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Use of silymarin for reducing nephrotoxicity caused by medicaments

V. A. Ivanov[1*], V. B. Slavova[1], D. P. Georgieva[1], V. H. Petrova-Tacheva[2], A. N. Tolekova[3]

[1]*Department of Neurology, Psychiatry and MDS, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria,*
[2]*Department of Molecular Biology, Immunology and Medical Genetics, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[3]*Department of Physiology, pathophysiology and pharmacology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*

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Seeds of *Silybum marianum (L) Gaertn (Carduus marianus L., Asteraceae)* have been used for more than 2 000 years to treat liver and gallbladder diseases, particularly in the treatment of hepatitis and cirrhosis. The growing interest in the plant is documented by the fact that nowadays the information platform PubMed has over 3 570 publications about it. There has been a steady increase in the number of publications on its use in diabetes, chemical weapons intoxication, its radio-protective effect. Silymarin, standardized extract of the plant is used as a chemoprotective and anticancer agent, especially as a protector against the toxic effects of some drugs used in oncology, as well as against the toxic action of antibiotics. The purpose of this publication is to examine silymarin's contribution to reducing nephrotoxicity induced by medicaments. We present some summarized examples of the nephroprotective effects of silymarin when applied with analgesics and non-steroid anti-inflammatory agents, with antibiotics, anti-tuberculosis agents, anticancer agents and immunosuppressive agents.

Keywords: silymarin, silybin, silibinin, nephrotoxicity

INTRODUCTION

The kidneys are often damaged by various toxic compounds - fungal poisons, heavy metals, organic solvents. Drug-induced nephrotoxicity (DIN) accounts for up to 60% of acute renal failures acquired at the hospital. Much effort is being made to reduce drug-induced renal impairment. However, DIN remains a problem that has a significant impact on patients and the health system. *Silybum marianum (L) Gaertn (Carduus marianus L., Asteraceae)* (milk thistle) is a medicinal plant which has been used for centuries in alternative and modern medicine for treatment of various diseases such as liver disorders and protecting the liver [1]. Silymarin is a standardized extract of *Silybum marianum* (milk thistle extract) consisting mainly of silybin, dehydrosilybin (DHSB), quercetin, toxifolin, silicristin, and a number of other compounds known to have numerous beneficial effects. The antioxidant, anti-inflammatory and anti-apoptotic properties of silymarin make it an interesting herb for medicines and these properties have included this agent as a potential renoprotective agent, similar to other plants [2, 3]. Silymarin exhibits significant protective effects against various toxic compounds. Whether the protective use of silymarin can be an effective clinical pharmacological strategy for preventing DIN is a question to be answered in clinical trials [1]. There is evidence of its role in reducing tumor growth, preventing liver toxicity, and protecting a number of organs against ischemic damage. A well-established fact is the hepatoprotective effect of silymarin, especially to prevent α-amanitin and alcohol intoxication causing liver damage. There is also strong evidence that silymarin has antimicrobial and anticancer effects [4]. The xanthine oxidase enzyme is involved in tissue oxidative damage after ischemia-reperfusion. The dehydrogenase/ oxidase ratio of homogenates in rats decreases during ischemia and reperfusion. Silymarin contains two flavonoids: quercetin and silybin, characterized as free radical scavengers and exerting a protective effect, preventing a decrease in the dehydrogenase / oxidase ratio during ischemia-reperfusion [5]. The purpose of this review is to discuss and summarize the information found in the literature regarding the potential for reducing the nephrotoxicity of various drugs using silymarin.

EXPOSITION

Paracetamol

Paracetamol (acetaminophen, N-acetyl-β-aminophenol, APAP) is the most widely used analgesic for acute pain and the most commonly used antipyretic agent [6]. Overdose with paracetamol causes severe damage to the liver and kidneys. Moreover, the APAP - induced liver

* To whom all correspondence should be sent:
E-mail: veskoasenov@abv.bg

damage is the most common cause of drug-induced liver failure [7].

