Pharmaceutical Sciences, 2020, 26(3), 287-295
doi:10.34172/PS.2020.33
https://ps.tbzmed.ac.ir/

Research Article

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

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Article Info

Article History:
Received: 3 February 2020
Accepted: 25 April 2020
ePublished: 20 September 2020

Keywords:
-Nephrotoxicity
-Gentamicin
-Silymarin
-Prevention

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 toimprove GEN-induced kidney damage. The aim of this clinical trial was to investigate the effect of SMon 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 profilesof 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 rateswere found to haveno effect on GEN nephrotoxicity. SM- and placebo-treated groups exhibited no significant differencesbetweenthe 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 placebowas significantly higher than those receivingSM(OR:12.69, 95%, CI: 1.38-116.74; *p*:0.03). Serum creatininewasfound to be significantly higher in the placebo-treated group than that in theSM-treatedgroup ($p<0.05$). 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 demonstratedthat SM could attenuate renal injury in GEN-treated patients.

Introduction

Aminoglycosides (AGs) are a class of antibiotics mainly usedfor 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.[3]

Gentamicin (GEN), belonging to the AGfamily, 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.

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Of note, nephrotoxicity isthe major adverse effect of GEN which develops in approximately 30% of the patients treated with GENfor more than 7 days.[4,7] Importantly, GEN was demonstrated to negatively affect the cochlea, kidneys, and vestibular apparatus,leading to limited clinical use of GEN.[8] 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 bacteriaresistantto other antibiotics, have made GEN a first-line antibiotic in a wide variety of clinical situations.[9-11]

Importantly, accumulating evidence demonstrated that antioxidant compounds play crucialroles in the reduction of renal damages caused by GEN. There are also studies suggesting the protective effect of Silymarin (SM)against antibiotic-induced nephrotoxicity.[12-14] SM is a flavonoid complex isolated from the seeds of milk thistle (*Silybummarianum*), 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.[9,15] The aim of the present study was to investigate the protective effects of SMonthe 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 usingSM and placebo. The study was conducted from April 2017 to October 2019on patientswho underwentGENtreatment hospitalizedin 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 consideringα=0. 05 and 80 % power (1-β=0. 8) and data from a relevant experimental study.[16] A minimum of 13 patients was calculatedfor each group. We considered 30 patients in the treatment and 30 patientsin 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 5mg/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 Crlevels 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 intramuscularGEN administrationduringthe past 14 days; no history of SMadministration over the past 7 days; no confirmed history of allergic reactions toSM; no concomitant use of antibiotics or compounds with antioxidant effects such as vitamin C, vitaminE, N-acetyl-cysteine,pentoxifylline, and fish oil extract; no concomitant use of antibiotics with high or prominent nephrotoxicity (e.g., vancomycin, amphotericin B, acyclovir, foscarnet, 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 thatthe 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 SMtablets (Livergol®, Goldaru PharmaceuticalLaboratories, Iran) administered orally three times per daywith meals up to completing the GEN treatment course. The dose used in the presentstudy was based on the protocol described byLivergol®for fatty liver. In contrast, patients in the control groupreceived, 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 profilesof patients were recorded in a form by reviewing the medical charts of the patientsas 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 used (name, medication form, dose, route and interval of administration, as well asstarting and ending use date), andindication and treatment regimen of GEN (dose, route and interval of administration, as

well asstarting and ending use date). Potential side effects associated with the administration of placebo and SM were also recordedduring the study.

GEN nephrotoxicity was defined by either a rise in the plasma Crconcentration of more than 0. 5 to 1 mg/dL or a 50%increase in plasma Crconcentrations from baseline.[17] ATNwasdefined as eitherfractional excretion of sodium > 2% or fractional excretion ofurea > 50% (in cases of diuretic co-administration).[18-20] The simplified MDRD(Modification of Diet in Renal Disease)formula was used to calculate patient GFRat different time points during the study.

Urine and blood samples (5 ml) were taken before GEN(baseline) as well ason days 1, 2, 3, 5 and 7 afterGEN administration. Followingblood 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 autoanalyser of "ParsafanAzmoon" and "NimaPouyesh" companies.

