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Effect of commercial (vimang) and hydroalcoholic extract of *Mangifera indica* (Mango) on gentamicin-induced nephrotoxicity in rat

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Abstract

Objectives: *Mangifera indica* (Mango) is used in folk medicine for treatment of different types of diseases, and its anti-inflammatory and free radical scavenging activities have been demonstrated. The present study evaluated the effects of commercial (vimang) and hydroalcoholic extract of Mango on gentamicin-induced nephrotoxicity in rat.

Materials and Methods: Female Wistar rats were treated with vimang (50 and 100 mg/kg) for 18 days, or hydroalcoholic extract (200 and 400 mg/kg) for 18 days as preventive groups and others with vimang (100 mg/kg) for 8 days, or hydroalcoholic extract (400 mg/kg) for 8 days as treatment groups and also gentamicin (GM) was used at 80 mg/kg/day for eight days, starting from day 10. At the end of treatment, blood and urine samples were taken for measurement of creatinine (Cr) and BUN. The kidney was prepared for histological evaluation.

Results: Serum Cr and urea concentrations as well as renal tissue injury increased significantly in GM group compared with the control group. Hydroalcoholic extract of Mango at 200mg/kg was able to reduce plasma Cr and urea concentrations significantly as well as kidney tissue necrosis. Vimang (50 and 100 mg/kg) and hydroalcoholic extract of Mango (200mg/kg) also prevented kidney tissue damage compared with the control group.

Conclusion: Mango products were able to improve kidney function in an established model of GM-induced nephrotoxicity in the rat. The beneficial effects of Mango on the rat kidney seem to be dose and time-dependent. However, more investigations are needed to elucidate Mango action on GM-induced renal toxicity.

Key words: Mango, Vimang, Gentamicin, Acute renal failure

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Introduction

Aminoglycosides, such as gentamicin (GM), are a class of clinically important antibiotics used extensively in the treatment of infections, particularly aerobic gram-negative bacteria (Nagai et al., 2004). However, nephrotoxicity and ototoxicity are the serious side effects in the use of aminoglycosides. GM- induced nephrotoxicity occurs in about 10–30% of patients receiving the drug (Mathew, 1992; Paterson et al., 1998). Nephrotoxicity induced by GM is a complex phenomenon characterized by an increase in plasma Cr and urea levels and proximal renal tubular necrosis, followed by deterioration of renal function and renal failure (Cuzzocrea et al., 2002; Al-Majed et al., 2002). The toxicity of aminoglycosides, including GM, is believed to be related to the generation of reactive oxygen species (ROS) in the kidney (Reiter et al., 2002; Al-Majed et al., 2002).

Several studies have demonstrated that various agents, including melatonin (Ozbek et al., 2000), garlic (Pedraza-Chaverri et al., 2000), arabic gum (Al-Majed et al., 2002), manganese chloride (Ates -s -ahin et al., 2003), lycopene (Karahan et al., 2005), aminoguanidine (Polat et al., 2006), spirulina platensis (Karadeniz et al., 2008) and fish oil (Priyamvada et al., 2008) can prevent GM-induced renal damage.

*Mangifera indica L. (*Mango*)* is one of the largest fruit crops in the world, which grows in tropical and subtropical regions. Mango contains: polyphenols such as gallic acid, and carotenoids that possess antioxidant, immunomodulatory, antimutagenic and anticancer activities (Pourahmad et al., 2010). It was also shown that the extract obtained from *Mangifera indica Kernel*, directly scavenged superoxide anions (Saito et al., 2008). Besides, several other groups have also reported the antioxidant activities for Mango stem bark aqueous extract (Vimang) (Pardo-Andreu et al., 2006). Many of the pharmacological properties of Mango fruit and stem bark may be attributed to the presence of phytochemicals such as polyphenols, carotenoids and vitamins (Singh et al., 2004). Because of their antioxidant properties, the phytochemicals present in Mango exhibit protection against oxidative damage.

