A Comparison between the Nephrotoxic Profile of Gentamicin and Gentamicin Nanoparticles in Mice

Akram Jamshidzadeh,[1,2] *Reza Heidari,*[1] *Soliman Mohammadi-Samani,*[3] *Negar Azarpira,*[4] *Asma Najbi,*[1,2] *Parisa Jahani,*[5] *and Narges Abdoli*[6]

[1]Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; E-mail: *rezaheidari@hotmail.com*

[2]Pharmacology and Toxicology Department, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

[3]Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

[4]Transplant Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[5]Pharmacology and Toxicology Department, Shiraz University of Medical Sciences International Branch (Kish), Shiraz, Iran

[6]Ministry of Health, Food and Drug Organization, Tehran, Iran

Received 22 July 2014; accepted 12 August 2014

ABSTRACT: Aminoglycoside antibiotics are widely used against Gram-negative infections. On the other hand, nephrotoxicity is a deleterious side effect associated with aminoglycoside therapy. Gentamicin is the most nephrotoxic aminoglycoside. Because of serious health complications ensue the nephrotoxicity induced by aminoglycosides, finding new therapeutic strategies against this problem has a great clinical value. This study has attempted to compare the nephrotoxic properties of gentamicin and a new nanosized formulation of this drug in a mice model. Animals were treated with gentamicin (100 mg/kg, i.p. for eight consecutive days) and nanogentamicin (100 mg/kg, i.p. for eight consecutive days). Blood urea nitrogen (BUN), plasma creatinine levels, and histopathological changes of kidney proximal tubule were monitored. It was found that gentamicin caused severe degeneration of kidney proximal tubule cells and an increase in serum creatinine and BUN. No severe injury was observed after nanogentamicin administration. This study proved that nanosized gentamicin is less nephrotoxic. © 2014 Wiley Periodicals, Inc. J. Biochem. Mol. Toxicol. 29:57–62, 2015; View this article online at wileyonlinelibrary.com. DOI 10.1002/jbt.21667

KEYWORDS: **Aminoglycoside Antibiotic; Kidney Injury; Nephrotoxicity; Nano-drug; Renal Failure**

INTRODUCTION

Nephrotoxicity is the most concerned side effect attributed to aminoglycoside antibiotic therapy [1]. However, in spite of their nephrotoxic reactions, aminoglycosides are widely used against Gram-negative bacteria and bacterial endocarditis [2]. Gentamicin is the most common nephrotoxic aminoglycoside [3]. Despite the accurate control of patients, the incidence of gentamicin-induced nephrotoxicity is high [4]. Kidney proximal tubule epithelial cells are the most convenient site of injury induced by aminoglycoside antibiotics [5]. The expression of a specific transporter in the proximal tubule seems to be responsible for drug accumulation and consequent nephrotoxicity induced by gentamicin (Figure 1) [6, 7]. Although the exact mechanisms of renal injury induced by aminoglycoside antibiotics are not fully clearly understood yet, but oxidative stress induction and the impairment of intracellular organelles functionality are supposed to be involved in this complication (Figure 1) [8–10]. Mitochondria, lysosomes, and endoplasmic reticulum are among the potential intracellular targets of aminoglycosides for cytotoxicity induction (Figure 1) [11, 12]. Moreover, drug accumulation in the plasma membrane of tubular cells might play a role in aminoglycosides-induced nephrotoxicity (Figure 1) [13, 14]. Some factors such as concurrent nephrotoxic drugs administration [15], and patients' underlying renal disease [16], might hasten the nephrotoxic properties of aminoglycosides.

The drug plasma concentration is another important factor, which is directly related to the gentamicin nephrotoxicity [17]. Fluctuation in serum levels of gentamicin and its relation to the kidney injury induced by this drug has been proven [18].

Nanoparticulate drug delivery systems have received considerable attention in recent years. Nanodrugs might show a better profile of pharmacokinetic

Correspondence to: Reza Heidari.
© 2014 Wiley Periodicals, Inc.

