Silymarin: A promising cardioprotective agent

Zeinab A. Zalat 1, Neeven A. Kohaf 1, Mohamed A. Alm El-Din 2, Hosny A. Elewa 3, Mohamed M. Abdel-Latif 4

1Department of Clinical Pharmacy, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.
2Department of Clinical Oncology, Faculty of Medicine, Tanta University, Tanta, Egypt.
3Department of Pharmacy Practice, Faculty of Pharmacy, Horus University, Dominate City, Egypt.
4Department of Clinical Pharmacy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.
*Correspondence: nevenabdoo@azhar.edu.eg.

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Abstract: Silymarin ‘milk thistle’ (Silybum marianum) plant, has been used for years for treatment of different diseases such as liver and gallbladder disorders, protecting liver against snake bite and insect stings, mushroom poisoning and alcohol abuse. Silymarin has antioxidant, anti-lipid peroxidative, antifibrotic, anti-inflammatory, immunomodulatory, and liver regeneration activities. The cardioprotective activities of silymarin were primarily shown in cisplatin-induced cardiotoxicity in rat models. These effects are due to replenishing endogenous antioxidant enzymes, suppressing neutrophil infiltration, and reducing serum malondialdehyde as the end product of myocardial lipid peroxide. Silymarin treatment protects against reperfusion damage and inflammation by confirming anti-inflammatory and antioxidant actions. Silymarin antioxidant properties is considered responsible for its cardio-protective activities. The mechanisms by which silymarin protects the heart remain largely unexplored. In this review, we will discuss in detail the cardioprotective properties of silymarin.

Keywords: Silymarin; Heart; Cardiotoxicity; Antioxidant; Chemotherapy.

1. INTRODUCTION

Cardiovascular diseases (CVD) are the main cause of death and morbidity worldwide 1. CVDs are a category of cardiovascular disorders, including heart disease, stroke, rheumatic heart disease, and other conditions. Five CVD deaths are caused by heart attacks and strokes, and one-third of these deaths occur prematurely in people under 70 years of age 2. Substantial evidence suggests that elevated oxidative stress plays a major role in the pathogenesis of cardiovascular disease, including atherosclerosis, hypertension, vascular endothelial dysfunction, and ischemic heart disease. Cellular oxidative stress results in endothelial cells and vascular smooth muscle cells, releasing toxic free radicals that interfere with cell components such as proteins, DNA, or lipids, contributing to cardiovascular pathology 3. Since the beginning of human civilization, plants have been used to alleviate human distress, and plants have been used for medicinal purposes for approximately thousands of years. Natural bioactive compounds called phytochemicals are obtained from 3. Silymarin is considered the most promising cardioprotective agent. Silymarin has antioxidant anti-cardiovascular disease activities and offers protection against oxidative stress-induced hypertension, atherosclerosis, and cardiac toxicity 4. In this review, the protective effect of silymarin and its mechanisms of prevention in cardiotoxicity will be discussed.

2. Silymarin morphology and structure

Modern alternative medicine is one of the most popular examples. Isolated Silybum marianum L, Germany Herb, also known as Mary Thistle and Mariendistel in English (Figure 1). Milk thistle is a family of Asteraceae (Compositae), one of the largest plant families. Initially as wild plants, it only grew in Europe and Asia, but are now widely grown and used for medicinal purposes and for food crops in North and South America 5.
Silylinn has five major compounds including four flavonolignans: silibinin (silybin A, silybin B, isosilybin B and A), silychristine, isosilichristine, silydianine and one flavonoid, taxifoline and silymarine. (Figure 2).

Figure (2): Structure of silymarin (silybin A, B) 

Milk thistle has been used in Europe for decades for hepatic and biliary disorders. It is used as an antidote to the toxicity of Amanita mushrooms and as an anti-drug protection for the liver and renal. Dyspeptic disorders, hepatic cirrhosis caused by toxins and hepatic therapy are advised by the German Commission E.

