Preventive and Curative Effects of Ginger Extract Against Histopathologic Changes of Gentamicin-Induced Tubular Toxicity in Rats

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Original Article

ABSTRACT

Background: Gentamicin (GM) is a commonly used aminoglycoside, however, renal toxicity has limited its usage. This study was designed to evaluate the curative and protective effects of Zingiber officinale (ginger) against gentamicin tubular toxicity in rats. The phenolic and flavonoid components and antioxidant activity of ginger were also evaluated. **Methods:** In a preclinical study, 50 male Wistar rats were designated into 5 groups of 10 and treated as follows: Group I: vehicle. Group II: 200 mg/kg/d of ginger for 3 days then, GM (80 mg/kg) for 7 days. Group III: 200 mg/kg ginger orally for 3 days, then ginger plus GM for 7 days. Group IV: GM for 7 days. Group V: GM for 10 days. Group VI: GM for 7 days, then 200 mg/kg ginger orally for 10 days. At the end of the study, the animals were sacrificed and their kidneys were histologically evaluated. **Results:** Ginger could prevent degeneration of the renal cells and reduce the severity of tubular damage caused by gentamicin. However, it could not regenerate the GM degeneration.

Conclusions: The results indicate that ginger is effective as a prophylaxis agent, but has not curative effect.

Keywords: Gentamicin, ginger, nephrotoxicity, tubular damage, zingiber officinale

INTRODUCTION

Gentamicin (GM) is probably the most commonly used and studied of all the aminoglycosides, however, it causes severe renal toxicity.[1-7] GM inhibits phosphorylation and reduces ATP levels in renal tubular cells.[2-8] Oxidative stress has also been reported in the tubular toxicity of gentamicin. Thus, gentamicin enhanced reactive oxygen species (ROS) formation[4-9] and ROS-induced cell death were found to have a role in GM-mediated acute renal failure.[5-10] Therefore, treatment with antioxidants might be effective to ameliorate the damage.[5-11] Several natural products have been used to protect the toxicities induced by drugs. Herbs are generally considered safe and proved to be effective against various human ailments and their medicinal uses have been gradually increasing in developed countries.[12] *Zingiber officinale* roscoe (ginger) belonged to Zingiberaceae family,[12,13] is a free radical scavenger and has been shown to be effective on ischemia/reperfusion (I/R) injury in the

rat's kidney.[12,13] Previous studies were shown the beneficial effect of ginger to inhibit cisplatin-induced nephrotoxicity.[12,13] However, the protective effect of *Z. officinale* against gentamicin-induced nephrotoxicity has not yet been fully investigated. Although, concurrent administration of *Zingiber officinale* has been shown to prevent GM toxicity.[14] However, it is not clear whether pretreatment with ginger is able to prevent GM toxicity or not. Furthermore, the regenerative activity of ginger on GM tubular toxicity is not yet investigated. The current study was therefore, designed to evaluate pretreatment and curative effects of *Z. officinale* against gentamicin-induced tubular toxicity in kidneys of Wistar rats. The phenolic and flavonoid components and antioxidant activity of ginger were also evaluated.

METHODS

Animals

Study samples included 50 male Wistar rats with a weight range of 200 to 250 g. Rats were purchased from Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. All animals were similarly handled in the animal house of the research center and had free access to food and water. They were housed at a controlled temperature (25 ± 3°C) and humidity (50 to 60%) environment with a 12 h dark-light cycle (lights on at 7 am) and allowed free access to pelleted diet and tap water. Their general health state and activity were monitored closely during the experiment. The animal experimentation was conducted in accordance with the National Institute of Health guidelines for the careful use of laboratory animals.

