**Natural Product Sciences**
20(4) : 251-257 (2014)

# Quatification of Flavonoid Contents in Chungsimyeonja-tang, a Multi-Herbal Decoction, and Its Protective Effect against Cisplatin-induced Nephrotoxicity

**Young-Jung Kim[1,†], Tae-Won Kim[1,†], Chang-Seob Seo[2], So-Ra Park[1], Hyekyung Ha[2], Hyeun-Kyoo Shin[2] and Ju-Young Jung[1,*]**

*[1]College of Veterinary Medicine & Institute of Veterinary Science, Chungnam National University, 220 Gung-dong, Yusung-gu, Daejeon 305-764, Republic of Korea*
*[2]Basic Herbal Medicine Research Group, Korea Institute of Oriental Medicine, 483 Expo-ro, Yusung-gu, Daejeon 305-811, Republic of Korea*

**Abstract** – Chungsimyeonja-tang (CSYJT) is an herbal decoction that consists with 12 herbal medicines. CSYJT has been shown to have anti-stress, anti-allergic and anti-oxidant effects. The aim of this study was to determine flavonoid contents in CSYJT and evaluate its protective effect against cisplatin induced nephrotoxicity using both *in vitro* (porcine renal epithelial cell; PK15 cell) and *in vivo* (Sprague Dawley rat) experiments. In the present study, thee mean contents of baicalin, wogonoside and baicalein in CSYJT were 14.65, 5.27 and 0.02 mg/g, respectively. The CSYJT extract treatment attenuated the following alteration in porcine renal epithelial (PK15) cell: the increase in reactive oxygen species (ROS), the glutathione depletion and the increase in p53 expression induced by cisplatin treatment. In the *in vivo* study, rats were orally treated with CSYJT extract once a day for 28 days. Five days before the last treatment, cisplatin (5 mg/kg) was intraperitoneally injected to induce acute renal failure. Increased blood urea nitrogen (BUN) and creatinine (CRE) levels after cisplatin treatment were ameliorated by pretreatment of CSYJT extract. In addition, lipid peroxidation was decreased and antioxidant enzyme (glutathione) was recovered in CSYJT pretreated kidney tissue. In histopathological examination, CSYJT pretreated group showed ameliorated pathological alteration after cisplatin injection with decreased apoptosis. Taken together, pretreatment of CSYJT could ameliorate cisplatin-induced nephrotoxicity.
**Keywords** – Chungsimyeonja-tang, Acute renal failure, cisplatin, p53

## Introduction

Acute renal failure (ARF) is a frequent complication in critically ill patients and the incidence of ARF was rising over the past two decades.[1] Preventions of the occurrence and progression of ARF have become a very important issue. In recent years, great effort has been focused on traditional and herbal medicine without toxic effects to provide a novel therapeutic agent of ARF.[2] Cisplatin (*cis*-Diammineplatium II dichloride) is widely used as a chemotherapy drug for solid tumors.[3] Despite its potency, cisplatin treatment has limitation due to its nephrotoxicity which leads to acute kidney injury and renal failure.[4]

CSYJT contains three major flavonoid including baicalin, baicalein and wogonoside. Traditionally, CSYJT was known to have anti-stress, anti-allergic and anti-oxidant effects.[5] In addition, CSYJT also has been prescribed to treat cerebral infaction.[6] Several studies have been conducted to explore its diverse therapeutic properties.[6-8] However, there is no report on the effects of the CSYJT extract on the cisplatin-induced acute renal failure. The main objective of the present study was to investigate the protective effect of the CSYJT extract on the cisplatin-induced nephrotoxicity. In addition, quantitative determination of three major compounds in CSYJT was carried out which is important for its quality control using the HPLC-PDA.

## Experimental

**Cheongsimyeonja-tang (CSYJT)** – Cheongsimyeonja-tang (CSYJT) is an herbal prescription that consisted with 12 herbal medicines (Table 1).

**General experimental procedures** – Baicalin (> 98%)

*Author for correspondence
Ju-Young Jung, D.V.M., Ph. D., College of Veterinary Medicine, Chungnam National University, 220 Gung-dong, Yusung-gu, Daejeon 305-764, South Korea
Tel: +82-42-821-8899, 7902; E-mail: jyjung@cnu.ac.kr