Statistical analysis

Statistical analyses were performed on data available to all individuals completingthe study (per protocol analysis). The Kolmogorov-Smirnov test was performed to evaluate the normality of the continuous variabledistribution. Continuous variables with normal and abnormal distributions areexpressed 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 SMgroupand 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 theplacebo-treated patients (p = 0. 04). SM- and placebo-treated groups exhibited no significant differencesbetween 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 SMgroup, but one (3. 3%) and two (6. 7%) casesdevelopednephrotoxicity in the placebo group (p> 0. 05). Seven days after GENadministration, 5 (16. 7%) and 16 (53. 3%) patients in the SMand placebo groups experienced nephrotoxicity, respectively (p = 0. 003) (Table 2). The overall rate of GEN nephrotoxicity in the

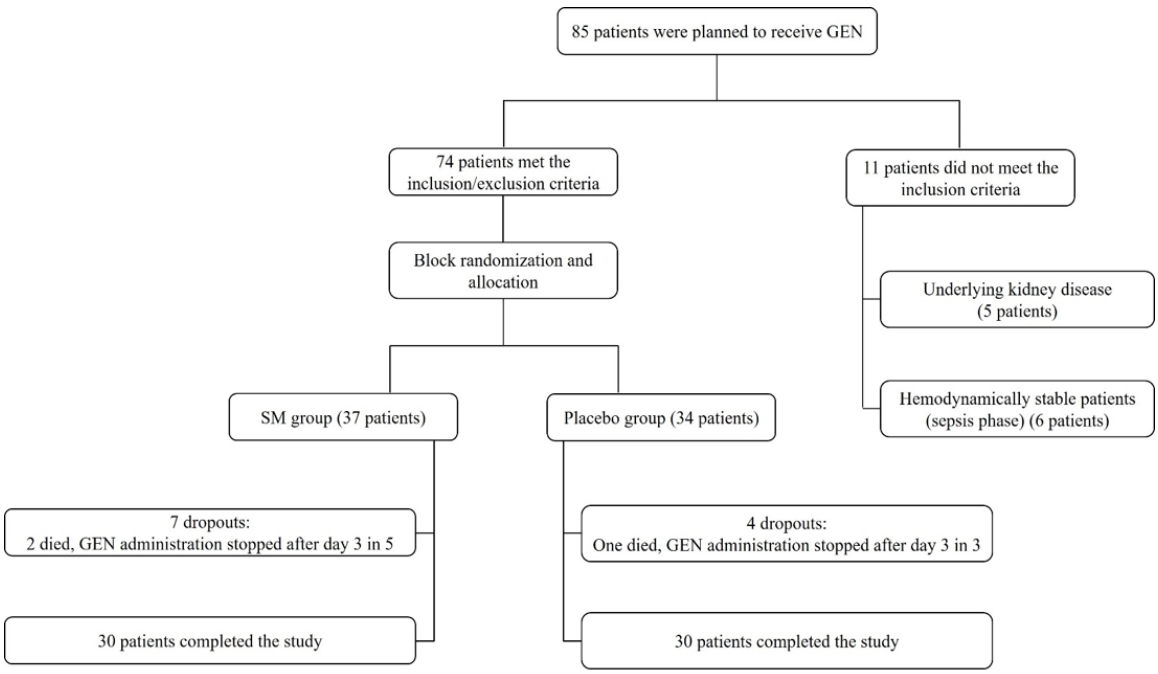

Figure 1. Consort flowchart of study recruitment and completion.

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

Variables	Group	Silymarin	Placebo	P value
Sex	Male (%) Female (%)	19 (63. 3) 11 (36. 7)	15 (50) 15 (50)	0.30*
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
Indication of the administration of gentamicin	Cellulitis Septic arthritis &Osteomyelitis UTI Brucellosis	4 (13.3) 7 (23.3) 13 (43.3) 6 (20)	10 (33. 3) 9 (30) 6 (20) 5 (16.7)	0.14
Gentamicin administration intervals	24 hours 8 hours	23(76.7) 7 (23.3)	17 (56.7) 13 (43.3)	0.10

The chi-square test was used for the statistical differences.
GFR: Glomerular filtration rate
SD:Standard deviation

Table 2. Comparison between thefrequency 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

SM group was significantly lower than that in the placebo group(16.7% and 53.3%, respectively; *p* value: 0.003). The mean ± SDtime 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 GENadministration exhibitedno significant differencesbetween SM- and placebo-treated patients ($p > 0.05$) (Table 3).

