Improvement of Cisplatin-induced renal dysfunction by *Schisandra chinensis* stems via anti-inflammation and anti-apoptosis effects

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ABSTRACT

Ethnopharmacological relevance: Schisandra chinensis (Turcz.) Baill is a frequently used traditional Chinese medicine, and modern pharmacological research has proven that *S. chinensis* has antioxidant, anti-hepatotoxity, anti-inflammatory, and anti-nephrotoxic effects. Cisplatin is widely used as antineoplastic drug at present, but the clinical application is limited owing to its nephrotoxicity.

Aim of the study: To demonstrate the renoprotective activity of the extract of the stems of *S. chinensis* (SCE) in mice established by cisplatin-triggering acute kidney injury (AKI). The possible molecular mechanism of nephroprotection exhibited by SCE was evaluated for the first time.

Materials and methods: Mice in SCE groups were pre-treated with SCE for 10 consecutive days, and on 7th day 1 h after final administration, following intraperitoneal injection of cisplatin with 20 mg/kg was treated to cisplatin group and SCE groups. On the 10th day, renal function, histopathological change, and oxidative stress markers were investigated.

Results: Renal oxidative stress level characterized by elevated heme oxygenase 1 (HO-1), cytochrome P450 E1 (CYP2E1) and 4-hydroxynonenal (4-HNE) expression was obviously reduced by SCE pre-treatment. In addition, SCE was found to suppress inflammatory response through the reduction of nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) expression and nuclear factor-kappa B (NF-κB) p65 activation. SCE treatment also inhibited activation of apoptotic pathways through down-regulating Bax, cleaved caspase-3, 8, 9 and up-regulating Bcl-2 expression levels.

Conclusion: These findings illustrated that SCE possessed powerful protective effect on AKI caused by cisplatin via amelioration of oxidative stress, inflammation and apoptosis.

1. Introduction

Cisplatin is one of the most effective and commonly used antitumor drugs in clinical practice. It was widely applied to the treatment of many solid tumors such as ovary, breast, lung, cervix and many other tissues (Miller et al., 2010). However, its side-effect includes nephrotoxicity, hepatotoxicity, neurotoxicity and ototoxicity in the clinics (Amidi et al., 2016; Fuertes et al., 2003). The antitumour effect of cisplatin is proportional to the dosage but the accompanied nephrotoxicity is mainly responsible for the dose limitation of cisplatin (Kim et al., 2014). According to the statistics, about 30% of patients receiving cisplatin chemotherapy experienced a severe decline in renal function (Pabla and Dong, 2008). The researches on pathogenic mechanism showed that oxidative stress, inflammatory and apoptosis mediators were involved in cisplatin-induced nephrotoxicity (Chhourou et al., 2016; Zhang et al., 2007; Zhu et al., 2017). These inflammatory mediators mainly include NF-κB p65, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS). In inflammatory responses of process in cisplatin-induced nephrotoxicity, NF-κB mediated the expression of iNOS and other inducible genes such as COX-2 (Ma et al., 2017). Oxidative stress, playing a pivotal role in the pathogenesis of cisplatin caused severe AKI, is characterized by over generation of reactive oxygen species (ROS) (Sahu et al., 2011; Wei et al., 2015). And oxidative stress often gives rise to overproduction of free radicals and...
the occurrence of lipid peroxidation (LPO), which ultimately resulted in cisplatin-induced renal dysfunction (Chirino et al., 2008). Excessive ROS induced by cisplatin leads to DNA damage and triggers caspases activation. Caspases are recognized as cell death proteases that play vital roles in the execution phase of apoptosis (Song et al., 2013).