†These authors contributed equally to this work

**Table 1.** Composition of CSYJT

| Scientific name | Latin name | Amount (g) | Source |
|---|---|---|---|
| *Nelumbo nucifera* | Nelumbinis Semen | 7.5 | HMAX (China) |
| *Dioscorea japonica* | Dioscoreae Rhizoma | 7.5 | Omniherb (Korea) |
| *Asparagus cochinchinensis* | Asparagi Tuber | 3.75 | HMAX (China) |
| *Liriope platyphylla* | Liriopis Tuber | 3.75 | Omniherb (Korea) |
| *Polygala tenuifolia* | Polygalae Radix | 3.75 | HMAX (China) |
| *Acorus gramineus* | Acoi Gramineri Rhizoma | 3.75 | HMAX (China) |
| *Zizyphus jujuba* | Zizyphi Semen | 3.75 | HMAX (China) |
| *Dimocarpus longan* | Longanae Arillus | 3.75 | HMAX (Vietnam) |
| *Thuja orientalis* | Thujae Semen | 3.75 | HMAX (China) |
| *Scutellaria baicalensis* | Scutellariae Radix | 3.75 | HMAX (Korea) |
| *Raphanus sativus* | Raphani Semen | 3.75 | HMAX (China) |
| *Chrysanthemum indicum* | Chrysanthemi Flos | 1.14 | HMAX (China) |
| Total | | 49.89 | |

and baicalein (> 98%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Wogonoside (> 98%) was from Tauto Biotech (Shanghai, China). HPLC-grade reagents, methanol, acetonitrile, and water were obtained from J.T.Baker (Phillipsburg, NJ, USA). Glacial acetic acid was of analytical reagent grade, procured from Junsei chemical (Tokyo, Japan). The herbal materials for CSYJT were purchased from Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea) and CSYJT was extracted from the Korea institute of Oriental Medicine. The origin of the samples was confirmed taxonomically by Prof. Je Hyun Lee, Dongguk University (Gyeongju, Korea). A voucher specimen (2008-KE15-1~12) was deposited at the Basic Herbal Medicine Research Group, Korea Institute of Oriental Medicine. PK 15 cell was from Korea cell line Bank. Lyophilized cisplatin (≥ 99.0%) and captopril (≥ 98.0%) were from Sigma-Aldrich (St. Louis, MO, USA). Reduced glutathione (GSH, NWK-GSH 01) and malondialdehyde (MDA, NWK-MDA01) assay kit and EZ-Cytox Cell Viability assay kit (EZ3000) were obtained from Northwest life science (Vancouver, WA, USA) and Daeil Lad Service (Seoul, Korea), respectively. Anti-p53 antibody and anti-mouse IgG were from Santa Cruz Biotechnology D (Santa Cruz, CA, USA). Enhanced chemiluminescence detection (ECL) kit was obtained from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Serum creatinine (CRE) and blood urea nitrogen (BUN) test kits were bought from IDEXX (Westbrook, ME, USA).

**CSYJT and HPLC sample preparation** – A CSYJT was prepared with 12 different chopped crude herbs (Table 1) and extracted in distilled water at 100 °C for 2 h. The solution was evaporated to dryness and freeze-dried (yield: 36.7%). For HPLC analysis, lyophilized CSYJT extract (200 mg) was dissolved in distilled water (20 mL) and mixed. The solution was filtered using a Smart Pore GHP syringe filter (0.2 μm, Woongki Science, Seoul, Korea). Standard stock solutions of compounds (baicalin, baicalein and wogonoside) were prepared in methanol (1,000 μg/mL) and stored below 4 °C. Working standard solutions were prepared by serial dilutions from stock solution with methanol.

**HPLC analysis of CSYJT** – A stock solution of compounds were prepared and diluted to the appropriate concentration range for the establishment of calibration curves. A Shimadzu LC-20A HPLC system (Shimadzu Co., Kyoto, Japan) consisting of a solvent delivery unit, an on-line degasser, a column oven, an auto sampler, and a PDA detector. The data processor employed LC solution software (Version 1.24). The analytical column used was a Luna C18 (250 × 4.6 mm; particle size 5 μm, Phenomenex, Torrance, CA, USA).The mobile phases consisted of 1.0% (v/v) aqueous acetic acid (A) and 1.0% (v/v) acetic acid in acetonitrile (B). The linear gradient elution system was performed as follows; 95 - 30% A (0 - 40 min), 30 - 0% A (40 - 45 min), 0% A (45 - 50 min), 0 - 95% A (50 - 55 min) and 95% finally 95% A isocratically (55 - 70 min). Analysis was performed at a flow-rate of 1.0 mL/min with detection wavelength at 275 nm. The injection volume was 10 μl. The linearity of the peak area (y) versus concentration (x, μg/mL) curve for compounds were used to calculate the contents of the main components in CSYJT.

