control, #$p < 0.05$, ##$p < 0.01$ vs. CP alone. A to C: N=4 rats/group.

1% DMSO diluted with saline). *In vivo* treatment of CP significantly reduced the increase in body weight (Fig. 3A), and increased the ratio of kidney to body weight (Fig. 3B). The ratio between body weight and isolated kidney weight, also known as the kidney index, is often used to reflect kidney damage because a kidney with impaired function has a greater weight due to edema [23]. Treatment with DP significantly rescued the change in body weight and the kidney/body weight ratio (Fig. 3A and 3B), suggesting that DP effectively reduced CP-induced nephrotoxicity *in vivo*. Indeed, kidney function, as determined by the level of blood urea nitrogen (BUN) and serum creatinine (sCreatinine), was significantly impaired in CP-treated rats, whereas administration of DP completely reversed the increase in BUN and sCreatinine (Fig. 3C and 3D). Treatment with DP CF (25 mg/kg) alone did not induce any changes in these parameters compared to rats in the control group (data not shown).

Concordant with the functional improvement, we also observed a protective effect of DP on CP-induced histopathologic damage in renal tissue (Fig. 4A). Renal tissues from CP-exposed rats exhibited acute tubular necrosis with epithelial cell desquamation in proximal and distal tubules, tubular dilation, epithelial flattening and sloughed epithelial cells. DP significantly improved these histopathologic changes, further indicating that DP had a protective function against CP-induced renal damage.

To investigate the mechanism underlying the *in vivo* DP protection, we examined the anti-oxidant capacity and caspase-3 activation in kidney samples. CP significantly decreased the level of renal SOD, a representative anti-oxidant enzyme. This decreased level of SOD was recovered by DP treatment, indicating that DP attenuated CP-induced oxidative stress in renal tissue (Fig. 4B). Consistent with our *in vitro* results, renal apoptosis was significantly enhanced in kidneys exposed to CP, as determined by the level of cleaved caspase-3.

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DP significantly decreased the activation of caspase-3 in renal tissue, suggesting that DP inhibited CP-induced renal apoptosis (Fig. 4B). Taken together, our findings demonstrate the protective effect of DP against CP-induced nephrotoxicity both in renal tubular cells and in rat animal models.

Discussion

In this study, we demonstrated that extracts from *Dendropanax morbifera* (DP) significantly recovered cisplatin (CP)-induced acute kidney injury (AKI) both in renal tubular *in vitro* models and rat *in vivo* models. Mechanisms underlying the nephroprotective effect of DP were shown to include anti-oxidant, mitochondrial preserving, and anti-apoptotic activities. Our findings provide new insight into novel biologic benefits of DP as a nephroprotective agent.

Despite its essential roles in maintaining homeostasis through clearance of endogenous/exogenous metabolites, control of fluid volumes, and maintenance of electrolytic balance, the kidney is a main target organ for xenobiotic toxicity because of its high perfusion rate and specialized uptake system [24, 25]. Notably, various nephrotoxicants, including many clinical drugs, can induce kidney damage including AKI, tubulopathies, proteinuric renal diseases, chronic kidney injury, and renal failure [26]. Considering the complexity of progressive renal damage, natural products that have multiple active ingredients with multiple actions may have beneficial activity against drug-induced nephrotoxicity. Drug-associated AKI is a serious complication of hospitalized patients and occurs in 25% of patients in intensive care unit [27]. Because CP is accumulated within the tubular epithelial cells to levels 5 times higher than those in blood and induces serious AKI, there have been many attempts to identify potential candidates that protect against CP-induced nephrotoxicity and elucidate their mechanism of action [28].

CP-induced renal damage is closely associated with an increase in oxidative stress in the kidney tissues [29]. CP triggers ROS generation in the kidney, and these ROS directly act on multiple cell components, including lipids, proteins, and DNA [30]. Interestingly, previous studies have shown that DP showed anti-oxidant activity [18], and our study demonstrated that DP could reverse CP-induced ROS generation in NRK-52E cells. Increased ROS production and impaired cellular redox status can enhance mitochondrial dysfunction and subsequent activation of the caspase cascade. Previous studies suggest that mitochondrial impairment is the major apoptotic pathway for renal tubular cell death induced by CP [3, 11]. Our study demonstrated a protective effect of DP against ROS generation and impairment of mitochondria membrane potential in renal tubular cells. It is generally accepted that recovery of impaired redox balance is critical in maintaining mitochondrial function, and we thought that anti-oxidant capacity of DP may represent the most principle bioactivity modulating nephrotoxicity. However, damaged mitochondria also aggravate ROS generation, and beneficial effect of DP on mitochondrial damage would contribute to reduced generation of ROS in CP-exposed tubular cells. Since mitochondrial dysfunction plays a key role in apoptosis, the protective effect of DP against mitochondrial impairment would underlie decreased apoptosis in CP-exposed kidney. We showed that induction of caspase-3 activation by CP was significantly reduced by DP extract both *in vitro* and *in vivo*, thus confirming the *in vivo* relevancy of the protective activity of DP.