Schisandra chinensis (Turcz. BAILl. (S. chinensis), which is commonly recognized as “Wu Wei Zi” due to that its fruit exhibits five tastes including sour, pungency, bitter, salty and fragrance, is a frequently used as traditional Chinese medicine with many effects such as astringent, tonifying qi, nourishing kidney and calming (Zhai et al., 2017). Modern pharmacological research has proven that S. chinensis has antioxidant (Kim et al., 2009), antitumor (Zhao et al., 2014), anti-hepatotoxic (Li et al., 2015), anti-inflammatory (Guo et al., 2008), and anti-nephrotic effects (Bunel et al., 2014). Previous studies have been reported that S. chinensis extract could attenuate cyclophosphamide induced kidney damage in rat (Zhai et al., 2018) and the fruits extract of S. chinensis could attenuate streptozotocin-induced diabetic nephropathy in mouse (Zhang et al., 2012). Also, it has been proven that the fruits and stems of S. chinensis have the similar chemical composition, such as lignans, phenolic, and flavonoid, of which lignans are considered as major bioactive constituents (Szopa and Ekiert, 2011). Moreover, schisandrin and schisandrin B from the fruits of S. chinensis were found to possess nephroprotective effects on kidney injury caused by cisplatin in vitro (Bunel et al., 2014). Schisandrin B also ameliorated renal damage caused by mercury and gentamicin in a rat model (Chiu et al., 2008; Stacchiotti et al., 2011). As we know, S. chinensis is a perennial vine. In order to improve the yield and quality of the fruits, about 30–40% of stems from S. chinensis were pruned every year. In China, the utilization of the stems is minimal and considered as waste products of the S. chinensis processing industry (Mocan et al., 2016).

Considering the constituents and potential medicinal value of the underutilized stems and nephroprotective effects of the fruits of S. chinensis, we made a decision to explore the potential improvement effect of the stems of S. chinensis against cisplatin-induced nephrotoxicity in the present work. Importantly, as far as we know, it is the first to report the potential mechanism underlying SCE’s renal protection.

2. Materials and methods

2.1. Chemicals and Reagents

The dried S. chinensis stems were obtained from Harbin and identified by Professor Wei Li. The voucher specimen (No. 160516) was reserved in our lab of College of Chinese Medicinal Materials, Jilin Agricultural University.

Commercial assay kits for detecting reduced glutathione (GSH), malondialdehyde (MDA), blood urea nitrogen (BUN), and creatinine (CRE) were all provided by Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). The primary antibodies for NF-κB p65, iNOS, COX-2, 4-HNE, Bax, Bcl-2, HO-1, and CP2ZE1 were recruited from BOSTER Biological Technology (Wuhan, China). The antibodies caspase-3, 8, 9 and cleaved-caspase-3, 8, 9 were bought from Cell Signaling Technology (Danvers, MA, USA). DyLight 488-labeled and Cy3-labeled secondary antibodies were obtained from BOSTER Bio-Engineer Ltd., Co. (Wuhan, China). Kits for Hoechst 33258 and Hematoxylin and eosin (H&E) dye kits were acquired from Shanghai Beyotime Co., Ltd. (Shanghai, China). The kit of TUNEL for detection apoptosis was purchased from Roche Applied Science (Roche Applied Science, Germany). Other chemicals reagents used in the experiment were analytical grade provided by Beijing Chemical Factory.