**Cell viability assay** – For all assays, PK15 cells were seeded at a density of $1 \times 10^4$ cells/mL in 96-well plates with regular growth medium (DMEM with FBS and antibiotics) and the experiments were carried out on the

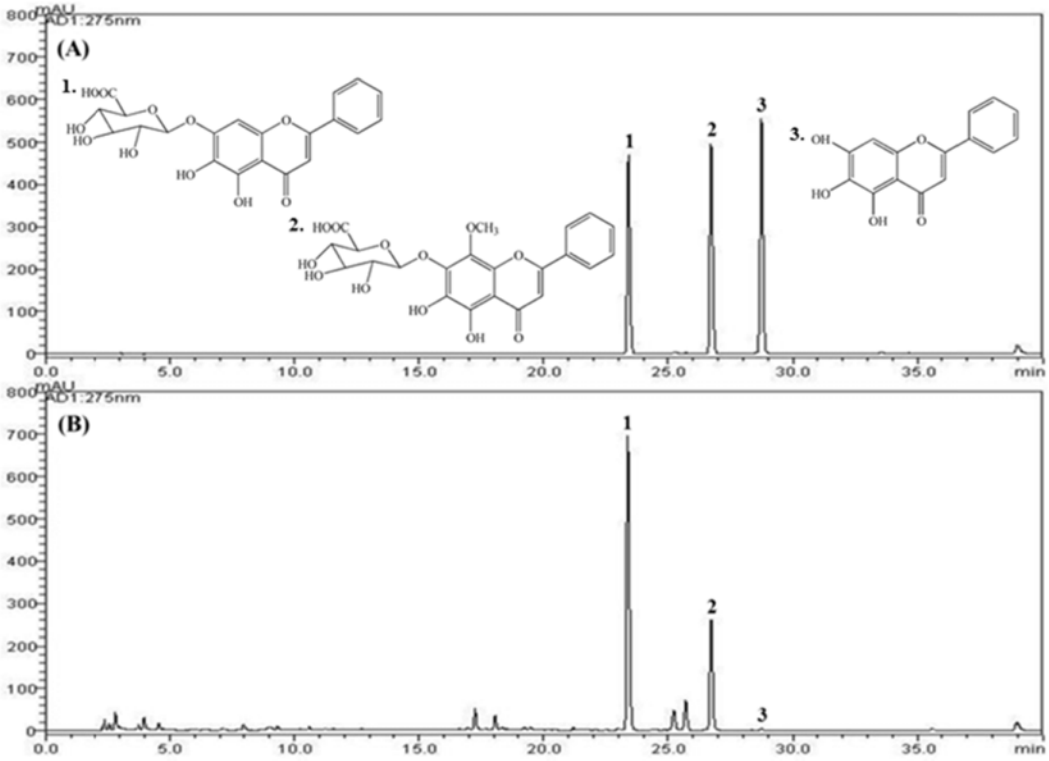


**Fig. 1.** Chemical structures of three major constituents in CSYJT (1. Baicalin, 2. Wogonoside and 3. Baicalein) and HPLC chromatogram of the standard mixture (A) and CSYJT sample (B).

following day. The effect of CSYJT on cisplatin treated PK15 cell was assessed using the MTT assays. PK15 cells were treated with the ascorbic acid (positive control, 1.7 mg/mL) and CSYJT (0, 20, 50 and 100 μg/mL) 2 h before cisplatin (25 μg/mL) treatment and after cisplatin treatment cells were incubated for 24 h. The MTT assay was performed using EZ-Cytox Cell Viability assay kit (Daeil) according to the manufacturer's protocol.

**ROS and GSH levels in PK15 cells** – For both the ROS and the GSH assay, the cells were pretreated with ascorbic acid (positive control, 1.7 mg/mL) and CSYJT (0 and 50 μg/mL). After 2 h, cells were treated with cisplatin (15 μg/mL) and incubated for 24 h. The ROS contents were determined using DCFH-DA method with some modification (LeBel and Bondy, 1990). The level of GSH was monitored by Glutathione Assay Kit (NWK-GSH01, Northwest) according to the manufacturer's protocol.

**Western blot of p53** – PK15 cells were prepared in the same manners as for the ROS and GSH assay. After incubation, the cells were lysed with lysis buffer (20 mM Tris-HCl pH 8, 150 mM NaCl, protease inhibitor cocktail). After the protein assay, 30 μg of protein were separated using 12% SDS-PAGE. The proteins were transferred onto a nitrocellulose membrane in a Semi-Dry Transfer system from Bio-Rad (Hercules, CA, USA). The membrane was blocked with TBS-T and incubated with primary antibody anti-p53 (1 : 300). Horseradish peroxidase-conjugated anti-mouse IgG (1 : 5000) was used for p53 detection. Immunoreactivity was visualized by an ECL kit.

