LABORATORY STUDY

Evaluation of the protective effect of agmatine against cisplatin nephrotoxicity with 99mTc-DMSA renal scintigraphy and cystatin-C

Yavuz Sami Salihoglu, Tarik Elri, Kanat Gulle, Murat Can, Mustafa Aras, Hale Sayan Ozacmak and Mehmet Cabuk

Department of Nuclear Medicine, School of Medicine, Bulent Ecevit University, Zonguldak, Turkey; Department of Histology and Embryology, School of Medicine, Bulent Ecevit University, Zonguldak, Turkey; Department of Medical Biochemistry, School of Medicine, Bulent Ecevit University, Zonguldak, Turkey; Department of Physiology, School of Medicine, Bulent Ecevit University, Zonguldak, Turkey

ABSTRACT
Background: The aim of the current study was to investigate whether agmatine (AGM) has a protective effect against cisplatin-induced nephrotoxicity.

Materials and methods: Thirty-two rats were randomly divided into four groups: (1) Saline (control); (2) Cisplatin (CDDP; 7.5 mg/kg intraperitoneally); (3) Agmatine (AGM; 10 mg/kg intraperitoneally); (4) Cisplatin plus agmatine (CDDP + AGM). Agmatine was given before and two consecutive days after cisplatin injection. All the animals underwent renal scintigraphy with 99mTc-DMSA. The levels of serum creatinine, cystatin C, and blood urea nitrogen (BUN) were measured in addition to examination of the tissue samples with light microscopy. Acute renal injury was assessed with biochemical analyses, scintigraphic imaging, and histopathological evaluation.

Results: In the cisplatin group, the levels of BUN, creatinine, and cystatin C were significantly higher than that of the controls. Histopathological examination showed remarkable damage of tubular and glomerular structures. Additionally, cisplatin caused markedly decreased renal 99mTc-DMSA uptake. AGM administration improved renal functions. Serum creatinine, BUN, and cystatin C levels had a tendency to normalize and, scintigraphic and histopathological findings showed significantly less evidence of renal toxicity than those observed in animals receiving cisplatin alone.

Conclusions: Our data indicate that AGM has a protective effect against cisplatin-induced nephrotoxicity. Therefore, it may improve the therapeutic index of cisplatin. In addition, the early renal damage induced by cisplatin and protective effects of AGM against cisplatin nephrotoxicity was accurately demonstrated with 99mTc-DMSA renal scintigraphy.

ARTICLE HISTORY
Received 1 May 2016
Revised 23 July 2016
Accepted 18 August 2016
Published online 7 September 2016

KEYWORDS
Agmatine; cisplatin; cystatin C; nephrotoxicity; scintigraphy; 99mTc-DMSA

Introduction
Cisplatin (cis-diaminedichloroplatinum II, CDDP) is one of the most important antineoplastic agents effectively used in the treatment of solid tumors. However, it has serious side effects, mainly nephrotoxicity, that limit its clinical use. Cisplatin causes proximal tubular injury, decrease in renal blood flow and disruption of glomerular filtration. The clinical manifestations of nephrotoxicity are decreased glomerular filtration rate, increased serum creatinine, and blood urea nitrogen (BUN) levels. Although protective measures as hydration and diuresis are routinely applied, renal injury is seen in about 20% of patients. Therefore, there is still a great interest in developing new protective agents to increase its therapeutic index.

Mechanisms of CDDP-induced nephrotoxicity have not been clearly understood. It has been suggested that CDDP leads nephrotoxicity by various cellular and molecular mechanisms. Oxidative stress has been shown to have an important role on the pathogenesis of nephrotoxicity. As a free radical formed through the oxidation of l-arginine by nitric oxide synthase (NOS), nitric oxide (NO) leads cellular damage by generating reactive oxygen species. Three isoforms of NOS were identified as inhibiting NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). Treatment with CDDP results in enhanced NO formation by activating iNOS in kidney and liver.

Previous studies have revealed that iNOS derived NO leads to inflammation and epithelial damage.

