Protective Effects of *Houttuynia cordata* Thunb. on Gentamicin-induced Oxidative Stress and Nephrotoxicity in Rats

Changgeun Kang[1], Hyungkyoung Lee[1], Do-Yun Hah[2], Jung Ho Heo[2], Chung Hui Kim[3], Euikyung Kim[1] and Jong Shu Kim[1]

[1]*Department of Pharmacology & Toxicology, College of Veterinary Medicine, Gyeongsang National University,*
[2]*Gyeongnam Livestock promotion Research Institute,*
[3]*Department of Animal Science and Biotechnology, Gyeongnam National University of Science and Technology, Jinju, Korea*

Development of a therapy providing protection from, or reversing gentamicin-sulfate (GS)-induced oxidative stress and nephrotoxicity would be of great clinical significance. The present study was designed to investigate the protective effects of Houttuynia cordata Thunb. (HC) against gentamicin sulfate-induced renal damage in rats. Twenty-eight Sprague-Dawley rats were divided into 4 equal groups as follows: group 1, control; group 2, GS 100 mg/kg/d, intraperitoneal (i.p.) injection; group 3, GS 100 mg/kg/d, i.p. + HC 500 mg/kg/d, oral; and group 4, GS 100 mg/kg/d i.p. + HC 1000 mg/kg/d, oral administration). Treatments were administered once daily for 12 d. After 12 d, biochemical and histopathological analyses were conducted to evaluate oxidative stress and renal nephrotoxicity. Serum levels of creatinine, malondialdehyde (MDA), and blood urea nitrogen (BUN), together with renal levels of MDA, glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were quantified to evaluate antioxidant activity. Animals treated with GS alone showed a significant increase in serum levels of creatinine, BUN, and MDA, with decreased renal levels of GSH, SOD, and CAT. Treatment of rats with HC showed significant improvement in renal function, presumably as a result of decreased biochemical indices and oxidative stress parameters associated with GS-induced nephrotoxicity. Histopathological examination of the rat kidneys confirmed these observations. Therefore, the novel natural antioxidant HC may protect against GS-induced nephrotoxicity and oxidative stress in rats.

Key words: *Houttuynia cordata* Thunb, Gentamicin sulfate, Oxidative stress, Nephrotoxicity, Antioxidant, Reactive oxygen species (ROS)

INTRODUCTION

A number of environmental contaminants, chemicals, and drugs, including antibiotics, dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, heart, and intestine (1). The kidney is a common target for toxic xenobiotics due to its capacity to extract and concentrate toxic substances, owing to its large share of blood flow (about 21% of cardiac output). The aminoglycoside antibiotics have been used either alone or in combination with other antibiotics that are cell wall synthesis inhibitors, as a treatment for severe and/or life-threatening infections caused by Gram-positive and Gram-negative microorganism (2,3). Gentamicin sulfate (GS) is the most commonly used and studied aminoglycoside antibiotic (4). Unfortunately, 30% of patients treated with GS for longer than 7 days show some signs of toxicity and nephrotoxicity. In some patients, the effects are so severe that clinical use is limited (5). GS nephrotoxicity is functionally characterized by increases in serum creatinine and blood urea nitrogen, along with decreases in glomerular filtration rate (GFR) (6,7). Morphological changes including proximal tubule epithelial desquamation, tubular necrosis, tubular fibrosis, epithelial edema, and glomerular hypertrophy have also been reported (8-10).

Several strategies and agents have been tested for their ability to protect from, or reverse GS-induced renal dam-

age, each with varying degrees of success (11,12). The level of cellular antioxidants determines susceptibility to oxidative damage, and is usually altered in response to oxidative stress (9,13). The toxicity of aminoglycosides such as GS is hypothesized to result from the generation of reactive oxygen species (ROS) in the kidney, including superoxide anions (14,15), hydroxyl radicals, hydrogen peroxide, and reactive nitrogen species (RNS), leading to renal injury (16). On the other hand, GS induces cellular injury and necrosis via several mechanisms, including peroxidation of membrane lipids and reduced efficiency of antioxidant enzymes in the kidney, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione (GSH) (4,14,17).