Table 3. Comparison between thefrequency 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 onsetof 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 placebowas significantly higher than those receivingSM (OR=12. 69, 95%CI: 1. 38-116. 74;*p*:0. 03). The averageRisk of nephrotoxicity in patients receiving GEN every 24 hours was found to be 0. 01 times lower thanthosereceiving every 8 hours(P<0. 001). In contrast, sex, GEN indication, baseline GFR and age had no significant associationwith GEN nephrotoxicity ($p > 0.05$) (Table 4).

Results from repeated measure analysis showed that serum Crlevels increased ($p<0.001$)in both SM- and placebo-treated patients, showing significant increases on days 2, 3, 5, and 7 as compared with thebaselinevalue. Importantly, this increase in serum Crwas significantly higher in the placebo groupthan that in theSMgroup (p <0. 05). No significant differenceswere found betweenthe baseline serum Crand serum Cron day 1 in both groups (p> 0. 05); however, the placebo group showed significantly higher serum Crvalues on days 2, 3, 5 and 7 afterGEN treatment than the SMgroup ($p<0.001$) (Figure 2 and Table 5).

Urinary Crlevels exhibited no significant differences in both SM- and placebo-treated patients on different days ($p > 0.05$). Additionally, no significant differences were

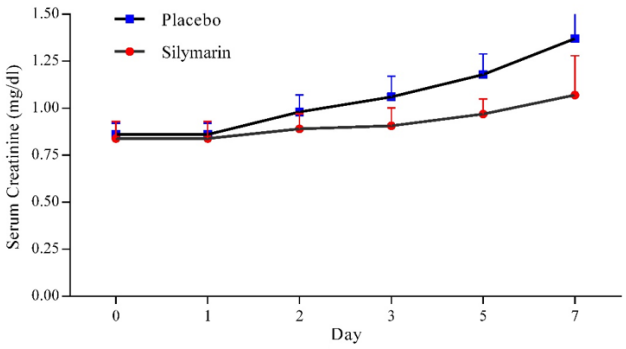

Figure 2. IComparison of serum creatinine levels on different days of gentamicin treatment between the SM- and placebo-treated patients.

Table 4. Logistic regression analysisfor 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

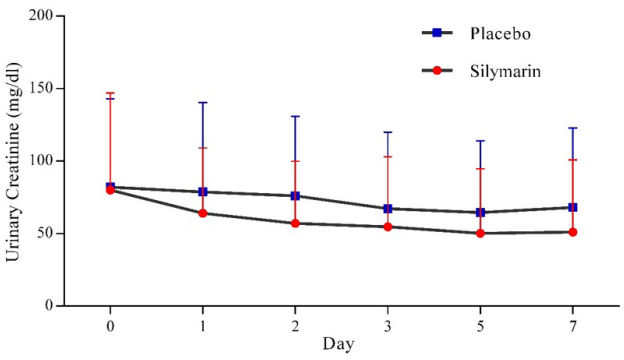

Figure 3. Comparison of urinary creatinine levels on different days of gentamicin treatment between silymarinand placebo-treated patients.

found in the mean change in urine Crlevelsat baseline as well as days 1, 2, 3, 5 and 7 afterGEN treatment in patients treated withSM and placebo (p> 0. 05) (Figure 3 and Table 5).

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 SMgroup (p <0. 05). This decrease inGFR valuesondays 1, 2, 3, 5, and 7 afterGEN treatment wassignificantly lower in the SM groupcompared withthe placebogroup (p <0. 05) (Figure 4 and Table 5). Importantly, no patients in the treatment group developed adverse effects associated with SM. All subjects tolerated SM and adhered to the treatment regimen.