CONTACT Tarik Elri, MD tarikelri@gmail.com Department of Nuclear Medicine, School of Medicine, Bulent Ecevit University, Esenkoy/Kozlu, 67600 Zonguldak/Turkey, Turkey

© 2016 Informa UK Limited, trading as Taylor & Francis Group
However eNOS derived NO was shown to inhibit both the expression of iNOS and the production of inflammatory cytokines.\(^8\) Some iNOS inhibitors were shown to be protective against CDDP-induced nephrotoxicity.\(^5,9–10\)

Agmatine (AGM), also known as decarboxylated arginine, is an aminoguanidine that was first identified in herring sperm. Then, it had been recognized in plants, bacteria, invertebrates, and various organs and cell types of mammals. It has been shown to have a modulatory role in neurotransmitter system, key ion channels, NO synthesis, and polyamine metabolism.\(^11,12\) It has been demonstrated that AGM plays an important role on the synthesis of NO by activating the eNOS while inhibiting iNOS.\(^13\) In a previous study, the authors demonstrated an increase in glomerular filtration and tubular reabsorption by direct injection of AGM into the renal interstitium via an NOS-dependent mechanism.\(^14\) Renoprotective effects of AGM have been shown in anti-Thy-1 glomerulonephritis (by inhibition of cell proliferation), cisplatinum-induced nephrotoxicity (by inhibition of oxidative phosphorylation), and acute ischemic renal injury (by enhancing renal blood flow and glomerular filtration rate).\(^15–17\) In addition, a recent report revealed that AGM ameliorate gentamicin-induced renal injury via its antioxidant and anti-inflammatory properties.\(^18\) Considering the mentioned advantages, we assume that AGM may have a protective effect against CDDP nephrotoxicity.

Various biochemical markers such as serum creatinine, BUN, and cystatin C (cys-C) have been used to evaluate the renal damage caused by CDDP. Cys-C was found to be more sensitive than the others in the assessment of CDDP-induced renal damage.\(^19\)

Radionuclide imaging is also widely used in evaluating renal functions. Technetium-99m 2–3 dimercaptosuccinic acid (99mTc-DMSA) scintigraphy is a well-known imaging modality that has been used for detecting parenchymal abnormalities and evaluating the functional condition of proximal renal tubular mass for many years. 99mTc-DMSA accumulates mainly in the proximal convoluted tubules depending on renal blood flow and cell membrane transport function of proximal tubules.\(^20–22\) Thus, it has been used in \textit{in vivo} imaging or \textit{in vitro} quantitative methods for evaluating renal tubular function in drug-induced nephrotoxicity.\(^23–24\)

The current study was undertaken to investigate the possible protective effects of AGM against CDDP-induced nephrotoxicity by 99mTc-DMSA renal scintigraphy, biochemical analysis, and histological examination.

### Materials and methods

#### Chemicals

Cisplatin vials (Cisplatin-Ebewe© 50 mg/100 mL, Sandoz, Istanbul, Turkey) were used. AGM was purchased from Sigma (St. Louis, MO) and freshly dissolved in normal saline with a concentration of 3 mg/mL prior to injection.

99mTc-sodium pertechnetate eluted from a 99 Molybdenum (99Mo)/99mTc generator (MON-TEK, Monrol Inc, Istanbul, Turkey) before radiolabeling procedure. DMSA (Renocis®, Cis Bio International, Cedex, France) was purchased as a freeze-dried commercial kit containing 1 mg dimercaptosuccinic acid. 99mTc-DMSA was prepared in accordance with instructions for the preparation of radiopharmaceutical recommended by the manufacturer. Quality control procedures were performed according to the manufacturer’s instructions, and labeling efficiency >95% of the radiopharmaceutical was used.

All other chemicals used in this study were of analytical grade and commercially available.

#### Animals

Female Wistar albino rats, weighing 230–280 g were used for the experimental procedures. Rats were maintained under controlled environmental conditions (20 ± 2°C, 55% relative humidity and 12-h light/dark cycle) and kept on a standard diet of pulverized rat pellet food and tap water \textit{ad libitum}. The protocol of this study was approved by the local ethical committee on animal experiments.

