Journal of Evidence-Based Complementary & Alternative Medicine
2015, Vol. 20(4) 292-301

DOI: 10.1177/2156587215571116
cam.sagepub.com
SAGE

Silybum marianum: Beyond Hepatoprotection

Mahmood Bahmani, PhD[1], Hedayatollah Shirzad, PhD[2], Samira Rafieian, PhD[3], and Mahmoud Rafieian-Kopaei, PhD[2]

Abstract

Silybum marianum is a medicinal plant that has long been used as hepatoprotective remedy. It has been used for the treatment of numerous liver disorders characterized by functional impairment or degenerative necrosis. Its hepatoprotective activity is unique and acts in different ways, including antioxidant and anti-inflammatory activities, cell permeability regulator and membrane stabilizer, stimulation of liver regeneration and inhibition of deposition in collagen fibers, which may lead to cirrhosis. Most of documented data with *Silybum marianum* are about liver disorders; however, recently several beneficial properties on a wide variety of other disorders such as renal protection, hypolipidemic and anti-atherosclerosis activities, cardiovascular protection, prevention of insulin resistance, especially in cirrhotic patients, cancer, and Alzheimer prevention. It is also used as a food remedy. This review article aims to present different aspects of *Silybum marianum*, especially the data in recently published articles about its effects on different diseases, apart from presenting the aspects of its hepatoprotection.

Keywords

Silybum marianum, Silymarin, hepatoprotective remedy, antioxidant

Received November 3, 2014. Accepted for publication January 6, 2015.

Silybum marianum is a medicinal plant whose therapeutic history dates back to 2000 years ago and was used as a hepatoprotective medication to treat jaundice and enlarged liver and spleen. Originally, *Silybum marianum* was a native of Asia and Southern Europe, but now it is found throughout the world.[1] The Food & Drug Administration in Germany has proposed this medicinal plant to treat digestive disorders, intoxication, and alcoholic liver and as a complement drug to treat enlarged liver.[2]

Numerous experimental and clinical studies have documented that *Silybum marianum* with its antioxidant activity and other liver protective properties is a unique hepatoprotective agent. Silymarin is the main component of *Silybum marianum* that has been documented to be highly hepatoprotective. It has been used for the treatment of numerous liver disorders characterized by functional impairment or degenerative necrosis. Although its mechanisms of action is not fully understood, it seems that it acts in different ways, including antioxidant and anti-inflammatory activities, cell permeability regulator and membrane stabilizer, stimulating liver regeneration and inhibiting deposition of collagen fibers, which may lead to cirrhosis. *Silybum marianum* and its major flavonoid, silymarin, are effective and well-tolerated antidotes for use against hepatotoxics, especially psychotropic drugs and the toxic agents produced by *Amanita phalloide*.[3]

Most of documented data about *Silybum marianum* are about liver disorders; however, it has beneficial property on a wide variety of other disorders such as hypoglycemic activity and reducing insulin resistance in patients with type 2 diabetes mellitus and its complications.[4,5] Silymarin is also able to protect kidneys against nephrotoxic agents.[6] The hepatoprotective effects of *Silybum marianum* have been well documented. However, recent researches have considered it as a key for all diseases. It is also used as a food remedy. This review article aims to present different aspects of *Silybum marianum*, especially the data in recently published articles about its effects on different diseases, apart from presenting the aspects of its hepatoprotection.[6]

Botany and Morphology

Silybum marianum is of Asteriaceae family and has various other names including milk thistle, Marian thistle, Mary thistle, Mary's thistle, Saint Mary's thistle, Blessed milk thistle, Mediterranean milk thistle, variegated thistle, *Cardus marianus*, and Scotch thistle. *Silybum marianum* has shiny pale green

[1] Food and Beverages Safety Research Center, Urmia University of Medical Sciences, Urmia, Iran
[2] Shahrekord University of Medical Sciences, Shahrekord, Iran
[3] Isfahan University of Medical Sciences, Isfahan, Iran

Corresponding Author:
Mahmoud Rafieian-Kopaei, PhD, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.
Email: rafieian@yahoo.com

Figure 1. *Silybum marianum*.

leaves and red to purple flowers with white veins. In Persian and Arabic countries, it is called Mary thiqhal.[7]