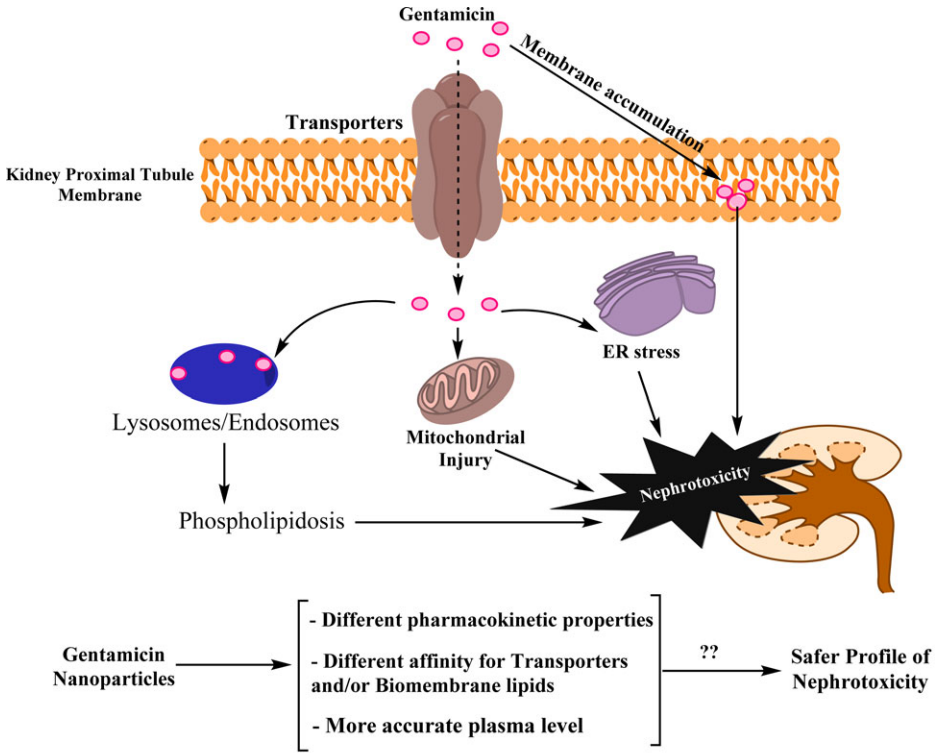

FIGURE 1. Mechanisms of nephrotoxicity induced by gentamicin as reported in previous investigations. The proposed reasons for safer profile of nephrotoxicity of gentamicin nanoparticles in current investigation are given. ER: endoplasmic reticulum.

/dynamics as compared to their conventional forms [19]. Nanodrugs are delivered to their targets by different carriers. Biodegrability and biocompatibility are properties of an ideal nanoparticle carrier [20]. Sodium alginate is a water soluble and biocompatible carrier for drug delivery [21]. Gentamicin nanoparticles were loaded on the alginate carrier in the current investigation.

Serious health complications might ensue the nephrotoxicity induced by aminoglycosides, hence finding new strategies against this problem might have a great clinical value. This study was designed to assess and compare the nephrotoxic properties of a newly synthesized nanosized gentamicin with its conventional form. Blood urea nitrogen (BUN) and serum creatinine levels were assessed as biomarkers of the kidney function. Furthermore, the histopathological changes in kidney were monitored.

MATERIALS AND METHODS

Chemicals

Gentamicin sulfate was obtained from Acros (Fair Lawn, NJ). Sodium alginate was purchased from Sigma Aldrich (St. Louis, MO). Formaldehyde was obtained from Merck (Dardamstd, Germany). Kits for evaluating plasma markers of kidney functionality (BUN and creatinine) were prepared from Pars Azmun (Tehran, Iran). All salts for buffer solutions were of the highest grade commercially available.

Animals

Male Swiss albino mice (25–35 g) were obtained from Shiraz University of Medical Sciences, Shiraz, Iran. Mice were housed in cages on wood bedding at a temperature of 25 ± 3°C. Animals had free access to food and water. The animals received humane care and use and were handled according to the animal handling protocol at Shiraz University of Medical Sciences, approved by a local ethics committee.