Most treatments focused on the use of natural plant extracts and synthetic anti-oxidants to reduce GS-induced nephrotoxicity have been effective for either preventing or ameliorating nephrotoxicity in rats. While the basis of the protective action of these plant extracts is not exactly known, it is postulated to result from their antioxidant properties (1,11,15, 18-20). *Houttuynia cordata* Thunb. (HC) is a perennial herb native to Southeast Asia, having a thin stalk and heart-like leaf called E-Sung-Cho in Korea. HC is a traditional medicinal plant containing bioflavonoids, and is known to possess a wide range of biological activities, such as antimicrobial, antileukemic (21), and antioxidant effects (22-25). In addition, HC has recently been investigated for its anti-obesity properties (26). Although the protective or curative mechanisms of HC have not been fully elucidated, this herb has attracted much attention due to its pharmacotherapeutic effects in the treatment of a variety of diseases (27,28), and because of this HC has been used for a long time in many parts of world (28,29). To our knowledge, there have been no comprehensive studies of the protective effects of HC in the modulation of oxidative stress associated with GS-induced nephrotoxicity in experimental animals. Consequently, the present study was conducted to evaluate the protective effects of herbal HC.

MATERIALS AND METHODS

Chemicals.   Gentamicin sulfate (GS) was purchased from Sigma chemical Co. (St. Lousi, MO, USA). All other chemicals of analytical grade were purchased from Sigma, Merck or Junsei (Japan).

Plants materials and extract preparation.   HC was obtained from the Research Institute of Traditional Medicine Plant of Gyeongnam (Hamyang, the province of Gyeongnam in southwestern Korea). The identification of the plant material was conducted in the same Research Institute. Voucher specimens were deposited in the same Research Institute. The HC leaves were air dried under shade, cut into small pieces and stored at 4°C until use. The HC leaves (300 g) were extracted with 900 ml of 80% methanol in a shaking incubator at 80°C for 12 hr. The residue was re-

extracted under the same conditions three times. The extracts obtained were combined and filtered. The combined methanol specimen was evaporated to dryness using a vacuum rotary evaporator (EYELA, New Rotary Vacuum Evaporator, AUTO JACK NAJ, Tokyo Rikakirai Co., LTD, Japan) and weighed (98.88 g W/W, dry base) to determine the yield of soluble constituents. The extract obtained was subject to evaluation of protective effects against GM-induced nephrotoxicity, and its chemical compounds that contribute to the aroma of *Houttuynia cordata* include β-myrcene and 2-undecanone (30-32).

Determination of HC extract doses for the protective effect of HC against GS-induced nephrotoxicity.   To evaluate the protective effect of HC against GS-induced nephrotoxicity, the HC doses were determined by a preliminary range finding test, in which, ten male rats were assigned to each group on the day before treatment administration. The animals were given the test items at levels of 500 and 1000 mg/kg for two consecutive days. No mortalities occurred, even at 1000 mg/kg. Because there were no significant toxicological differences among the rats, these doses were used for the protective effects of HC against GM-induced nephrotoxicity and conducted in accordance with the principles and specific guidelines presented in the guideline for toxicity tests of drugs and chemicals of the KFDA (33). The plant extracts were dissolved in 2.5% dimethyl sulfoxide (DMSO) and were diluted to a final concentration of 1000 mg/ml for the protective effects of HC against GS-induced nephrotoxicity. Next, two-fold serial dilutions were made to a concentration range of 500~1000 mg/kg for protective effects of HC against GS-induced nephrotoxicity. The solvent DMSO (2.5%) was used as the negative control for all the experiments.

Animals.   Forty male Sprague-Dawley rats (180~220 g) were obtained from the Animal Department of SAMTA CO Company, Seoul, Korea, and were quarantined and allowed to acclimate for a week prior to experimentation. The animals were handled under standard laboratory conditions of a 12-hr light/dark cycle in a temperature- and humidity-controlled room. All animals were fed with commercial chow diet (Superfeed Co., Seoul, Korea) and tap water *ad libitum*. All procedures were in accordance with the guiding principles established by the Animal Care Committee and the institutional ethical guidelines of Gyeongsang National University. Animals were divided into four equal groups randomly including 10 rats each as follows:

Group 1 (control group): The animals received intraperitoneal injection of saline (0.5 ml/day) for 12 days.