#### Experimental design

The rats were divided randomly into four groups, each consisting of eight animals. Group 1 (control) received vehicles used for CDDP (physiological saline solution, i.p.). Group 2 (CDDP) was administered a single dose of CDDP (7.5 mg/kg, i.p.). Group 3 (AGM) was injected AGM (10 mg/kg, i.p.) for three successive days. Group 4 (AGM + CDDP) was administered AGM before and two consecutive days after CDDP injection. All rats were also hydrated with a daily injection of saline of 5 mL/kg for 3 days. On the fourth day, all rats were subjected to anesthesia with ketamine hydrochloride and xylazine hydrochloride, and then 99mTc-DMSA administered intravenously by tail vein. After completion of renal scintigraphy, blood samples were taken by heart puncture into nonheparinized tubes. Serum was separated by centrifugation at 3000 rpm for 15 min. and stored at \(-20\)°C until analysis. The animals were then sacrificed.

| Experimental design | Group 1 (control) | Group 2 (CDDP) | Group 3 (AGM) | Group 4 (AGM + CDDP) |
|---------------------|------------------|----------------|--------------|---------------------|
| Control vehicles    | Physiological saline solution | Physiological saline solution | Physiological saline solution | Physiological saline solution |
| CDDP dosage        | 7.5 mg/kg         | 7.5 mg/kg       | 7.5 mg/kg     | 7.5 mg/kg |
| AGM dosage         | 10 mg/kg          |                |               | 10 mg/kg |
| Administration     | 3 days            | 1 day          | 3 days        | 3 days |
| Hydration           | 5 mL/kg/day       |                |               | 5 mL/kg/day |
| Anesthesia         | Ketamine hydrochloride and xylazine hydrochloride | Ketamine hydrochloride and xylazine hydrochloride | Ketamine hydrochloride and xylazine hydrochloride | Ketamine hydrochloride and xylazine hydrochloride |
| Blood sampling     | Heart puncture    |                | Heart puncture | Heart puncture |
| Serum storage       | -20°C             |                | -20°C         | -20°C |
| Animals sacrificed  | All              |                |               | All |

The study was approved by the local ethical committee on animal experiments.
and their kidneys were removed and specimens were fixed in formaldehyde solution for histological examination.

**Biochemical analysis**

Serum creatinine and BUN concentrations were measured colorimetrically using Advia 2400 auto analyzer (Siemens Diagnostics, Tarrytown, NY) with same brand of diagnostic kits. Cys-C levels were determined by using a rat cystatin C ELISA kit (ICL, Portland, OR).

**Scintigraphic study**

99mTc-DMSA (14.8 MBq/0.4 mL) was given by tail vein via 24 G cannula. Three to four hours after radiopharmaceutical injection scintigraphic images were obtained with a dual-headed gamma camera (Siemens, Symbian S). Anterior and posterior static images of kidneys were obtained by using a pinhole and parallel hole collimator (25 x 256 matrix, 2x magnification, 5 min). Renal uptake of each kidney was calculated separately on scintigraphic images by dedicated software program. A semi-quantitative functional evaluation was performed using scintigraphic images. Total renal function was calculated by the arithmetic mean of the two kidneys. Calculation of the renal uptake on scintigraphic images was performed by drawing a region of interest around each kidney and background with isocontour technique. As an index of renal function, calculated 99mTc-DMSA renal/background mean count ratios (MCR) were compared. Renal uptake was calculated using the following formula.

\[
\text{Mean count ratio (MCR)} = \frac{\text{mean count of kidney}}{\text{mean count of background histological analysis}}
\]

Kidney specimens were fixed in 10% neutral buffered formalin and blocked with paraffin inclusion. Then, specimens were sectioned at 5 µm and stained with Hematoxylin and eosin stain (HE). They were examined with a light microscope (Axio Lab A1, Carl Zeiss Microscopy GmbH, Jena, Germany).

