Protective Effects of Silymarin on Gentamicin-Induced Nephrotoxicity in Infectious Patients: A Randomized Double Blinded Placebo-Controlled Clinical Trial

Motahareh Mahi-Birjand1,2, Iman Karimzadeh4, Asghar Zarban4, Mehghad Abdollahpour-Alitappeh5, Seyed Alireza Saadatjoo6, Masood Ziaee1*

1Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran.
2Student Research Committee, Department of Clinical Pharmacy, School of Pharmacy, Shiraz University of Medical Science, Shiraz, Iran.
3Department of Clinical Pharmacy, School of Pharmacy, Shiraz University of Medical Science, Shiraz, Iran.
4Department of Clinical Biochemistry, faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.
5Cellular and Molecular Biology Research Center, Larestan University of Medical Sciences, Larestan, Iran.
6Department of Nursing, Birjand University of Medical Sciences, Birjand, Iran.

Abstract

Background: Nephrotoxicity is one of the most important side effects of gentamicin (GEN). Accumulating evidence demonstrated the crucial roles of antioxidant compounds in the reduction of GEN-induced renal injuries. Silymarin (SM), an antioxidant agent, was demonstrated to improve GEN-induced kidney damage. The aim of this clinical trial was to investigate the effect of SM on GEN-induced nephrotoxicity.

Methods: This randomized, double-blinded, placebo-controlled clinical trial was conducted from April 2017 to October 2019 on patients diagnosed with infectious diseases receiving GEN at least for 7 days. After approving the study and obtaining informed consents, 60 patients were included in this study. Patients in the treatment (30) and control (30) groups were given injectable GEN along with 140 mg of SM tablets or placebo orally three times a day, respectively. Demographic, laboratory, and therapeutic profiles of the patients were recorded. Urine and blood samples were collected before and on days 1, 2, 3, 5 and 7 after GEN administration and intervention.

Results: Sex, age, GEN indication and baseline glomerular filtration rates were found to have no effect on GEN nephrotoxicity. SM- and placebo-treated groups exhibited no significant differences between the indications and intervals of GEN administration. The overall rate of GEN nephrotoxicity in the SM group was significantly lower than that in the placebo group (16.7% and 53.3%, respectively; p: 0.003). In addition, the risk of GEN nephrotoxicity in patients receiving placebo was significantly higher than those receiving SM (OR:12.69, 95%, CI: 1.38-116.74; p:0.03). Serum creatinine was found to be significantly higher in the placebo-treated group than that in the SM-treated group (p<0.05). However, the frequency of acute tubular necrosis on days 2, 3, 5, and 7 after GEN administration exhibited no significant differences between SM- and placebo-treated patients.

Conclusion: This study demonstrated that SM could attenuate renal injury in GEN-treated patients.

Introduction

Aminoglycosides (AGs) are a class of antibiotics mainly used for the treatment of gram-negative infections through inhibition of protein synthesis. However, nephrotoxicity is the main concern regarding the use of AGs.1,2 Acute kidney injury (AKI), a relatively common complication of AG therapy caused by acute tubular necrosis (ATN), is a major clinical complication. AKI occurs in 10-20% of the patients treated with AGs, presumably resulting in increased mortality and morbidity.1 Gentamicin (GEN), belonging to the AG family, is an important antibiotic used to treat a wide variety of bacterial infections, especially those caused by life-threatening gram-negative bacteria.4,6 However, ototoxicity and nephrotoxicity remain the serious side effects of GEN.
Of note, nephrotoxicity isthe major adverse effect of GEN which develops in approximately 30% of the patients treated with GEN for more than 7 days. Importantly, GEN was demonstrated to negatively affect the cochlea, kidneys, and vestibular apparatus, leading to limited clinical use of GEN. Despite the above-mentioned issues as well as the introduction of newer and less toxic antibiotics, the unique characteristics of GEN, including broad-spectrum activity, rapid bactericidal action, post-antibiotic effects, chemical stability, low cost, and efficacy against bacteria resistant to other antibiotics, have made GEN a first-line antibiotic in a wide variety of clinical situations. Importantly, accumulating evidence demonstrated that antioxidant compounds play crucial roles in the reduction of renal damages caused by GEN. There are also studies suggesting the protective effect of Silymarin (SM) against antibiotic-induced nephrotoxicity. SM is a flavonoid complex isolated from the seeds of milk thistle (Silybum marianum), which has been widely used as a natural remedy for the treatment of liver and gall bladder disorders, including hepatitis, cirrhosis, jaundice, and protection of liver from injuries caused by ischemia, radiation, alcohol abuse, iron overload, and environmental toxins. The aim of the present study was to investigate the protective effects of SM on the prevention of GEN-induced nephrotoxicity in patients admitted to university-affiliated hospitals.