Group 2 (GS group): GS 100 mg/kg/day, intraperitoneal injection (34,35) for 12 days.

Group 3: GS (100 mg/kg/day, intraperitoneal injection) + HC (500 mg/kg/day, oral administration) for 12 days.

Group 4: GS (100 mg/kg/day, intraperitoneal injection) +

HC (1000 mg/kg/day, oral administration) for 12 days.

Sample collection and biochemical assays.   HC was administrated orally by feeding daily 1 hr before GS injection. This study approved by the animal Ethics Committee of Gyeongsan National University. At the end of designed day, the rats were anesthetized with a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally and blood samples were collected via cardiac puncture method at the end of these 24 hr. The serum was rapidly separated and processed for determination of serum creatinine, malondialdehyde (MDA) and blood urea nitrogen (BUN) using commercially available kits (Bio Assay Systems, Hayward, CA, USA). Both of the kidneys were collected and one of the both fixed with 10% buffered formalin solution in the room temperature for histopathological examination. Tissue samples from kidney were stored at -70°C liquid nitrogen for enzymatic analysis. Kidney samples were thawed and homogenized (10% w/v) in 0.5 M KCl at 4°C then centrifuged at 10,000 ×g for 90 min. The supernatant was used as the source of experimental product for determination of oxidative stress biomarkers. Protein content of all samples was determined by the method of Lowry *et al.* (36) using bovine serum albumin as standard.

Determination of oxidative stress biomarkers in renal tissues.   Glutathione (GSH) content of kidney tissues homogenate was determined using a dithionitrobenzoic acid recycling method described by Tietze (37) and Anderson (38) and was expressed as μmol/g protein. SOD activity was determined by method of Sun *et al.* (39) and was expressed as U/mg protein. Rat kidney homogenate lipid peroxide levels were measured by colorimetric determination of MDA is based on the reaction of one molecule of MDA with 2 mol of thiobarbituric acid (TBA) at low pH (2-3) according to the method of Mihara and Uchiyama (40) and was expressed as n mol/g protein. The catalase activity was estimated in the rat kidney tissue depending on the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide by catalase according to the method of Maehly and Chance (41) and Aebi (42). All the measurements were carried out by using a spectrophotometere (Shimadzu spectrophotometer, UV, 1201, Japan).

Histopathological evaluations.   Pieces of kidney from each groups were fixed immediately in 10% neutral formalin for a period of at least 24 hr, dehydrated in graded (50~100%) alcohol and embedded in paraffin, cut into 4~5 μm thick sections and stained with Hematoxylin-Eosin. The sections were evaluated for the pathological lesions of nephrotoxicity. Histopathlogical evaluations were evaluated according to Table 1.

Statistical analysis.   The results are expressed as the mean standard deviation (S.D). Differences between groups were assessed by one-way analysis of variance using the SAS software package for Windows. If a significant F-value of $p < 0.05$ was obtained in a one-way analysis of variance test, a Dunnett's multiple comparison test between the treated and control group was conducted. Differences between two groups were statistically evaluated by t-tests.

RESULTS

Effect of HC on GS-induced changes in serum biochemical parameters.   The animals treated with GS (100 mg/kg/d, i.p.) for 12 d showed a significant increase in

Table 1. Evaluation criteria of the histopathological parameters

Score	Structural change
(−)	The lack of any structural changes
(+)	Slight structural changes
(++)	Moderate structural changes
(+++)	Severe structural changes

Table 2. Effect of crude extract of *Houttuynia cordata* Thunb. on serum MDA, creatinine and BUN levels in gentamicin-sulfate treated rats

