Drug and Chemical Toxicology

ISSN: 0148-0545 (Print) 1525-6014 (Online) Journal homepage: https://www.tandfonline.com/loi/idct20

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To cite this article: Burak Dumludag, Mehmet Kursat Derici, Osman Sutcuoglu, Betul Ogut, Ozge Tugce Pasaoglu, Ipek Isık Gonul & Ulver Derici (2020): Role of silymarin (*Silybum marianum*) in the prevention of colistin-induced acute nephrotoxicity in rats, Drug and Chemical Toxicology, DOI: 10.1080/01480545.2020.1733003

To link to this article: https://doi.org/10.1080/01480545.2020.1733003

Published online: 16 Mar 2020.

DRUG AND CHEMICAL TOXICOLOGY
https://doi.org/10.1080/01480545.2020.1733003

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ORIGINAL PAPER

Role of silymarin (*Silybum marianum*) in the prevention of colistin-induced acute nephrotoxicity in rats

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ABSTRACT

Silymarin (Silybum marianum) has some protective effects against drug toxicity (cisplatin, acetaminophen, adriamycin, gentamicin etc.). Colistin is a strong antimicrobial, which is frequently used in the treatment of resistant gram-negative bacterial infections in recent years although it has nephrotoxic potential. This study was aimed to determine the role of silymarin against colistin-induced acute nephrotoxicity (CIN). Rats were randomly divided into four groups. The control group was treated with tap water whereas groups 2 and 3 received silymarin (orally, 100 mg/kg/day) and colistin (intraperitoneally, 750.000 IU/kg/day) for seven days, respectively. Group 4 received both 750,000 IU/kg/day colistin and 100 mg/kg/day silymarin for seven days. After euthanasia, histopathological and biochemical examinations were completed for the kidney tissue specimens and blood samples. All parameters of the control and silymarin groups were similar. Severe weight loss was seen in the groups receiving colistin (groups 3 and 4). Silymarin significantly increased glutathione peroxidase and superoxide dismutase levels when administered with colistin in group 4 only. Acute tubular injury, tubular necrosis, meduller congestion, interstitial inflammation and apoptotic indices of colistin group were significantly higher than the control group. The administration of colistin with silymarin (group 4) was able to make some improvements in tubular necrosis and significant increase in antioxidant capacity. Silymarin increased antioxidant enzyme activity only when used in combination with colistin. The effects of silymarin may become more pronounced when used at higher doses or with a longer duration of treatment and may prevent nephrotoxicity.

ARTICLE HISTORY
Received 15 November 2019
Revised 3 February 2020
Accepted 10 February 2020

KEYWORDS
Silymarin; colistin; nephrotoxicity; apoptosis; antioxidant

Introduction

Drug-related nephrotoxicity is responsible for 14%−26% of all acute kidney injuries in adults and 16% in pediatric patients (Gai et al. 2019). Some factors may facilitate the development of nephrotoxicity; these factors include concomitant nephrotoxic drug use, obesity and age. The affected area of the kidneys can involve any of the nephron segments, such as the glomeruli, proximal or distal tubule. Drug-related kidney injury can be the result of glomerular and/or tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis and thrombotic microangiopathy (Gai et al. 2019). Some antibiotics (like colistin) produce acute kidney damage by causing tubular cell toxicity (Ghlissi et al. 2013). Some potential therapeutic agents, such as antioxidants, have been studied to help prevent cellular toxicity in the affected areas.

Silymarin is an extract from the seeds of *Silybum marianum*, a plant belonging to the Asteraceae family. It is used to treat liver and gallbladder diseases as well as poisoning because of its beneficial effects on the prevention of liver dysfunction. It is one of the most researched plants in the treatment of liver disease (Morazzoni et al. 1993). Eighty percent of silymarin is silibinin, the most effective component; other components include isosilybinin, taxifolin, silychristine, dihydrosilibinin and silydianine (Choppin and Desplaces 1975, Vogel et al. 1984, Morazzoni et al. 1993). Hepatoprotective properties have been proven. Moreover, these substances are thought to have also anti-inflammatory, antifibrotic and anti-apoptotic effects (Vaid and Katiyar 2010). The causes of silymarin's hepatoprotective properties include antioxidant and strong detoxification properties, and ability to reduce lipid peroxidation and increase glutathione (Halim et al. 1997).