Due to these previously published reports, we planned to study protective effects of hydroalcoholic extract of *Mangifera indica L.* fruit and commercial extract (Vimang) on gentamicin- induced nephrotoxicity in rats.

Materials and Methods

Animals

Adult female Wistar albino rats, weighing 150-220 g, were obtained from the Razi Vaccine and Serum Research Institute, Iran. They were housed in an animal holding room at a constant temperature of 24 ± 2℃, with a relative humidity of 70% ± 5% and a 12-h light/dark cycle. All experimental animals had free access to standard rat pellet food and tap water. The protocol for the present study was performed in accordance with the guide for the care and use of laboratory animals approved by Mashhad University of Medical Sciences.

All groups of rat were studied during a time-course of 18 consecutive days. Rats were divided randomly into eight equal groups including five animals each, groups III-VI considered as preventive groups and groups VII-VIII considered as treatment groups:

Group I (control group): normal saline (0.5 ml, i.p) was given for 18 consecutive days.

Group II (GM group): GM (100 mg/kg, i.p) was injected to rats for 8 consecutive days (Ates -s -ahin et al., 2003; Karadeniz et al., 2008), starting from day 10.

Groups III and IV: vimang extract (50 and 100 mg/kg i.p) was injected for the first ten days of experiment and then continued

one hour before daily injection of GM (100mg/kg, i.p), during the last 8 days.
Groups V and VI: were the same as groups III and IV; only the dosage of Mango was 200 and 400 mg/kg, respectively.

Group VII: the rats received vimang extract (100 mg/kg, i.p) one hour before daily GM injection for the last 8 consecutive days of experiment.

Group VIII: was the same as group VII; only the dosage of hydroalcoholic extract of Mango was 400 mg/kg.

At the end of experiment (day 18), anesthetized rats were sacrificed by guillotine. The kidney was removed, rinsed with water and preserved in 10% formalin solution for histological examination. After preparation and staining with hematoxylin and eosin (H&E), specimens were blindly examined by a pathologist with light microscopy.

Sample collection and biochemical assay:

Blood samples were collected from ocular sinus at day 0 and 18th for the measurement of Cr and urea concentration. 24-hour urine sample from metabolic cage were collected for measuring of albumin at days 0 and 18. Biochemical factors of urine and serum were measured spectrophotometrically using autoanalyzer and respective kits.

Statistical analysis

Differences between values (expressed as mean ± SEM; n =5) were evaluated by one-way analysis of variance (ANOVA) followed by the LSD multiple comparison test and paired *t* test. $P \leq 0.05$ was taken to indicate statistical significance.

Results

Serum creatinine concentration

As depicted in figure 1, GM increased the serum Cr concentration in group II compared to group I on the 18th day of experiment ($p<0.01$). The serum Cr concentration increased in groups II ($p<0.001$), III ($p<0.01$), IV ($p<0.001$), V ($p<0.05$), VI ($p<0.05$), VII ($p<0.05$) and VIII ($p<0.001$) on the 18th day of experiment compared to day 0 in the same groups. All groups except for group V showed a significant increase in serum Cr concentration on the 18th day of experiment compared with the same day of control group, (Figure 1). There was no significant difference in serum Cr concentration between control group and group V on day 18. On the other hand, there was increase in serum Cr concentration in group VII ($p<0.001$), but decrease in group V ($p<0.01$) in comparison with GM group on day 18.