Materials and Methods

Study design and setting

The study was designed as a randomized, double-blinded, placebo-controlled clinical trial using SM and placebo. The study was conducted from April 2017 to October 2019 on patients who underwent GEN treatment hospitalized in an educational center affiliated to Birjand University of Medical Sciences, Vali-e-Asr teaching hospital, Southern Khorasan Province (East Iran).

Ethical approval

The study was approved by the Ethics Committee of the Shiraz University of Medical Sciences (IR. SUMS. REC. 1397. 330). Written informed consent was obtained from patients or their family members before entering the study. The trial was registered in the Iranian Registry of Clinical Trials (IRCT20161010030246N3). The study was in accordance with the 1975 Helsinki Declaration as revised in 2008.

Sample size

Sample size of the present study was calculated by considering α=0.05 and 80% power (1-β=0.8) and data from a relevant experimental study. A minimum of 13 patients was calculated for each group. We considered 30 patients in the treatment and 30 patients in the placebo groups.

Exclusion and inclusion criteria

Patients were included in this study if they met the following criteria: hemodynamic stability (mean arterial blood pressure above 70 mmHg and/or systolic blood pressure above 90 mmHg); willingness to participate in the study; receiving GEN intravenously or intramuscularly for at least one week (a dose of 5 mg/kg/day or 80-100 mg/TDS, depending on the type of infection, GEN can be given once daily or several times a day); no confirmed history of AKI (including increased serum creatinine (Cr) levels equal to or greater than 0.3 mg/dL within 48 hours, increased Cr levels equal to or more than 1.5 fold compared with baseline over the past 7 days and urinary output less than 0.5 mL/kg/hour for 6 hours); no confirmed history of chronic kidney disease (including calculated glomerular filtration rate (GFR) less than 60 ml/min/1.73 m2 or history of peritoneal dialysis or hemodialysis for more than 3 months); no history of intravenous or intramuscular GEN administration during the past 14 days; no history of SM administration over the past 7 days; no confirmed history of allergic reactions to SM; no concomitant use of antibiotics or compounds with antioxidant effects such as vitamin C, vitamin E, N-acetyl-cysteine, pentoxifylline, and fish oil extract; no concomitant use of antibiotics with high or prominent nephrotoxicity (e.g., vancomycin, amphotericin B, acyclovir, foscarin, cisplatin, and nonsteroidal anti-inflammatory drugs (NSAIDs)); and tolerance for oral medications.

Intervention

Patients included in this study were assigned into either placebo or treatment groups by the blocked randomization method. Subjects in both groups were given injectable GEN according to the treatment protocol. It is important to note that the GEN regimen, including daily dose and duration of treatment, was the same in both placebo and SM groups. Patients in the treatment group received, in addition to GEN, 140 mg of SM tablets (Livergol, Goldaru Pharmaceutical Laboratories, Iran) administered orally three times per day with meals up to completing the GEN treatment course. The dose used in the present study was based on the protocol described by Livergol for fatty liver. In contrast, patients in the control group received, in addition to GEN, 140 mg of placebo orally three times with the similar pattern. Placebo tablets were also manufactured by the same company and were similar in size, shape, weight, color, and taste.

Measurements and study outcomes

Demographic, clinical, laboratory, and therapeutic profiles of patients were recorded in a form by reviewing the medical charts of the patients as well as interviewing patients, if required. The information obtained from the patients included age, sex, weight, height, early diagnosis at admission, final diagnosis, history of disease, medical history, antibiotic use (name, medication form, dose, route and interval of administration, as well as starting and ending use date), and indication and treatment regimen of GEN (dose, route and interval of administration, as
well as starting and ending use date). Potential side effects associated with the administration of placebo and SM were also recorded during the study.