2.2. Sample extraction and HPLC analysis

100 g of Dried and powdered stems of S. chinensis were ultrasonically-assisted extracted with 1.0 L of 100% ethanol in a water bath at 45 °C for three times. Subsequently, the combined extract was concentrated by evaporating dish and freeze dryer to get the crude extract (the extract of S. chinensis, SCE). The five major lignans in SCE was analyzed using HPLC analysis on a Waters e2695 system with UV detector. HPLC analysis were performed through a Hypersil ODS2 column (250 × 4.6 mm, 5 µm) with the chromatographic condition as follows: column temperature of 30 °C, flow rate of 1.0 mL/min, detection at 217 nm, and the mobile phase of acetonitrile (A) and water (B). A programmed gradient eluting was used as follows: 0–16 min, 48% A; 16–24 min, 48–55% A; 24–35 min, 55–70% A; 35–40 min, 75% A; 40–45 min, 75–48% A. The chromatographic peaks of five lignans were confirmed by their retention times compared to the reference standards containing Schisandrol A, Schisandrol B, Deoxyxyschisandrin, Schisandrin B and Schisandrin C. Quantification was performed by the integration of the peaks using the external standard method. The proposed HPLC method was validated with the tests including linearity range, precision and recovery. The limit of detection (LOD) of Schisandrol A, Schisandrol B, Deoxyxyschisandrin, Schisandrin B and Schisandrin C is 0.04 µg/mL, 0.03 µg/mL, 0.02 µg/mL, 0.02 µg/mL, and 0.03 µg/mL, respectively. The limit of quantification (LOQ) of Schisandrol A, Schisandrol B, Deoxyxyschisandrin, Schisandrin B and Schisandrin C is 0.012 µg/mL, 0.010 µg/mL, 0.006 µg/mL, 0.006 µg/mL, and 0.008 µg/mL, respectively. The methodological study of a good linear relationship ranged from 25 µg/mL to 800 µg/mL for five lignans. Intra-day precision was performed in triplicate on the same sample extracted on a single day, while the inter-day precision was carried out in triplicate on three different days. Variations were expressed by the relative standard deviations (RSD). The RSD values of the peak areas for five lignans were less than 2.0%. The recovery rates of five lignans ranged from 98.6% and 100.2% with their RSD values were less than 3.1%. These above results indicated that the present HPLC method could be applied to the determination of Schisandrol A, Schisandrol B, Deoxyxyschisandrin, Schisandrin B and Schisandrin C in the stems of S. chinensis. The content of individual lignan was expressed as mg/g, and the contents of Schisandrol A, Schisandrol B, Deoxyxyschisandrin, Schisandrin B and Schisandrin C are 5.81 mg/g, 7.26 mg/g, 1.99 mg/g, 4.56 mg/g, 0.39 mg/g, respectively. Fig. 1 shows chromatograms of SCE.

2.3. Animals

Male SPF grade ICR mice (weighing about 22–25 g) used for cisplatin-induced AKI were purchased from Changchun YISHI Experimental Animal Ltd., Co. with a Certificate of Quality No. of SCXK (Jl) 2016–0003 (Changchun, China). All experimental mice were acclimatized for 1 week before formal experiment, and housed under a controlled condition (22–24 °C, 55–60% relative humidity, 12 h light-dark cycle and were feed with a standard rodent chow and clean tap water). The animal experiments were approved by Jilin Agricultural University Animal Welfare and Research Ethics Committee (Permit Number: ECLA-JLAU-2016026).

2.4. Experimental design

The mice selected for the experiments were randomly divided into four groups (n = 8 animals per group), normal group, cisplatin group, and two treatment groups. SCE was dissolved in 0.05% carboxymethylcellulose sodium (CMC-Na) and was gavaged to the mice for ten consecutive days at doses of 300 and 600 mg/kg, respectively. The mice in normal and cisplatin groups were administered with only 0.9% saline. One hour after final treatment on day 7, the mice in normal group were gavaged with equal volume of 0.9% saline and other mice were given a single nephrotoxic dose of cisplatin (20 mg/kg, i.p.). At 72 h post-cisplatin challenge (Day 10), mice were sacrificed. Subsequently, the serum was collected and separated by centrifuge at 3500 rpm for 15 min at 4 °C after standing for 1 h and stored at −20 °C for determination of kidney function indicators. At the same time, the
Kidneys were harvested. 10% formalin was used to fix the left kidney section for histopathological assay, and the right kidney was stored at −80 °C until further analysis. Kidney index = kidney weight/ body weight (mg/g, × 100).

2.5. Biochemical assay

Serum was used for quantifying CRE and BUN with commercial kits according to the manufacturer’s protocols.

The antioxidant activity assay was conducted as previously described with slight modification (Li et al., 2015). The content of GSH in kidney tissues were measured based on the method provided by commercial kits. The production of lipid peroxidation was assayed via determining the hepatic MDA content using a commercial kit.

2.6. H&E and Hoechst 33258 staining

5 μm-thickness were made from formalin-fixed paraffin-embedded kidney tissues, which were stained with H&E dye kits following the manufacturer’s protocol. H&E-stained kidney sections were used to assess kidney damage using a light microscope for histopathological examination. Tubular injure scores were semi-quantitatively analyzed by counting the percent of tubules that displayed inflammatory infiltration and tubular necrosis as follows: 0, no damage; 1, < 10%; 2, 10–25%; 3, 25–75%; 4, > 75% (Li et al., 2016).