Preparation of the extract

Zingiber officinale (ginger) was purchased from a grocery in Isfahan, Iran, and authenticated in Medical Plants Research Center of Shahrekord University of Medical Sciences, Iran. A voucher specimen was deposited in its herbarium department (No: 289). The hydro-alcoholic extract of ginger was prepared by maceration method. Three kg of the ginger was chopped in a suitable container and 2000 ml ethanol 80% was added and left at room temperature for 72 h. Then, it was filtered and the solvent was evaporated using a rotary evaporator. Finally, it was concentrated in 37°C in oven and kept in a fridge until use.

EVALUATION OF THE TOTAL FLAVONOIDS

Aluminum chloride colorimetry and Rutin method was used to measure the total flavonoids.[15] First, standard solutions of Rutin with concentration levels of 25, 50, 100, 250 and 500 ppm were prepared. Then 1 ml from these solutions was transferred into test tubes and mixed with 1 ml of chloride aluminum 2%. Then, 6 ml potassium acetate 5% was added and the optical density level was read after 40 min at 415 nm wavelength. The concentration levels of the standard solutions were assayed in three intervals. In order to measure the overall level of flavonoids in the extracts, 0.01-0.02 g of the extracts was dissolved in methanol 60%, reaching 10 ml. Then, using chloride aluminum colorimetry the total level of flavonoids was measured. However, instead of using the standard solution, 1 ml of the extract was added. The total flavonoid level was calculated in mg per one gram extract, equivalent to Rutin.

Evaluation of total phenolic compounds

Total phenolic compounds were measured equivalent to gallic acid using Folin-Ciocalteu colorimetry.[16] The standard solutions were prepared with concentrations of 12.5, 25, 50, 62.5, 100 and 125 ppm of gallic acid in methanol 60%. Then, 0.1 ml from each sample was transferred into a test tube and 0.5 ml Folin-Ciocalteu 10% was added as reactive agent. The solutions were left for 8 min at room temperature and then 0.4 ml sodium carbonate 7.5% was added. The tubes were maintained for 30 min at the laboratory temperature and then assayed in three intervals by a spectrophotometer (Unico uv 2010) at 765 nm wavelength. To measure the overall phenol in the extracts, 0.01-0.02 g of the extracts was solved in 60% methanol, reaching 10 ml and then, using Folin-Ciocalteu method, the overall level of phenol was measured. However, instead of using the standard solution, 0.1 ml extract solution was added. Finally, the overall phenol level was obtained from the read optical density in mg/g extract in gallic acid equivalent.

Determination of antioxidant activity in the extract

β-carotene model was employed to evaluate the antioxidant activity of the extract.[17] 0.5 ml chloroform, 5 ml β-carotene (0.2 mg), 20 ml linoleic acid (20 mg) and 0.2 ml Tween 40 were

mixed in a suitable container and incubated at 50°C for 10 min. The solution was diluted with distilled water and mixed with 4 ml of aliquots in the following manner. The control solution was prepared including 0.2 ml ethanol and 0.2 ml of the extract sample with 0.15 ml ethanol and 0.05 ml turmeric extract. The optical density of the control group was recorded at t = 0 and t = 90 at 470 nm wavelength and similar to the standard group. The samples were incubated in a bain-marie at 50°C. The antioxidant activity was measured on the basis of the ability of the samples in preventing the washing of β-carotene. The antioxidant activity was calculated through formula 1 below.

(1) $AA = 100\ [1-(A_o-A_t)/(A^o_o-A^o_t)]$

Where,

A_o: the optical density at t = 0

A_t: optical density of the sample at t = 90

A^o_o and A^o_t: as optical density values in the control samples at t = 0 and t = 90, respectively.

Drugs

In this study, rats were received 80 mg/kg body weight/day of GM, which have been previously reported.[18]

Determination of nephroprotection of *Z. officinale*

The animals were divided into 6 groups (10 rats each) as follows:

Group I (Sham group)

They kept in the same condition as others without receiving drugs for 10 days and then sacrificed. They treated with vehicle (distilled water) was kept as normal.