Silymarin has low water solubility. It is used orally as a standard encapsulated plant extract. No side effects have been observed in human studies to date. Silymarin reduces elevated liver enzymes, improves liver histology and prolongs survival in patients with Amanita mushroom poisoning, alcoholic hepatitis and cirrhosis (Choppin and Desplaces 1975, Salmi and Sarna 1982, Hruby et al. 1983, Vogel et al. 1984, Ferenci et al. 1989). Data on the renal effects of silymarin are not as well-known as the hepatic effects. Some studies have reported positive effects of silymarin in ischemia reperfusion

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and drug nephrotoxicity models in rats (Tan et al. 2015, Soodvilai et al. 2018). Data on the effects of silymarin on renal functions have been obtained from studies evaluating hepatic effects. Although it has been reported that there is no deterioration in renal function, there is little study data evaluating renal histology. Considering the hepatoprotective properties of this substance with strong antioxidant properties, its kidney protective effect should be evaluated.

Colistin is a polymyxin group antibiotic. The target of colistin is the bacterial cell membrane. It interacts electrostatically with bacterial outer membranes, passes into the race with divalent cations (calcium and magnesium) in the negatively charged phosphate groups in membrane lipids, causes damage to the outer membrane, increases permeability, releases cell content and causes the death of bacteria (Falagas et al. 2005, Li et al. 2005, Li et al. 2006). Nowadays, antibiotic resistance is increasing. Although the side-effect profile is high, colistin treatment must be used especially to treat *Pseudomonas aeruginosa and Acinetobacter baumanii* infections due to this resistance. The most common limiting effect of colistin on clinical use is its nephrotoxicity (Falagas et al. 2005, Li et al. 2005, Li et al. 2006). Colistin-induced-nephrotoxicity (CIN) is a consequence of intracellular colistin accumulation, its necrotic nature at the level of the proximal tubule cells and its detergent activity on the cell membrane. It is thought that this damage can be prevented by drugs that feature antioxidant activity (Ghlissi et al. 2013, Gai et al. 2019). In this context, silymarin may be effective in preventing colistin nephropathy due to its antioxidant and anti-inflammatory properties.

This study was designed with the hypothesis that silymarin, having been previously shown to have hepatoprotective properties and positive effects on cisplatin nephrotoxicity, may also aid in the prevention of nephrotoxicity due to colistin.

Method

Twelve-week-old Sprague Dawley male rats, weighing 210–350 grams, were used in this study. Rats were obtained from the experimental animal laboratory at Gazi University. Rats were randomly divided into four groups. Each group included six rats, according to power analysis. The rats were kept at adjusted laboratory conditions (temperature = 25 ± 1 °C, humidity = 60 ± 10%, and a 12/12 h light/dark cycle). They were fed a standard diet and water *ad libitum*. The study was approved by the ethics committee of the university. In the study, *GNC brand Milk Thistle Capsule* was used as silymarin, which contains 160 mg of active substance. Each capsule was suspended with 5 ml tap water (32 mg/ml stock solution) and given to each rat by gavage, according to its weight at a dose of 100 mg/kg/day silymarin. Colimycin® 150 mg IM/IV (Kocak Farma) vials containing lyophilized powder were used. One vial of Colimycin® contained 150 mg colistin base ~365 mg colistimethate sodium), which is the equivalent of 4.500.000 IU colistin. Colimycin® was dissolved with 6 ml of distilled water to form a 750 000 IU colistin/ml stock solution. Colistin was administered intraperitoneally at a dose of 750 000 IU/kg/day.

Study protocol

The body weight and amount of liquid consumed by all rats were monitored daily. The rats in group 1 (control group, C) were treated with 0.4 ml tap water gavage (equivalent volume to the amount of silymarin solution) twice a day for 7 days. In group 2 (silymarin group, SILY), silymarin was administered at a dose of 100 mg/kg/day with gavage as two divided doses for 7 days. The C and SILY groups received 0.3 ml of distilled water intraperitoneally in two divided doses, like the colistin injection volume in groups 3 (colistin group, COL) and 4 (colistin-silymarin group, COL/SILY). In the COL group, colistin was administered intraperitoneally at a dose of 750.000 IU/kg/day in two divided doses, and 0.4 ml tap water by gavage was applied twice daily. In the COL/SILY group rats were administered two divided doses of 750.000 IU/kg/day of colistin intraperitoneally, and silymarin was administered 100 mg/kg/day by gavage in two divided doses for 7 days. Intraperitoneal and oral gavage applications were made every 12 h. On the eighth day of the study, deep anesthesia was performed with ketamine and xylazine in the presence of a veterinarian. Intracardiac blood sampling was performed an the anesthetized rats. Blood samples were allowed to clot for about 15 min and then centrifuged at 4000 rpm for 10 min for serum samples. Serums were separated and stored at −80 °C.