The sections were stained by Hoechst 33258 dyeing kits according to previous the procedure in our laboratory (Li et al., 2016). Briefly, the paraffin-embedded tissue samples were stained with Hoechst 33258 solution with 10 μg/mL. The stained nuclei was detected under UV detector and photographed under fluorescence microscopy (Leica TCS SP8, Germany) following washed three times with PBS.

2.7. TUNEL staining

TUNEL assay was carried out as previously described with minor modification (Domitrović et al., 2013). The TUNEL staining technique using an In Situ Cell Death Detection kit (Roche Applied Science, Germany) according to manufacturer’s introduction. Kidney sections were counterstained with hematoxylin prior to analysis by light microscopy.

2.8. Immunohistochemistry and immunofluorescence

Immunohistochemical assays for Bax, Bcl-2, COX-2 and iNOS were performed as previously described (Zhang et al., 2009). In short, deparaffinized kidney tissues slices were incubated overnight at 4 °C with the primary antibodies of COX-2(1:200), iNOS (1:200), Bax (1:200), and Bcl-2 (1:200), and then washed thrice with PBS. Subsequently, the samples were incubated with secondary antibody for 1 h at 37 °C, and then washed thrice with PBS. Substrate was added to the sections for 30 min and stained with 3,3′-diaminobenzidine (DAB), then

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Fig. 1. HPLC chromatogram of lignans from the stems of S. chinensis. Structures of 5 lignans in S. chinensis (A) reference standard (B) and sample (C). Schisandrol A (1), Schisandrol B (2), Deoxyschisandrin (3), Schisandrin B (4), Schisandrin C (5).
counterstained with hematoxylin. The immunostaining intensity was analyzed by light microscopy (Olympus BX-60, Tokyo, Japan).

Immunofluorescence was performed as indicated for immunohistochemistry. The kidney tissues were incubated overnight at 4 °C with primary antibodies on CYP2E1 (1:200), HO-1 (1:50), 4-HNE (1:200). After 12 h, the kidney tissues were covered with DyLight 488-labeled and Cy3-labeled goat anti-rabbit IgG and visualized under the Leica fluorescence microscope equipped with a camera (Leica TCS SP8, Germany) and immunofluorescence intensity were analyzed by Image-Pro Plus 6.0 software.

2.9. Immunoblotting

The western blotting analysis were slightly modified as previously described (Mitazaki et al., 2011). Kidneys samples were homogenized in RIPA buffer. Protein samples (50 µg) were resolved by denatured 12% SDS-PAGE, transferred to PVDF membranes, which were probed with primary antibodies iNOS (1:100), COX-2 (1:100), NF-κB p65 (1:1000), caspase-3, 8, 9 (1:1000), cleaved-caspase-3, 8, 9 (1:1000), Bcl-2 (1:2000), and Bax (1:2000) overnight at 4 °C, followed by incubation with secondary antibody. Blots were probed for GAPDH as a loading control. Signals were detected using ECL substrate (Pierce Chemical Co., Rockford, IL, USA). Protein band intensities were analyzed using densitometry.

2.10. Statistical analysis

Data were presented as Means ± S.D (n = 8 per group). All statistical analysis was carried out with SPSS software (SPSS, Chicago, IL, USA). Differences between the groups were evaluated with one-way analysis of variance (ANOVA). It was considered that the probability value less than 0.05 or 0.01 was significant.
3. Results

3.1. Effects of SCE on body weight and organ index

Body weights were evaluated in mice. As shown in the Fig. 2A, the mice exposure to cisplatin with 20 mg/kg caused remarkable weight loss than those of normal mice, while SCE reversed these changes. As depicted in the Fig. 2B, the increase in organ index of the kidneys evaluated in mice at 72 h after cisplatin exposure was blunted after SCE treatment ($P < 0.05$, $P < 0.01$).