GEN nephrotoxicity was defined by either a rise in the plasma Cr concentration of more than 0.5 to 1 mg/dL or a 50% increase in plasma Cr concentrations from baseline.17 ATN was defined as either fractional excretion of sodium > 2% or fractional excretion of urea > 50% (in cases of diuretic co-administration).18-20 The simplified MDRD (Modification of Diet in Renal Disease) formula was used to calculate patient GFR at different time points during the study.

Urine and blood samples (5 ml) were taken before GEN (baseline) as well as on days 1, 2, 3, 5, and 7 after GEN administration. Following blood centrifugation, serum and urine samples were stored at -80°C. After completing the study, Cr, urea, sodium, and potassium were measured in both blood and urine samples. Biochemical measurements in both urine and serum samples were conducted by using an autoanalyzer of "ParsaanAzmoon" and "NimaPouyesh" companies.

Statistical analysis
Statistical analyses were performed on data available to all individuals completing the study (per protocol analysis). The Kolmogorov-Smirnov test was performed to evaluate the normality of the continuous variable distribution. Continuous variables with normal and abnormal distributions are expressed as mean ± standard deviation (SD) and median with interquartile ranges, respectively. In contrast, categorical variables were reported as a percentage. To investigate the research hypotheses, paired t-test, intra-group variance analysis, Benferon's follow-up, and Wilcoxon and Friedman tests were used for normal distribution. Stepwise logistic regression analysis with odds ratio (OR) and 95% confidence interval (CI) was exploited to determine the factors associated with GEN nephrotoxicity. P values less than 0.05 were considered to be statistically significant. All of the above descriptive-analytical statistical analyses were performed using the IBM SPSS Statistics Version 20 software.

Results

Demographic characteristics
A total of 60 patients were included in this study, including 30 in the SM group and 30 in the placebo group (Figure 1). There was no significant difference between two groups in terms of sex, age, and weight (p > 0.05). Our results showed no significant differences between SM- and placebo-treated groups (p > 0.05) in the mean laboratory findings including urine as well as serum Cr, sodium, potassium and magnesium. In contrast, the mean baseline GFR in the SM-treated patients was significantly higher than that in the placebo-treated patients (p = 0.04). SM- and placebo-treated groups exhibited no significant differences between the indications and intervals of GEN administration (p > 0.05) (Table 1).

GEN nephrotoxicity
No nephrotoxicity episode was observed on the first and second days after GEN administration. On days 3 and 5 of GEN treatment, no nephrotoxicity was found in the SM group, but one (3.3%) and two (6.7%) cases developed nephrotoxicity in the placebo group (p > 0.05). Seven days after GEN administration, 5 (16.7%) and 16 (53.3%) patients in the SM and placebo groups experienced nephrotoxicity, respectively (p = 0.003) (Table 2). The overall rate of GEN nephrotoxicity in the

Figure 1. Consort flowchart of study recruitment and completion.
SM group was significantly lower than that in the placebo group (16.7% and 53.3%, respectively; \( p \) value: 0.003). The mean ± SD time onset of GEN nephrotoxicity in the SM- and placebo-treated patients was 7.00± 0.00 and 6.63± 1.09 days, respectively, showing no statistically significant differences (\( p = 0.46 \)).

The frequency of ATN on days 2, 3, 5, and 7 after GEN administration exhibited no significant differences between SM- and placebo-treated patients (\( p > 0.05 \)) (Table 3).

### Table 1. Comparison of demographic characteristics, baseline laboratory findings, as well as gentamicin administration indication and intervals of patients in silymarin and placebo groups.