Serum creatinine was measured using the Beckman Coulter auto analyzer. Cystatin C, Glutathione Peroxidase (GSH-Px) and Superoxide dismutase (SOD) parameters were measured using commercially available ELISA kits (Shanghai Sunred Biological Technology), according to the manufacturer's protocol. Malondialdehyde (MDA), an indicator of lipid peroxidation, was evaluated as thiobarbituric acid-reactive substances (TBARS) in a previously described method. Peroxidized membranes produce fragments that give a color reaction with thiobarbituric acid (TBA). This formation is the basis of the TBARS test. The amount of substance in the samples is calculated based on known standards of concentration (Hunter et al. 1985).

Rat nephrectomy samples were fixed in 10% buffered formaldehyde for 24 h. Following routine tissue processing, sections with a 4-micron thickness were cut from paraffin-embedded tissue blocks. Hematoxylin-eosin (H&E) stained sections were examined under a light microscop for the following features: (a) interstitial inflammation, (b)interstitial capillary congestion, and (c) tubular injury, including early and late features and tubular dilatation. The localization of capillary congestion, as medullary, cortical, or mixed (medullary and cortical) was noted. The degree of medullary congestion was semiquantitatively graded as absent or insignificant (score 0: congestion recognizable under ×400 magnification), mild (score 1: congestion recognizable under ×200 magnification), moderate (score 2: congestion recognizable under ×100 magnification) and severe (score 3: congestion recognizable under ×40 magnification). Interstitial

inflammation, tubular necrosis, cytoplasmic swelling and tubular dilatation were semiquantitatively scored as follows; 0: absent; 1: mild, <25% of the renal parenchyma affected; 2: moderate, 26%−50% of the renal parenchyma affected; 3: severe, > 50% of the renal parenchyma affected.

Apoptosis (DNA fragmentation) was studied on paraffine embedded tissue sections by use of the TUNEL (terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling) assay method (Apoptag reagent, Q-Biogene, Strasbourg, France). Briefly, kidney sections were digested by proteinase K followed by H_2O_2- inactivation of endogenous peroxidase. The sections were incubated with residues of digoxigenin nucleotide and terminal deoxynucleotide transferase (which catalyzes a template-independent addition of deoxyribonucleotide triphosphate to the 39-end of double single-stranded DNA). After that, the sections were incubated with the antidigoxygenin antibody coupled to peroxidase. The cells with evidence of nuclear DNA fragmentation could be identified, and the labeling index calculated, after incubating the sections with diaminobenzidine and H_2O_2. The number of TUNEL positive cells was determined in the tubular epithelial cells, both in the cortex and medulla. More than 1000 tubular epithelial cells per patient were counted, and cells were considered to be positive when the staining intensity was moderate to strong.

Statistics

All data obtained during this study were evaluated in SPSS 21. Descriptive statistics were presented as means ± standard deviations (SD) or medians. The quantitative data were evaluated using the Shapiro-Wilk test to examine whether they conformed to normal distribution. For comparison among the groups with normal distribution, a one way analysis of variance (ANOVA) and *post hoc* Tukey's test were performed. A Kruskal-Wallis test was used for the abnormal distribution form of data. A Mann-Whitney U test was then applied with Bonferroni correction for comparison between the two groups. $p \leq 0.05$ was considered significant.

Results

The rats' body weight differences

After seven days of treatment, the final weight of each rat was recorded. The rats in the the C group and SILY group gained an average of 6.03 g and 7.9 g respectively, whereas the rats in the COL group lost a mean of 35.8 g, and those in the COL/SILY group lost an average of 22.8 g. There was a statistically significant difference between the groups ($p = 0.004$). The mean weight loss in the COL group was higher than that of the COL/SILY group, but not significant ($p = 0.26$) (Table 1).

Biochemical and histopathological evaluation

While the mean serum cystatin C level of the COL group was higher than the C and SILY groups, these differences did not reach statistical significance. Serum cystatin C levels were decreased in the COL/SILY group compared to the COL group, but it was not statistically significant (Table 1). Serum creatinine and MDA levels were not different in the four groups. However, SOD activity was significantly low in the COL group compared to the C and SILY groups; it was significantly increased in the COL/SILY group. When the serum GSH-Px activity was evaluated, the C, SILY and COL groups were not different from one another, whereas significantly higher enzyme activity was detected in the COL/SILY group (Table 1). This significant increase in GSH-Px and SOD levels was seen only in the COL/SILY group, but not in the SILY group. In other words, the effect of silymarin on GSH-Px and SOD activity is more pronounced in the presence of colistin.

The histopathological results were similar for the C and SILY groups. Silymarin did not change renal pathology; its effects were similar to those of the C group. Tubular necrosis was not found in the C and SILY groups, while it was found to be mild to moderate (mean score; 1.6 ± 0.89) in the COL group and mild in the COL/SILY group (mean score; 0.4 ± 0.5). Tubular necrosis was less apparent when silymarin was administrated in CIN ($p = 0.03$). Acute tubular injury was not detected in the C and SILY groups, whereas in COL and

Table 1. The results of the analyses of body weights and biochemical parameters between groups.

