Protective effect of silymarin against chemical-induced cardiotoxicity

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Abstract
Cardiac disorders remain one of the most important causes of death in the world. Oxidative stress has been suggested as one of the molecular mechanisms involved in drug-induced cardiac toxicity. Recently, several natural products have been utilized in different studies with the aim to protect the progression of oxidative stress-induced cardiac disorders. There is a large body of evidence that administration of antioxidants may be useful in ameliorating cardiac toxicity. Silymarin, a polyphenolic flavonoid has been shown to have utility in several cardiovascular disorders. In this review, various studies in scientific databases regarding the preventive effects of silymarin against cardiotoxicity induced by chemicals were introduced. Although there are many studies representing the valuable effects of silymarin in different diseases, the number of researches relating to the possible cardiac protective effects of silymarin against drugs induced toxicity is rather limited. Results of these studies show that silymarin has a broad spectrum of cardiac protective activity against toxicity induced by some chemicals including metals, environmental pollutants, oxidative agents and anticancer drugs. Further studies are needed to establish the utility of silymarin in protection against cardiac toxicity.

Keywords: Cardiotoxicity, Metals, Oxidative stress, Silybum marianum, Silymarin

Introduction

Milk thistle (Silybum marianum) is the most ancient and broadly used therapeutic plant for its useful effects on liver and other organs (1). This plant is native to the Mediterranean and grows throughout Europe and North America. It is also cultivated in China, India, South America, Africa, Iran and Australia (2).

Silymarin, a polyphenolic flavonoid, is isolated from the milk thistle (3). Silymarin is a combination of some bioflavonoids found in the fruit, seeds and leaves of this plant including silybin, isosilybin, silydianin and silychristin (4).

Silybin, the most common constituent and the main active phytochemical of the silymarin complex (50-60% of silymarin) is responsible for the beneficial properties of the silymarin (5). It is orally absorbed, but due to its low water solubility, poor bioavailability has been observed (5). Besides the antioxidant effect (6), silymarin indicates effective anti-inflammatory (7), antifibrotic (4), antineoplastic (8), immunomodulating (9) and membrane stabilizing (10) properties in different animal and human studies. Furthermore, according to the literature, protective effects of silymarin in different tissues including brain (11), heart (9), liver (12), kidney (13, 14), lung (15), pancreas (16) and skin (17) have been reported against some toxic materials and different disorders. It is established that silymarin has been utilized medicinally to cure liver diseases including viral hepatitis, cirrhosis and alcoholic liver disorders (18). Inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane; increase in the level of reduced glutathione in the liver, stimulatory effect on ribosomal RNA polymerase and finally protein synthesis leading to increased hepatocyte regeneration, are considered as its hepatoprotective mechanisms of action (5). In addition to hepatoprotective activity, the other organ protective effect could be related to its antioxidant activity via free radical scavenging and increasing endogenous antioxidant defense such as reduced glutathione (19).

Based on pharmacological studies, silymarin has been established as a safe herbal product. Animal studies indicated that silymarin is nontoxic. It has been shown that silymarin is not teratogen and had no post-mortem toxicity (4). Some adverse effects including gastroenteritis, headache and dermatological symptoms have been reported. Among them gastrointestinal symptoms are the most prevalent (20).

Heart disease remains one of the most important causes of death in the world (21), and the quest for
new treatment options has recently directed the attention to herbal therapy because of the safety, efficacy and cultural acceptability (22). According to several important documents, administration of antioxidants may be useful in ameliorating drug-induced cardiac toxicity (4, 6, 9, 10, 23, 24).

Silymarin, as a potent antioxidant, has been shown to have utility in several heart disorders (9, 21). Hence, in this review, different in vivo and in vitro studies in scientific databases regarding the protective effect of silymarin against cardiotoxicity induced by chemicals were discussed. According to the literature these chemicals include metals, environmental pollutants, oxidative agents and anticancer drugs.