**Statistical analysis**

Statistical analysis was performed using Statistical Package for the Social Sciences (IBM SPSS version 19.0, Chicago, IL). Data are presented as medians (minimum–maximum). Comparisons of groups were made by using Kruskal–Wallis analysis of variance for biochemical, histological, and scintigraphic variables. Bonferroni-corrected Mann–Whitney U test was used for binary comparison of subgroups. Correlation between two quantitative variables was examined by Spearman’s correlation analysis. The results were evaluated at 95% confidence intervals and level of \( p < .05 \) was considered as statistically significant.

**Results**

Two animals in CDDP group died before scintigraphic procedure, but we were able to get blood and tissue samples.

**Biochemical results**

The serum creatinine, BUN and cys-C levels were significantly higher in group 2 (\( p \leq .01 \)). Administration of CDDP caused a significant deterioration in renal function. Supplementation of AGM improved the renal function tests. Serum creatinine, BUN, and cys-C were tended to normalize and there was no statistically significant difference among other groups. However, serum cys-C values showed a mild increase in group 3 and 4 compared to the control group (Table 1).

**Scintigraphic findings**

Visual assessment of scintigraphic images revealed markedly reduced renal 99mTc-DMSA uptake and increased background activity in CDDP group. Administration of AGM significantly improved scintigraphic findings manifested as slightly reduced renal uptake and increased background uptake compared to control group. Scintigraphic findings of group 3, which received AGM alone were similar to control rats (Figure 1).

In semi quantitative functional evaluation of the groups, the MCR values were significantly lower in CDDP group (\( p = .005 \)). When compared, the difference among other three groups was statistically not significant but MCR value of CDDP + AGM group was slightly lower than that of control (Table 2).

| Table 1. Serum creatinine, BUN, and cystatin C values (median and range). |
|---------------------------|---------------------------|---------------------------|
| Group                  | Serum Creatinine (mg/dL) | BUN (mg/dL) | Cystatin C (mg/L) |
| Control (n = 8)         | 0.55 (0.5–0.7)           | 17.0 (13–30) | 3.31 (1.62–6.03) |
| CDDP (n = 6)            | 1.90 (1.5–2.6)           | 105.0 (81–200) | 7.96 (5.27–8.98) |
| AGM (n = 8)             | 0.60 (0.4–0.7)           | 19.0 (12–23)  | 4.78 (2.63–6.67) |
| AGM + CDDP (n = 8)      | 0.65 (0.6–0.8)           | 17.5 (10–25)  | 4.69 (3.50–6.11) |
| \( p < .001 \)          | \( p = .001 \)           | \( p = .001 \)  |

Data are presented as medians with minimum–maximum values in parentheses.

AGM: agmatine; BUN: blood urea nitrogen; CDDP: cisplatin.
Histological findings

In the evaluation of glomerular structure, Bowman's capsule, Bowman's space, interstitial space, proximal and distal convoluted tubules of the kidney by light microscopy; findings were normal in control group and group 3. Cisplatin treatment induced serious pathological changes that are indicative for nephrotoxicity and the most striking changes were observed in tubules. Oval, long, or spiral-shaped tubules with enlarged lumen and vacuolated tubular cells were observed in cortical region. Nuclear pyknosis as a finding of apoptosis and necrosis was also seen. A large number of renal corpuscles which lost their normal morphology with partially or fully corrupted glomeruli and widened bowman's space were detected. In group 4, supplementation of AGM was markedly ameliorated the pathological changes induced by CDDP. Most of the renal corpuscles were close to normal size but glomerular disruption was observed in a small number of renal corpuscles. In addition, affected tubules of cortical region showed an improvement as evidenced by mild degeneration and cystic formation (Figure 2).