	Group 1 (C)	Group 2 (SILY)	Group 3 (COL)	Group 4 (COL/SILY)	*p Anova*
ΔWeight *(g, mean ± SD)*	6.03 ± 9.06	7.9 ± 6.7$^{\$}$	−35.8 ± 12.3^{+}	−22.8 ± 13.9$^{+,\&}$	0.004
Creatinine *(mg/dl, mean ± SD)*	0.29 ± 0.66	0.29 ± 0.38	0.31 ± 0.2	0.32 ± 0.23	0.44
Cystatin C *(mg/L, mean ± SD)*	1.8 ± 0.3	1.9 ± 0.3	2.07 ± 0.37	1.6 ± 0.35	0.11
SOD *(ng/ml, mean ± SD)*	14.1 ± 2.1	13.6 ± 0.37$^{\$}$	8.9 ± 3.4^{+}	17.04 ± 2.9*	0.002
GSH-Px *(ng/ml, mean ± SD)*	29.2 ± 3.7	32 ± 3.9$^{\$}$	26.9 ± 4.11$^{\$}$	36.5 ± 5.09$^{*,\beta}$	0.01
MDA *(nmol/ml, mean ± SD)*	31.2 ± 1.12	26.8 ± 9.36	33.8 ± 4.96	34.4 ± 6.41	0.46

$^{+}p < 0.01$ vs control.
$^{*}p < 0.01$ vs group 3.
$^{\$}p > 0.05$ vs control.
$^{\&}p > 0.05$ vs group 3.
$^{\beta}p < 0.05$ vs control.
SOD: Superoxide dismutase; GSH-Px: Glutation peroxidase; MDA: Malondialdehide.
ΔWeight: rat's weight at the end of study (g) − rat's weight at the beginning of the study(g).
g: gram.

COL/SILY groups, the average score of the injured cells was 1.4 (±0.5) and 1(±0), respectively. Interstitial inflammation was not detected in the C and SILY groups, whereas there was mild interstitial inflammation in both the COL and COL/SILY groups (Figures 1 and 2). The significant increase in scores of acute tubular injury, tubular necrosis, medullar congestion and interstitial inflammation in the COL group (group 3), as well as the apoptotic index formed by colistin administration, were found to be significant compared to both the C and SILY groups ($p < 0.00$). Apoptosis was not detected in the C and SILY groups, while an average of 15 epithelial cells was found in the COL group and six in the COL/SILY group. There was a significant difference between the C and COL groups ($p = 0.004$); colistin administration was found to have a significant apoptotic effect on renal epithelial cells (Figure 3). In the COL/SILY group, the application of silymarin was found to decrease the apoptotic effect of colistin, but no significant difference was found when compared to the COL group ($p = 0.31$) (Figure 4).

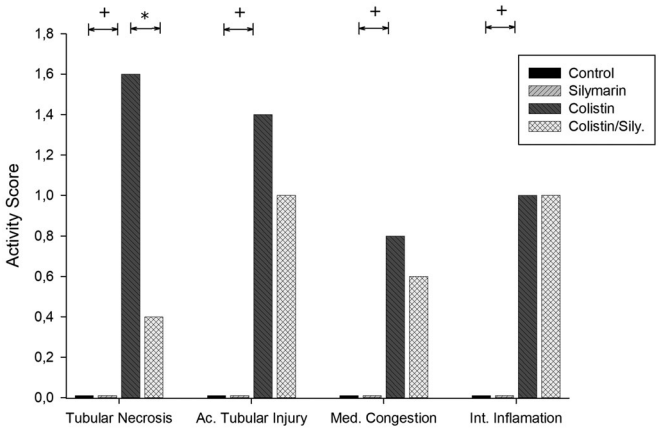

Figure 1. Colistin induced tubular necrosis, acute tubular injury, medullar congestion and interstitial inflammation. All parameters were represented as mean score. Kruskal Wallis and then Mann Whitney U test was applied for comparisons. $+p < 0.03$ vs control $*p = 0.03$ vs group 3.