| Variables                        | Group       | Silymarin | Placebo | \( p \) value |
|----------------------------------|-------------|-----------|---------|--------------|
| Sex                              | Male (%)    | 19 (63.3) | 15 (50) | 0.30**       |
|                                  | Female (%)  | 11 (36.7) | 15 (50) |              |
| Age (years)                      | Mean ± SD   | 49.70 ± 21.28 | 57.53 ± 18.91 | 0.14"       |
| Weight (kg)                      | Mean ± SD   | 66.38 ± 12.28 | 69.53 ± 10.17 | 0.28*       |
| Serum Creatinine (mg/dl)         | Mean ± SD   | 0.84 ± 0.09 | 0.86 ± 0.06 | 0.33        |
| Serum Sodium (meq/lit)           | Mean ± SD   | 138.37 ± 3.11 | 137.73 ± 3.36 | 0.45        |
| Urine Creatinine (mg/dl)         | Mean ± SD   | 80.07 ± 66.99 | 82.13 ± 60.78 | 0.90        |
| Urine Sodium (meq/lit)           | Mean ± SD   | 64.17 ± 49.87 | 86.70 ± 54.30 | 0.10        |
| GFR (ml/min)                     | Mean ± SD   | 91.16 ± 19.78 | 81.20 ± 15.88 | 0.04        |

The chi-square test was used for the statistical differences.

### Table 2. Comparison between the frequency of gentamicin nephrotoxicity in patients in silymarin and placebo groups.

| Day | Silymarin | Placebo | \( p \) value |
|-----|-----------|---------|--------------|
| 3   | 0 (0)     | 1 (3.3) | 1.00*        |
| 5   | 0 (0)     | 2 (6.7) | 0.49*        |
| 7   | 5 (16.7)  | 16 (53.3) | 0.003**     |

*The Fisher Exact test was used for the statistical differences. ** chi-square

### Table 3. Comparison between the frequency of ATN on different days in patients treated with silymarin and placebo.

| Day | Silymarin | Placebo | \( p \) value |
|-----|-----------|---------|--------------|
| 2   | 0 (0)     | 1 (3.3) | 1.00*        |
| 3   | 1 (3.3)   | 5 (16.7) | 0.20*       |
| 5   | 6 (20)    | 8 (26.7) | 0.76*       |
| 7   | 9 (30)    | 14 (46.7 ) | 0.18**     |

*The Fisher Exact test was used for the statistical differences. ** chi-square

ATN: acute tubular necrosis

The mean ± SD time onset of ATN was 5.44 ± 1.33 and 4.21 ± 2.22 days in SM- and placebo-treated patients, respectively, representing no statistically significant differences (\( p = 0.15 \)). Results from logistic regression analysis showed that the risk of nephrotoxicity in patients receiving placebo was significantly higher than those receiving SM (OR=12.69, 95%CI: 1.38-116.74; \( p = 0.03 \)). The average risk of nephrotoxicity in patients receiving GEN every 24 hours was found to be 0.01 times lower than those receiving every 8 hours (\( P<0.001 \)). In contrast, sex, GEN indication, baseline GFR and age had no significant association with GEN nephrotoxicity (\( p > 0.05 \)) (Table 4).

Results from repeated measure analysis showed that serum Cr levels increased (\( p<0.001 \)) in both SM- and placebo-treated patients, showing significant increases on days 2, 3, 5, and 7 as compared with the baseline value. Importantly, this increase in serum Cr was significantly higher in the placebo group than in the SM group (\( p < 0.05 \)). No significant differences were found between the baseline serum Cr and serum Cr on day 1 in both groups (\( p > 0.05 \)); however, the placebo group showed significantly higher serum Cr values on days 2, 3, 5, and 7 after GEN treatment than the SM group (\( p<0.001 \)) (Figure 2 and Table 5).

Urinary Cr levels exhibited no significant differences in both SM- and placebo-treated patients on different days (\( p > 0.05 \)). Additionally, no significant differences were
Effects of Silymarin on Gentamicin-Induced Nephrotoxicity