Table 2. Tc-99m DMSA renal/background uptake ratios (median and range).

| Group          | L-MCR     | R-MCR     | Cumulative |
|----------------|-----------|-----------|------------|
| Control (n = 8) | 32.48 (21.15–39.72) | 28.01 (18.17–47.28) | 30.29 (24.08–34.22) |
| CDDP (n = 6)   | 12.13 (5.05–14.90)   | 9.45 (6.92–11.54)   | 10.42 (5.98–12.87)  |
| AGM (n = 8)    | 28.67 (26.07–47.45) | 29.58 (15.52–40.50) | 31.47 (22.05–40.95) |
| AGM + CDDP (n = 8) | 22.88 (5.05–49.75) | 24.27 (7.27–37.92) | 25.57 (6.23–43.84) |
|                | p = .009   | p = .006  | p = .005   |

Data are presented as medians with minimum–maximum values in parentheses.
AGM: agmatine; CDDP: cisplatin; L-MCR: left kidney mean count ratio; R-MCR: right kidney mean count ratio.

*Arithmetic mean of L-MCR and R-MCR.

Figure 1. Posterior images of 99mTc-DMSA scintigraphy obtained by a pinhole collimator. Scintigraphic image of a rat in the CDDP group showed markedly decreased renal 99mTc-DMSA uptake with increased background activity (b), compared to the control group (a). Renal scintigraphy of a rat in the AGM + CDDP group demonstrated quite good renal 99mTc-DMSA uptake that slightly reduced and very low background activity that minimally increased as compared with control group (d). Scintigraphic image of a rat in the AGM group showed similar findings with control group (c).
Discussion

CDDP is one of the widely used antineoplastic drugs and, nephrotoxicity is its major dose-limiting side effect. There is still a great interest in developing new agents to overcome its nephrotoxic effects. In the present study, we aimed to highlight the protective ability of AGM against CDDP-induced renal damage by using Tc-99m DMSA scintigraphy, biochemical, and histological analysis.

In our study a single dose of CDDP (7.5 mg/kg, i.p.)-induced nephrotoxicity, manifested in the serum by a significant elevation of creatinine, BUN and cys-C levels as predicted. Histological findings confirmed the nephrotoxicity. CDDP has led to the proximal tubule damage as well as deterioration in glomerular structure. Biochemical and histological results were consistent with those noted previously. Nephrotoxicity was also well reflected by semi quantitative scintigraphic evaluation as manifested by a markedly decreased renal uptake ratio of 99mTc-DMSA. Scintigraphic results were compatible with the previous studies that showed negative effect of cisplatin on renal uptake of 99mTc-DMSA albeit in different methods.

Supplementation with AGM before and two consecutive days after single dose of CDDP markedly reduced the nephrotoxic effects as evidenced in the histological evaluation. The protective effect of AGM was demonstrated by a significant decrease in serum creatinine, BUN, and cys-C concentrations. This protection was also demonstrated by scintigraphic evaluation as the decrease of 99mTc-DMSA uptake was markedly lowered below that caused by CDDP. These results were in accordance with previous studies that showed the protective effect of AGM against renal damage induced by some other nephrotoxic agents such as iphosphamide and gentamicin.

The CDDP procedure selected in our study was based on the known onset of the minimal dose (7.5 mg/kg) that induced acute renal failure in rats within three days after injection. Nevertheless, scintigraphic evaluation and biochemical analysis including serum creatinine, BUN, and cys-C successfully reflected either severe renal damage caused by CDDP and improvement due to the protection of AGM. However, serum creatinine and BUN levels showed any significant difference when compared with control rats, and was insufficient to reflect...
the slight nephrotoxic effects in AGM supplemented group. This result may be related with low sensitivity of aforementioned parameters on detecting mild renal damage as argued in the literature.26

Cys-C was discussed as a more sensitive marker than creatinine to detect mild reductions in GFR and early assessment of renal damage in previous studies.27,28 It was concluded that cys-C levels are less dependent on factors other than renal injury as sex, age, race, and muscle mass.28 In the current study, co-administration of AGM with CDDP resulted in a nonsignificant mild elevation of cys-C while serum creatinine and BUN levels were normal as in control group suggesting the superiority of cys-C as noted before.19,27,28 However, a similar increase of cys-C was seen in AGM administered alone group which revealed a completely normal kidney morphology in histological examination. Moreover, administration of AGM alone did not induce significant changes in serum creatinine and BUN consistent with findings in the literature.15