Discussion

Colistin is often used in gram-negative bacterial infections, but the nephrotoxic effect of this agent limits its usage. Therefore, the prevention of nephrotoxicity caused by this agent will bring a significant advantage to its use. In this study, we aimed to investigate the effect of silymarin, which has been shown to have hepatoprotective effects against different toxic agents, preventing CIN. The COL and COL/SILY groups had significant weight loss, whereas the C and SILY groups had weight gain. This may be a clinical indicator of nephrotoxicity caused by colistin in rats. The weight loss in the COL/SILY group was less than the COL group and showed that silymarin could partially reduce stress and weight loss due to colistin. There was no difference in creatinine levels as an indicator of renal function among the groups. Cystatin C levels, an accepted early marker in the evaluation of glomerular filtration rate, were not different between the groups. Serum MDA levels, as an indicator of lipid peroxidation formation, and SOD and GSH-Px levels, as indicators of antioxidant enzyme activities, were measured. Serum SOD activity was similar in the C group and in the SILY group; it was was significantly decreased in the COL group. Serum SOD activity in the COL/SILY group was significantly higher than the COL group. Serum GSH-Px activity was not significantly different between the control and silymarin

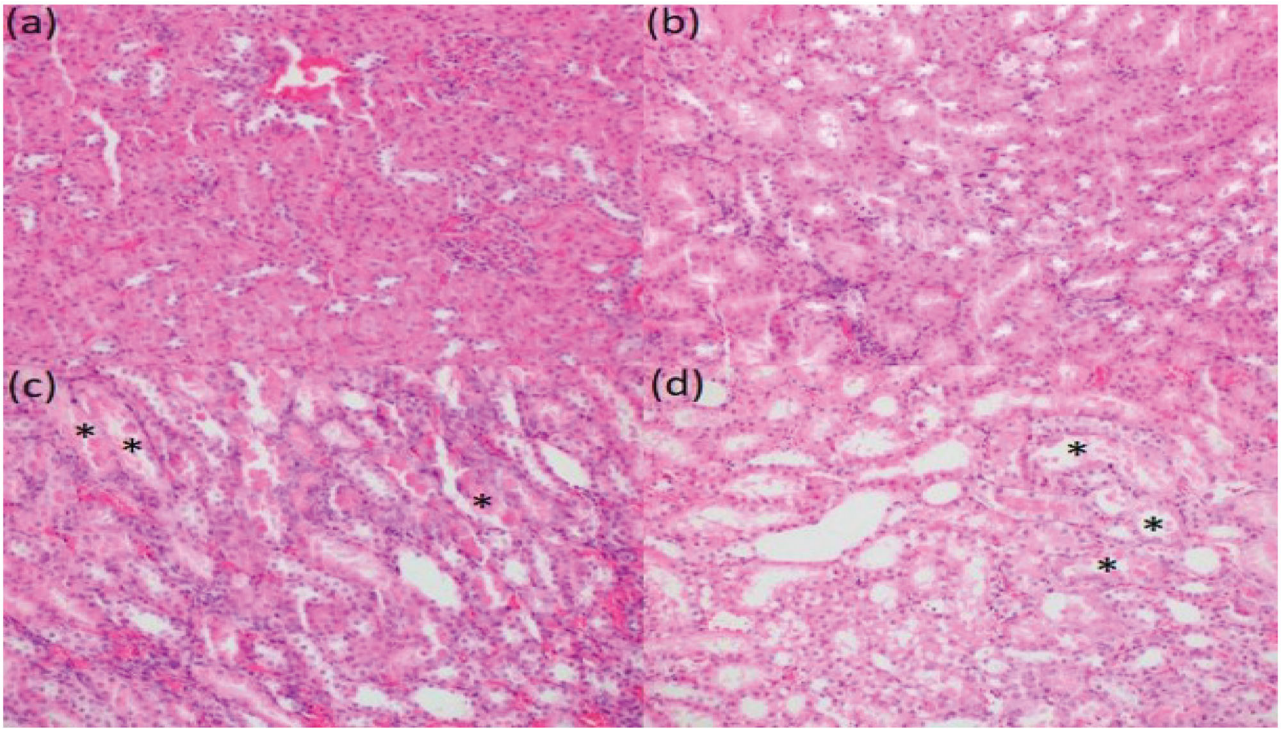

Figure 2. Light microscopic changes in kidney parenchyma and nuclear in 4 different groups, H&E X 200: (a) Control group; light microcopy is unremarkable (b) Silymarin group; unremarkable kidney parenchyma (c) Colistin group; prominent tubular necrosis is present (* necrobiotic cells denuded into the tubular lumina) (d) Colistin/Silymarin group; focal acute tubular necrosis is present (* necrobiotic cells denuded into the tubular lumina).