3. Absorption and metabolism of silibinine

Silymarin (SM) has already been thoroughly examined for its metabolism and bioavailability with its major silibinin sources. Silibinin was shown to be comparatively low after oral consumption, for example, in rats, it is only approximately 0.95%. The oral administration of the standardized milk thistle extract Legalon, which lasted 6 h for half-life for silibinine, quickly absorbed and removed Flavonolignan. Conjugation has been reported for silybin and derivatives as the main biotransformation route. Ironically, the body recognizes silibinin as a foreign product and easily metabolizes other flavonoids with phase II enzymes. Oral silibinin intake was correlated with substantial increases in both liver, heart, stomach, skin and small bowel function, Glutathione S-transferase (GST) and quinone reductase (QR) activity, both in glutathione. Silibinin was found in conjugated form in the systemic circulation. Indeed, after oral administration of SM, it has been found that 10 to 16% of total silibinin in plasma has no conjugation with healthy volunteers. In addition, it has been demonstrated that mono-, di- and sulpho-glucuronides shape and 31 metabolites described. In fact, both free and conjugated forms of silibinin in humans and rats are quickly eliminated, with a mean elimination half-life of 6.32 h. Therefore, the key component of SM after oral silibinin intake, comparatively low quality, strong metabolism, and plasma concentration, is primarily within the range of nano-molars and exceeds micro-molar levels in certain cases only.

4. Antioxidant properties of silymarin (SM)

Silymarin contributes to antioxidant defenses in various ways. First, by direct radical scavenging. Second, by preventing the free formation of radical materials by inhibiting specific enzymes, producing free radical materials, or maintaining integrity in stress conditions in the mitochondrial electron transportation chain. Third, it contributes mainly to the preservation of the cell’s optimal redox state by triggering several antioxidant enzymes and non-enzymatic antioxidants, including Nrf-2 and NF-SB. Finally, the activation of various defensive molecular vitagens responsible for synthesis, such as HSP, thioredoxin (Trx), sirtuins and additional stress safety.

5. Direct free radical scavenging

For the formation of reactive oxygen species (ROS) and eicosanoids, silibinin was studied on human platelets, white blood, and endothelial cells. Silibinin was a strong HOCI scavenger (IC50 7 μM), but not human granulocyte O2· (IC50 > 200 μM). The dose dependent production inhibitors for O2· and NOs in isolated rats have also been reported to occur with an IC50 of 80 μM. Silibinin has not been able to scavenge super oxides but has reduced oxidation by low-density lipoprotein (LDL) (after 20 μM) significantly. The levels of silybin are regulated by diffusion (1,8 to 1010 dm / mol / s), showing that silybin is a good free radical scavenger. Indeed, in Aβ–42 neurons stressed, Silibinine (2.5 μM) significantly reduced H2O2 levels and prevented injuries in oxidation. In addition, defensive function was also found in the model DNA mitigation system in silybin (10 μM or higher).

Different experiments have observed antioxidant activity (equivalent to 62 μM silybin). For example, IC50 was 38 μM for H2O2 and 266 μM for NO. Linolealic acid emulsions were prevented from fat peroxidations by SM by 82.7 percent while BHA, BHT, alpha-tocopherol, and trolox blocked linolenic acid.
acid peroxidation by 83.3, 82.1, 68.1 and 61.3 percent, respectively. In a further analysis in vitro, the free radical scavenging and antioxidant properties of SM were demonstrated by four distinct tests (> 200 μM). It should also be noted that the free radical scavenging behaviors, which are 2 to 10 times stronger than silibinine and SM significantly, are 8 times stronger than silibinine and free radical scavengers, are substantially different between pure SM and silydianin compounds.

6. Protective effects of silybin on mitochondria

The major user of oxygen in the cell is mitochondria, which is rich in redox enzymes that can convert lone pair electrons to oxygen generating the ROS superoxide (O$_2^-$). The mitochondrial enzymes that produce ROS include the tricarboxylic acid (TCA) cycle enzymes aconitase and α-ketoglutarate dehydrogenase; the electron-transport chain (ETC) complexes I, II and III; pyruvate dehydrogenase and glycerol-3-phosphate dehydrogenase; dihydroorotate dehydrogenase; the monoamine oxidases (MAO) A and B; and cytochrome b5 reductase.