Mice were randomly divided equally into four groups of six animals. The treatments were as follows: (A) control (vehicle-treated mice, 0.5 mL of normal saline, i.p. for eight consecutive days), (B) gentamicin-treated animals (100 mg/kg/day, i.p. for eight consecutive days) [22], (C) sodium alginate, nanoparticles carrier (200 mg/kg/day, i.p. for eight consecutive days), and (D) gentamicin nanoparticles-treated mice (100 mg/kg/day, i.p. for eight consecutive days).

Plasma Biochemical Analysis

Blood was collected from the abdominal vena cava under pentobarbital anesthesia (50 mg/kg, i.p.) and transferred to heparinized tubes. Then, the kidneys were removed. Plasma samples were used to measure creatinine and BUN as the standard parameters of evaluating the kidney function. Creatinine and BUN were assessed by commercial kits available (Pars Azmun, Tehran, Iran).

Kidney Histopathological Evaluation

For histopathological evaluation, samples of kidney were fixed in formalin (10%). Paraffin–embedded sections of tissue were prepared and stained with hematoxylin and eosin before analyzing with a light microscope [23].

Nanoparticle Preparation and Loading

The ionic gelation between gentamicin sulfate and sodium alginate was used to design desired nanoparticles in adequate particle size range [24]. An aqueous solution of alginate was prepared and while being mixed gentamicin sulfate solution was added to the polymer containing solution and in different proportions of these solutions appropriate size range was obtained. The preparation method was optimized with respect to the drug volume, polymer volume (constant concentration) at different mixing rates. Then the effect of each group of variables on particle size distribution and release profiles was assessed. To optimize the particle size distribution and to get optimum average particle size, an orthogonal factorial design was considered using Minitab 16 software. The average particle size of nanoparticle in the optimized formulation was 80 nm. Gentamicin was assayed in pharmaceutical samples using a HPLC method precolumn derivatization with phenylisosianide. Loading efficiency of the nanoparticles in the optimized formulation was 85%. The physical stability of the particles at room temperate and in refrigerated samples during the 3 months study was acceptable.

Statistical Analysis

Results are shown as mean ± SE for at least six animals. Comparisons between multiple groups were made by a one–way analysis of variance followed by Turkey's test as post hoc. Differences between experimental groups were considered significant when $P < 0.05$.

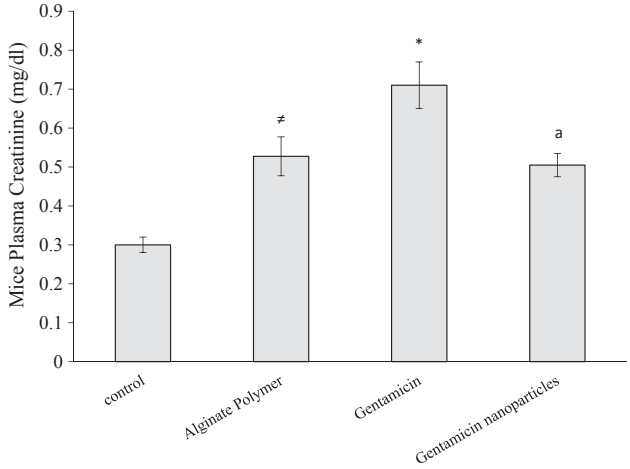

FIGURE 2. Mice plasma creatinine level as a marker of kidney functionality. Data are shown as mean ± SEM for six animals. * indicates a significant difference as compared with control animals ($P < 0.05$). [a] indicates significantly lower than the gentamicin-treated group ($P < 0.05$). ≠ indicates a slight elevation in the plasma creatinine level was occurred in alginate-treated mice.