Metals
Iron
Tissue iron deposition may induce organ dysfunction. Cardiac iron deposition is the leading cause of death in the patients with sickle cell disease and thalassemia, possibly due to cell apoptosis (25, 26). It is reported that silybin has an important role in the chelation therapy of chronic iron overload, as occurs in the treatment of Cooley's anemia (Beta thalassemia major). The polyphenol structure of silymarin allows both the scavenging of free radicals, with concomitant formation of fairly stable aroxyl radicals and the chelation of transition metals, including iron (27, 28).

The protective effect of silymarin on iron overload-induced hepatotoxicity has been shown (29). In another study, the effect of deferoxamine (a synthetic iron chelator) and silymarin against heart iron deposition in an iron overload rat model (100 mg/kg every other day for two weeks) were investigated. Results showed that the serum levels of ferritin and malondialdehyde (MDA) in silymarin or deferoxamine group were less than those that received combination of these agents. Furthermore, co-administration of silymarin and deferoxamine did not attenuate the intensity of iron deposition in heart probably due to the pharmacokinetic interaction. The results confirmed that silymarin and deferoxamine may reduce the level of iron and oxidative stress in the subjects (30).

Although silymarin is well known as antioxidant, it may paradoxically affect cells by inducing intracellular oxidative stress. Silymarin reduce Fe (III) to Fe (II) before making iron complexes through Fenton type reactions with production of hydroxyl radicals, or Haber–Weiss reactions with superoxide anions. It can exert prooxidant effects, by generation of oxygen radicals in the presence of metal ions and inducing lipid peroxidation, protein modification and DNA damage (26, 27). It seems that treatment of iron overload by silymarin is challenge to physicians that exposes them to great dilemma regarding handling of problem.

Arsenic (As)
Inorganic arsenic is a naturally occurring toxic metalloid. The important sources of arsenic exposure are contaminated drinking water and food. Approximately 100 million people in the world exposed to arsenic at levels above 50 µg/l (31). Chronic arsenic exposure induces ROS mediated oxidative stress and plays an important role in the pathogenesis of cardiac toxicity, which is associated with myocardial injury, cardiac arrhythmias and cardiomyopathy (32, 33). arsenic-induced ROS generation causes lipid peroxidation, enzymes inactivation and DNA damage in the heart tissue (34).

It has been demonstrated that silibinin (75 mg/kg/day, 4 week, orally) attenuated arsenic-induced cardiotoxicity and dyslipidemia in rats. Muthumani and Milton Prabu (2014) showed that consumption of sodium arsenite (5 mg/kg, orally) for 4 weeks could induce cardiotoxicity as evidenced by increasing the activity of serum cardiac markers, such as creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH). Moreover, mitochondrial enzymes activities such as isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), α-ketoglutarate dehydrogenase (α-KDH) and NAD dehydrogenase were decreased in arsenic-intoxicated rats. Reduction in cardiac SOD activity and glutathione content in arsenic exposed animals were also observed. Arsenic disturbed the mitochondrial phospholipid bilayer which leads to an elevation in lipid peroxidation. Arsenic decreased the level of cardioliopin via the increased level of lipid peroxidation in heart mitochondria. Plasma total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) significantly increased in the arsenic treated rats followed by a significant decrease in the high density lipoprotein (HDL). These alterations in lipid profile are indication of arsenic-induced hyperlipidemia. A significant decrease in the activity of Na+/K+-ATPase and Mg2+ ATPase and a significant increase in the activity of Ca2+ ATPase in the heart were observed in arsenic-treated rats. Besides, arsenic up-regulated myocardial NADPH (NOX) oxidase sub units such as NOX2 and NOX4 and down-regulated of Nrf2 and HO-1 (heme oxygenase 1) protein expressions. Nrf2 is found to have an important role in protecting the cell against oxidative stress. Silibinin, activates Nrf2 resulting in the activation of antioxidant response elements within the cells such as HO-1 (heme oxygenase 1) and SOD (superoxide dismutase). Therefore, Nrf2 activators such as silibinin are useful in protecting oxidative cardiotoxicity induced by arsenic. Also, arsenic...
induced histopathological changes in the cardiac tissue such as necrosis, mononuclear inflammatory cell infiltration, myofibroblast derangement and hemorrhage (10). Arsenic-intoxicated rats demonstrated swelling of heart mitochondria together with loss of cristae, irregular shape and size. Silybin remarkably improved all these altered markers and abnormalities (10). As a result, silybin could protect the Arsenic-induced free radicals in mitochondria and increased the amount of NADPH due to its free radical scavenging activity of hydroxyl and methoxy groups and facilitates the dismutation of arsenic-induced free radicals because of the presence of C=O in silybin structure. The up-regulation of Nrf2 expression and the prevention of lipid peroxidation are also involved in the mechanisms of silybin protection against arsenic-induced cardiotoxicity.