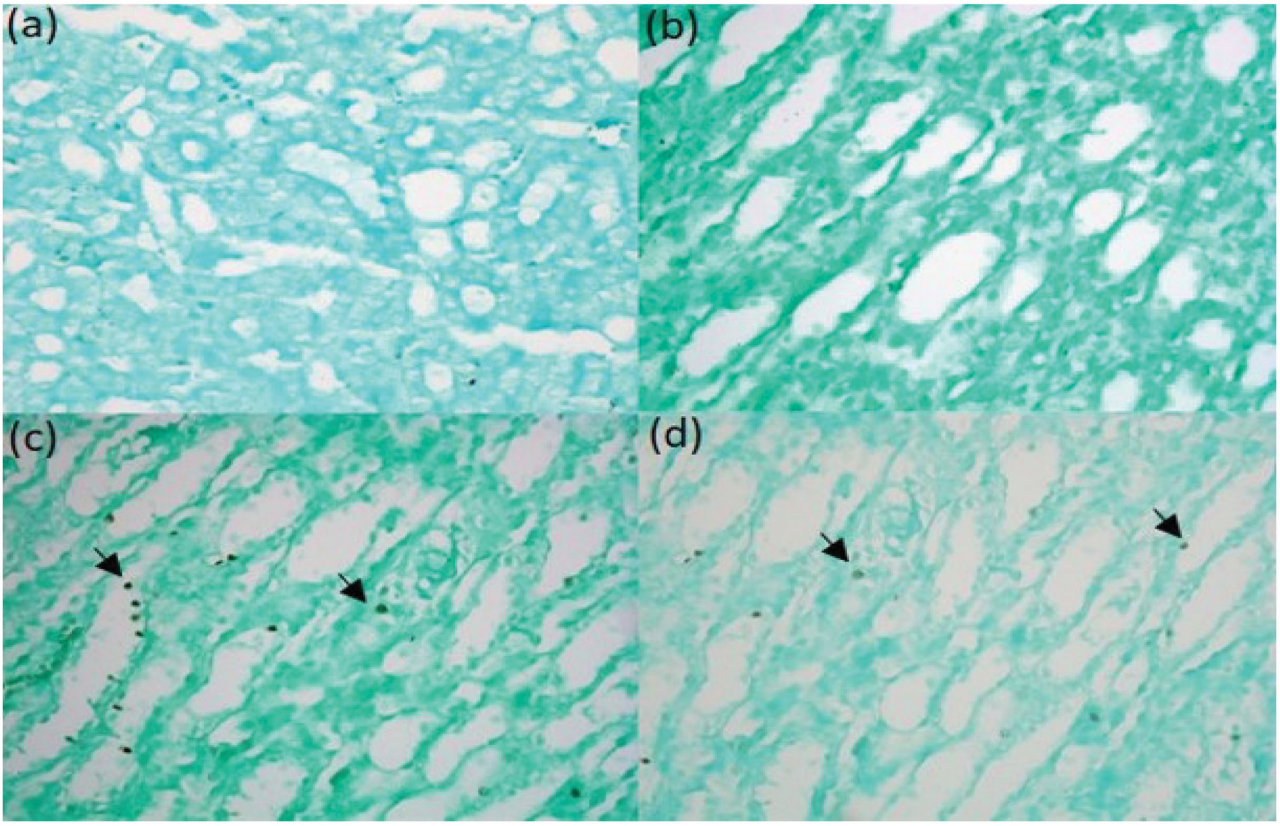

Figure 3. DNA fragmentation by immunoperoxidase staining seen as black to brown nuclear staining of tubular epithelial cells (arrows); (a) Control group (b) Silymarin group (c) Colistin group (d) Colistin/Silymarin group.

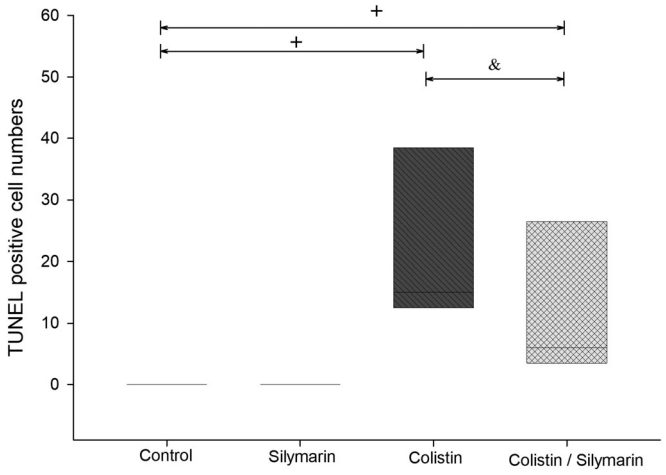

Figure 4. Colistin-induced tubular apoptotic renal damage and the effect of silymarin on this damage. Colistin-induced apoptosis was assessed by TUNEL assay. The median TUNEL-positive cell count in colistin treated rats was 15 cells/1000 tubular cells. With the application of silymarin, the median of TUNEL positive cells was found to be 6/1000 cells but this decrease was not significant statistically. There were no apoptotic cells in the control and silymarin groups. Kruskal Wallis and then Mann Whitney U test was applied for comparisons. + $p<0.004$ vs control group, & $p > 0.05$ vs colistin group.

groups, and it showed a non-significant decrease in the group treated with COL. Similar to the increase in SOD activity in the group treated with COL/SILY, GSH-Px was also significantly higher in the COL/SILY group than the C and COL groups. These data show that silymarin does not activate the antioxidant systems when stress factors (like colistin administration) are absent, and the antioxidant systems can be activated by silymarin when used with colistin. Similar to our findings, this antioxidant activity of silymarin has been shown in the presence of a toxic agent in animal models of hepatotoxicity (Sonnenbichler et al. 1986, Miguez et al. 1994).