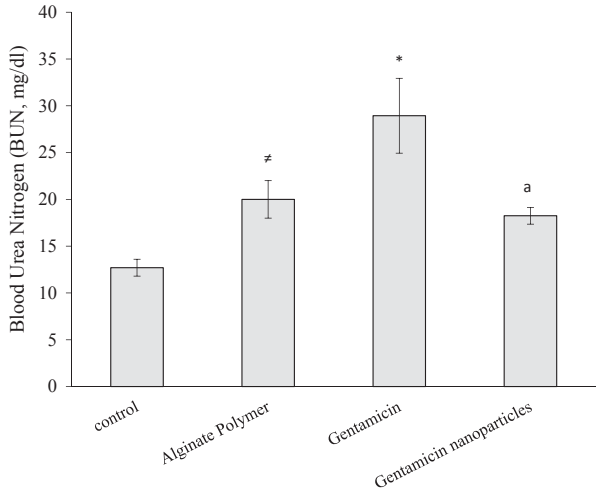

FIGURE 3. Plasma BUN in gentamicin-treated mice. Data are expressed as mean ± SEM for six animals. * indicates a significant difference as compared with control animals ($P < 0.05$). [a] indicates significantly lower than the gentamicin-treated group ($P < 0.05$). ≠ indicates a slight increase in BUN was seen in alginate-treated animals.

RESULTS

Gentamicin caused nephrotoxicity in mice as revealed by an increase in plasma creatinine (Figure 2) and BUN (Figure 3). When animals were treated with nanogentamicin in the current investigation, it was found that serum plasma creatinine was at a lower level as compared with gentamicin-treated mice (Figure 2). Furthermore, BUN levels were lower in the nanogentamicin group in comparison with the gentamicin-treated ones (Figure 3), which might

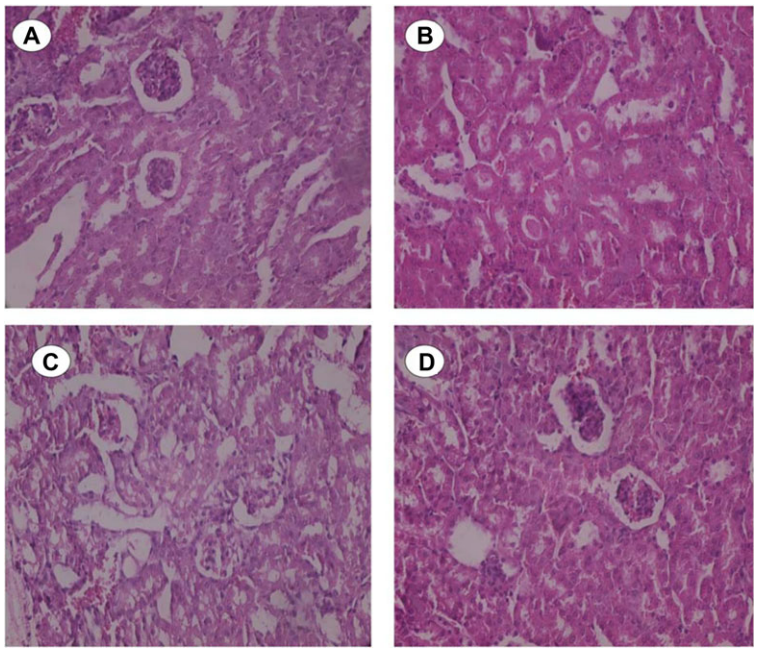

FIGURE 4. Histopathological evaluation of proximal tubule of mice's kidney in different experimental groups. The normal proximal tubule of mice liver with no significant injury (A). Tubular casts and mild vacuolization are seen in alginate polymer–treated mice (200 mg/kg, i.p. for eight consecutive days) (B). Gentamicin-treated mice (100 mg/kg, i.p. for eight consecutive days) showed a severe tubular cells vacuolization (C). No severe and significant changes in kidney pathology was found when animals were treated with gentamicin nanoparticles (D).

indicate its lower level of nephrotoxicity in this investigation.

It was found that the alginate polymer (the nanoparticle carrier in this study) caused a slight increase in serum creatinine (Figure 2) and/or BUN (Figure 3). These results might indicate a slight toxicity for the alginate polymer as a drug carrier.