### Table 4. Logistic regression analysis for determining risk factors of gentamicin nephrotoxicity.

| Variables                                      | Nephrotoxicity | OR (CI 95%) | P value |
|------------------------------------------------|----------------|-------------|---------|
|                                                | NO             | YES         |         |
| **Group (%)**                                  |                |             |         |
| Silymarin                                      | 25 (64.1)      | 5 (23.8)    | 1       |
| Placebo                                        | 14 (35.9)      | 16 (76.2)   | 12.69 (1.38-116.74) | 0.03 |
| **Sex (%)**                                    |                |             |         |
| Male                                           | 22 (56.4)      | 12 (57.1)   | 1       |
| Female                                         | 17 (43.6)      | 9 (42.9)    | 1.93 (0.13-28.54) | 0.63 |
| **Age, years (Mean ± SD)**                     |                |             |         |
|                                                | 51.21±20.65 (12-90) | 58.10±19.47 (21-91) | 1.01 (0.95-1.07) | 0.79 |
| **Indication of the administration of gentamicin (%)** |                |             |         |
| Brucellosis                                    | 6 (15.4)       | 5 (23.8)    | 1       |
| Cellulitis                                     | 11 (28.2)      | 3 (14.3)    | 0.28 (0.02-3.71) | 0.33 |
| Septic arthritis & osteomyelitis               | 11 (28.2)      | 5 (23.8)    | 0.39 (0.03-4.43) | 0.44 |
| UTI                                            | 11 (28.2)      | 8 (38.1)    | 0.59 (0.06-5.42) | 0.64 |
| **Gentamicin administration intervals**        |                |             |         |
| 24 hours                                       | 36 (92.3)      | 4(19)       | 1       |
| 8 hours                                        | 3 (7.7)        | 17(81)      | 0.01 (0.001-0.1) | <0.001 |
| **Baseline GFR, mL/min/1.73 m² (Mean ± SD)**   |                |             |         |
|                                                | 87.55±18.04 (54.20-119.80) | 83.63±19.46 (54.66±132.42) | 1.01 (0.93-1.10) | 0.81 |

GFR: Glomerular filtration rate, UTI: Urinary tract infection.

### Table 5. Comparison between the mean serum creatinine levels, urinary creatinine and glomerular filtration rate levels on different days in silymarin- or placebo-treated patients.

| Variables | Day       | Silymarin | Placebo | P value | Mean Changes (95% Confidence Interval) |
|-----------|-----------|-----------|---------|---------|---------------------------------------|
| **Serum Creatinine**                            | Baseline  | 0.84 ± 0.09 | 0.86 ± 0.06 | 0.33 | -0.02 (-0.06 - 0.02) |
| Day 1     | 0.84 ± 0.09 | 0.86 ± 0.06 | 0.10 | -0.03 (-0.07 - 0.01) |
| Day 2     | 0.89 ± 0.08 | 0.98 ± 0.09 | < 0.001 | -0.09 (-0.14 - -0.05) |
| Day 3     | 0.91 ± 0.09 | 1.06 ± 0.11 | < 0.001 | -0.15 (-0.20 - -0.10) |
| Day 5     | 0.97 ± 0.08 | 1.18 ± 0.11 | < 0.001 | -0.21 (-0.26 - -0.16) |
| Day 7     | 1.07 ± 0.21 | 1.37 ± 0.16 | < 0.001 | -0.30 (-0.40 - -0.20) |

**Urine Creatinine**

| Baseline | 80.07 ± 66.99 | 82.13 ± 0.09 | 0.90 | -2.07 (-35.12 - 30.99) |
| Day 1    | 64.03 ± 45.03 | 78.80 ± 61.67 | 0.29 | -14.77 (-42.67 - 13.14) |
| Day 2    | 57.00 ± 42.90 | 76.03 ± 54.84 | 0.14 | -19.03 (-44.48 - 6.41) |
| Day 3    | 54.71 ± 48.22 | 67.20 ± 52.69 | 0.34 | -12.49 (-38.59 - 13.61) |
| Day 5    | 50.27 ± 44.40 | 64.60 ± 49.26 | 0.24 | -14.33 (-38.57 - 9.90) |
| Day 7    | 49.92 ± 51.53 | 68.10 ± 54.84 | 0.19 | -18.18 (-45.68 - 9.32) |

**GFR (mL/min/1.73 m²)**

| Baseline | 91.16 ± 19.78 | 81.20 ± 15.88 | 0.04 | 9.96 (0.69 - 19.23) |
| Day 1    | 91.30 ± 20.96 | 79.97 ± 16.73 | 0.02 | 11.33 (1.53 - 21.13) |
| Day 2    | 85.82 ± 18.97 | 70.52 ± 14.90 | 0.001 | 15.29 (6.48 - 24.11) |
| Day 3    | 83.32 ± 19.48 | 64.42 ± 13.33 | < 0.001 | 18.90 (10.32 - 27.49) |
| Day 5    | 77.66 ± 16.96 | 56.91 ± 11.90 | < 0.001 | 20.74 (13.77 - 28.32) |
| Day 7    | 70.83 ± 19.48 | 48.29 ± 12.01 | < 0.001 | 22.54 (14.18 - 30.90) |

GFR: Glomerular filtration rate

Importantly, no patients in the treatment group developed adverse effects associated with SM. All subjects tolerated SM and adhered to the treatment regimen.