Group II

Rats in this group received 200 mg/kg/d only for 3 days and ginger discontinued, then rats received GM (80 mg/kg; intraperitoneally) for 7 days.

Group III

Rats in this group received 200 mg/kg ginger orally for 3 days and then ginger plus GM for 7 days.

Group IV

Rats in this group received GM (80 mg/kg; intraperitoneally) for.

Group V

Rats in this group received GM (80 mg/kg; intraperitoneally) for 10 days.

Group VI

Rats in this group were received GM (80 mg/kg; intraperitoneally) for 7 days and discontinued, then rats received 200 mg/kg ginger orally for 10 days.

At the end of the experiment rats were sacrificed by injecting ketamine (i.p.) under general anesthesia and the kidneys were removed immediately for histological examinations.

Histopathological evaluations

The kidneys of each animal were dissected out and fixed in buffered formalin for 12 h and processed for histopathological examination. Three μm-thick paraffin sections were stained with hematoxylin and eosin (H and E) for light microscope examination using conventional protocol.[19] Histopathological studies were performed under a light microscope. Slides were coded and examined by a histopathologist who was blinded to the treatment groups. All specimens were examined for six morphologic parameters including epithelial cell vacuolization, degeneration, tubular cell flattening, hyaline cast, tubular dilatation and debris materials in tubular lumen on a semi-quantitative score from 1 to 5, while the score of zero was assigned to the normal tissue without damage.[20,21]

Statistical analysis

The pathology damage score in each groups of 4 and 5 were compared with the groups 1 and 3 by Mann-Whitney U-test. The Bonferroni correction was applied for statistical *P* value, and accordingly $P < 0.05$ was considered statistically significant.

RESULTS

Three Kg *Zingiber officinale* root yielded 29 g hydro-alcoholic extract. The levels of flavonoid and phenolic compounds were 16.17 ± 1.9 mg/g Rutin equivalent and 31.09 ± 2.88 mg/g gallic acid equivalent, respectively, and its antioxidant activity was 37.66%.

The pathology damage scores for all groups of experiments are demonstrated in Table 1. The intensity of nephrotoxicity from the Group 2 that received ginger as prophylaxis was not significantly different from the group of sham (Group I) and from the group treated with ginger without GM (group III). This finding reveals that ginger as prophylaxis could potentially attenuate the GM-induced nephrotoxicity.

Table 1: The pathology damage score in all experimental groups

Group	*n*	Minimum	Maximum	Median
I	6	0.0	1.0	0.5
II	9	0.0	2.0	1.0
III	5	0.0	1.0	0.0
IV	6	2.0	4.0	2.0*
V	8	1.0	4.0	2.0*
VI	8	2.0	2.0	2.0*

*Significant difference from groups I or III ($P < 0.005$), Group I = vehicle; Group II = 200 mg/kg/d of ginger for 3 days then, GM (80 mg/kg) for 7 days; Group III = 200 mg/kg ginger orally for 3 days, then ginger plus GM for 7 days, Group IV = GM for 7 days; Group V = GM for 10 days, Group VI = GM for 7 days, then 200 mg/kg ginger orally for 10 days

However the rats treated with GM for 7 days and then ginger for 10 days demonstrated high intensity of nephrotoxicity, which was not significantly different form treated rats with GM alone (groups 4 and 5).