**Animal study** – Male Sprague Dawley (SD) rats, 4 weeks old, were obtained from Orient Bio (Seongnam, Korea) and acclimated to the laboratory condition (25 ± 0.2 °C, 50% relative humidity, 12 h light/dark cycle) for 1 week before the experiments. Healthy male SD rats (n = 30) were randomly allocated into 6 groups (n = 5/group) and orally treated for consecutive 28 days as follows: group 1 (control), group 2 (cisplatin), group 3 (100 mg/kg of captopril), group 4-6 (100, 300 and 500 mg/kg of CSYJT). On day 23, cisplatin (5 mg/kg) was intraperitoneally injected to induce ARF. One day 27, the urine of each animal was collected over a 24 h for urine volume analysis and 3 h after the last treatment, the animals were anesthetized with a combination of zolazepam and tiletamine (Zoletil; Virbac) injection. The experimental protocols were approved (No. CNU-00072) by the Institutional Animal Care and Use Committee of Chungnam National University (Daejeon, Korea). Blood samples were collected from the caudal vena cava and separated

by centrifugation at 800 *g* for 15 min, and the serum CRE and BUN were determined on a dry chemistry system, the Vettest 8008 blood chemistry analyzer (IDEXX Laboratories, Westbrook, ME, USA). The left kidney was quickly removed for histopathological studies. The other kidney was removed and used in the GSH (NWK-GSH01) and the MDA (NWK-MDA01) assay. The assays were conducted according to the manufacturer's protocol.

**Histopathological examination** – The left kidney was fixed immediately in a 10% buffered formalin phosphate solution, embedded in paraffin and cut into 5 μm sections and processed for histological staining. These serial tissue sections were either stained with haematoxylin and eosin (H&E) for histopathological examination or subjected to TUNEL staining. Apoptotic nuclei were detected by the TUNEL method using an apoptosis detection kit (ApopTag Peroxidase In Situ Apoptosis Detection Kit; Millipore, Billerica, MA, USA) according to the manufacturer's protocol. All of the stained slides were analyzed under the light microscope.

**Statistical analysis** – Data were presented as mean values ± standard error of mean (SEM). The significances of differences among experimental groups were determined using the one way analysis of variance (ANOVA) test followed by Tukey's post hoc analysis. $P < 0.05$ was considered to be statistically significant.

## Results and Discussion

Cisplatin induced acute renal failure is classified as an intrinsic renal failure, which is related to circulatory problem for restoring the normal glomerulus filtration rate (GFR) caused by tubular necrosis.[9] The cisplatin-induced nephrotoxicity showed histologic destruction of intracellular organelles, such as loss of microvilli, desquamation of cell lining, and mitochondrial vacuolization which result from GSH depletion, lipid peroxidation increment, and protein synthesis inhibition.[10] Recently, many studies focused on ROS generation as a main factor in the pathophysiology of cisplatin-induced nephrotoxicity.[11,12] In this study, cisplatin alone treated group showed decreasesd PK15 cell viability and histopathological alteration with disrupted antioxidant status in kidney tissue.

Traditional medicinal herbs are well known to have many therapeutic effects with few side effect. Several traditional medicinal plants inhibit the increment of the ROS generation.[5,13,14] CSYJT is a compounding herbal medicine with 12 different herbal materials. Recently, CSYJT treatment revealed to have therapeutic effect on cerebral infarction and obesity.[5,8] In the present study, effect of CSYJT treatment on cisplatin-induced nephrotoxicity was investigated. To standardize CSYJT, HPLC analysis was performed. Although the CSYJT is a mixture of various herbal compounds, we choosed relatively well known flavonoid (baicalin, baicalein and wogonoside) to performe quantitative determination of CSYJT. The retention times of the main compounds were 23.40, 26.71, and 28.73 min for baicalin, wogonoside, and baicalein, respectively. Reproducibility was assessed by repeatedly measuring retention times and peak areas for six independently prepared samples of analyze. Relative standard deviation (RSD) for reproducibility of compounds were less than 0.3% for peak responses and less than 0.03% for retention times (data not shown). The results from the calibration curves and the identified contents of three major compounds (baicalin, baicaelin and wogonoside) in CSYJT were summarized in Table 2.