**Oxidative agents**

**Copper-ascorbate**

Copper-ascorbate produces reactive oxygen species (ROS) via the following reaction:

\[
\text{Cu}^{2+} + \text{ascorbic acid} \rightarrow \text{Cu}^{+} + \text{dehydroascorbic acid} + \text{H}_2\text{O}_2 (\text{hydrogen peroxide})
\]

\[
\text{Cu}^{2+} + \text{O}_2 \rightarrow \text{Cu}^{2+} + \text{O}_2^- + \text{e}^-
\]

\[
\text{Cu}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{4+} + \text{O}_2 + \text{H}_2\text{O}
\]

According to a study by Dutta et al. (2014), silymarin was used as a protective agent in mitochondrial oxidative stress due to presence of copper-ascorbate. In this study, copper-ascorbate was acted as an inducer of oxidative stress in goat heart mitochondria. It was indicated that incubation of isolated goat heart mitochondrial with copper-ascorbate (0.2 mM Cu²⁺ and 1 mM ascorbic acid for 1 hr) increased the levels of MDA and protein carbonylation of the mitochondrial membrane, reduced the content of mitochondrial glutathione and altered the status of antioxidant enzymes. Moreover, copper-ascorbate reduced the activity of Kreb's cycle enzymes and disturbed mitochondrial morphology. Co-incubation with silymarin (0.05-0.50 mg/ml) ameliorated all cardiac mitochondria damages induced by copper-ascorbate. It seems that silymarin may protect against copper-ascorbate induced mitochondrial oxidative stress through different mechanisms including improvement the alteration in mitochondrial TCA cycles and respiratory chain enzyme activities, reduction of mitochondrial swelling and mitochondrial NO concentration as well as mitochondrial dityrosine level as a marker of protein oxidation (35).

**Sodium fluoride**

Fluoride (F) is usually distributed in the environment in various forms. Fluoride anions naturally exist in water sources and drinking water. F is released from the runoff of F containing rocks and soils and leak into groundwater. In some places, drinking water is the largest supplier to daily F intake. Moreover, F is found in various insecticide formulations, fluoridated food stuffs, dentifrices, drugs and vapors emitted from industries using F containing compounds (36). Increased F concentration accumulates in different soft tissues and organs, such as the heart. It was indicated that experimental fluorosis causes changes in electrocardiogram such as P-Q interval prolongation and sinus bradycardia due to hypofunction of the thyroid gland. Also, mean duration and amplitude of the T wave were higher and longer in chronic fluorosis as a result of a decrease in the level of blood potassium. Moreover, chronic fluorosis induces severe pathological damages in the heart of animals including degeneration and disruption of cardiac myofibers, wide cardiac fibrillar spaces, cardiac inflammation and marked necrosis (37, 38). ROS play an important role in the pathogenesis of myocardial tissue damage induced by F (23, 39). Furthermore, it has been reported that some cardiac abnormalities including atherosclerosis and hypertension are related to the chronic exposure to F. It was reported that expression of some inflammatory genes including vascular cell adhesion molecule-1 (VCAM-1), P-selectin, monocyte chemotactic protein-1 (MCP-1), IL-8, and IL-6 at the RNA and protein levels were increased following chronic F exposure (40). As long term F exposure produces oxidative stress in cardiac tissue; therefore antioxidants could protect myocardial damage induced by F (39). It was indicated that silymarin can improve damages induced by sodium fluoride in rat cardiac tissue via its antioxidant activity. Silymarin (10 and 20 mg/kg/day), one week prior to sodium fluoride (600 ppm through drinking water, 1 week) could decrease MDA level, increase SOD and catalase activities and reduce GSH content in heart tissues of rats treated by sodium fluoride. Vitamin C (10 mg/kg/day) was used as a positive control. The effect of silymarin (20 mg/Kg) was similar to that of vitamin C (39). According to the results of this study, silymarin-rich foods can prevent cardiac tissues from fluoride-induced oxidative stress which needs further studies to understand the exact mechanism of action.