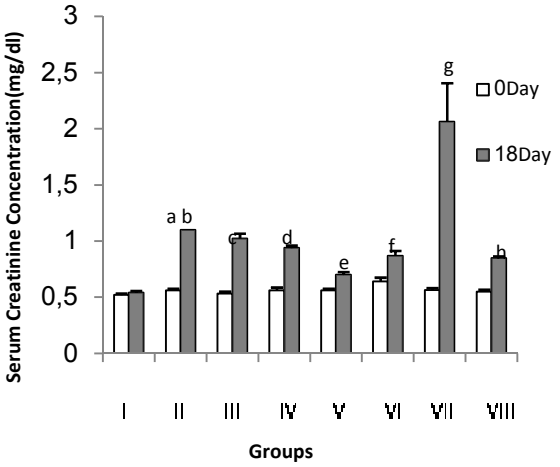

Figure 1. Comparison of serum Cr concentration (mg/dl) in different groups of rats (n= 5). I: Control group, II: GM group (100 mg/kg), groups III and IV: treated with vimang extract (50 and 100 mg/kg, respectively), groups V and VI: treated with mangifera extract (200 and 400 mg/kg, respectively), group VII: treated with vimang extract (100 mg/kg), group VIII: treated with mangifera extract (400 mg/kg). Data are presented as mean±SEM, [a] $p<0.01$ as compared to group I on day 18, [b,d,h] $p<0.001$ as compared to day 0 in same group, [c] $p<0.01$ as compared to day 0 in same group, [e,f,g] $p<0.05$ as compared to day 0 in same groups.

Serum urea concentration

As illustrated in figure 2, serum urea concentration increased in groups II ($p<0.001$), III ($p<0.001$), IV ($p<0.001$), VI ($p<0.05$), VII ($p<0.001$) and VIII ($p<0.05$)

on the 18th day compared with the same day in the control group. In group II, GM injection increased the serum urea concentration on the 18th day compared to day 0 ($p<0.001$). There wasn't any significant difference in serum urea concentration on the 18th day as compared with the same day of control group in group V. As shown in figure 2, serum urea concentration increased significantly in group IV ($p<0.001$) and group VII ($p<0.001$), but decreased significantly in group V ($p<0.01$) compared with group II.

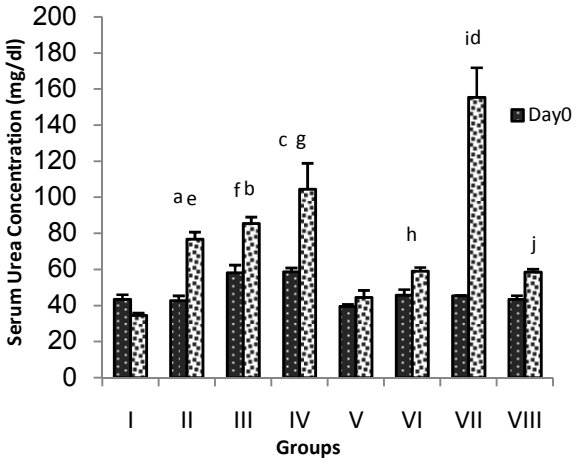

Figure 2. Comparison of serum urea concentration (mg/dl) in different groups of rats (n=5). I: Control group, II: GM group (100 mg/kg), groups III and IV: treated with vimang extract (50 and 100 mg/kg, respectively), groups V and VI: treated with mangifera extract (200 and 400 mg/kg, respectively), group VII: treated with vimang extract (100 mg/kg), group VIII: treated with mangifera extract (400 mg/kg). Data are presented as mean±SEM, [a, b, c, d] $p<0.001$ as compared to group I on day 18, [e] $p<0.01$ as compared to day 0 in the same group, [f, g, i] $p<0.05$ as compared to day 0 in the same groups, [h,j] $p<0.01$ as compared to day 0 in the same groups.

Albumin excretion rate

As shown in figure 3, the albumin excretion rate increased in groups VII ($p<0.001$) and VIII ($p<0.05$) compared with the control group on the 18th day of experiment. Albumin excretion rate also increased significantly in group VII ($p<0.001$) and VIII ($p<0.01$) compared with group II on day 18. Albumin excretion rate significantly increased in groups III, VII and VIII ($p<0.05$) on the 18th day of experiment compared with day 0 in the same groups.