Because the geographic distribution of DP as an endemic species is limited to southwestern regions of South Korea and some other areas of Asia, there are few scientific evaluations of this plant. Despite its traditional use in folk medicine for the treatment of pain and infectious diseases [15], the biologic activities of DP remain to be established. An anti-oxidant activity of DP has previously been proposed, implicating beneficial effects through anti-complement, anti-atherogenic, anti-cancer, and anti-diabetic activities. Moon et al. (2011) observed that DP improved diabetes-associated renal dysfunction in streptozotocin-

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induced diabetic rats [19], but these results may stem from anti-diabetic rather than nephroprotective effects. Here, we provide new evidence for the biologic activity of DP against drug-induced nephrotoxicity and a novel protective mechanism through preservation of mitochondrial function. Considering that mitochondrial damage is involved in diverse pathologic states, including ischemic damage, the effect of DP against these disease states also needs to be determined. Our current findings support the pleiotropic multimodal action of DP and expand its potential applications.

Because of the limited available resources with respect to active ingredients in DP, total methanol or ethanol extracts from DP have been commonly used to evaluate the biological benefits of DP [17, 31]. Here, we used total methanol extracts as well as successive sub-fractions prepared using n-hexane, $CHCl_3$, EtOAc, n-BuOH, and H_2O, and found that the DP-$CHCl_3$ fraction (DP CF) showed the most potent protective activity. As we focused on demonstrating the nephroprotective benefits of DP and elucidating the underlying mechanisms using *in vitro* and *in vivo* system, we could not expand our study to isolate a principle ingredient. Although several recent studies have proposed dendropanoxide and rutin as active ingredients in DP responsible for its anti-diabetic and neuroprotective activity, respectively [19, 31], these compounds are not likely to be present in DP CF on the basis of their molecular structure or polarity. Considering that natural products and their derivatives have been invaluable resource for drug discovery [32], further studies on the purification/identification of active compounds in DP CF are obviously necessary. Of note, the nephroprotective-bioactivity of pure compound from DP may demonstrate a high efficacy, since most of the target compounds from natural products represent much less than 1% of the crude extract [32] and we observed significant protection at concentrations of 10 μg/mL *in vitro* and 25 mg/kg BW *in vivo* with extracts. Identification of the nephroprotective active components of DP will be valuable for development of these compounds as adjuvants for the treatment of drug-induced AKI.

In summary, our data show that DP protected renal proximal tubular epithelial cells against CP-induced cytotoxicity through multiple mechanisms, including anti-oxidant, mitochondrial protecting, and anti-apoptotic activities.

Conclusion

Notably, we demonstrated *in vivo* relevancy of DP nephroprotection in CP-exposed rat models, with histopathologic, functional, and mechanistic supporting evidence. These findings support the biologic benefits of DP as an adjuvant therapy against drug-induced kidney damage.

Abbreviations

BUN, blood urea nitrogen; CP, cisplatin; DMSO, dimethyl sulfoxide; DP, *Dendropanax morbifera*; DP CF, chloroform fraction of DP extract; EDTA: ethylenediaminetetraacetic acid; FBS, fetal bovine serum; NRK-52E, *Rattus norvegicus* kidney tubular epithelial cells; ROS, reactive oxygen species; SOD, superoxide dismutase

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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Acknowledgments

References

1   Schetz M, Dasta J, Goldstein S, Golper T: Drug-induced acute kidney injury. Curr Opin Crit Care 2005;11:555-565.

2   Arany I, Safirstein RL: Cisplatin nephrotoxicity. Semin Nephrol 2003;23:460-464.

3   Pabla N, Dong Z: Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int 2008;73:994-1007.

4   Siddik ZH: Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003;22:7265-7279.

5   Wang D, Lippard SJ: Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 2005;4:307-320.

6   Chirino YI, Trujillo J, Sanchez-Gonzalez DJ, Martinez-Martinez CM, Cruz C, Bobadilla NA, Pedraza-Chaverri J: Selective iNOS inhibition reduces renal damage induced by cisplatin. Toxicol Lett 2008;176:48-57.

7   Yao X, Panichpisal K, Kurtzman N, Nugent K: Cisplatin nephrotoxicity: a review. Am J Med Sci 2007;334:115-124.

8   Kuhlmann MK, Burkhardt G, Kohler H: Insights into potential cellular mechanisms of cisplatin nephrotoxicity and their clinical application. Nephrol Dial Transplant 1997;12:2478-2480.