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GFR decreased on different days (p<0.001) in both SM- and placebo-treated patients. In particular, GFR significantly decreased on days 2, 3, 5 and 7 when compared with baseline, representing significantly higher in the placebo group compared with the SM group (p <0.05). This decrease in GFR values on days 1, 2, 3, 5 and 7 after GEN treatment was significantly lower in the SM group compared with the placebo group (p <0.05) (Figure 4 and Table 5).
In the present study, we investigated the protective effects of SM on the prevention of GEN-induced nephrotoxicity in hospitalized patients. Patients treated with SM, in contrast with those treated with placebo, exhibited no nephrotoxicity on days 3 and 5. In addition, patients treated with SM showed renal toxicity three times less than those treated with placebo on day 7, showing the preventive effects of SM against GEN nephrotoxicity. The overall risk of GEN nephrotoxicity in patients treated with SM was found to be 12.69 times lower than those received placebo. In addition, an increase in serum Cr values on days 2, 3, 5, and 7 of the GEN treatment course in the placebo group was higher than that in the SM group. However, frequency of ATN on days 2, 3, 5, and 7 after GEN administration exhibited no significant differences between SM- and placebo-treated patients.

Several studies indicated the potential role of antioxidant compounds in reducing the possibility of GEN-induced nephrotoxicity. SM is a mixture of 4 isomeric flavonoids, including silybinin, isosilybinin, silydianin, and silychristine, among which silybin is the major and most active compound, constituting between 60% and 70% of SM. It has also been reported that SM has beneficial effects in illnesses of different organs and could be useful in treating diabetes and a wide range of cancers. A number of studies have suggested that SM has antifibrotic, anti-lipid-peroxidative, anti-inflammatory, immunomodulating, and dose-dependent anti-apoptotic effects and serves as a strong antioxidant and free radical scavenger.

There are studies demonstrating that SM and silybin extracts exhibit a number of pharmacological activities, including anticancer, antioxidative, and radical-scavenging properties. Importantly, the antioxidant and anti-inflammatory properties of SM may play a protective role on nephropathic processes. A number of clinical studies demonstrated the beneficial effects of SM supplementation alone or in combination with vitamin E in patients with different kidney diseases including diabetic nephropathy, hemodialysis, and peritoneal dialysis. Decreased TNF-α, TGF-β, and Malondialdehyde (MDA) levels demonstrated the beneficial effects of SM supplementation against both glomerular and tubular aspects of cisplatin nephrotoxicity including urine electrolyte wasting and renal function impairment. In addition, Voroneanu et al. reported that the addition of SM to renin–angiotensin system blockers in normotensive patients with type 2 diabetes mellitus and proteinuria had no significant effects on both primary (adecrease in eGFR > 50% or development of ESRD) and secondary outcomes (changes in eGFR and proteinuria).

Four experimental studies have specifically assessed the protective effects of SM alone against AG-induced nephrotoxicity. For example, Ghaznavi et al. evaluated the protective effects of SM and melatonin on serum Cr and urea levels in GEN-treated rats. Their findings showed that SM and melatonin pretreatment significantly lowered the elevated serum urea and Cr concentrations, kidney weight, renal reactive oxygen species (ROS), as well as MDA levels. In addition, SM and melatonin significantly enhanced the renal glutathione (GSH) level and superoxide dismutase (SOD) activity. Naji Al-Shawi also demonstrated that pre-treatment with SM (250 mg/kg twice a day orally for 7 days) attenuates some aspects of GEN-induced renal injury. These included a decrease in free calcium and copper levels as well as an increase in zinc levels in the kidney tissue. In 2007, Varzi et al. analyzed the protective effect of SM alone (20 mg/kg/day orally for 9 days) or in combination with vitamin E on a model of GEN nephrotoxicity in dogs. SM could effectively attenuate GEN nephrotoxicity indexes including increased serum Cr, urea, and MDA levels with decreased total serum antioxidant (TSAO) activity as well as GFR.