A previous study demonstrated that some factors other than renal functions as smoking and levels of C-reactive protein might influence serum cys-C levels.29 Oc et al. investigated the correlation between Cys-c and radionuclide-based measurement of glomerular filtration rate in lung cancer patients receiving cisplatin chemotherapy. They concluded that cys-c might not be reliable in determining renal functions after chemotherapy.30 Therefore, this nonsignificant increase in cys-C levels in our study was thought to be due to the chemotherapy or presence of another factors acting on cys-C levels that likely to be AGM.

Unlike biochemical tests including cys-C, scintigraphic findings successfully demonstrated even mild renal damage that is consistent with histological findings. In addition, in patients receiving chemotherapy, scintigraphic evaluation of renal functions is more useful than other clinical methods since it gives information about both split renal function and parenchymal pathologies.

In conclusion, our results indicate that AGM is a protective agent against CDDP-induced nephrotoxicity confirmed by scintigraphic, biochemical, and histological findings. In addition, the semiquantitative scintigraphic method used in current study appears to be a useful and sensitive tool to evaluate nephrotoxicity even in mild impairment of renal functions and efficacy of potential protective agents.

Acknowledgements
We thank Dr Furuzan Kofturk (Department of Medical Statistics, Bulent Ecevit University School of Medicine) for his help in statistical analysis.

Disclosure statement
The authors declared no potential conflict of interest with respect to the authorship, and/or publication of this study.

Funding
Scientific Research Project Coordination Unit of Bulent Ecevit University [2012-20-00-31].

ORCID
Tarik Elri http://orcid.org/0000-0001-5850-914X

References
1. Arany I, Safirstein RL. Cisplatin nephrotoxicity. Semin Nephrol. 2003;23:460–464.
2. Saleh S, El -Demerdash E. Protective effects of l-arginine against cisplatin-induced renal oxidative stress and toxicity. Role of nitric oxide. Basic Clin Pharmacol Toxicol. 2005;97:91–97.
3. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: A review. Am J Med Sci. 2007;334:115–124.
4. Chirino YI, Pedraza-Chaverri J. Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. Exp Toxicol Pathol. 2009;61:223–242.
5. Mansour MA, Mostafa AM, Nagi MN, Khattab MM, Al-Shabanah OA. Protective effect of aminoxyguanidine against nephrotoxicity induced by cisplatin in normal rats. Comp Biochem Physiol C Toxicol Pharmacol. 2002;132:123–128.
6. Flak TA, Goldman WE. Autotoxicity of nitric oxide in airway disease. Am J Respir Crit Care Med. 1996;154:202–206.
7. Schuiling M, Meurs H, Zuidhof AB, Venema N, Zaagsma J. Dual action of iNOS-derived nitric oxide in allergen-induced airway hyperreactivity in conscious, unrestrained guinea pigs. Am J Respir Crit Care Med. 1998;158:1442–1449.
8. Ten Broeke R, De Crom R, Van Haperen R, et al. Overexpression of endothelial nitric oxide synthase suppresses features of allergic asthma in mice. Respir Res. 2006;7:58.
9. Srivastava RC, Farookh A, Ahmad N, Misra M, Hasan SK, Husain MM. Evidence for the involvement of nitric oxide in cisplatin-induced toxicity in rats. Biometals. 1996;9:139–142.
10. Chirino YI, Trujillo J, Sánchez-González DJ, et al. Selective iNOS inhibition reduces renal damage induced by cisplatin. Toxicol Lett. 2008;176:48–57.
11. Piletz JE, Arcioglu F, Cheng JT, et al. Agmatine: Clinical applications after 100 years in translation. Drug Discov Today. 2013;18:880–893.
12. Uzbay TI. The pharmacological importance of agmatine in the brain. Neurosci Biobehav Rev. 2012;36:502–519.
13. Auguet M, Viossat I, Marin JG, Chabrier PE. Selective inhibition of inducible nitric oxide synthase by agmatine. Jpn J Pharmacol. 1995;69:285–287.
14. Schwartz D, Peterson OW, Mendonca M, et al. Affects glomerular filtration via a nitric oxide synthase-dependent mechanism. *Am J Physiol.* 1997; 272: F597–F601.