In the present study, cell viability and ROS and GSH values were monitored after cisplatin treatment together with p53 expression (Fig. 2). In the present study, ascorbic acid (positive control, poten antioxidant) and CSYJT treated cells showed enhanced viability compared to cisplatin alone treated group. Moreover, decreased ROS level and enhanced GSH content were observed in CSYJT pretreated cell compared with cisplatin alone treated cell, which indicates improved antioxidative status (Fig. 2; $p < 0.05$). This was in line with the previous study which reported free radical scavenging and antioxidant activities of baicalin flavonoids.[15] Furthermore, although the cell type was different, the flavonoid baicalein prevented cisplatin-induced cell death in human glioma cells.[16] p53 is a tumor suppressor protein, which plays important role in preventing inappropriate cell proliferation.[17,18] In cisplastin-induced nephrotoxicity, p53 activation is related

**Table 2.** Regression data, linear range and correlation coefficient for compounds and its contents in CSYJT (n = 3)

| Compound | Linear range (μg/ml) | Slope | Intercept | Correlation coefficient ($r^2$) | Content (mg/g) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Mean | SD | RSD (%) |
| 1. Baicalin | 3.91-500.00 | 34990.27 | 65010.15 | 1.0000 | 14.65 | $1.56 \times 10^{-2}$ | 0.11 |
| 2. Wogonoside | 1.26-200.00 | 38458.09 | 43483.69 | 0.9999 | 5.27 | $1.39 \times 10^{-3}$ | 0.03 |
| 3. Baicalein | 0.08-10.00 | 66906.28 | 2780.34 | 0.9999 | 0.02 | $4.21 \times 10^{-4}$ | 2.17 |

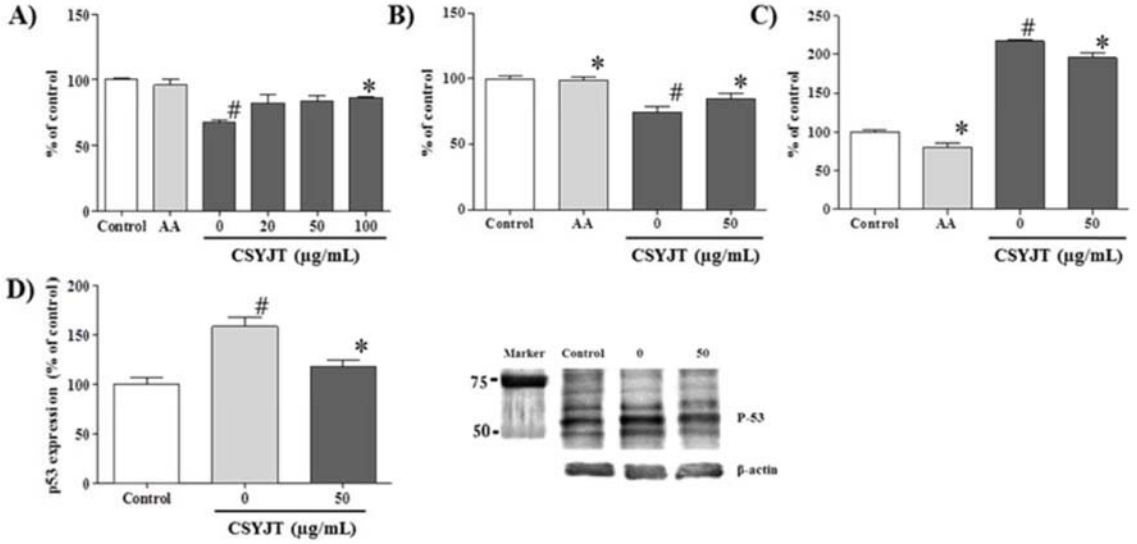


**Fig. 2.** The effect of standardized CSYJT on cisplatin treated PK15 cells. A) for MTT assay, the cells were treated with ascorbic acid (AA, 1.7 mg/mL) and different concentration of CSYJT (0, 20, 50, and 100 μg/mL) 2 h before cisplatin (15 μg/mL) treatment and incubated for 24 h before assessment. For glutathione (B), reactive oxygen species (C) and p53 expression (D) assessment, the cells were treated with ascorbic acid (AA, 1.7 mg/mL) and CSYJT (0 and 50 μg/mL) 2 h before cisplatin (15 μg/mL) treatment and incubated for 24 h before assessment. Values are expressed as mean ± SEM for triplicate experiments, $*p < 0.05$, a significant difference in comparison with the cisplatin-treated group, $^{\#}p < 0.05$, a significant difference in comparison with the control group.