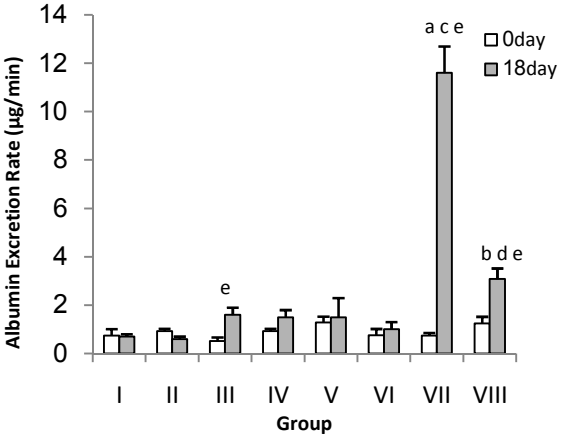

Figure 3. Comparison of albumin excretion rate (µg/min) in different groups of rats (n=5). I: Control group, II: GM group (100 mg/kg), groups III and IV: treated with vimang extract (50 and 100 mg/kg, respectively), groups V and VI: treated with mangifera extract (200 and 400 mg/kg, respectively), group VII: treated with vimang extract (100 mg/kg), group VIII: treated with mangifera extract (400 mg/kg). Data are presented as mean±SEM, [a] $p<0.001$ as compared to group I on day 18, [b] $p<0.05$ as compared to group I on day 18, [c] $p<0.001$ as compared to group II on day 18, [d] $p<0.05$ as compared to group II on day 18, [e] $p<0.05$ as compared to day 0 in the same groups.

Histopathology parameters

As shown in figure 4, the percentage of necrosis increased in GM group compared to the control group on the 18th day of study ($p<0.05$) (Figure 5&6). Necrosis showed a significant reduction in group V when compared to group II ($p<0.05$) on day 18, while this parameter increased in groups VI ($p<0.05$), VII ($p<0.01$), and VIII ($p<0.05$) compared to GM group on the 18th day of

investigation. Kidney tissue injury showed a reduction in groups III and IV compared with GM group, but it was not significant.

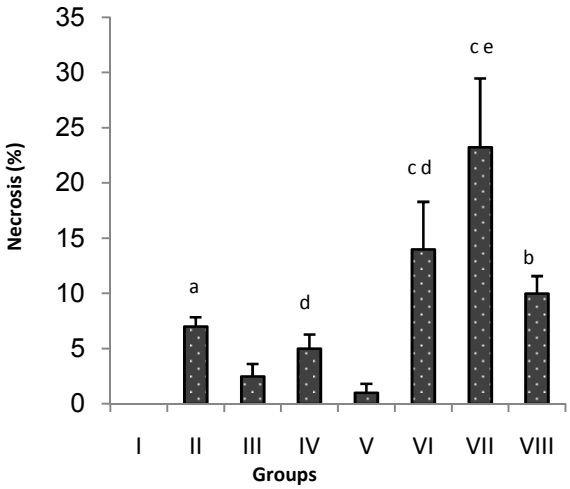

Figure 4. Comparison of necrosis (%) in different groups of rats (n= 5). I: Control group, II: GM group (100 mg/kg), groups III and IV: treated with vimang extract (50 and 100 mg/kg, respectively), groups V and VI: treated with mangifera extract (200 and 400 mg/kg, respectively), group VII: treated with vimang extract (100 mg/kg), group VIII: treated with mangifera extract (400 mg/kg). Data are presented as mean±SEM, [a] $p<0.05$ as compared to group I, [b] $p<0.01$ as compared to group I. [c] $p<0.001$ as compared to group I, [d] $p<0.05$ as compared to group II, [e] $p<0.001$ as compared to group II, [b] $p<0.001$ as compared to group I on day 18, c $p<0.01$ as compared to group II on day 18.