In addition, mitochondrial offenses, such as oxidative damage, can lead to a net imbalance in ROS production from growth to disposal. For example, ROS can lead in the event of mitochondrial dysfunction, change the protein, lipid peroxide and damage DNA. The harmful effects of ROS are reported in many studies. However, it is now apparent that mitochondrial-generated ROS also control the transduction of intracellular signals that help change cell stress.

One of the mechanisms responsible for reducing oxidative stress is the useful role of mitochondrial SM/silibinine in structure and function. In addition, with the activation of pro-survival cell signals, SM protects mitochondria from disease. For example, silibinine has increased the electron transport network, reduced electron leakage and ROS output and decreased ROS with explicit input. A significant loss of mitochondrial bioenergy compared to the control group was observed in the rats under ischemic / reperfusion (I/R). Significant advances were prevented by SM in mitochondrial IC/R (lower ATPs, membrane potential and respiratory diseases) and cell dysfunction causing these diseases.

7. Inhibition of free-radical producing enzymes by silybin

7.1. Xanthine Oxidase (XO)

Heart attack, stroke, spinal trauma, myocardial or kidney hypoxia and infarction disturbance are the main causes of free radicals and oxidative stress. In the steady state, XO activation is often independent of silibinine concentration and can influence XO conduct. When the system of xanthin / xanthin oxidase was involved in O$_2^-$ production, silybin inhibited the production of uric acid with a 32.2 μM IC50.

7.2. NADPH Oxidase

The ROS generation NADPH oxidase complex plays a key role in host protection, modification of posttransduction proteins, cell distinction and gene expression regulation. Silybin and its derivatives block NADPH oxidase activity in activated concentration-dependent cell lysate in PMA. Out of 5,7,4′ trimethylsilybin and 10 μM final silybin, 50 % of NADPH oxidase's activity appears to be inhibited. The effect of silybin in mouse podocytes and OVE26 mouse was investigated in model type 1 diabetes mellitus and diabetic nephropathy. Glucose exposure Podocytes increased the production of intracellular superoxide by 60%, NADPH oxidase activity by 90%, NOx4 expression by 100% and the number of apoptotic cells by 150%.

8. Physiological roles of cardiac redox signaling pathways

8.1. Differentiation and proliferation

A crucial differentiation and proliferation regulator for several cell types is cellular redox balance, including cardiomyocytes. Nevertheless, the percentage of cardiomyocytes beating embryos associated with increased intracellular ROS increases both mechanical and electrical stimulation. On the other hand, the formation of cardiomyocytes is influenced by agents that scavenge or decrease ROS. The NADPH oxidases ROS family Nox are recommended as second messengers for cell growth and differentiation control. In particular, the downregulation of Nox4 inhibited differentiation of the cardiomyocyte, the dominant Nox isoform expressed in the early stages of differentiation in embryonic stem cells and was remedied by a weak H$_2$O$_2$ pulse. In addition, ROS-dependent signaling pathways include activation of the myocyte enhancer factor of mitogen activated protein kinase (MAPK).
p38 and nuclear translocation (MEF2C)\textsuperscript{38}. Phosphatidylinositol 3 kinases (PI-3-kinases), which are also apparently involved in the control of intracellular redox status and in the cardiomyocytes of embryonic stem cells, are de-regulated as well and cardiac involvement is abolished in embryos because PI-3 kinase inhibitors Ly294002 and wortmannin; PI-3-kinase is also a crucial downstream effector for β1 integrated signaling\textsuperscript{39}.

8.2. Coupling of the excitation contraction

The excitation-contraction pair (ECC) is the key mechanism for transforming electrical activity into a heart contraction. ECC events have been well designed and mainly depend on intracellular Ca\textsuperscript{2+} levels through the participation of Ca\textsuperscript{2+} channels and carriers. Depolarization of the action potentials stimulates membrane and T-tubular L-type Ca\textsuperscript{2+} channels. The subsequent injection of Ca\textsuperscript{2+} in limited amounts leads to a substantial increase in the calcium release of [Ca\textsuperscript{2+}] in dyadic space (T-tubular area and sarcoplasmic [SR]) by the release channel SR Ca\textsuperscript{2+} (ryanodine [ryR2]), resulting in a much higher amount of Ca\textsuperscript{2+} by SR. The addition of Ca\textsuperscript{2+} to troponin C causes actomyosin cross-bridge rotation and contraction\textsuperscript{40}.