3.2. SCE inhibits cisplatin-induced renal dysfunction

In this work, CRE and BUN levels were detected at 72 h after cisplatin exposure in all groups. As is shown in the Figs. 2C and 2D, a single injection of cisplatin caused the overexpression of nephrotoxic markers such as serum CRE and BUN ($P < 0.01$), revealing a severe nephrotoxicity injury. However, the CRE and BUN levels were obviously reduced after SCE pretreatment compared with the cisplatin-treated mice ($P < 0.05$, $P < 0.01$).

3.3. Effects of SCE on cisplatin-induced oxidative stress

The previous studies indicated that oxidative stress is involved in the mechanisms of cisplatin caused kidney toxicity (Fernandez-Rojas et al., 2015). As shown in Figs. 2E and 2F, cisplatin treatment caused a dramatically decrease of GSH companied with the increased MDA level compared with those of normal mice ($P < 0.01$). However, treatment with SCE could obviously reduce overproduction of MDA and inhibited cisplatin-induced GSH decrease ($P < 0.05$). In addition, the overexpression of the CYP2E1 and HO-1 caused by cisplatin were reversed by SCE dose-dependently ($P < 0.01$, $P < 0.05$) (Fig. 3 A-B). Our data suggested that pretreatment with SCE could protect the kidneys from cisplatin caused serious oxidative stress. Lipid peroxidation was verified by 4-HNE staining to confirm whether there is a correlation between oxidative stress and the occurrence of cisplatin-induced kidney toxicity. At 72 h after cisplatin exposure, strong 4-HNE fluorescence intensities were analyzed in the tubular epithelium of the kidney tissues in the cisplatin group ($P < 0.01$). However, SCE-treatment could significantly reduce these fluorescence intensities ($P < 0.05$) (Fig. 3 C).
3.4. Histopathological studies

Histological changes of kidney slices in each treatment group are shown in Fig. 4 A, and a quantitative assessment of kidney tubules injury is shown in Fig. 4 D. Cisplatin exposure resulted in inflammatory infiltration and severe necrosis (P < 0.01). In contrast, the administration of SCE prevented some alterations evoked by cisplatin in kidney tissues (P < 0.05).

To determine whether supplement with SCE reduced renal tubular cell apoptosis in the presence of cisplatin, Hoechst 33258 staining was employed. As shown in Fig. 4 B, in the cisplatin group, a large number of blue positive cells were visualized, showing large area of renal tubular cell apoptosis caused by cisplatin. In contrast, pretreatment with SCE obviously decreased the number of positive cells.

Tubular cell apoptosis plays an important pathogenic role for cisplatin-induced AKI. Kidney tissues sections of 5 µm-thickness were stained using TUNEL colorimetric assay to assess the ability of SCE to inhibit cisplatin-induced apoptosis in vivo. As depicted in Figs. 4C and 4E, the kidney slices in the normal group almost no apoptosis cells were observed. Compared with the normal group, TUNEL positive cells were markedly elevated in cisplatin group (P < 0.01). However, treated with SCE reduced the expressions of TUNEL positive cells in kidney slices (P < 0.05, P < 0.01). Moreover, western blotting was used to analyze the apoptosis protein cleaved caspase-3, 8, 9, and these protein expression levels were obviously elevated after cisplatin injection, but SCE treatment reverse this change (P < 0.01, P < 0.05) (Fig. 6B).

3.5. SCE ameliorates cisplatin-induced renal inflammation

In the present work, we determined COX-2 and iNOS expression...
levels in each treatment group using immunohistochemical analysis to determine anti-inflammatory effects of SCE. As shown in Fig. 5, the positive expression of COX-2 and iNOS in the kidney tissues of the normal group was negligible, whereas were dramatically increased in the cisplatin treated mice \((P < 0.01)\). Importantly, administration of SCE caused dose-dependent decrease of iNOS and COX-2 expression \((P < 0.05)\). Next, we further examined iNOS, COX-2 and NF-κB p65 protein expression by western blotting. The results indicated that the protein expression levels including iNOS, COX-2 and NF-κB p65 was obviously up-regulated by cisplatin and reversed by SCE treatment \((P < 0.01, P < 0.05)\) (Fig. 6A).