DISCUSSION

In this study we found, ginger could serve as a preventive agent against GM-tubular toxicity. This study showed that, rats treated with GM for 7 days and then ginger for period of 10 days demonstrated high intensity of nephrotoxicity, which was not significantly different form treated rats with GM alone (groups 4 and 5), it means that ginger possess a preventive, but not a curative property against gentamicin tubular toxicity. Various herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases resulting from lipid peroxidation.[22-24] In the fresh ginger rhizome, the gingerol (polyphenol) was identified as the major active component.[22-25] The volatile oil consists of mainly mono sesquiterpenes; camphene, beta-phellandrene and curcumin.[22-25] The present study showed high levels of polyphenolic and flavonoid compounds with high antioxidant activity for ginger. The presence of polyphenols and flavonoids in the *Z. officinale* extract might be responsible for the antioxidant and nephroprotective activities.[22-25] To determine ginger effect on kidney function, Modaresi *et al*. conducted a study on male mice, which received ginger extract for a period of 20 days. They found that, administration of ginger extract markedly decreased the BUN concentrations in experimental mice. They concluded that ginger might have a beneficial effect for removal of urea from plasma and it should be considered as a therapeutic herb to manage renal function in patients with uremia.[26] To examine the preventive effect of ginger against renal ischemia-reperfusion (I-R) injury, a study was conducted by Maghsoudi *et al*. on 30 adult male rats.[13] They found that, ginger was a useful agent for the prevention of renal ischemia reperfusion-induced injury.[13] To investigate the effect of ginger administration on altered blood glucose levels, intra-and extra-mitochondrial enzymes and tissue injuries in streptozotocin (STZ)-induced diabetic rats, Ramudu *et al*. found that, activities of intra-and extra-mitochondrial enzymes such as glucose-6-phosphate dehydrogenase (G6PD), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH) were significantly decreased in the kidneys of the diabetic rats, while these were significantly reversed by 30 days of ginger treatment.[27] They also observed consistent renal tissue damages in the diabetic rats; however, these injuries recovered in the ginger-treated diabetic rats as shown in histopathological studies. They demonstrated that an ethanolic extract of ginger could lower the blood glucose levels and improve activities of intra-and extra-mitochondrial enzymes in diabetic rats. They suggested that ginger extracts could be used as a nephro-protective supplement particularly to reverse diabetic-induced complications.[27] Similarly, Mahmoud *et al*. conducted a study to evaluate the effects of Zingiber officinale on both acute and chronic renal failure (CRF) and the mechanisms underlying their effects.[28] Acute renal failure was induced by 30 min ischemia followed by 24 h reperfusion, while CRF was induced by adenine feeding for 8 weeks. Prophylactic oral administration of ginger was started 3 days before and along with adenine feeding in different groups or 7 days before ischemia-reperfusion. Ginger showed renoprotective effects in both models of renal failure.[28] These protective effects may be attributed at least in part to their anti-inflammatory properties as evident by attenuating serum C-reactive protein levels and antioxidant effects as evident by attenuating lipid peroxidation marker, malondialdehyde levels, and increasing renal superoxide dismutase activity.[29-31]

Hence, we envisage that ginger could be beneficial adjuvant therapy in patients with acute renal failure

and CRF to prevent disease progression and delay the need for renal replacement therapy. In a similar study, Uz *et al*. analyzed the possible protective effect of dietary ginger against the damage inflicted by reactive oxygen species (ROS) during renal I/R on thirty rats using histopathological and biochemical parameters.[32] Ginger supplementation in the diet before I/R injury resulted in higher total antioxidant capacity and lower total oxidant status levels than I/R group. The ginger supplemented diet prior to I/R process demonstrated marked reduction of the histological features of renal injury. Their findings imply that ginger exerts renoprotective effects probably by the radical scavenging and antioxidant activities.[32] Hence, it is assume that oxidative stress due to abnormal production of ROS is believed to be involved in the etiology of renal toxicities.[27-39]

All the above mentioned studies demonstrated that concurrent administration of ginger could prevent nephrotoxicity induced by oxidative stress. The results of the present study showed that pretreatment with ginger (preloading of blood antioxidant capacity), could also prevent nephrotoxicity induced by gentamicin. Furthermore, our results demonstrated that if nephrotoxicity was induced by GM, ginger would not be able to regenerate tubular cell damages. It means that ginger might have preventive, but not curative properties against GM nephrotoxicity.