Silybum marianum is a biennale, glabrous, pale green, spinescent plant with straight stems. Leaves of this plant are big and have white spots around the veins. It has several pinnate parts as triangular-ovary parts. *Silybum marianum* has simple or a little branched or relatively thick branches that terminate to a green mass and with longitudinal rakes. It grows up to 200 cm in height, with the stem of cottony, having an overall conical shape. Its leaves are oblong to lanceolate, and the stem is usually hollow. *Silybum marianum* is either lobate or pinnate, with spiny edges and hairless, shiny green, with milk-white veins. The flower heads of this plant are up to 12 cm long and wide, with red-purple color (Figure 1).[7]

Growth Regions

This plant grows in European, Asian, and American countries. In Iran, it is distributed widely in Noode, Kelardasht, Poshtkooh, Hezar Valley, Moghan, Poshtkooh, Gonbad-e Kāvus, Gorgan, and Mollathani in Ahvaz, as well as Shoush, Hamidiyeh, Ramhormoz, Izeh and Kazeroun in other regions of Iran.[7]

Chemical Compounds

The seeds of this plant contain many compounds such as silybin, silibinin A and B, silicristin, silidianin, apigenin, dehydrosilybin, deoxysilyn cristin, deoxysilyn dianin, among other. Extract of dried seed of this plant contains up to 4% silymarin. Silymarin is a combination of flavonoids such as silibinin A and B, silidianin, silicristin, and dihydroxysilibin.[8,9] Other flavonolignans present in extract of this plant include sylandrin, silybinom, silyhermin, and myristic, palmitic, and stearic acids, which may have hepatoprotective properties.[4] In addition, dried seeds of the plant contain up to 20% oil without medicinal properties.[7]

Silymarin is readily absorbed from the gastrointestinal tract and then reaches its maximum blood concentration after 2 to 4 hours. Its half-life excretion is 6 hours. Eighty percent of this drug is excreted from the bile. Bioavailability of this drug depends on the type of formulation. Sylibin is the most effective antioxidant and hepatoprotective substance present in silymarin, and its concentration in bile is 60 times more than the other components.[2]

Pharmacology and Therapeutic Effects

Various beneficial effects of *Silybum marianum* or its derivatives have been reported including hepatoprotection, renal protection, hypolipidemic and anti-atherosclerosis activities, cardiovascular protection, prevention of insulin resistance, especially in cirrhotic patients, cancer, and Alzheimer prevention. Silymarin in the traditional medicine is consumed in European countries to treat different types of diseases and liver disorders.[10,11]

Liver Diseases

The uses of *Silybum marianum* as hepatoprotective dates back to 2000 years ago. Different clinical and laboratory studies have also documented that silymarin protects the liver against toxicities resulting from various toxins such as carbon tetrachloride, acetaminophen, and tetrachloromethane.[7]

It has been reported that silymarin provides the hepatoprotective effect by different mechanisms including antioxidant activity and scavenging free radicals, increase of cellular glutathione concentration, stimulation of DNA polymerase, and stabilization of hepatocellular membrane.[7]

Numerous studies have strongly suggested that the hepatoprotective effects of silymarin are mainly due to its antioxidant activity and free radical scavenging property. This effect is reflected by glutathione modulation and the membrane stabilization that it produces.[12]

Stimulation of DNA polymerase by silymarin results in increase in the synthesis of ribosomal RNA and reconstruction of liver cells. Increase of cellular glutamine concentration stabilizes superoxide dismutase and glutathione peroxidase. Silymarin decreases the enlarged liver by inhibiting 5-lipoxygenase cycle and inhibiting production of leukotrienes and free radicals in Kupffer cells of liver. Moreover, silybin in the mouse's hepatocyte cells inhibits production of peroxidation lipid and cellular damage.[13]

Actually the effect of silymarin on cellular permeability is associated with alterations of membrane lipids including cholesterol and phospholipids. Silymarin also act on other lipid compartments in the liver that may influence the uptake and secretion of lipoproteins.[14]

Data on the influence of silymarin on triglyceride metabolism in the liver are scanty. It is known that in rats silibinin is able to partly antagonize the increase in total lipids and triglycerides produced in the liver by carbon tetrachloride and, probably, to activate fatty acid β-oxidation. It has also been suggested that silymarin may diminish triglyceride synthesis in the liver.[15]