Onolapo *et al.* [8] treated rats intraperitoneally (i.p.) at a dose of 800 mg/kg/day for 3 days.

Acetaminophen overdose leads to impaired motor activity, memory impairment, anxiety, impaired liver and kidney biochemistry, antioxidant balance, and histological changes in the liver, kidney, and cerebral cortex. Preliminary treatment with silymarin at a dose of 25 mg/kg per day for 14 days and then intraperitoneal (i.p.) administration of acetaminophen at 800 mg/kg per day for 3 days counteracted behavioral changes. This leads to improved biochemical indicators of liver and kidney damage and improves antioxidant activity.

In another study, acetaminophen overdose resulted in elevated levels of aspartate aminotransferase (AST), alanine transaminase (ALT), nitric blood nitrogen (BUN), and creatinine (SCr) in serum, as well as levels of nitric oxide in the liver and kidney. There are also significant histologic changes, including decreased body weight, hepatocyte edema, cellular infiltration, dilation and congestion, necrosis and apoptosis in the liver, and dilatation of Bowman's capsule space and glomerular capillaries, pale colored tubules, cellular infiltration and apoptosis in the kidney. Treatment with silymarin 1 hour after APAP injection for 7 days significantly normalized body weight, histologic lesions, serum ALT, AST, BUN, SCr and tissue NO levels. Silymarin has been hypothesized to improve the toxic effects of APAP-induced hepatotoxicity and nephrotoxicity in mice. The protective role of silymarin against AAPP-induced lesions may be due to its antioxidant and anti-inflammatory effects [9].

In an experimental model with Wistar albino rats, the nephroprotective effect of the ethanol extract of *Scrophularia hypericifolia* (stems, leaves), a plant grown in Saudi Arabia, was investigated. Toxic doses of paracetamol were used to induce renal toxicity, while the standard drug silymarin was used as a reference. Renal impairment was investigated by measuring serum urea, serum creatinine, and sodium and potassium levels [10].

Propacetamol is a medicine that is administered intravenously and is metabolized by the body to paracetamol. This suggests that if silymarin is protecting against the toxic effect of paracetamol, it is likely to have such an effect against propacetamol [11, 12].

Antibiotics and agents against tuberculosis

Gentamicin is used clinically against Gram negative bacteria because of its efficacy. However, it causes kidney damage. Silymarin could eventually reduce the kidney damage [13].

Drug-induced nephrotoxicity is an important cause of renal failure in dogs. Aminoglycoside antibiotics, such as gentamicin, can produce nephrotoxicity in dogs, due in part to imbalance of pro- and antioxidants (oxidative stress). Silymarin has potentially useful antioxidant properties. Dogs were given gentamicin by intramuscular injection at a dose of 20 mg/kg once daily for 9 days [14]. Renal function was evaluated using serum biochemical markers (creatinine and urea). Malondialdehyde (MDA) concentration is measured as a lipid peroxidation marker. The activity of total serum antioxidants (TSAO) is evaluated as a marker of antioxidant protections [15]. Concentrations of serum creatinine and urea increased significantly and TSAO decreased significantly due to the administration of gentamicin. Silymarin reduces gentamicin-induced nephrotoxicity in dogs [14]. In such experiments, rats were treated with gentamicin. Compared to rats in the control group, all rats injected with the antibiotic showed significantly elevated serum creatinine and urea levels, which was accompanied by an increase in renal relative weight, increased levels of reactive oxygen species (ROS), and MDA, and a decrease in the level of renal glutathione (GSH) and superoxide dismutase activity (SOD). Preliminary treatment with silymarin significantly lowers elevated serum urea and creatinine concentration, kidney weight, and kidney ROS and MDA levels. In addition, silymarin significantly increases the level of renal GSH and SOD activity [14]. According to Ghaznavi and co-workers [16] silymarin can reduce kidney damage in rats treated with gentamicin, possibly by reducing the level of ROS. Jedage and Manjunath [17] induced nephrotoxicity in male Wistar rats of gentamicin at 100 mg/kg/per day for 10 days, and silymarin (50 mg/kg, p.o.) was used as a lesion reducing drug. The renal biochemical markers creatinine, urea, uric acid, albumin, protein, and other parameters - kidney weight, body weight, and urine volume and kidney histopathology - were used to assess injuries. The results of the study suggest that gentamicin damages the kidneys, and silymarin reduces the extent of this damage.