CONCLUSIONS

Ginger significantly protects the renal cells and reduces the severity of tubular damage caused by gentamicin. Ginger was effective as a prophylaxis agent, but it has not curative activity. However, further detailed studies are required to establish its clinical application.

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REFERENCES

1. Kopple JD, Ding H, Letoha A, Ivanyi B, Qing DP, Dux L, *et al*. L-carnitine ameliorates gentamicin-induced renal injury in rats. Nephrol Dial Transplant 2002;17:2122-31.
2. Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: A new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. J Exp Med 2000;192:1001-4.
3. Ali BH, Al Za'abi M, Blunden G, Nemmar A. Experimental gentamicin nephrotoxicity and agents that modify it: A mini-review of recent research. Basic Clin Pharmacol Toxicol 2011;109:225-32.
4. Rafieian-Kopaei M, Nasri H, Nematbakhsh M, Baradaran A, Gheissari A, Rouhi H, *et al.* Erythropoietin ameliorates genetamycin-induced renal toxicity: A biochemical and histopathological study. J Nephropathology 2012;1:109-16.
5. Kadkhodaee M. Erythropoietin; bright future and new hopes for an old drug. J Nephropathology 2012;1:81-2.
6. Khajehdehi P. Turmeric: Reemerging of a neglected Asian traditional remedy. J Nephropathology 2012;1:17-22.
7. Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights in to the mechanism of aminoglycoside nephrotoxicity: An integrative point of view. Kidney Int 2011;79:33-45.
8. Quiros Y, Vicente-Vicente L, Morales AI, López-Novoa JM, López-Hernández FJ. An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. Toxicol Sci 2011;119:245-56.
9. Simmons CF, Bogusky RT, Humes HD. Inhibitory effects of gentamicin on renal mitochondrial oxidative phosphorylation. J Pharmacol Exp Ther 1980;214:709-15.
10. Tavafi M. Inhibition of gentamicin–induced renal tubular cell necrosis. J Nephropathology 2012;1:83-6.
11. Kiritoshi S, Nishikawa T, Sonoda K, Kukidome D, Senokuchi T, Maztsuo T, *et al*. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: Potential role in diabetic nephropathy. Diabetes 2003;52:2570-7.
12. Heeba GH, Abd-Elghany MI. Effect of combined administration of ginger (Zingiber officinale Roscoe) and atorvastatin on the liver of rats. Phytomedicine 2010;17:1076-81.
13. Maghsoudi S, Gol A, Dabiri S, Javadi A. Preventive effect of ginger (Zingiber officinale) pretreatment on renal ischemia-reperfusion in rats. Eur Surg Res 2011;46:45-51.
14. Lakshmi BV, Sudhakar M. Protective effect of *Zingiber officinale* on gentamicin-induced nephrotoxicity in rats. Int J Pharmacol 2010;6:58-62.
15. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. J Med Food 2011;14:969-74.
16. Bahmani M, Rafieian-kopaei M, Parsaei P, Mohsenzadegan A. The anti-leech effect of Peganum harmala L. extract and some anti-parasite drugs on