**Table 3.** Effect of CSYJT on serum biochemical parameters, oxidative status and body weight in cisplatin-induced acute renal failure rats

| Group | BUN (mg/dL) | CRE (mg/dL) | Body weight gains (g) | Urine volume (mL) | GSH (nmol/g tissue) | MDA (nmol/g tissue) |
|---|---|---|---|---|---|---|
| Control | 16.3 ± 6.4 | 0.4 ± 0.1 | 19.4 ± 10.0 | 9.4 ± 1.0 | 17.0 ± 1.7 | 14.6 ± 2.2 |
| Cisplatin | 83 ± 12.7$^{\#}$ | 6.9 ± 1.7$^{\#}$ | −11.3 ± 7.1$^{\#}$ | 19.6 ± 1.6$^{\#}$ | 9.0 ± 1.9$^{\#}$ | 69.5 ± 7.6$^{\#}$ |
| Captopril + cisplatin | 18.0 ± 4.2* | 1.8 ± 0.7* | −10.5 ± 6.5 | 10.3 ± 1.2* | 15.7 ± 2.0* | 15.2 ± 5.7* |
| CSYJT (100) + cisplatin | 28.5 ± 9.19* | 1.9 ± 0.8* | −16.5 ± 3.5 | 18.0 ± 4.6 | 16.6 ± 2.0* | 56.1 ± 14.4 |
| CSYJT (300) + cisplatin | 91.5 ± 29.69$^{\#}$ | 1.1 ± 0.8* | −13.7 ± 4.8 | 13.4 ± 2.7 | 19.0 ± 1.8* | 47.6 ± 6.6* |
| CSYJT (500) + cisplatin | 33.7 ± 15.07* | 3.0 ± 0.7* | −8.0 ± 2.0* | 11.0 ± 1.7* | 18.0 ± 3.9* | 52.8 ± 6.9* |

Rats (n = 6/group) were given orally distilled water (DW), captopril (100 mg/kg) and CSYJT extracts (100, 300 and 500 mg/kg) once daily for 28 days for each group. After 23 days of treatment, cisplatin (5 mg/kg) was intraperitoneally injected except in saline injected control group. Values are expressed as mean ± SEM, $*p < 0.05$, a significant difference in comparison with the cisplatin alone treated group. $^{\#}p < 0.05$, a significant difference in comparison with the control group.

to oxidative stress, specifically to the hydroxyl radical accumulation.[17] Caspase dependent apoptosis is a main feature of p53 signal related cisplatin toxicity process which was confirmed by p53 null mouse model.[19,20] In this study, p53 expression was moderately decreased in CSYJT treated cell compared to cisplatin alone treated PK15 cell (Fig. 2; $p < 0.05$). Furthermore, CSYJT reported to have inhibitory effect on inflammatory cytokine production by inhibiting NF-κB.[6] Many studies demonstrated that decrease in apoptotic cells may occur by decreasing NF-κB activation leads to expression of many pro-inflammatory cytokine genes.[21,22]

The effect of CSYJT pretreatment on cisplatin-induced nephrotoxicity was also examined using *in vivo* animal study. The MDA, index for lipid peroxidation, content was considerably increased after cisplatin injection which indicates increased ROS generation. Decreased GSH level was also observed in cisplatin alone treated group. In CSYJT pretreated group, GSH level was recovered and MDA level was decreased if compared with cisplatin alone treated group (Table 3). Pretreatment of CSYJT significantly prevented serum BUN and CRE levels increment and markedly decreased cisplatin-induced renal injury (Table 3). However, the reason is unclear, significant individual differences were obserbed in the BUN index from 300 mg/kg CSYJT treated group with large SEM value. In renal system, water uptake is mainly takes place in the proximal tubule. Damaged by cisplatin to proximal