Polymyxins were detected from different species of *Bacillus polymyxa*. Their efficacy against most Gram-negative bacteria has not been called into question, but their early use has been associated

with reports of adverse renal effects in a significant number of patients. This class of antibiotics consists of five chemically different compounds, polymyxin A, B, C, D, and E (colistin); but only polymyxins B and E have been used in clinical practice. After reports of its nephrotoxicity polymyxin E is discontinued. According to Hasan *et al*. [18] in a rat study, silybin had the potential to protect the kidney from polymyxin E. Rats were treated with polymyxin E and the other group was pretreated with silybin and the same antibiotic for 7 days. Histological, ultrastructural and morphometric analyzes were performed on rat kidney tissues. The results indicated that administration of silibin reduced the neomyotoxicity induced by polymyxin E in rat kidney.

In such a study [19], urine was examined. It was collected daily for 7 days to test for N-acetyl-beta-D-glucosaminase (NAG). Serum was collected after rat euthanasia on day 7 for a renal function test. The results indicate that polymyxin E affected the renal glomerulus and tubercles, as well as the possible protective effect of silybin against polymyxin E-induced nephrotoxicity.

The main drawback is the toxic side effect of isoniazid. Adverse reactions caused by the administration of INH (50 mg/kg) on haematological parameters, markers of oxidative status, markers of liver and renal function and their improvement were examined by administration of silymarin treated at a dose of 50 mg/kg for 1 hour with INH for 30 days in rats. The results showed that silymarin reduced the isoniazid toxicity [20].

In a similar study, rats were treated concomitantly with isoniazid and rifampicin (RIF) orally at a dose of 50 mg/kg/ per day for 28 days. Addition of silymarin at a dose of 25 mg/kg/ per day significantly reduced the toxic effects on the liver and kidneys. Treatment with INH and RIF resulted in a significant decrease in antioxidant levels and a significant increase in creatinine, urea and uric acid levels, which indicate impaired renal function. Silymarin treatment improved these effects. Moreover, histological studies of the kidney supported these findings and showed that the renal structure was almost normal [21]. Cecen *et al*. [22] conducted a study with doxorubicin at a single intraperitoneal dose of 10 mg/kg. Serum is secreted to determine SOD, GSH Px, CAT, MDA, NO, creatinine, urea, AST, ALT, lactate dehydrogenase (LDH) and creatine phosphokinase activity (CPK). Histopathological and electron microscopic examinations of the heart, kidneys and liver were performed. In the second group the rats were treated with a combination of doxorubicin and silymarin. The results indicated that doxorubicin caused a significant increase in serum NO levels compared to controls. This pointed out that silymarin significantly protected the renal and hepatic toxicity induced by doxorubicin in the rat, and suggested its use as a supportive treatment during anticancer treatment with doxorubicin.

Silymarin and renal toxicity of anticancer agents and immunosuppressants

There is a lot of evidence which considered that anticancer agents damaged the kidneys. Despite several preventive conditions, the nephrotoxicity of cisplatin remains a clinical problem. *In vitro* and *in vivo* studies addressed the protective effects of silymarin against the nephrotoxicity of cisplatin. Shahbazi *et al*. [23] evaluated the effect of silymarin on cisplatin nephrotoxicity as the first human study. During this pilot, randomized, double-blind, placebo-controlled clinical trial, the effect of oral silymarin 420 mg daily was studied in three divided doses beginning from 24 -48 hours prior to initiating the cisplatin infusion and continuing until the end of the 32nd day. Acute renal impairment associated with cisplatin was observed in 8% of patients; no side effects with silymarin have been reported. Prophylactic administration of a conventional form of silymarin tablets could not prevent cisplatin-induced impairment of renal function.