Limnatis nilotica. Afr J Microbiol Res 2012;6:2586-90.
17. Mirzaei MG, Sewell RD, Kheiri S, Rafieian-Kopaei M. A clinical trial of the effect of St. John's Wort on migraine headaches in patients receiving sodium valproate. J Med Plants Res 2012;6:1519-23.
18. Said MM. The protective effect of eugenol against gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Fundam Clin Pharmacol 2011;25:708-16.
19. Nasri H, Mortazavi M, Ghorbani A, Shahbazian H, Kheiri S, Baradaran A, *et al*. Oxford-MEST classification in IgA nephropathy patients: A report from Iran. J Nephropathology 2012;1:31-42.
20. Nematbakhsh M, Ashrafi F, Safari T, Talebi A, Nasri H, Mortazavi M, *et al*. Administration of vitamin E and losartan as prophylaxes in cisplatin-induced nephrotoxicity model in rats. J Nephrol 2012;25:410-7.
21. Eshraghi-Jazi F, Nematbakhsh M, Nasri H, Talebi T, Haghighi M, Pezeshki Z *et al*. The protective role of endogenous nitric oxide donor (L-arginine) in cisplatin-induced nephrotoxicity: Gender related differences in rat model. J Res Med Sci 2011;16:1389-96.
22. Nakatani N. Phenolic antioxidants from herbs and spices. Biofactors 2000;13:141-6.
23. Ho CT, Wang M, Wei GJ, Huang TC, Huang MT. Chemistry and antioxidative factors in rosemary and sage. Biofactors 2000;13:161-6.
24. Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J Agric Food Chem 2005;53:7749-59.
25. Policegoudra RS, Aradhya SM, Singh L. Mango ginger (Curcuma amada Roxb.), a promising spice for phytochemicals and biological activities. J Biosci 2011;36:739-48.
26. Masuda Y, Kikuzaki H, Hisamoto M, Nakatani N. Antioxidant properties of gingerol related compounds from ginger. Biofactors 2004;21:293-6.
27. Ma J, Jin X, Yang L, Liu ZL. Diarylheptanoids from the rhizomes of Zingiber officinale. Phytochemistry 2004;65:1137-43.
28. Rouhi H, Ganji F, Nasri H. Effects of Ginger on the improvement of asthma [the evaluation of Its treatmental effects]. Pakistan J Nutr 2006;5:373-6.
29. Mehrdad M, Messripour M, Ghobadipour M. The effect of ginger extract on blood urea nitrogen and creatinine in mice. Pak J Biol Sci 2007;10:2968-71.
30. Ramudu SK, Korivi M, Kesireddy N, Lee LC, Cheng IS, Kuo CH, *et al*. Nephro-protective effects of a ginger extract on cytosolic and mitochondrial enzymes against streptozotocin (STZ)-induced diabetic complications in rats. Chin J Physiol 2011;54:79-86.
31. Mahmoud MF, Diaai AA, Ahmed F. Evaluation of the efficacy of ginger, Arabic gum, and Boswellia in acute and chronic renal failure. Ren Fail 2012;34:73-82.
32. Hamed MA, Ali SA, El-Rigal NS. Therapeutic potential of Ginger against renal injury induced by carbon tetrachloride in rats. ScientificWorldJournal 2012;2012:840421.
33. Ramudu SK, Korivi M, Kesireddy N, Chen CY, Kuo CH, Kesireddy SR. Ginger feeding protects against renal oxidative damage caused by alcohol consumption in rats. J Ren Nutr 2011;21:263-70.
34. Sharkovska Y, Kalk P, von Websky K, Relle K, Pfab T, Alter M, *et al*. Renoprotective effects of combined endothelin-converting enzyme/neutral endopeptidase inhibitor SLV338 in acute and chronic experimental renal damage. Clin Lab 2011;57:507-15.
35. Uz E, Karatas OF, Mete E, Bayrak R, Bayrak O, Atmaca AF *et al*. The effect of dietary ginger (Zingiber officinale Rosc) on renal ischemia/reperfusion injury in rat kidneys. Ren Fail 2009;31:251-60.
36. Tayebi Khosroshahi H. Short history about renal transplantation program in Iran and the world: Special focus on world kidney day 2012. J Nephropathology 2012;1:5-10.
37. Tolou-Ghamari Z. Nephro and neurotoxicity, mechanisms of rejection: A review on Tacrolimus and Cyclosporin in organ transplantation. J Nephropathology 2012;1:23-30.
38. Assadi F. The epidemic of pediatric chronic kidney disease: the danger of skepticism. J Nephropathology 2012;1:61-4.
39. Ardalan MR, Samadifar Z, Vahedi A. Creatine monohydrate supplement induced interstitial nephritis. J Nephropathology 2012;1:117-20.

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