Ca\textsuperscript{2+} is distributed from the cytoplasm, following cytoplasmic and mitochondrial ion changes, by SR Ca-ATPase (SERCA) and other sarcolemmal ion pumps, including sodium calcium exchangers (NCX). Redox-sensitive targets and protein modifications are various ECC cardiomyocyte devices, and include disulfide thresholds, thiol nitrosylation and glutathiolation, tyrosine nitrate, and phosphorylation\textsuperscript{41}. The first modifications include redox-activated protein kinases and later changes. Protein kinase A (PKA), for example, is recognized as the two catalytic tetramer and two regulatory sub-units (RI and RII)\textsuperscript{42}.

9. Silymarin as a cardioprotective agent in chemotherapy-induced cardiotoxicity

9.1. Against Doxorubicin

Doxorubicin (DOX) is the active chemotherapy agent. However, the adverse consequences are oxidative stress, mitochondrial dysfunction, and apoptosis cardiotoxicity\textsuperscript{43}. DOX-induced biochemical and histopathological changes have been found to be reversed by SM (50 mg / kg / BW) or covered. Protection tests in the SM and prevention trials were also demonstrated to reduce the stress of carbohydrate DOX, phosphokinase (CPK), LDH, creatinine, urea, myocardial MDA, MDA, and GSH\textsuperscript{44}. In rats treated with DOX by MDA fragmentation and DNA SM (16 mg / kg / BW) decreased significantly even after oxidative stress\textsuperscript{45}. The prevention of increased ADT and CK serum of SM in heart and liver tissues (60 mg / kg / BW) against DOX toxicity\textsuperscript{4} (Figure 3).

Figure (3): Cardioprotective effect of silymarin on dox-induced cardiotoxicity\textsuperscript{5}.

9.2. Against Cisplatin

Cisplatin is an effective chemical therapy medicine for different cancers. Cisplatin and steady tumors resistant to other treatment regimens can be managed by many tumors, including testicular, breast, ovarian, prostate and lung cancers. Studies have shown that cardiotoxicity is typically consistent with cisplatin treatment like arrhythmias, cardiomyopathy, and congestive heart failure. It has been shown that oxidative stress and apoptosis play an important role in cisplatin-induced cardiotoxicity that restricts the clinical use of this drug\textsuperscript{46}. Silymarin (100 mg / kg orally, over a period of ten days) was used as an anti-oxidant per oxidant to protect the heart from myocardial damage caused by cisplatin by reducing serum biochemical marker activity, including LDH and CK-MB\textsuperscript{47}. Silymarin then made the heart membranes stable and stopped the leakage of the heart enzyme. Lipid peroxidation was also inhibited by silymarin because free hydroxyl groups react with peroxide radicals at C5 and C7. In addition, silymarin increased the activity of endogenous antioxidant enzymes such as SOD\textsuperscript{48}. It has contributed to a significant increase in the protection of cellular antioxidants. Silymarin reduced damage to oxidative mitochondrial DNA caused by the free radical scavenging process\textsuperscript{49}. Silymarin can therefore become a potential therapeutic agent to prevent the cardiotoxic effects of anti-cancer drugs mainly cis-platin and doxorubicin.
10. Molecular mechanism of silymarin cardioprotective activities