**H₂O₂-Phenylephrine**

Recent in vitro and in vivo studies indicate that reactive oxygen species involve in the hypertrophic response. Increase of heart mass (cardiac hypertrophy) is considered as an important adaptive reaction for maintaining or increasing heart hemodynamic output in conditions such as arterial hypertension and familial hypertrophic cardiomyopathy which is a requirement for increased workload (41, 42). Chronic hypertrophy can accordingly lead to heart failure that is the important cause of morbidity and mortality in many countries. Besides mechanical stress, some stimulatory
mechanisms that are associated with cardiac hypertrophy include stimulation via α1-adrenergic agonists, endothelin-1 (ET-1) and angiotensin II (AngII), as well as peptidic growth factors (epidermal growth factor, insulin growth factor, fibroblast growth factor) (41, 43).

Recently, several lines of evidence suggested that oxidative stress plays an important role in pathologic cardiac hypertrophy. So, inhibition of the increased ROS generation could be considered as a promising therapeutic approach for treating of cardiovascular diseases including cardiac hypertrophy (44). Phenylephrine is an α1-adrenergic drug which can induce cardiac hypertrophy. It is well known that activation of the several signaling pathways including ERK/MAPK and the PI3K/Akt are involved in the regulation and progression of cardiac hypertrophy. One study showed that silybinin is able to inhibit the development of cardiac hypertrophy in embryonic rat heart-derived H9c2 cells. Results showed that phenylephrine phosphorylated ERK1/2 after 15 and 30-min of incubation times, whereas silybinin co-incubation inhibited ERK1/2 phosphorylation. This study also revealed that although slight elevation in the Akt phosphorylation was observed in H9c2 cells following phenylephrine treatment, however, incubation with silybinin completely reversed this modest activation of Akt by phenylephrine in H9c2 cells (45). Therefore, Akt phosphorylation has a minor role in cardiac hypertrophy induced by phenylephrine.

As a result, silybinin could protect cardiac against hypertrophy induced by phenylephrine probably due to the inhibition of ERK1/2 and Akt phosphorylation (45).

H2O2, as a physiologically appropriate form of oxidative stress (46), stimulates H9c2 cells death as verified by decrease in the cell viability. Results indicated that increased cell viability was observed following preincubation with silybinin. Furthermore, it was demonstrated that silybinin pretreatment alleviated significantly apoptosis in H9c2 cells induced by H2O2. Results showed that silybinin prevented H9c2 cells from H2O2-induced oxidative stress and apoptotic cell death (45).

Isoproterenol

Isoproterenol is a beta-adrenergic agonist. Evidences have shown that beta-adrenergic agonists can induce apoptosis in cultured neonatal cardiac myocytes (47). So, it is suggested that myocardial cell injury in heart failure might be induced by this factor in animal studies. Several studies showed that silymarin could protect cardiomyocytes against isoproterenol induced cytotoxicity. According to a study by zhou et al, (2006), silybinin prevented isoproterenol-induced oxidative stress and apoptosis in rat cardiac myocytes. Silybinin (0.05-0.7 mM) was added 1 hour before 10 μM isoproterenol. Results showed that significant morphological changes were observed in rat cardiac myocytes treated by 10 μM isoproterenol for 48 hr. Most of cardiac myocytes exposed to isoproterenol had become round. However, injury effects were not observed in the cells of the control group and silybinin (0.5 mM) pre-treated group. In addition, silybinin significantly reduced LDH release and MDA production. Silybinin also reversed the increase in [Ca2+]i and increased mitochondrial membrane potential. This study showed that silybinin induced myocyte Bcl-2 protein expression, which prevents permeability transition pore opening, and therefore cytochrome c release reduced. These actions could be considered as one of the mechanisms of silybinin-mediated stabilization of the mitochondrial membrane. Furthermore, silybinin can up-regulate SIRT1. SIRT1, is a NAD+-dependent histone deacetylase. The increased level of SIRT1 might deacetylate Ku70 factor, a DNA repair factor, and subsequently prevents Bax from moving to the mitochondria. So, silybinin inhibited the translocation of Bax from cytoplasm to mitochondria by up-regulation of SIRT1 (48).