Ibrahim *et al.* [24] treated rats with cisplatin at a dose of 5 mg/kg for 5 days to cause acute renal failure. Silymarin was pretreated 6 hours before cisplatin. Functional kidney tests and histopathological examinations were performed. The results from the study showed a significant improvement in renal function tests and renal histopathology by using silymarin as a protective mechanism for cisplatin -induced acute renal failure.

Divya *et al.* [25] administered cisplatin once at 16 mg/ kg i.p. in Wistar rats. The dose is sufficient to cause nephrotoxicity. To some of the animals was given a methanolic extract of *Apodytes dimidiata* for 5 consecutive days before/after injection of cisplatin at a dose of 250 mg/kg. Blood and kidney parameters were analyzed. The results showed a significant protective effect of the extract on cisplatin –induced nephrotoxicity in the pretreated animals. Urea, creatinine and lipid peroxidation were reduced by 58.31%, 42.19% and 60%, respectively, and hemoglobin and leucocytes increased by 28.25% and 42.91%, respectively. GSH, GPx, SOD and catalase increased by 35.64%,

18.14%, 74.42% and 35.46% respectively. Tissue architecture of the kidneys is almost normal in animals treated with the extract. According to the authors, the results are comparable to the standard medicine, silymarin.

Prabhu *et al*. [26] previously administering 1,2-diazole alkaloid significantly reduced cisplatin-induced nephrotoxicity. Biochemical studies such as GPX, GSH and LPO levels, as well as urine volume, kidney weight, body weight, and histopathological studies confirmed that 1,2-diazole (10 mg/kg) possessed nephroprotective activity. These results were similar to those with the standard drug silymarin at a dose of 50 mg/kg. In addition, the results have shown that 1,2-diazole could be used as a neoprophylaxant in combination with silymarin.

Ninsontia *et al*. [27] also induced apoptosis and necrosis by cisplatin in NK-2 cells and caused cell viability to be reduced by ~ 40% and 60% at doses of 25 and 100 μM, respectively. Pretreatment with 25-200 μM silymarin significantly protected against cisplatin-induced cell death in a dose-dependent manner. Pretreatment of silymarin (25-100 μM) did not cause a significant change in cisplatin-induced cell death in H460 cells, but significantly enhanced cisplatin-induced apoptosis in G361 cells. These findings revealed the selectivity of silymarin in protecting kidney cells from cisplatin-induced cell death and might be useful for the development of the compound as a re-prophylactic agent. In a rat model, kidney damage was induced by a single dose of cisplatin (5 mg/kg). The protective effect of silibinin was studied in rats having received flavonoid at a dose of 200 mg/kg (i.v.) for 1 hour prior to the cisplatinate administration. Renal function was monitored by analyzing the urinary markers for glomerular and tubular function over a period of 11 days. The animals from a second identical treatment were sacrificed 4 days after drug administration to assess tubular microscopy light microscopy. Administration of cisplatin caused a reduction in renal function within one day of treatment. The observed symptoms were: a decrease in creatinine clearance and an increase in proteinuria. The effects of cisplatin on creatinine clearance and proteinuria were completely prevented by pretreatment of the animals with silibinin. Reduced damage to proximal tubular function. Silibinin itself did not affect renal function. Treatment with silibinin clearly reduced the morphologic changes seen in the S3-segment of the proximal tubule 4 days after administration of cisplatin. The effects of cisplatin on glomerular and proximal tubular function as well as proximal tubular morphology could be fully or partially improved by silibinin. In conclusion, silibinin might act as a non-prophylactic agent and it is believed that it may have an effect on the kidneys in the clinical setting [28]. Bokemeyer *et al.*, [29] conducted such study on an animal model in rats *in vitro* and in three human cancer cell lines of the testicular system. Cisplatin is one of the most active cytotoxic agents in the treatment of testicular cancer, but its clinical application is associated with side effects such as nephrotoxicity. The results show significant nephrotoxicity of cisplatin. Pre-infusion of silibinin decreases the toxicity of cisplatin. Silibinin alone did not affect renal function. The *in vitro* data excluded significant inhibition of the anti-tumor activity of the major nephrotoxic components, cisplatin and 4-hydroperoxyphosphamide, by co-administering silibinin in a human cell tumor cell line model of human germ cells.