Groups / Parameters	Serum malondialdehyde (nmol/dl)	Serum creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
Control	0.063 ± 0.02	0.67 ± 0.04	17.55 ± 1.52
Houttuynia cordata Thunb (1000 mg/kg/day)[b]	$0.060 \pm 0.04^{\#}$	$0.77 \pm 0.06^{\#}$	$19.21 \pm 1.48^{\#}$
Gentamicin sulfate (100 mg/kg/day)[a]	$0.104 \pm 0.03^{*}$	$1.99 \pm 0.35^{*}$	$46.74 \pm 2.81^{*}$
Gentamicin sulfate (100 mg/kg/day)[a] + HC (500 mg/kg/day)[b]	$0.065 \pm 0.06^{\#}$	$0.85 \pm 0.18^{\#}$	$31.57 \pm 2.74^{*\#}$
Gentamicin sulfate (100 mg/kg/day)[a] + HC (1000 mg/kg/day)	$0.051 \pm 0.035^{*\#}$	$0.80 \pm 0.08^{\#}$	$21.88 \pm 1.70^{\#}$

$^{*}p < .05$ as compared with control group.
$^{\#}p < .05$ as compared with gentamicin group.
[a]Intraperitoneal injection.
[b]Oral administration.

Table 3. Effect of crude extract of *Houttuynia cordata* Thunb. on antioxidant enzyme in kidney of gentamicn sulfate-treated rats

Parameters / Groups	GSH (μmol/g protein)	CAT (U/mg protein)	SOD (U/mg protein)	MDA (nmol/g protein)
Control	19.89 ± 1.09	25.23 ± 2.41	32.34 ± 1.10	67.9 ± 3.3
Gentamicin sulfate (100 mg/kg/day)[a]	14.25 ± 0.22*	16.51 ± 1.60*	15.71 ± 0.29*	116.45 ± 4.06*
Gentamicin sulfate (100 mg/kg/day)[a] + HC (500 mg/kg/day)[b]	18.43 ± 0.19[#]	21.89 ± 1.90[#]	24.9 ± 0.9[#]	80.8 ± 3.19[#]
Gentamicin sulfate (100 mg/kg/day)[a] + HC (1000 mg/kg/day)[b]	20.15 ± 1.01[#]	23.16 ± 2.13[#]	30.61 ± 1.09[#]	76.4 ± 3.25[#]

*$p < .05$ as compared with control group.
[#]$p < .05$ as compared with gentamicin group.
[a]Intraperitoneal injection.
[b]Oral administration.

Table 4. Effect of crude extract *Houttuynia cordata* Thunb. on histopathological lesions the kidney of gentamicin sulfate-treated rats

Treatment / Lesions	Histopathological lesions			
	Tubular necrosis	Infiltration of inflammatory cells	Hyaline casts	Disintegrated nucleus
Control	−	−	−	−
Gentamicin sulfate (100 mg/kg/day)[a]	+++	++	+++	+++
Gentamicin sulfate (100 mg/kg/day)[a] + HC (500 mg/kg/day)[b]	++	−	++	++
Gentamicin sulfate (100 mg/kg/day)[a] + HC (1000 mg/kg/day)[b]	+	−	+	+

[a]Intraperitoneal injection.
[b]Oral administration.

serum creatinine, MDA, and BUN levels compared to the control group (Table 2). However, elevations in serum creatinine, MDA, and BUN were significantly attenuated by pre-treatment with HC ($p < 0.05$), indicating a reduction in GS-induced nephrotoxicity. Treatment of animals with HC alone did not alter renal function tests, compared to controls (Table 2).

Effects of HC treatments on kidney GSH, CAT, SOD, and MDA levels. Table 3 summarizes the protective effects of HC on the levels of GSH, CAT, SOD, and MDA in the kidney. Compared to the control group (GSH: 19.89 ± 1.09; CAT: 25.23 ± 2.41; SOD: 32.34 ± 1.10; MDA: 67.9 ± 3.3), GS-induced renal damage caused a significant increase in renal MDA levels (116.45 ± 4.06), while GSH (14.25 ± 0.22), SOD (15.71 ± 0.29), and CAT (16.51 ± 1.60) levels significantly decreased.