15. Ishizuka S, Cunard R, Poucell-Hatton S, et al. Agmatine inhibits cell proliferation and improves renal function in anti-Thy-1 glomerulonephritis. *J Am Soc Nephrol.* 2000;11:2256–2264.

16. Nissim I, Horyn O, Daikhin Y, et al. Ifosfamide-induced nephrotoxicity: Mechanism and prevention. *Cancer Res.* 2006;66:7824–7831.

17. Sugiuira T, Tsutsui H, Takaoka M, et al. Protective effect of agmatine on ischemia/reperfusion-induced renal injury in rats. *J Cardiovasc Pharmacol.* 2008;51:223.

18. El-Kashef DH, El-Kenawi AE, Rahim MA, Suddek GM, Salem HA. Agmatine improves renal function in gentamycin-induced nephrotoxicity in rats. *Can J Physiol Pharmacol.* 2015;94:278–286.

19. Benohr P, Grenz A, Hartmann JT, Müller GA, Blaschke S. Cystatin C – a marker for assessment of the glomerular filtration rate in patients with cisplatin chemotherapy. *Kidney Blood Press Res.* 2006;29:32–35.

20. Merrick MV, Uttley WS, Wild SR. The detection of pyelonephritic scarring in children by radioisotope imaging. *Br J Radiol.* 1980;53:544–556.

21. Willis KW, Martinez DA, Hedley WE, Uttley WS. Renal localization of Tc-99m stannous glucoheptonate and Tc-99m dimercaptosuccinate in the rat by frozen section autoradiography: the efficiency and resolution of technetium-99m. *Radiat Res.* 1977;69:475–488.

22. Enlander D, Weber PM, dos Remedios LV. Renal cortical imaging in 35 patients: superior quality with 99mTc-DMSA. *J Nucl Med.* 1974;15:743.

23. Yürekli Y, Ünak P, Ertay T, Müftüler FZB, Medine El, Acar C. Radiopharmaceutical model using 99mTc-DMSA to evaluate amifostine protection against cisplatin nephrotoxicity in rats. *Turk J Nucl Med.* 2010;19:105–109.

24. Yamada M. Assessment of 99mTc-DMSA renography and uptake compared with creatinine clearance in rats with drug-induced nephrotoxicity – II. Cisplatin-induced nephrotoxicity. *Kaku Igaku.* 1991;28:347–354.

25. Kunworarath N, Muangnil P, Itharat A, Hiranyakchattad S. Acute and subchronic treatment of *Hibiscus sabdariffa* Linn. Extract on renal function and lipid peroxidation in cisplatin-induced acute renal failure rats. *J Physiol.* 2014;27:5–12.

26. Hartshorn EA, Anand AJ, Bashey B. Newer insights into cisplatin nephrotoxicity. *Ann Pharmacother.* 1993;27:1519–1525.

27. Ozer BA, Dursun B, Baykal A, Gultekin M, Suleymanlar G. Can cystatin C be a better marker for the early detection of renal damage in primary hypertensive patients? *Ren Fail.* 2005;27:247–253.

28. Coll E, Botey A, Alvarez L, et al. Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. *Am J Kidney Dis.* 2000;36:39–39.

29. Knight EL, Verhave JC, Spiegelman D, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int.* 2004;65:1416–1421.

30. Oc MA, Demir H, Cekmen MB, Isgoren S, Gorur GD, Bilgili U. Correlation of cystatin-C and radionuclidic measurement method of glomerular filtration rate in patients with lung cancer receiving cisplatin treatment. *Ren Fail.* 2014;36:1043–1050.