The histopathological findings of mice kidney proximal tubular cells was in accordance with aforementioned markers of nephrotoxicity. It was found that gentamicin administration (100 mg/kg, i.p. for eight consecutive days) caused a severe proximal tubule cells vacuolization in addition of casts (Figure 4C), where nanogentamicin administration was not accompanied by severe histopathological changes in mice kidney (Figure 4D).

DISCUSSION

Gentamicin caused a severe renal injury in mice as revealed by a marked elevation in plasma BUN and creatinine and histopathological changes in proximal tubule cells. When animals were treated with nanogentamicin in a same course, significant amelioration in a kidney function profile was observed and pathological damages were alleviated.

Different strategies are employed to prevent and/or ameliorate the nephrotoxicity induced by aminoglycosides. The inhibition of tubular accumulation [25, 26], cotreatment with renoprotective agents [27–29], blockade of the immune system [30], and preventing intracellular targets dysfunction [11] are tested strategies against aminoglycosides nephrotoxicity. On the other hand, different antibiotics are formulated as nanoparticles in an attempt to enhance their antimicrobial activity [31, 32]. Moreover, it has been reported that some other drugs with nephrotoxic properties became less toxic when they were formulated as nanoparticles [33, 34]. In this study, we attempted to investigate whether nanogentamicin is less nephrotoxic than gentamicin.

Gentamicin (in general aminoglycosides) is transported to kidney cells by endocytosis and some transporters in kidney proximal tubules membrane [7, 35]. Furthermore, it has been shown that gentamicin is accumulated in tubular cells' plasma membrane lipids, an event which finally leads to cell death (Figure 1) [36, 37]. The distribution of nanosized gentamicin to tubular cells might be different from the conventional forms. Endocytosis and/or binding to phospholipids, which are required for gentamicin nephrotoxicity (Figure 1), might be affected by the size of the administered drug. It is possible to speculate that nanogentamicin may not accumulate in tubular cells as a gentamicin dose (Figure 1). Hence, the small size, customized surface, and improved solubility of gentamicin nanoparticles all might contribute to its lower rate of renal injury. The

therapeutic range of 5–8 μg/mL is suggested for peak serum levels of gentamicin and that caused less nephrotoxicity [18]. As nanosized pharmaceuticals maintain a more stable serum level of drugs [38], another possible factor, which might contribute to less nephrotoxic properties of nanogentamicin, could be related to the effect of this formulation on the drug plasma level. Less fluctuation in drug plasma levels and consequently less frequency of kidney injury might occur after administration of nanogentamicin.

Although alginic acid is known as a biocompatible and nontoxic polymer [39], we found that a slight change in serum BUN and creatinine occurred in alginate-treated animals (Figures 2 and 3). Furthermore, tubular casts were found in the kidney (Figure 4). Hence, a limitation of the current investigation and a possible reason for a slight toxicity of alginate carrier might be attributed to the procedure used to induce nephrotoxicity. If other doses and routes (with lower doses of the drug and drug carrier) were used, the nephrotoxicity of the alginate carrier may not have ensued. Although alginate polymers showed a mild nephrotoxic effect in this study, it was found that gentamicin nanoparticles carried with the alginate polymer were extremely safer than conventional gentamicin.

In conclusion, we found that nanogentamicin is less nephrotoxic than gentamicin. Owing to its less nephrotoxic properties and enhanced antibiotic efficacy of nanodrugs [40], nanogentamicin could be the subject of future investigations in laboratory and clinical tests. Furthermore, future investigations on the tubular accumulation of nanosized gentamicin will expand our understanding of the mechanism(s) by which nanogentamicin reduces renal injury. The proposed mechanisms for the lower profile of toxicity of nanogentamicin are summarized in Figure 1.

ACKNOWLEDGMENTS

The authors thank Pharmaceutical Sciences Research Center of Shiraz University of Medical Sciences for providing instrumental facilities to carry out this investigation. The authors declare no conflict of instruction.