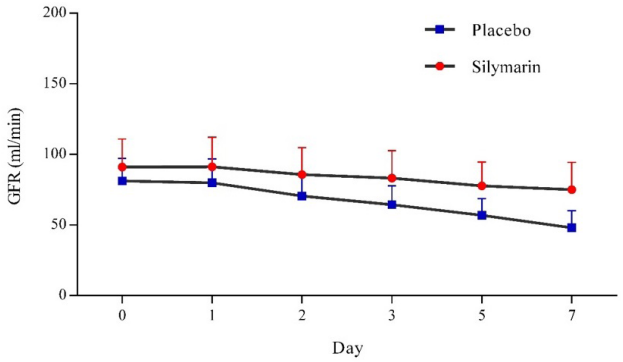

Figure 4. Comparison of glomerular filtration rate values on different days between silymarin and placebo groups.

Discussion

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 placeboon day 7, showing the preventive effects of SM against GEN nephrotoxicity. The overall risk of GENnephrotoxicity in patients treated with SM was found to be 12. 69 times lower than those received placebo. In addition, an increase in serum Crvalues 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-inducednephrotoxicity.[4,21,22] SM is a mixture of 4 isomeric flavonoids, including silibinin, isosilibinin, silydianin, andsilychristine, among whichsilibinin is the major and most active compound, constituting between 60% and 70%of SM.[9,23] 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.[9,24]

There are studies demonstrating that SM and silybin extracts exhibit a number of pharmacological activities, including anticancer, antioxidative, and radical-scavenging properties.[25,26] Importantly, the antioxidativeand anti-inflammatory properties of SMmay play a protective role on nephropathic processes. A number of clinical studies demonstrated the beneficialeffects of SM supplementation alone or in combination with vitamin E in patients with different kidney diseases including diabetic nephropathy, hemodialysis, and peritoneal dialysisthroughdecreased TNF-α, TGF-β, and Malondialdehyde (MDA) levels.[27-29] Dashti-Khavidaki *et al.* in a literature review reported and discussed experimental studies on nephroprotective effects of SM against drug-induced kidney injury. These agents includeddoxorubicin, cyclosporine, acetaminophen, and aminoglycosides.[30] However, apilot, randomized, double-blinded, placebo-controlled clinical trial failed to demonstrate any preventive effects of SMagainst both glomerular and tubular aspects of cisplatin nephrotoxicity including urine electrolyte wasting and renal function impairment.[31] In addition, *Voroneanu et al.* reported that the addition ofSM to renin–angiotensin system blockersin normotensive patients with type 2 diabetes mellitusand proteinuria had no significant effects on both primary (adecrease in eGFR ≥ 50% or development of ESRD)and secondary outcomes (changes in eGFR and proteinuria).[32] Four experimental studies have specificallyassessed the protective effects of SM alone against AG-inducednephrotoxicity.[22] For example, Ghaznavi *et al.* evaluated the protective effects of SM and melatonin on serum Crand urea levels in GEN-treated rats. Their findings showed that SM and melatonin pretreatment significantly lowered the elevated serum urea and Crconcentrations, 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 levelsas well as an increase in zinc levels in the kidney tissue. In 2007, *Varziet al.* analyzed the protective effect of SM alone (20 mg/kg/dayorally for 9 days) or in combination with vitamin E on a model of GEN nephrotoxicityin dogs. SM could effectively attenuateGEN nephrotoxicity indexes includingincreasedserum Cr, urea, andMDAalong with decreased total serum antioxidant(TSAO) activity as well as GFR.[33] In addition, *Mashayekhi*, in a study carried out in 2012, showed that co-treatment of SM (80 mg/ three-times a 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.[34] 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.[14]

The SM dosage used in the present study was shown tobe both safe and effective in the treatment of liver diseases.[26] 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).[27-29,31,32] 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.[35] Findings from our study showed that patients well-tolerated SMandimportantly,exhibitedno adverse effects, includingheadache or gastrointestinal symptomsincluding

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.[36] However, low SM bioavailability was even described with modified formulation in soy phosphatidyl choline, showing to be able to improve its bioavailability.[37] 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 SM 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.

Acknowledgments

This study was supported by a grant from the Shiraz University of Medical Sciences.

Conflict of Interests

The authors claim that there is no conflict of interest.

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