In another study, the antiapoptotic effect of silymarin against isoproterenol-induced apoptosis and DNA damage in rat cardiac myocytes has been established. The increased NO and iNOS mRNA levels induced by isoproterenol, reduced after treatment by silybinin. The decrease of NO in silybinin-treated cardiomyocytes might be attributed to the activation of SOD. In addition, the increase of iNOS was obviously reversed by silybinin treatment, which could decrease the production of NO. Silybinin also down-regulated p53 phosphorylation and increased the expression of procaspase-3. In addition, silybinin inhibited the cleavage of inhibitor of caspase-activated DNase (ICAD) and poly-(ADP-ribose) polymerase (PARP) that lead to the cell survival. In conclusion, according to this study, caspase pathway and the expression of p53 are involved in silybinin protective effect against isoproterenol induced DNA damage in rat cardiac myocytes (49). It is reported that tyrosine kinase pathway is involved in the protective effect of silymarin against isoproterenol-induced cardiomyocyte toxicity. Results showed that isoproterenol (10 μmol/l, for 48 hr), reduced the protein expressions of Ras, Raf-1 and the adaptor protein, Grb2 whereas silybinin reversed their expression. Also, silybinin (0.05-0.5 mM) increased PKC activity which was attenuated by isoproterenol. This study revealed that silybinin protected isoproterenol-induced apoptosis in rat cardiac myocytes via the activation of PKC involving Ras, Raf-1 and the phosphorylation of ERK (50).

Taken together, according to the above mentioned studies, silybinin could protect rat cardiomyocytes against isoproterenol-induced
apoptosis through several mechanisms including the decrease of cytochrome c release from mitochondria, increasing the level of Bcl-2 protein, inhibition the translocation of Bax from cytoplasm to mitochondria and upregulation of SIRT1 (48). Siliibinin also down-regulated p33 phosphorylation, increased the expression of procaspase-3, inhibited the cleavages of ICAD and PARP (49), activated tyrosine kinase pathway and finally increased PKC activity and phosphorylated ERK (50).

**Environmental pollutant**

**Acrolein**

Acrolein, an ubiquitous environmental pollutant, has been utilized as an intermediate for production of some organic materials (51, 52). Incomplete burning of plastic, petrol, wood, gasoline and diesel fuel, paraffin wax, tobacco, and frying of foods in oils can produce acrolein (51, 52). Various acrolein levels (10 to 600 \( \mu \)g/kg) have been found in some foods including cheese, donuts, fish, bread, potatoes, and alcoholic beverages. It has been proved that acrolein is toxic for different tissues including heart via production of reactive oxygen species (51). The protective effect of silymarin (25, 50 and 100 mg/kg/day, IP) against cardiotoxicity induced by acrolein (7.5 mg/kg/day, gavage) was evaluated in mice. Treatments were continued for 3 weeks. Results showed that acrolein increased the levels of malondialdehyde (MDA) and decreased glutathione (GSH) content, superoxide dismutase (SOD), and catalase (CAT) activities in mice heart tissue. Serum cardiac markers such as troponin I (cTnI) and creatine kinase-MB (CK-MB) were increased in acrolein treated animals. Acrolein also induced abnormalities in normal mice heart structure. Pretreatment by silymarin improved the changes induced by acrolein. Furthermore, silymarin reduced cardiac histopathological damages. Results also revealed that acrolein induced apoptosis in mice heart via increasing Bax/Bcl-2 ratio, cytosolic cytochrome c content, and cleaved caspase-3 level. Silymarin also inhibited apoptosis induced by acrolein (9). Based on these results silymarin could be considered as a potent protective agent against some environmental pollutants like acrolein through alleviating oxidative stress and anti-apoptotic properties.