Histopathological observations. The changes in histopathological lesions in the kidney of all treated rats are summarized in Table 4. Light microscopic examination of kidneys from control and HC treated rats showed mild structural alterations in renal tissues (Fig. 1C and Fig. 1D). Massive and diffuse cellular necrosis was observed in the proximal tubules of kidneys from rats injected with GS alone. In addition, the lumen of these tubules were filled with degenerated and desquamated epithelial cells, massive hyaline

casts, and several infiltrated inflammatory cells in the form of mononuclear cells (Fig. 1B). Kidneys showed reparative tendencies in the GS + HC treatment in both groups 3 and 4 (Fig. 1C and Fig. 1D). There was a significant reduction in lesions caused by epithelial and nuclear changes typically associated with tubular necrosis, observed only in the animals that were pretreated with HC. This was evidenced by preservation of tubular morphology compared to the group treated with GS alone (Fig. 1C and 1D).

DISCUSSION

Gentamicins still have been used effectively regardless of adverse side effects. Oxygen free radicals are considered to be important mediators of GS-induced acute renal failure in others paper (16,34). Characters of GS nephrotoxicity are oxidative stress and inflammation. Generation of reactive oxygen metabolites may be the basis of a variety of insults, such as lipid peroxidation. The nephrotoxicity of GS is associated with its accumulation in the renal cortex, which is leading cell damage and death. Treatment with several natural and synthetic antioxidant substances has been extensively studied and shown to be useful for either the prevention or amelioration of nephrotoxicity in experimental rats (8,10,15,17,34,35,43-45). We evaluated the efficacy of HC, a potent antioxidant and free-radical scavenger, on the GS-induced renal damage and oxidative injury induced by GS
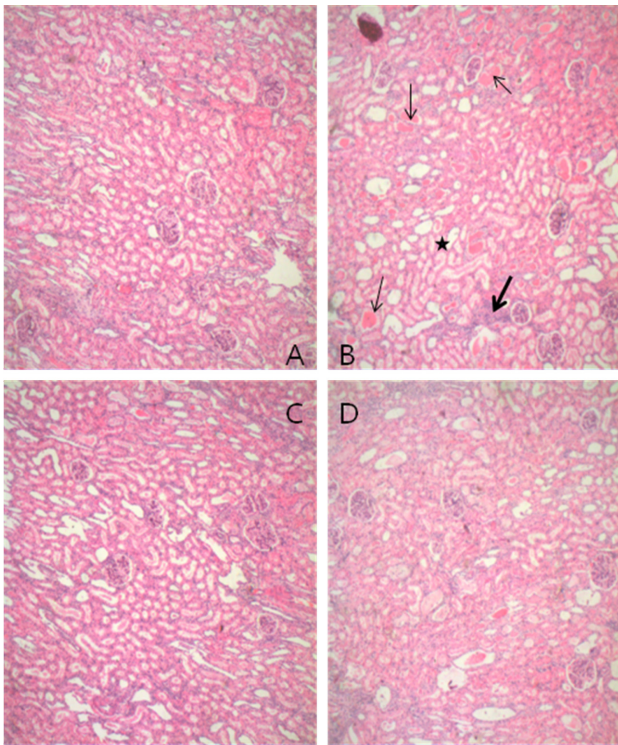
Fig. 1. Photomicrograph of renal tissue of untreated, gentamicin and gentamicin plus *Houttuynia cordata* Thunb. (HC) treated rats (H & E stain, ×200). Normal histopathological view of renal section in control rats (A), The treatment with Gentamicin sulfate (100 mg/kg/day) showd degeneration, desquamation and extensive and marked necrosis in tubules (asterisk), massive hyaline casts in tubules (thin arrows), and inflammatory cells infiltration in intertubular area (thick arrow) (B), Section from rat treated with GS (100 mg/kg) plus HC (1000 mg/kg) reveal near to normal structure having mild hyaline cast (C), Section from rat treated with GS (100 mg/kg) plus HC (500 mg/kg) show near to normal structure having moderate hyaline cast (D).