Following occlusion of the blood supply, ischemic tissue will eventually die by necrosis. It follows that reperfusion became the main form of intervention for myocardial infarction. This led to the discovery of ischemia reperfusion (IR) injury of the heart, due to prolonged period of ischemia, blood supply is restored to the ischemic tissue, paradoxically causing a rise in cell death. This is proposed to occur because the kick-starting of respiration, in cells where most of the ion gradients have all but collapsed, sets up the perfect conditions for the opening of the mitochondrial permeability transition pore (mPTP) and the subsequent induction of apoptosis. In accordance with this model, ischemic cells rapidly become hypoxic and switch to glycolysis for their source of adenosine triphosphate (ATP) hence becoming acidified. At the same time, levels of reactive oxygen species (ROS) increase, and levels of ATP drop along with the activity of the Na⁺ /K⁺ ATPase. Due to the increased proton concentration, i.e. intracellular acidification and reduced activity of the Na⁺ /K⁺ ATPase, the Na⁺ /H⁺ "exchanger causes an influx of Na⁺. This reverses ion-flux through the Na⁺ /Ca²⁺ antiporter, increasing the intracellular concentration of Ca²⁺. Under normal conditions this increase in ROS and Ca²⁺ would be sufficient to open the mPTP and induce apoptosis, however, as low pH inhibits mPTP opening, apoptosis does not occur in ischemic cells. Instead, the damage occurs upon reperfusion, when the mitochondrial pH begins to normalise with the restoration of the mitochondrial H⁺ gradient and all the conditions for the opening of the mPTP have been met. Pre- and post-conditioning, must therefore function either by reducing calcium concentrations in the cells, limiting over-production or accumulation of ROS or increasing the mPTP threshold. Silymarin as a multicomponent extract has a single molecular target. This is exemplified by studies whose findings highlight quercetin's ability to protect various tissue types against IR Injury. As quercetin and taxifolin are relatively minor components of silymarin, the importance of their contribution to preconditioning by silybin remains unclear but may be attributed to silybin strong ROS scavenging properties.

11. Cardioprotective effects of silymarin in animal studies

Several studies have shown the cardioprotective properties of silymarin during the animal stage. Rao et al. proposed cardio protection in ischemic myocardial infarction in rats of the phytochemical silymarin. Silymarin was administrated to Wistar albino rats by gastric gavage for a week in three different dose doses orally (100 mg / kg, 250 mg / kg and 500 mg / kg). At the end of this time test, the silymarin-treated groups were exposed to the anterior left coronary artery and reperfused to 4 H for a 30-minute occlusion. This period is done. The reduction in mean blood pressure and cardiac rate at the end of reperfusion is covered by the silymarin-rat heart. Research by Rašković et al. reported that silibin-rich silymarin, caused by doxorubicin, has a possible cardioprotective and hepatoprotective effect at a dose of 60 mg / kg by mouth for an oral 12-days dose. The in vivo model investigated whether silymarin could prevent intraperitoneal damage of doxorubicin for twelve days to the liver and cardiac tissue. The analysis investigated an increase in weight, ECG shifts, biochemical parameters of oxidative stress, production of alanine and aspartate serum, lactate dehydrogenase, creatine kinase and cardiovascular histological and liver preparation animal samples. Silymarin was tested for its protective impact on both the heart and tissue against doxorubicin-driven toxicity based on physiologic, pharmacological, microscopic, and biochemical findings. Cecen et al have studied the doxorubicin’s effect on rat kidney, heart and liver toxicity. A single dose of 10 mg / kg of doxorubicin was given intraperitoneally in doxorubicin (i.p). Silymarin (100mg / kg) has been given on a regular basis. For five days, silymarin and doxorubicin was injected with 100 mg / kg i.p before doxorubicin (10 mg / kg single ip injection) and continued to be regularly euthanized thereafter. Eight animals from each party were decapitated on the seventh day after doxorubicin injection, and heart and liver samples were taken. Every other day, the remaining eight animals in each party received silymarin before euthanized within the first 20 days. Serum dismutase (SOD), glutathione peroxidase (GSHPx), catalase (CAT), malondialdehyde (MDA), creatinine, urea, AST, ALT, lactate dehydrogenase (LDH), and creatinine phosphokinase (PK) are isolated for the determination of superoxide dismutase. Microscopic analyses of the histopathological and electron and have also been performed from parts of the heart, kidney, and liver. Doxorubicin caused substantial changes in NO serum levels relative to controls. These have been lowered by silymarin's pretreatment community. Doxorubicin was shown to cause myocardial and renal damage, as shown by histopathological and electron microscopic studies on the kidneys, heart and liver, for silymarin rats.
The results of this study showed that silymarins significantly prevented doxorubicin-induced toxicity to rat kidneys, heart, and liver, indicating the use of silymarins as a supporting agent during treatment with doxorubicin anti-cancer 55. Work by Kumaş et al. on the safety effect of silymarin on heart damage due to a large dose of isotretinoin (ISR) histopathologically and biochemically. Thirty-two mice Blab / c divided into four groups: electricity, ISR, SM and ISR + SM. Masson hematoxyloseine and trichrome were treated for histopathological treatment in both sections. Biologically defined oxidative stress markers were catalas (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione reductase (GSH red) , S-specific transfere glutathione (GST) , lipid peroxidation (E-MDA) and plasma malondialdehyde (PMDA). The behavior of the ISR + SM antioxidant (p=0.015, p=0.05, p=0.002 and p=0.018 was the cause. EMDA (p=0.003) and P-MDA (p=0.005) concentrations within the ISR group have increased significantly and decreased in the SM group. SM, thus improved antioxidant production stopped, but oxidation and decreased apoptosis. SM is an antioxidant caused by ISR cardiovascular oxidative damage 56.