**Anticancer drugs**

The cytotoxic agents and drugs that used to treat cancer could affect the cardiovascular system (53). Cardiotoxicity is one of the most important cancer treatment adverse effects and is responsible for noticeable morbidity and mortality. The most prevalent and severe adverse effects of chemotherapeutic agents on the cardiovascular system are heart failure with ventricular systolic dysfunction. Other toxic effects are hypertension, thromboembolic disease, arrhythmias and myocardial ischemia (53). The best examples of cardiotoxicity of anticancer treatment are anthracycline-related cardiomyopathy, which induce permanent damage at the cellular level (54). Cardiotoxicity has limited the clinical use of these drugs (53, 54). Silymarin can significantly ameliorate cisplatin and doxorubicin-induced cardiotoxicity.

**Doxorubicin**

Doxorubicin is a broad spectrum anthracycline antibiotic and used for the treatment of different tumors including uterine, ovarian, breast, lung and some other cancer types (55). It is supposed that oxidative stress and the free radicals formation play essential roles in the mechanism of doxorubicin toxicity. Although doxorubicin is toxic to most organs, its cardiotoxicity is a limiting factor in cancer therapy (56). Cardio protective effect of silymarin against doxorubicin-induced toxicity has been shown through cell membrane stabilization, radical scavenging and iron chelating effects (57).

It was established that a single dose of doxorubicin (10 mg/kg) caused marked acute cardiotoxicity 72 hr after injection. Doxorubicin-induced cardiotoxicity was manifested by increased plasma CPK and LDH activities and verified by severe histopathological damages in heart including sporadic early necrotic fibers, vascular congestion and intravascular hemolysis. Results showed that these alterations were associated with hyperlipidemia, significant elevation of heart MDA level and reduction of reduced glutathione content. Pretreatment with silymarin (50 mg/kg, IP, for 30 days, 7 days before doxorubicin injection) significantly improved all toxic effects of doxorubicin in rat heart tissue except hyperlipidemia. Thus, further investigations with orally administered silymarin phospholipids complex to increase its bioavailability has been suggested (56). Another study revealed that doxorubicin (10 mg/Kg, IP) administration increased serum NO level significantly after 7 and 21 days. Pretreatment with silymarin (100 mg/kg, IP, 5 days before doxorubicin injection) reduced NO level. There were no significant differences in the serum levels of MDA, GSH, GPx and SOD among different groups after silymarin treatment. In light microscopic examination, cytoplasmic vacuole formation and interstitial edema were seen in doxorubicin-treated rats. Myofibrillar disorganization, disintegration and dilatation of sarcoplasmic reticulum were observed using electron microscopic. These histopathological damages were restored in silymarin treated rats (58).
Silymarin has attracted great attention because of its antioxidative, antitumor and anti-inflammatory properties. In this review article, efforts have been made to introduce some animal and in vitro studies in scientific databases on the topic of the protective effect of silymarin against cardiotoxicity induced by chemicals. Although there are an increasing number of studies indicating the beneficial actions of silymarin in various diseases, the number of studies relating to the potential cardiac protective effects of silymarin against chemical- induced toxicity is rather limited. Based on the results of some important investigations, silymarin acts as a potent protective agent in different types of intoxication induced by metals, environmental pollutants, oxidative agents and drugs such as doxorubicin. Some mechanisms including anti-inflammatory, free radical scavenging, improvement of antioxidant defense systems, membrane stabilizing, iron chelating activity and inhibition of apoptosis are involved in silymarin cardiac protective effects (Figure 1).

In conclusion, based on the current review, silymarin has a broad spectrum of cardiac protective activities against toxicities induced by chemicals. Because human reports are rare, further studies are required to establish the utility of silymarin in protection against cardiac toxicity.

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