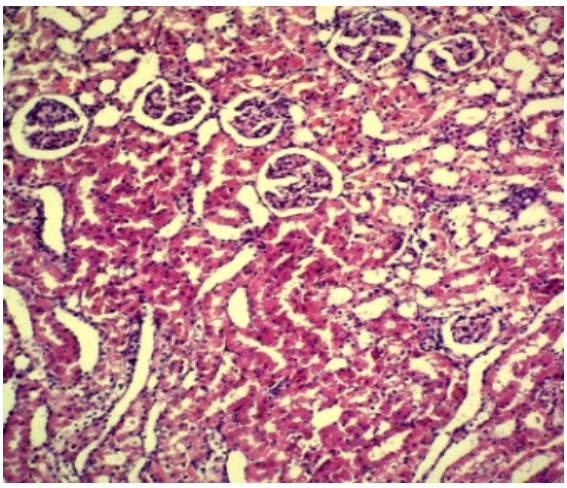

Figure 5. Microscopic section of kidney from group I (control). H&E. Magnification×100.

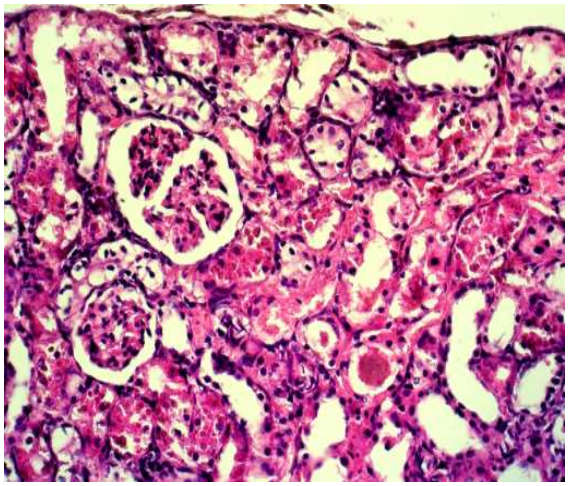

Figure 6. Microscopic section of kidney from group II (Gentamicin). H&E. Magnification×200.

Discussion

Drug-induced nephrotoxicity is an important cause of renal failure (Perazella, 2003). Aminoglycosides throughout the endocytic pathway are taken up into the epithelial cells of the renal proximal tubules and stay there for a long time, which leads to nephrotoxicity (Ajami et al., 2010). Although the aminoglycoside antibiotic can cause nephrotoxicity; despite of availability of other antibacterial drugs with better sensitivity and safety profiles than aminoglycosides, they remain an important group of antibiotics against several Gram-negative life-threatening infections. Therefore, the goal of reducing or preventing the development of GM-induced nephrotoxicity has attracted considerable efforts (Sayed-Ahmed et al., 2007).

Plasma Cr concentration is an important indicator than the urea concentration in the first phases of kidney disease. Furthermore, urea concentration begins to increase only after parenchymal tissue injury (Gilbert et al., 1989).

The present results showed that serum urea and Cr increased significantly in GM group compared to the control group on the 18th day. In agreement with present results, Safa et al. (2010) showed that Serum Cr and BUN levels were highest in the rats of gentamicin-received group. Also Ajami et

al. (2010) showed that treatment with gentamicin for five days induced renal functional deficiency as demonstrated by significant increase in serum Cr and urea levels. Yaman et al. (2010) revealed that GM injection during 6 days, induced a significant increase in plasma Cr and urea levels. Sayed-Ahmed et al. (2007) demonstrated that administration of GM (80 mg/kg) for 8 days resulted in significant 139 and 258% increases in serum Cr and BUN, respectively.

The current investigation of histopathology results also showed that administration of GM (100 mg/kg/day) created significant degenerative changes in kidney tissue including cast formation, vacuolation; loss of brush border in large parts of proximal tubules and tubular obstruction in association with dominant tubular necrosis of proximal tubules and epithelial cells dissociation.