According to the data obtained from the examination of the pathology samples, administering colistin to the rats produced significant tubular necrosis, acute tubular injury, interstitial inflammation and apoptosis compared to the control and silymarin groups. The administration of colistin with silymarin (group 4) was able to make some improvements in tubular necrosis, but the activity scores of tubular injury, medullar congestion and interstitial inflammation did not change significantly. The antioxidant activity of silymarin had a limited effect on either the biochemical results or the pathological appearance. In a nephrotoxicity study performed by Ghaznavi et al. gentamicin was administrated to rats for 8 days and biochemical parameters (urea, creatinine) were found to increase. When silymarin was combined with gentamicin, there was a significant decrease in urea and creatinine (Ghaznavi et al. 2016).

A similar study was performed by Hassan et al. They investigated the protective effects of silymarin (100 mg/kg/day, as two divided doses, similar to ours) against colistin nephrotoxicity in rats and found no significant difference between creatinine levels in colistin and colistin-silymarin groups, similar to our findings. In Hassan et al.'s study, the levels of N-acetyl beta-D glycosaminidase produced by proximal tubule cells, a lysosomal enzyme which is exposed to the toxic effect of colistin, were evaluated. The enzyme levels were found to be

lower in the colistin-silymarin group than in colistin-only group; it was demonstrated that silymarin protects proximal tubular cells (Hassan et al. 2017). In the current study, although there was no significant difference between the groups showing glomerular filtration function in groups 3 and 4, there was an increase in the COL/SILY group in the parameters evaluating the antioxidant capacity (GSH-Px, SOD). In the pathological evaluation of the kidney tissues in Hassan et al.'s study, the colistin group was found to have, an increased number of lysosomes, abnormal mitochondria with swelling and multiple shapes and sizes, shortened microvilli, irregular nuclear outline with nuclear indentation, lipid droplets and thickened and highly irregular basal lamina, according to a transmission electron microscope. Additionally, tubular degeneration and intratubular hyaline casts, peritubular vascular congestion and interstitial exudation were observed under a light microscope. On the contrary, in the COL/SILY group, the basal lamina was of normal thickness, the integrity of the nuclear membrane was preserved, and the numbers of mitochondria remained unchanged and had a normal microvilli structure. However, tubular toxicity can not be completely reversed by silybin because tubular atrophy was still present, although improved. This ameliorating effect of silymarin was attributed to its antioxidant effects (Hassan et al. 2017). In our study, there were no pathological findings in the control and silymarin groups, but the scores of the apoptotic index, acute tubular injury, tubular necrosis, and interstitial inflammation were higher in the COL group. In the group treated with colistin and silymarin, tubular necrosis occurred less frequently. The scores of the apoptotic index, acute tubular injury and medullar congestion were reduced but did not differ significantly from those of the COL group. The appearance of interstitial inflammation was the same as the colistin group. Both the colistin-induced nephrotoxicity and the partial curative effects of silymarin on kidney injury were observed histopathologically, without significant recovery in biochemical markers (creatinine, cystatin C).

Animal and human studies have revealed that silymarin may be effective in tubular damage caused by the use of chemotherapeutic agents, such as cisplatin and methotrexate (Dabak and Kocaman 2015, Shahbazi et al. 2015). In a human study, 15 patients with cancer diagnoses were treated with cisplatin-based chemotherapy and the other 15 patients with cisplatin and silymarin (420 mg daily) together (Shahbazi et al. 2015). This was the first human study in which silymarin was used to prevent chemotherapy-induced nephrotoxicity.and had disappointing results. Serum creatinine, urine neutrophil gelatinase-associated lipocalin to urine creatinine ratio (NGAL/Cr) and urinary magnesium and potassium wasting were compared between two groups. The results did not show that the application of silymarin had a remedial effect on the tubular functions deteriorating in cisplatin nephrotoxicity.