REFERENCES

1. Mingeot-Leclercq M-P, Tulkens PM. Aminoglycosides: nephrotoxicity. Antimicrob Agents Chemother 1999;43(5):1003–1012.
2. Avent ML, Rogers BA, Cheng AC, Paterson DL. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. Intern Med J 2011;41(6):441–449.
3. Luft FC, Bloch R, Sloan RS, Yum MN, Costello R, Maxwell DR. Comparative nephrotoxicity of aminoglycoside antibiotics in rats. J Infect Dis 1978;138(4):541–545.
4. Bertino JS, Booker LA, Franck PA, Jenkins PL, Franck KR, Nafziger AN. Incidence of and significant risk factors for aminoglycoside-associated nephrotoxicity in patients dosed by using individualized pharmacokinetic monitoring. J Infect Dis 1993;167(1):173–179.
5. Luft FC, Patel V, Yum MN, Patel B, Kleit SA. Experimental aminoglycoside nephrotoxicity. J Lab Clin Med 1975;86(2):213–220.
6. Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int 2011;79(1):33–45.
7. Wedeen RP, Batuman V, Cheeks C, Marquet E, Sobel H. Transport of gentamicin in rat proximal tubule. Lab Invest 1983;48(2):212–223.
8. Walker PD, Barri Y, Shah SV. Oxidant mechanisms in gentamicin nephrotoxicity. Ren Fail 1999;21(3/4):433–442.
9. Yang C-L, Du X-H, Han Y-X. Renal cortical mitochondria are the source of oxygen free radicals enhanced by gentamicin. Ren Fail 1995;17(1):21–26.
10. Sandoval R, Leiser J, Molitoris BA. Aminoglycoside antibiotics traffic to the Golgi complex in LLC-PK1 cells. J Am Soc Nephrol 1998;9(2):167–174.
11. Zorov DB. Amelioration of aminoglycoside nephrotoxicity requires protection of renal mitochondria. Kidney Int 2010;77(10):841–843.
12. Hostetler KY, Hall LB. Inhibition of kidney lysosomal phospholipases A and C by aminoglycoside antibiotics: possible mechanism of aminoglycoside toxicity. Proc Natl Acad Sci U S A 1982;79(5):1663–1667.
13. Williams PD, Hottendorf GH, Bennett DB. Inhibition of renal membrane binding and nephrotoxicity of aminoglycosides. J Pharmacol Exp Ther 1986;237(3):919–925.
14. Williams PD, Holohan PD, Ross CR. Gentamicin nephrotoxicity II. Plasma membrane changes. Toxicol Appl Pharmacol 1981;61(2):243–251.
15. Appel GB. Aminoglycoside nephrotoxicity. Am J Med 1990;88(3):S16-S20.
16. Moore RD, Smith CR, Lipsky JJ, Mellits ED, Lietman PS. Risk factors for nephrotoxicity in patients treated with aminoglycosides. Ann Intern Med 1984;100(3):352–357.
17. Walker RJ, Duggin GG. Drug nephrotoxicity. Annu Rev Pharmacol Toxicol 1988;28(1):331–345.
18. Barza M, Lauermann M. Why monitor serum levels of gentamicin? Clin Pharmacokinet 1978;3(3):202–215.
19. Singh R, Lillard JW Jr. Nanoparticle-based targeted drug delivery. Exp Mol Pathol 2009;86(3):215–223.
20. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 2001;70(1):1–20.
21. Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery. Adv Drug Deliv Rev 2008;60(15):1638–1649.
22. Eslami SH, Ebrahimzadeh MA, Moghaddam HA, Nabavi SF, Jafari N, Nabavi SM. Renoprotective effect of *Eryngium caucasicum* in gentamicin-induced nephrotoxic mice. Arch Biol Sci 2011;63(1):157–160.
23. Moezi L, Heidari R, Amirghofran Z, Nekooeian AA, Monabati A, Dehpour AR. Enhanced anti-ulcer effect of pioglitazone on gastric ulcers in cirrhotic rats: The role of nitric oxide and IL-1b. Pharmacol Rep 2013;65(134):134–143.