In vitro experiments with kidney cells injured by acetaminophen, cisplatin and vincristine, showed that the administration of silibinin before or after chemically induced damage might reduce or avoid the nephrotoxic effects [30]. Single and multiple treatment with toxic doses of cyclophosphamide activates peroxidation of lipids in kidney cell membranes. Silymarin inhibits the cyclophosphamide prooxidant effect, suggesting additional antioxidant studies as a means of counteracting the adverse effects of cytostatic drugs [31].

Methotrexate is widely used in the treatment of various malignancies and non-cancer diseases, but its use is limited by its nephrotoxicity. A study was conducted to determine whether silymarin exhibited a protective effect against methotrexate-induced nephrotoxicity. The rats were injected with methotrexate at a dose of 20 mg/kg, i.p. single injection. Histopathological changes, including apoptotic changes in the kidneys, have been evaluated. Injection with methotrexate shows extended Bowman space, infiltration of inflammatory cells, glomerular and peritubular vascular congestion and edema of renal tubular epithelial cells. Apoptotic cell death was also markedly elevated in the renal tubules after methotrexate administration. Treatment with silymarin 300 mg/kg i.p. daily for 5 days resulted in a statistically significant improvement in histological changes and reduced the number of TUNEL –positive cells compared to methotrexate – treated rats ($p < 0.05$). In conclusion, treatment with silymarin resulted in a reduction in methotrexate – induced renal impairment in rats [32]. Adriamycin

is a potent anticancer agent, but its clinical use is limited due to pronounced cardiotoxicity and nephrotoxicity. El-Shitany *et al.* [33] performed rat studies with adriamycin and combination of adriamycin and silymarin. The first group of rats was treated with adriamycin at a dose of 10 mg/kg, the same dose of adriamycin was administered to the second group that was pretreated with silymarin at a dose of 50 mg/kg. On the third day after treatment was determined LDH, CPK, cholesterol and total lipids. Thirty days after injection creatinine and urea levels were determined. To evaluate the lipid peroxide and GSH content, frozen heart samples (72 h) and frozen kidney samples (30 days) were used. Histopathological examinations of cardiac and renal sites were also performed. Serious reduction in plasma CPK, LDH, creatinine and urea was observed in sildarin treated rats. On the other hand, silymarin treatment does not alter adriamycin-induced hyperlipidemia. Silymarin treatment significantly reduces myocardial MDA. In addition, the silymarin administration normalized the level of MDA and GSH in the kidney tissue. Histopathological examination of heart and kidney segments revealed that adriamycin caused only mild myocardial damage in silymarin–treated rats. Moreover, the silymarin administration inhibits adriamycin–induced renal tubular damage in rats [33]. The immunosuppressive drug cyclosporine A (CsA) is metabolised by cytochrome P-450 IIIA, and caused acute reversible and chronic irreversible nephrotoxic effects [34]. The effect is based on vasoconstriction of afferent and efferent glomerular arterioles, resulting in a reduction in glomerular plasma flow and glomerular filtration rate. Silibinin is the main ingredient of silymarin, and inhibits lipid peroxidation. The possibility of silibin to posses a protective effect due to its radical scavenging properties has also been investigated.

CONCLUSIONS

The investigated literature suggested that use of silymarin might reduce the nephrotoxicity of a number of medications. Silymarin appears to be one of the most promising nephroprotectors. Despite intensive studies, there are still a number of uncertainties. This requires more detailed studies of its mechanisms and effective doses to extend its practical application to prevent and treat the side effects of the above –mentioned drugs and these silymarin properties should be studied in greater detail and put into practice for treatment of intoxications.

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