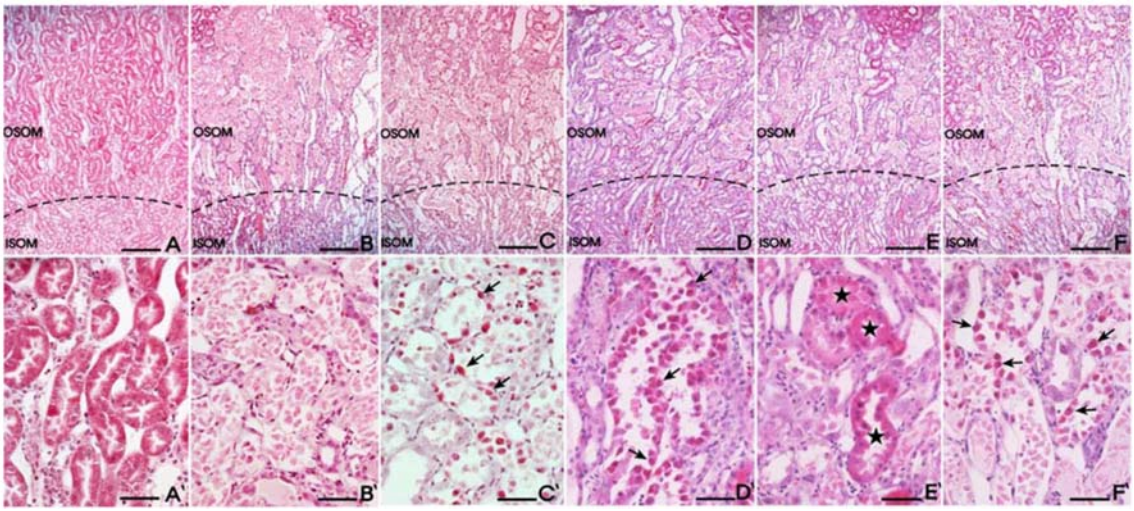


**Fig. 3.** The standardized CSYJT improves renal histopathology in cisplatin-injected rats. Representative images of the renal pathology (hematoxylin-eosin staining) in the S3 segments of proximal tubules of the outer stripe of the outer medulla (OSOM) on day 5 after administration of cisplatin. (A, A') Control rats, (B, B') cisplatin, (C, C') cisplatin + captopril, (D, D') cisplatin + CSYJT (100 mg/kg), (E, E') cisplatin + CSYJT (300 mg/kg) and (F, F') cisplatin + CSYJT (500 mg/kg). A, A'; Control groups show normal renal structure. B, B'; Cisplatin-injected kidneys show marked injury with sloughing of tubular epithelial cells, loss of the brush border and dilation of the tubules. C, C'; These change were less pronounced in captopril treated groups. A few tubular epithelial cells remained in the proximal tubules (arrows). D', F'; The detachment of the proximal tubule cells was significantly reduced by treatment with CSYJT and many epithelial cells remained in the S3 segments of the proximal tubules (arrows). E'; note, A few proximal tubules show almost normal morphology in the high dose group (stars). Bar; 200 μm (A~F), 100 μm (A'~F').

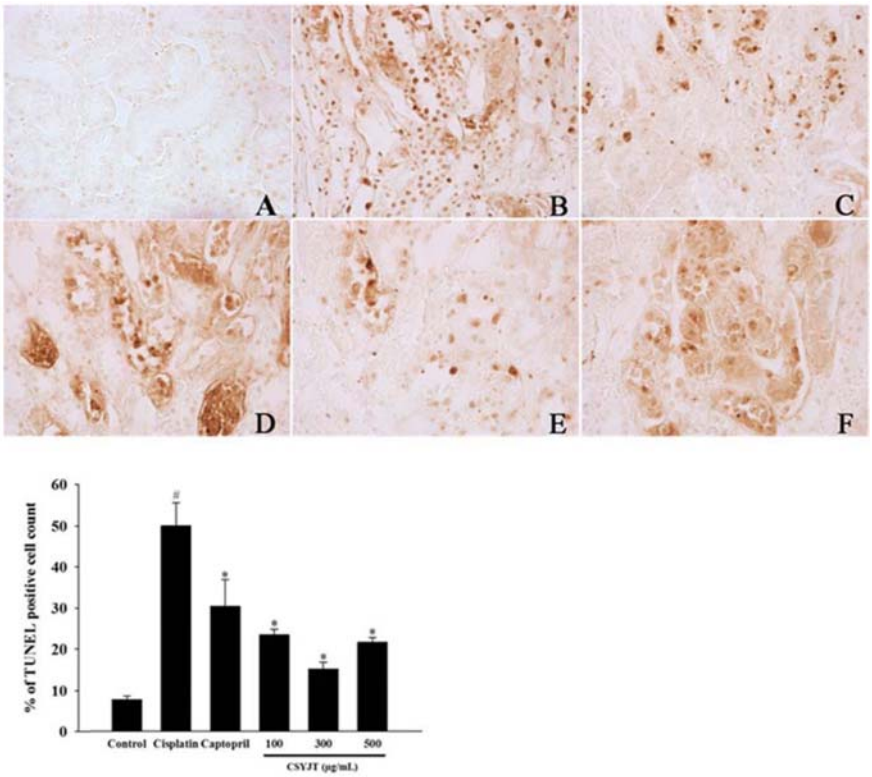


**Fig. 4.** The standardized CSYJT ameliorated cisplatin induced apoptosis in kidney tissue. (A) Control; (B) cisplatin alone; (C) captopril (100 mg/kg) pretreated; (D) CSYJT (100 mg/kg) pretreated; (E) CSYJT (300 mg/kg) pretreated; (F) CSYJT (500 mg/kg) pretreated group. The presence of TUNEL-positive cells were measured by the image analyzer. Values are expressed as mean ± SEM, $^{*}p < 0.05$, a significant difference in comparison with the cisplatin-treated group, $^{\#}p < 0.05$, a significant difference in comparison with the control group.

tubule may decrease water uptake. In this study, the urination volume was increased in the cisplatin alone treated group, whereas CSYJT pretreated group showed decreased urine volume. These results may derive from protective effect of CSYJT in proximal tubule and structural alterations were confirmed by histopathological examination. The kidney from the control group showed normal histology (Fig. 3). Cisplaltin treatment caused a severe proximal tubular necrosis, desquamation and parenchyma degeneration (B). Pretreatment with CSYJT decreased the tubular necrosis intensity and showed relatively mild degenerative signs compared to cisplatin alone treated group (C). Moreover, TUNEL-positive stained cells were decreased in CSYJT pretreated groups, although the decrement was not dose proportional, if compared with cisplatin alone group, which indicates decreased apopotosis (Fig. 4).