In addition, Mashayekhi, in a study carried out in 2012, showed that co-treatment of SM (80 mg/3 times/day for 20 days) considerably decreased biochemical markers (serum Cr, uric acid, blood urea nitrogen (BUN), urea, and glutamyltransferase) of GEN nephrotoxicity in a sheep model. Finally, Alavijeh et al. recently reported that SM (200 mg/kg/day for 3 days a week for 6 weeks) only along with aerobic exercise (5 days in week for 6 weeks), but not alone, significantly protected against GM-induced nephrotoxicity.

The SM dosage used in the present study was shown to be both safe and effective in the treatment of liver diseases. The relevant clinical investigations of SM in different kidney diseases or antibiotic-induced AKI have also exploited the similar treatment regimen (140 or 150 mg three times a day). Duration of treatment with SM in these studies varied from 3 weeks to 2 years. Only one clinical investigation used a single dose of silymarin (280 mg) 2 hours before the administration of the contrast media. Findings from our study showed that patients well-tolerated SM and importantly, exhibited no adverse effects, including headache or gastrointestinal symptoms including...
nausea, vomiting, dyspepsia, or bloating. Other relevant studies also confirmed the safety profile of SM in their study populations.24–28

Our study had several strengths. To the best of our knowledge, this investigation was the first clinical trial assessing the effect of SM on AG-induced nephrotoxicity. Importantly, a majority of confounding factors and conditions potentially involved in GEN nephrotoxicity such as concomitant nephrotoxic medications (e.g., vancomycin, amphotericin B, furosemide, radiocontrast media, cyclosporine, cisplatin), co-morbidities (e.g., hepatorenal syndrome, underlying kidney disease, hypotension, and shock syndrome), and habitual as well as dietary behaviors were absent, excluded, or matched between two groups. In addition, the sample size was relatively acceptable.

Although great advancements have been made in our study, the potential limitations of the present study should be considered. They are as follows: First, the duration of patient’s follow-up was short and limited to 1 week. This may underestimate the rate of GEN nephrotoxicity and the real pattern of kidney function may not reflect our study population. Second, conventional formulation of SM, the only product of this agent currently available in the Iranian pharmaceutical market, was used in this study. Conventional formulation of SM was also used in other studies.31 This formulation may not be suitable due to low oral bioavailability, low permeability across intestinal epithelial cells, extensive metabolism, low water solubility, as well rapid excretion in the bile.30 However, lower SM bioavailability was even described with modified formulation in soy phosphatidyl choline, showing to be able to improve its bioavailability.32 Therefore, measuring the SM level in the plasma and urine seems to be necessary. However, several studies demonstrated that only low amount of SM flavonolignans, a component responsible for the potential renoprotective effects of SM, were detected in urine by conventional SM formulation. Third, the possible mechanisms of S against GEN nephrotoxicity were not determined. Fourth, other features of GEN nephrotoxicity such as electrolyte abnormalities were not considered in the present study.

**Conclusion**

Our results showed that SM co-treatment (140 mg orally three times per day) during the course of GEN treatment (1 week) was well-tolerated and significantly attenuated or prevented the nephrotoxicity of GEN in patients with different infectious diseases. Further clinical studies with longer duration of follow-up, along with determining serum and urine levels of SM, seem to be necessary in this regard. The plausible mechanisms of SM against GEN nephrotoxicity and the effects of SM on other aspects of GEN nephrotoxicity are essential questions that should be addressed in future clinical trials.

**Ethical Issues**

The study was approved by the Ethics Committee of the Shiraz University of Medical Sciences. The trial was registered in the Iranian Registry of Clinical Trials (www. irct. ir, registration code: IRCT20161010030246N3).

**Data Sharing**

Applicants can obtain data by contacting the corresponding author.

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**Conflict of Interests**

The authors claim that there is no conflict of interest.

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