24. Coviello T, Matricardi P, Marianecci C, Alhaique F. Polysaccharide hydrogels for modified release formulations. J Control Release 2007;119(1):5–24.
25. Josepovitz C, Pastoriza-Munoz E, Timmerman D, Scott M, Feldman S, Kaloyanides GJ. Inhibition of gentamicin uptake in rat renal cortex in vivo by aminoglycosides and organic polycations. J Pharmacol Exp Ther 1982;223(2):314–321.
26. Antoine DJ, Srivastava A, Pirmohamed M, Park BK. Statins inhibit aminoglycoside accumulation and cytotoxicity to renal proximal tubule cells. Biochem Pharmacol 2010;79(4):647–654.
27. Kotnis MS, Patel P, Menon SN, Sane RT. Renoprotective effect of Hemidesmus indicus, a herbal drug used in gentamicin-induced renal toxicity. Nephrology 2004;9(3):142–152.
28. Pedraza-Chaverrı́ J, González-Orozco AE, Maldonado PD, Barrera D, Medina-Campos ON, Hernández-Pando R. Diallyl disulfide ameliorates gentamicin-induced oxidative stress and nephropathy in rats. Eur J Pharmacol 2003;473(1):71–78.
29. Nitha B, Janardhanan KK. Aqueous-ethanolic extract of morel mushroom mycelium, *Morchella esculenta*, protects cisplatin and gentamicin induced nephrotoxicity in mice. Food Chem Toxicol 2008;46(9):3193–3199.
30. Zhang B, Ramesh G, Uematsu S, Akira S, Reeves WB. TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. J Am Soc Nephrol 2008;19(5):923–932.
31. Mohammadi G, Nokhodchi A, Barzegar-Jalali M, Lotfipour F, Adibkia K, Ehyaei N, Valizadeh H. Physicochemical and anti-bacterial performance characterization of clarithromycin nanoparticles as colloidal drug delivery system. Colloids Surf B Biointerfaces 2011;88(1):39–44.
32. Azhdarzadeh M, Lotfipour F, Zakeri-Milani P, Mohammadi G, Valizadeh H. Anti-bacterial performance of azithromycin nanoparticles as colloidal drug delivery system against different gram-negative and gram-positive bacteria. Adv Pharm Bull 2012;2(1):17–24.
33. Uchino H, Matsumura Y, Negishi T, Koizumi F, Hayashi T, Honda T, Nishiyama N, Kataoka K, Naito S, Kakizoe T. Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. Br J Cancer 2005;93(6):678–687.
34. Sengupta P, Basu S, Soni S, Pandey A, Roy B, Oh MS, Chin KT, Paraskar AS, Sarangi S, Connor Y, Sabbisetti VS, Kopparam J, Kulkarni A, Muto K, Amarasiriwardena C, Jayawardene I, Lupoli N, Dinulescu DM, Bonventre JV, Mashelkar RA, Sengupta S. Cholesterol-tethered platinum II-based supramolecular nanoparticle increases antitumor efficacy and reduces nephrotoxicity. Proc Natl Acad Sci U S A 2012;109(28):11294–11299.
35. Fanos V, Cataldi L. Renal transport of antibiotics and nephrotoxicity: a review. J Chemother 2001;13(5):461–472.
36. Kovács E, Savopol T, Iordache M-M, Săplăcan L, Sobaru I, Istrate C, Mingeot-Leclercq M-P, Moisescu M-G. Interaction of gentamicin polycation with model and cell membranes. Bioelectrochemistry 2012;87:230–235.
37. Nagai J, Komeda T, Yumoto R, Takano M. Effect of protamine on the accumulation of gentamicin in opossum kidney epithelial cells. J Pharm Pharmacol 2013;65(3):441–446.
38. Allen C, Maysinger D, Eisenberg A. Nano-engineering block copolymer aggregates for drug delivery. Colloids Surf B Biointerfaces 1999;16(1):3–27.
39. Sundar S, Kundu J, Kundu SC. Biopolymeric nanoparticles. Sci Tech Adv Mat 2010;11(1):1–13.
40. Dornelles Cherobim M, Correa Amaral A, Campos Dias S, Luiz Franco O. Nanoformulated Antibiotics: the Next Step for Pathogenic Bacteria Control. Curr Med Chem 2013;20(10):1232–1240.