12. Cardioprotective effect of silymarin in human studies

Cardioprotective effect of silymarin is a promising point of research since there is a lack of clinical studies in this topic. Randomized controlled trial by Zalat et al aimed to investigate the benefits of the addition of l-carnitine / silymarin to anthracycline chemotherapy in patients with breast cancer since 83 patients were recruited from Clinical Oncology Department, Tanta University, Egypt, then prospectively randomized to receive their anthracycline-containing therapeutic regimen, control group (n=33), or anthracycline plus l-carnitine, l-carnitine group (n=25), or anthracycline plus silymarin, silymarin group (n= 25). Blood samples were collected at the beginning and after 6 months to measure LDH, CK-MB, cTn I, Anticardiolipin IgG, Fe, ferritin, and TIBC and % of saturation. % EF was documented. Data were statistically analyzed by ANOVA and paired t-test. And the results demonstrated that, the supplementation with silymarin to anthracycline chemotherapy had a statistically significant decrease in Anticardiolipin IgG (P=0.000), iron (P=0.001), ferritin (P= 0.001), TIBC (P=0.007), and % saturation (P=0.001), so they concluded that co-administration of silymarin with anthracycline chemotherapy represents a new therapeutic strategy for better control of anthracycline-induced cardiotoxicity 57.

Another study by Mehdi Ghaderian et al aimed to evaluate the protective effects of SM on hepatotoxicity and cardiotoxicity of chemotherapy drugs in leukemic children. In this study, 71 children, aging 2-14 years old, going through the maintenance phase of acute lymphoblastic leukemia (ALL) treatment at Children's Cancer Center of Isfahan University of Medical Sciences (Iran) in 2015 were studied. The patients were divided into intervention and control groups. Intervention group received Mercaptopurin and Methotrexate plus Silymarin 140mg/day and control group were taking only Mercaptopurin and Methotrexate. Liver enzymes, Coagulation tests, Creatine kinase- Muscle and Brain (CK-MB), Ejection fraction and Systolic function (SF) were measured at the first, and 3- and 6-months’ intervals from the commencement of study. Changes in CK-MB were not significant in intervention and controls (P=0.07, 0.10, respectively) but ejection fraction and (shortening fraction) SF decreased in both groups, especially among controls. They concluded that silymarin can be useful in prevention of hepatotoxicity and cardiotoxicity of chemotherapy drugs for ALL children 58.

13. CONCLUSIONS

In conclusion, through several possible mechanisms, SM can improve the body’s antioxidant protection mechanisms. First of all, extreme, free radical scavenging are often successful. Second, it is important to prevent free radical formation by inhibiting the different enzymes produced by ROS or by improving the integrity of the mitochondrial transmission chain in stressful circumstances caused by SM consumption. Third, SM's AO action is likely primarily driving to ensure the optimal redox balance of the cells by enabling a number of antioxidants and non-enzymatic antioxidants, mainly by the activation of Nrf2. Fourth, cardioprotective effects of silymarin is reported on chemotherapeutics such as doxorubicin and cisplatin. Finally, silymarin and its components influence the signaling pathways involved. Silymarin therefore has a promising cardioprotective effect.

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REFERENCES


https://aijpms.journals.ekb.eg/


43. Saleh D, Abdelbaset M, Hassan A, Sharaf O,