3.6. SCE attenuates cisplatin-induced tubular apoptosis

Immunohistochemical and western blotting analyses were performed to explore the mechanisms for cisplatin-induced nephrotoxicity and to determine the effect of SCE on the Bax and Bcl-2 protein expression. As depicted in Fig. 5, we observed increasing expression of Bax in cisplatin group compared with normal group, clearly demonstrating cisplatin induced apoptosis in tubular cells \((P < 0.01)\). SCE treatment in dose-dependently obviously decreased Bax positive expressions of cell nucleus compared to the cisplatin-treated mice \((P < 0.05)\). Bcl-2 positive expression was surveyed in the nucleus of tubular cells. Our data showed that SCE inhibited the decreasing expression of Bcl-2 notably compared to cisplatin group \((P < 0.05)\). Western blotting results also confirmed that pretreatment with SCE down-regulated the protein expression of Bax and up-regulated the protein expression of Bcl-2 \((P < 0.05)\) (Fig. 6B).

Fig. 5. Effects of SCE on the protein expression levels of: Bcl-2 (A), Bax (B), and COX-2 (C), iNOS (D). Protein expressions were measured by immunohistochemistry in kidney tissues. Percentage of stained area (E, F, G, H). All data were expressed as mean \(\pm\) S.D., \(n = 8\). **\(P < 0.01\) vs. normal group; #\(P < 0.05\) vs. cisplatin group.
4. Discussion

Cisplatin is widely applied in clinical treatment of various solid tumors of chemotherapy drugs (Chitourou et al., 2016). Numerous studies during the past 10 years has revealed many cellular mechanisms of the cisplatin induced renal cell death (Peres and da Cunha, 2013). It has become obviously that generation of free radicals, inflammation and apoptosis and other mechanisms were involved in cisplatin-induced renal tubular damage (Domitrovic et al., 2013b; Zirak et al., 2014).

Although the fruits are highly nutritional, the stems of *S. chinensis* are wasted and the reports on its chemical composition and bioactivities is little. A recent study indicated that Wuzhi tablet (an ethanol extract of *S. sphenanthera*), Schizandrin and Schizandrin B could ameliorate cisplatin-induced nephrotoxicity (Bunel et al., 2014; Jin et al., 2015). Hence, we supposed whether the stems of *S. chinensis* extract could attenuate cisplatin-induced AKI.

In the present work, the results clearly indicated that cisplatin exposure caused nephrotoxicity followed severe renal tissue damage and failure of renal function, including the decrease of body weights and the elevations in kidney index, and serum CRE as well as BUN levels. However, pretreatment with SCE obviously decreased the kidney index, serum levels of CRE and BUN and increased the body weights, indicating that SCE treatment significantly ameliorated renal dysfunction caused by cisplatin via biochemical analysis and kidney tissue morphological examination.

Oxidative stress plays a vital role in the pathogenesis of cisplatin caused kidney toxicity (Qi et al., 2017). Oxidative stress generated by the production of ROS following cisplatin challenge, which decreases GSH content and reduces activities of antioxidant enzymes. MDA, a marker of oxidative stress, is a product of lipid peroxidation (Rafieian-Kopaei et al., 2014). It is the most studied biologically relevant free radical reaction (Niki, 2008; Song et al., 2016), increased in the cisplatin-intoxicated mice kidney. In this study, cisplatin exposure caused the decrease of GSH and increase in MDA level. Pretreatment with SCE reduced lipid peroxidation, and restored antioxidant ability via inhibiting GSH decrease. The results of our study were in line with a previous study that Schisandrin B suppressed ICV-infused amyloid β caused oxidative injuries (Giridharan et al., 2015). Furthermore, the process of oxidative stress can cause the damage of tissues and organs. Drug-metabolizing enzyme CYP2E1 mediated biotransformation of cisplatin to generate ROS, including hydrogen peroxide and hydroxyl radical, which play a vital role in cisplatin caused AKI (Liu et al., 2002). Heme oxygenase (HO) is a stress-responsive enzyme that can reduce highly deleterious free heme and produce anti-oxidants, anti-inflammatories, and modulators of cell death thereby maintaining homeostasis under pathological conditions (Fan et al., 2012; Soares and Bach, 2009). 4-HNE is one of the final products of lipid peroxidation, which was measured to determine oxidative stress in the kidneys (Domitrovic et al., 2014). In this work, the expression in CYP2E1, HO-1 and 4-HNE were low in the normal group, while significantly elevated after cisplatin exposure. Interestingly, pretreatment with SCE reduced these expression levels, suggesting significant ameliorative effect. These results indicated that SCE might decrease the nephrotoxicity induced by cisplatin in mice effectively by reducing the excessive oxidative stress and lipid peroxidation.