9   Kuhlmann MK, Horsch E, Burkhardt G, Wagner M, Kohler H: Reduction of cisplatin toxicity in cultured renal tubular cells by the bioflavonoid quercetin. Arch Toxicol 1998;72:536-540.

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

11 Cummings BS, Schnellmann RG: Cisplatin-induced renal cell apoptosis: caspase 3-dependent and -independent pathways. J Pharmacol Exp Ther 2002;302:8-17.

12 Francescato HDC, Coimbra TM, Costa RS, Bianchi MLP: Protective effect of quercetin on the evolution of cisplatin-induced acute tubular necrosis. Kidney Blood Press Res 2004;27:148-158.

13 Yousef MI, Saad AA, El-Shennawy LK: Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food Chem Toxicol 2009;47:1176-1183.

14 Han S, Jung Y, Ko M, Oh Y, Koh S, Kim M, Oh M: Phylogenetic relationships of the Dendropanax morbifera and D-trifidus based on PCR-RAPD. Korean J Genet 1998;20:173-181.

15 Bae K: The medicinal plants of Korea. Kyo-Hak Publishing Co Ltd: Seoul, Korea, 2000, p. 364.

16 Park BY, Min BS, Oh SR, Kim JH, Kim TJ, Kim DH, Bae KH, Lee HK: Isolation and anticomplement activity of compounds from Dendropanax morbifera. J Ethnopharmacol 2004;90:403-408.

17 Chung IM, Kim MY, Park SD, Park WH, Moon HI: In vitro evaluation of the antiplasmodial activity of Dendropanax morbifera against chloroquine-sensitive strains of Plasmodium falciparum. Phytother Res 2009;23:1634-1637.

18 Hyun TK, Kim MO, Lee H, Kim Y, Kim E, Kim JS: Evaluation of anti-oxidant and anti-cancer properties of Dendropanax morbifera Leveille. Food Chem 2013;141:1947-1955.

19 Moon HI: Antidiabetic effects of dendropanoxide from leaves of Dendropanax morbifera Leveille in normal and streptozotocin-induced diabetic rats. Hum Exp Toxicol 2011;30:870-875.

20 Kang K, Jung H, Lim JS: Cell death by polyvinylpyrrolidine-coated silver nanoparticles is mediated by ROS-dependnet signaling. Biomol Ther (Seoul) 2012;20:399-405.

21 Mitchell T, Saba H, Laakman J, Parajuli N, MacMillan-Crow LA: Role of mitochondrial-derived oxidants in renal tubular cell cold-storage injury. Free Radic Biol Med 2010;49:1273-1282.

Kidney Blood Press Res 2015;40:1-12

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22 Melnikov VY, Faubel S, Siegmund B, Lucia MS, Ljubanovic D, Edelstein CL: Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. J Clin Invest 2002;110:1083-1091.

23 Pandir D, Kara O, Kara M: Protective effect of bilberry (Vaccinium myrtillus L.) on cisplatin induced ovarian damage in rat. Cytotechnology 2014;66:677-685.

24 Deguchi T, Kusuhara H, Takadate A, Endou H, Otagiri M, Sugiyama Y: Characterization of uremic toxin transport by organic anion transporters in the kidney. Kidney Int 2004;65:162-174.

25 Naud J, Michaud J, Beauchemin S, Hebert MJ, Roger M, Lefrancois S, Leblond FA, Pichette V: Effects of chronic renal failure on kidney drug transporters and cytochrome P450 in rats. Drug Metab Dispos 2011;39:1363-1369.

26 Perazella MA: Renal vulnerability to drug toxicity. Clin J Am Soc Nephrol 2009;4:1275-1283.

27 Panesso MC, Shi M, Cho HJ, Paek J, Ye J, Moe OW, Hu MC: Klotho has dual protective effects on cisplatin-induced acute kidney injury. Kidney Int 2014;85:855-870.

28 Fouad AA, Morsy MA, Gomaa W: Protective effect of carnosine against cisplatin-induced nephrotoxicity in mice. Environ Toxicol Pharmacol 2008;25:292-297.

29 Atessahin A, Yilmaz S, Karahan I, Ceribasi AO, Karaoglu A: Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. Toxicology 2005;212:116-123.

30 Song J, Liu D, Feng L, Zhang Z, Jia X, Xiao W: Protective Effect of Standardized Extract of Ginkgo biloba against Cisplatin-Induced Nephrotoxicity. Evid Based Complement Alternat Med 2013;2013:846126.

31 Chung IM, Song HK, Kim SJ, Moon HI: Anticomplement activity of polyacetylenes from leaves of Dendropanax morbifera Leveille. Phytother Res 2011;25:784-786.

32 Koehn FE, Carter GT: The evolving role of natural products in drug discovery. Nat Rev Drug Discov 2005;4:206-220.