in experimentally intoxicated rats. In this study, the known histopathological lesions, biochemical findings and antioxidant enzyme levels associated with GS-induced nephrotoxicity in rats were also observed. Plasma creatinine concentrations are a more reliable indicator than BUN concentrations in the initial phases of kidney disease. Furthermore, BUN concentrations begin to increase only after parenchyma tissue injury has commenced (46). In this study, plasma creatinine and BUN levels were higher ($p < 0.05$) in the GS group compared to the control group. These findings were confirmed by observation of histopathological lesions such as epithelial desquamation, tubular necrosis, tubular casts, and infiltration of inflammatory cells, indicating that the kidney is very sensitive to GS toxicity. A significant decrease in biochemical parameters, however, was observed in the GS + HC groups (groups 3 and 4) when compared with the GS alone group. These data indicate that HC

at both doses has exerted a protective effect against GS nephrotoxicity. These data are also supported by another study (8,10,15,35,37,43-45). A relationship between nephrotoxicity and oxidative stress has been demonstrated in many experimental models (37,43,45). Lipid peroxidation is one of the main indicators of oxidative damage, and has been found to play an important role in nephrotoxicity (27,34). Significantly elevated levels of MDA, a marker for lipid peroxidation, were observed in the GS treatment group compared with the control group. However, the elevated MDA levels were efficiently decreased by HC treatment, suggesting that formation of reactive oxygen species in GS-induced nephrotoxicity were scavenged, and lipid peroxidation was attenuated by HC. Although the antioxidants used in each experiment possessed different components, these results are consistent with previous studies (35,43,45). Oxidative stress appears to play a key role in the development and progression of degeneration diseases, including GS toxicity (14,16). Glutathione is one of the essential compounds required for maintaining cell integrity and participation in the cell metabolism, and is also a critical indicator for evaluating oxidative stress in cells (22,38). The decreased GSH, SOD, and CAT activity in GS-induced nephrotoxicity shown in this study indicates an increase in the generation of free radicals. This decrease in activity indicates that antioxidant enzymes were depleted during the process of combating oxidative stress. When compared with the GS-only, HC supplementation in both groups 3 and 4 significantly increased antioxidant levels in the kidney. However the decrease in MDA and increase in GSH, SOD, and CAT activities in the GS + HC in both groups 3 and 4 could be due to the antioxidant properties of HC. These results correlate with results reported by other researchers (17,34,35,43,45). However, other papers reported contrasting results with regard to SOD levels (34,47) when compared with the current study. The discrepancy between our results and those of others is not yet understand, because all of the compounds used in each study and this study are derived from a natural medicinal plant, all of them having antioxidant activity. As indicated by the extensive explanation of this discrepancy, the mechanisms underlying xenobiotic or peroxide-dependent alterations in tissue antioxidant enzyme activities are currently a controversial issue.

The histopathological lesions were assessed in parallel with the serum, antioxidant, and lipid peroxidation assays. Rats treated with GS had extensive and massive renal lesions including tubular necrosis, hyaline casts, and infiltration of inflammatory cells. These marked and massive lesions were predicted because it has been well established that GS injected to animals intraperitoneally at a dose of 100 mg/kg, for six consecutive days, causes significant nephrotoxicity in rats (13,48). These findings are in agreement with previous studies where animals were treated with different antioxidant agents (35,43,45). The implication of a role of

reactive oxygen species in GS nephrotoxicity was supported by the finding that many free radical scavengers provided marked functional and histopathological protection against GS nephrotoxicity (8,10,15,35,43-45).

In conclusion, HC protects against GS-induced nephrotoxicity, possibly by inhibiting lipid peroxidation, enhancing renal glutathione content and activity of antioxidant enzymes. Thus, we suggest that oxidative stress is a common mechanism contributing to initiation and progression of renal damage, and that HC has protective effects against GS-induced nephrotoxicity. These protective effects may result, at least in part, from its antioxidant activity. While it remains unknown as to which of the ingredients of HC mediates an antioxidant effect, research shows that the herb HC acts primarily in the kidney.

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