Taken together, CSYJT pretreatment ameliorates oxidative stress with p53 expression in cisplatin induced nepohrotoxicity.

## Acknowledgments

This work was supported by the Korea Institute of Oriental Medicine (KIOM) under Grant 'Evidence-based Medicine for Herbal Formula' (No. K12031).

## References

(1) Dennen, P.; Parikh, C. R. *Clin. Nephrol.* **2007**, *68*, 269-278.
(2) Zhang, H. A.; Wang, M.; Zhou, J.; Yao, Q. Y.; Ma, J. M.; Jiang, C. L. *Physiol Res*. **2010**, *59*, 61-70.
(3) Siddik, Z. H. *Oncogene* **2003**, *22*, 7265-7279.
(4) Arany, I.; Safirstein, R. L. *Semin Nephrol*. **2003**, *23*, 460-464.
(5) Hong, S. C.; Ko, B. H.; Song, I. B. *J. Sasang Const. Med.* **1995**, *7*, 227-240.
(6) Jeong H. J.;,Choi, I. Y.; Kim, M. H.; Kim, H. M.; Moon, P. D.; Hong, J. W.; Kim, S. H. *J. Neuroinflamm.* **2010**, *7*, 85.
(7) Park, S. C. *J. Sasang Const. Med.* **2003**, *15*, 166-179.
(8) Seo, C. S.; Jeong, S. J.; Kim, J. H.; Yoo, S. R. Shin, H. K. *J. Sasang Const. Med.* **2013**, *25*, 51-61.
(9) Lote, C. J.; Harper, L.; Savage C. O. *Br. J. Anaesth*. **1996**, *77*, 82-89
(10) Kellum, J. A. *Minerva Anestesiol.* **2004**, *70*, 181-188.
(11) Kawai, Y.; Nakao, T.; Kunimura, N.; Kohda, Y.; Gemba, M. *J. Pharmacol. Sci*. **2006**, *100*, 65-72.
(12) Chirino, Y.I.; Pedraza-Chaverri, J. *Exp. Toxicol. Pathol*. **2009**, *61*, 223-242.
(13) Cai, Y.; Luo, Q.; Sun, M.; Corke, H. *Life Sci*. **2004**, *74*, 2157-2184.
(14) Kim, T. W.; Song, I. B.; Lee, H. K.; Lim, J. H.; Cho, E. S.; Son, H. Y.; Park, S. J.; Kim, J. W.; Yun, H. I. *Food Chem. Toxicol*. **2012**, *50*, 4254-4259.
(15) Gao, Z.; Huang, K.; Yang, X.; Xu, H. *Biochim. Biophys. Acta.* **1999**, *1472*, 643-650.
(16) Lee, S. W.; Song, G. S.; Kwon, C. H.; Kim, Y. K. *Neurosci. Lett.* **2005**, *382*, 71-75.
(17) Jiang, M.; Wei, Q.; Pabla, N.; Dong, G.; Wang, C. Y.; Yang, T.; Smith, S. B.; Dong, Z. *Biochem. Pharmacol*. **2007**, *73*, 1499-1510.
(18) Dong, G.; Luo, J.; Kumar, V.; Dong, Z. *Am. J. Physiol. Renal Physiol.* **2010**, *298*, F293-F300.
(19) Yano, T.; Itoh, Y.; Matsuo, M.; Kawashiri, T.; Egashira, N.; Oishi, R. *Apoptosis* **2007**, *12*, 1901-1909.
(20) Yang, C.; Kaushal, V.; Haun, R. S.; Seth, R.; Shah, S. V.; Kaushal, G. P. *Cell Death Differ.* **2007**, *15*, 530-544.
(21) Francescato, H. D.; Costa, R. S.; Scavone, C.; Coimbra, T. M. *Toxicology* **2007**, *230*, 64-75.
(22) Pabla, N.; Dong, Z. *Kidney Int.* **2008**, *73*, 994-1007.

Received July 11, 2014
Revised August 22, 2014
Accepted August 24, 2014