Ajami et al. (2010) also showed that treatment with gentamicin 80 mg/kg/day for five days induced moderate to severe histological damage. Yaman et al. (2010) demonstrated vacuolation, hydrophic degeneration, desquamation and necrosis in epithelial cells of the proximal tubules in rats of GM group. Furthermore, hyaline casts in tubular lumen, tubular brush border loss, severe inflammatory infiltrate in the form of mononuclear cells and intertubular hemorrhage were observed in the renal sections of this group. There were also some changes in the glomerulus such as congestion and swelling and some alterations in the basement membrane. Histopathological results demonstrating aminogylcoside-induced structural changes in renal tissue were also reported by other researchers (Nakakuki et al., 1996; Kumar et al., 2000; Yaman et al., 2010). These results confirm that kidney is sensitive to GM toxicity (Sayed-Ahmed et al., 2007; Safa, 2010).

As treatment with some antioxidants protects against GM-induced nephrotoxicity, in the current study we evaluated the effect of *Mangifera indica*, which is a potent antioxidant and free-radical scavenger (Sanchez et al., 2000; Marquez et al., 2010; Kim et al., 2011). In this study, *Mangifera indica* extract displayed a protective effect action against GM-induced renal injury. Group V manifested a significant decrease in plasma Cr and urea levels when compared with the GM group on the 18th day of experiment. Also, plasma Cr and urea concentrations in group V did not demonstrate a significant change in comparison with control group on day 18. Other extract-treated groups including groups III, IV, VI and VIII were also able to reduce serum Cr concentration after GM utilization on the 18th day of experiment compared with the GM group, though their differences were not statistically significant. The mechanism of safeguarding action of Mango against GM-induced kidney toxicity remains to be elucidated. A relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models (Al-Majed et al., 2002).

Several studies have reported that oxygen-free radicals are considered to be important mediators of GM-induced acute renal failure (ARF). Accordingly, among the main approaches used to ameliorate GM-induced nephrotoxicity is the use of agents with antioxidant properties (Yaman et al., 2010).

Recent studies have reported that some natural products are useful in ameliorating GM nephrotoxicity (Ali, 2004; Sayed-Ahmed et al., 2007; Yaman et al., 2010). Mangoes are a source of bioactive compounds with potential health promoting activity including ascorbic and dehydroascorbic acids, carotenoids, phenolics compounds, fiber and terpenoids. Several studies have demonstrated the biological properties of compounds found in all parts of the Mango plant, suggesting their beneficial effects on human health, particularly as antioxidant and immunomodulatory agents. Hereby, aqueous Mango leaves extract which was found to be rich in total phenols and total

flavonoides is also considered as a powerful antioxidant (Sônia Machado Rocha Ribeiro, 2010).

The histopathology results also confirmed that the *Mangifera indica* extract protection against GM-induced nephrotoxicity. Renal tissue necrosis was significantly higher in GM group but not in vimang or *Mangifera indica*-treated groups including groups III, IV and V compared with the control rats. Indeed, Mango extract was able to reduce kidney injury significantly caused by GM in group V compared with GM group. These findings are additionally in agreement with the above mentioned biochemical results. Consistent with our results, Bibu et al. (2010) demonstrated that treatment with ethanolic extract of *Mangifera indica* inhibited gentamicin-induced proximal tubular necrosis. The Mango products can prevent the increase of nephrotoxicity indices and histopathological lesions induced by GM, suggesting that Mango has a protective effect against GM-induced ARF. However, in group VII commercial product vimang at 100mg/kg and groups VI and VIII high dose of Mango extract at 400mg/kg caused a significant increase in renal tissue necrosis compared with the GM group. The exact reason needs more investigations to be clarified, but it may indicate that Mango extract benefits the kidney function against GM in a dose-dependent manner. In other word, high dose of Mango utilization may accelerate GM-induced renal toxicity. This finding was also supported with significant increase in urine albumin excretion rate in groups VII and VIII compared with the control and GM groups on day 18.

In conclusion, the present study demonstrates that GM increases nephrotoxicity indices including plasma Cr and urea concentrations as well as renal tissue toxicity. It seems that *Mangifera indica* products are able to improve kidney function against GM-induced nephrotoxicity. These beneficial effects of Mango may work in a dose and time-dependent style. Further investigations with different doses and time-courses are advised to elucidate Mango action on GM-induced nephrotoxicity.

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