Moreover, inflammation plays a vital role in the pathogenesis of cisplatin caused kidney toxicity (Miller et al., 2010). As we know, NF-κB signaling pathway plays a key role in the oxidative stress induced inflammatory reaction. It is a ubiquitous protein complex that participates in the regulation of cellular signaling in a variety of conditions...
of renal damage caused by cisplatin (Ansari, 2017). The Bcl-2 family was associated with apoptosis, which is one of the crucial mechanisms activated, specifically including COX-2, is considered as another important mechanism in cisplatin-induced apoptosis. Moreover, in order to further demonstrate the anti-apoptotic effects of SCE on cisplatin-induced kidney damage through inhibition of oxidative/nitrosative stress, inflammation, autophagy and apoptosis. Food Chem. Toxicol. 62, 397–406.

In conclusion, these data from the present work clearly suggested that SCE attenuates cisplatin-induced oxidative stress and nephrotoxicity. Toxicology 245, 18–23.

Numerous evidence shown that cisplatin-induced nephrotoxicity was associated with apoptosis, which is one of the crucial mechanisms of renal damage caused by cisplatin (Ansari, 2017). The Bcl-2 family contains both pro-apoptotic and anti-apoptotic protein factors, which is a well-known critical intracellular checkpoint for apoptosis within a common cell death pathway (Lee and Gustafsson, 2009). Bax, a protein of the Bcl-2 family that predispose cells to apoptosis. Bcl-2, another member of the Bcl-2 family, could antagonize the apoptosis signaling (Kroemer et al., 2007). Increased Bax expression supported the physiological role of this proapoptotic protein in cisplatin-induced kidney toxicity (Wei et al., 2007). Caspases, cysteine-dependent aspartate-specific proteases, are notable for typical regulations of apoptosis. Upon receiving the death signals from dysfunctional mitochondria, the initiator caspases (caspase 8 and 9) gets activated which initiate the activation of downstream caspases-3, which activates the form of cleaved caspase-3 and exhibits a crucial function in apoptosis (Song et al., 2013; Uchiyama et al., 2002). Moreover, previous study suggested that schisandrin B from S. chinensis inhibited the cleaved caspase-3 expression caused by cisplatin (Giridharan et al., 2012). In this work, the protein expression levels of Bax, Bcl-2, and cleaved caspase-3, 8, 9 were analyzed by IHC and western blotting analysis. Our data showed that cisplatin exposure leads to the increase of protein expression of Bax, cleaved caspase-3, 8, 9, with concomitant reduction of Bcl-2 expression, ultimately resulting in apoptotic cell death. Interestingly, supplement with SCE significantly reversed these changes caused by cisplatin exposure, indicating the ameliorative effects of SCE on cisplatin-induced apoptosis. Moreover, in order to further demonstrate the anti-apoptotic effects of SCE on cisplatin-induced nephrotoxicity, the results from Hoechst 33258 and TUNEL staining suggested that SCE treatment could suppress apoptosis of cisplatin-induced nephrotoxicity.

5. Conclusions

In conclusion, these data from the present work clearly suggested that SCE exert important therapeutic benefits against cisplatin-induced nephrotoxicity, evidenced by inhibiting oxidative stress, inflammation, and apoptosis. Importantly, the findings indicated that the stems of S. chinensis could be considered as natural dietary supplements for protecting renal functions.

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Conflicts of interest

The authors declare no conflict of interest.

References