Colistin serves to damage to target cell membranes. Many studies on CIN have shown that colistin damages the proximal tubule cell membrane in particular: it accumulates in the intracellular area and causes dysfunction by promoting mitochondrial damage (Gai et al. 2019). In a meta-analysis of 7911 patients to evaluate colistin nephrotoxicity, the prevalence was 26.7% (Oliota et al. 2019). Despite these high rates, life-threatening infections caused by gram-negative opportunistic bacteria, especially in multidrug-resistant and critically ill patients, required colistin use. In a study evaluating serum creatinine, cystatin C and NGAL levels for early detection of colistin nephrotoxicity, it was reported that only serum cystatin C and NGAL levels increased, especially in high-dose colistin exposure, and could be used to diagnosis acute kidney injury, serum creatinine did not increase (Ghlissi et al. 2013). In a recent study, urine glucosuria, as well as NGAL and kidney injury molecule-1 (KIM-1), proved to be an early marker of colistin nephrotoxicity and correlated with the histopathological appearance of acute kidney injury (Samodelov et al. 2019). In our study, according to histopathological data, tubular necrosis, apoptotic activity and acute tubular injury were found to be more prominent in the colistin group than in the control group. However, neither serum cystatin C nor serum creatinine levels correlated with the pathological appearance of colistin-induced acute renal injury. Therefore, they did not seem suitable as early markers of acute kidney injury. We require an early marker like NGAL, KIM-1, and glucosuria, and their absence is an important limitation of this study. However, histopathological evaluation is still of great importance in the early detection and presentation of nephrotoxicity findings, as stated in Ghlissi's study (Ghlissi et al. 2013).

Various agents (dexmedetomidine, grape seed extract, n-acetylcysteine, luteolin, old black garlic aqueous extract, etc.) have been used to prevent colistin nephrotoxicity (Ozyilmaz et al. 2011, Ozkan et al. 2013, Lee et al. 2015, Arslan et al. 2016, Talih et al. 2018, Lee et al. 2019). In some of these studies, oxidative damage and caspase dependent apoptosis were identified as playing an important role in the development of colistin nephrotoxicity (Ozkan et al. 2013, Arslan et al. 2016, Talih et al. 2018). Although there was no difference between the groups in terms of MDA level in our study, which is an indicator of lipid peroxidation, the number of TUNEL positive cells was significantly higher in the colistin-treated group; interstitial inflammation, acute tubular injury and tubular necrosis were more prominent. Our study emphasizes the development of apoptosis in the formation of colistin nephrotoxicity.

Another study was performed by Lee et al. They reported the results using old black garlic aqueous extract (ABGE) in colistin nephrotoxicity. They found that ABGE decreased the levels of 8-hydroxydeoxyguanosine and MDA, which are indicators of oxidative stress in colistin-treated rats, and decreased BUN and creatinine levels with these antioxidant effects. The levels of IL-1β and TNF-α increased with colistin application in renal tissue, whereas SOD, catalase and glutathione levels in renal tissue were significantly increased with ABGE. It has been reported that the antioxidant and anti-inflammatory properties of ABGE provide an improvement in colistin nephrotoxicity (Lee et al. 2019). In our study, silymarin increased the antioxidant SOD and GSH-Px activity in rats with CIN, which is demonstrated histopathologically, but the positive effects on this antioxidant activity did not result in prominent histopathological improvement. Although a

significant increase in antioxidant activity was detected in our study, it may be speculated that this is not sufficient for the development of the expected histopathological recovery. In this context, nephrotoxicity should be examined both pathologically and biochemically with the application of higher doses of silymarin.

A limitation of this study is our use of only one dose level in order to prevent nephrotoxicity; it would be interesting to assay higher doses of silymarin (e.g., 200 or 400 mg/kg/day) if they are well tolerated. In a recent study, Guzel et al. studied the effects of low (50 mg/kg/day) and high doses's (200 mg/kg/day) of silymarin in vancomycin-induced nephrotoxicity. They found that pretreatment with silymarin especially at increased doses, significantly reduced caspase activities causing apoptosis and displayed potential renoprotective effects against vancomycin-induced nephrotoxicity due to its antioxidant, anti-inflammatory, and anti-apoptotic properties (Guzel et al. 2019). Therefore, the hypothesis that earlier use of silymarin may increase its protective capacity on colistin-induced nephropathy, will be investigated in our next study.

Conclusion

In this study, silymarin was administered to treat colistin nephrotoxicity in rats. It was found to have ameliorating effects on tubular necrosis and enhancing effects on antioxidant molecules, such as SOD and GSH-Px. This increase in the levels of antioxidant molecules leads to the expectation that the effects of silymarin may become more pronounced when used at higher doses or with a longer duration of treatment (e.g., a few days before and during colistin treatment) and may prevent nephrotoxicity.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by Turkish Society of Hypertension and Kidney Diseases.

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