Lettéron et al studied the mechanisms of action of silymarin that provide protection against lipid peroxidation and the hepatotoxicity of carbon tetrachloride in mice, and they came to the conclusion that silymarin works by reducing metabolic activation by carbon tetrachloride and by acting as an antioxidant that prevents chain rupture.[16]

Other authors have shown that silymarin affords hepatoprotection against specific injury induced by microcystin (a hepatotoxin), paracetamol, halothane, and alloxan in several experimental models.[17]

Different researchers on live animals suggest that silymarin protects liver cells against different types of diseases including viruses, chemical substances, and natural toxic substances. Pretreatment of laboratory animals with silymarin protects them against intoxication from *Amanita muscaria*. Administration of silybin (50 mg/kg) in dogs has protected them against the intoxication resulting from a lethal dose of *Amanita muscaria* even after 40 hours after administration of silybin.[18] Moreover, pretreatment of laboratory animals with silymarin inhibits occurrence of liver intoxication and production of peroxidation lipid resulting from administration of halothane, thallium tetrachloride, carbon tetrachloride, and acetaminophen in these animals significantly.[19] Administration of silymarin or silybin to rats inhibited the activity of the liver enzymes including γ-glutamyl transpeptidase, alanine transaminase, and aspartate transaminase, indicating inhibition of hepatotoxicity resulting by the alcohol effect.[20] Silymarin inhibits alcoholic liver resulting from biliary atresia in rat.[21]

Clinical researches indicate that consumption of 120 mg silybin twice a week for 2 months decreased aspartate transaminase and alanine transaminase levels in blood serum of patients with liver diseases.[20] In a study that was conducted on 2637 patients with chronic liver disorders, administration of silymarin extract for 8 weeks caused significant decrease in the amount of liver enzyme in 88% of patients. Minor side effects of the drug were observed only in less than 1% of patients.[22]

Silymarin is also used in the treatment of intoxication resulting from *Amanita muscaria*. This treatment decreased morality up to 80% in patients.[18]

Administration of intravenous silybin (20-50 mg/kg/day for 3-4 days) inhibited liver damage completely up to 48 hours after intoxication with *Amanita muscaria*. In a study conducted on 250 people intoxicated by *Amanita muscaria*, 46 deaths were reported in the nontreated group. However, no mortality was observed in the group comprising 16 individuals who received silybin.[23] In another study, 18 people who had been poisoned with *Amanita muscaria* were treated with silybin. The results revealed that only one person who took *Amanita* mushroom to commit suicide and was not treated for 60 hours had died.[24]

Other authors have shown that silymarin affords hepatoprotection against specific injury induced by microcystin (a hepatotoxin), paracetamol, halothane, and alloxan in several experimental models.[25]

Administration of silymarin in the treatment of alcohol-induced liver disease indicates contradictory results. A multiple controlled double-blind study conducted on 300 patients with alcohol-induced liver disease showed that silymarin (420 mg/day) led to significant reduction in enzyme levels and liver histology evaluation following 4 weeks.[26] An additional study was conducted on 170 patients with alcohol-induced cirrhosis who were administered silymarin for a period of 4 years. The result showed reduction in mortality when compared with the control group.[27] Inversely, in a double-blind study of administration of silymarin at 280 and 150 mg doses (3 times daily) on patients with cirrhosis of the liver did not reduce mortality compared with the control group.[28] In another investigation performed on 116 people with alcohol-induced hepatitis, the use of 420 mg/day silymarin for 3 months appeared to show no significant improvement compared with control group; however, 46% of the patients were able to stop drinking alcohol.[29]

It is known that silibinin is capable of neutralizing 2 effects of ethanol in rats: the reduction in labeled glycerol incorporation into isolated hepatocytes lipids and the inhibition of phospholipid synthesis.[30] In addition, silibinin stimulates synthesis of phosphatidylcholine and increases cholinephosphate cytidylyltransferase activity in rat liver.[31] It is well established that silymarin acts as an antioxidant, preventing chain rupture and reducing metabolic activation by carbon tetrachloride.[15,32]

Administration of silymarin demonstrates contradictory results in patients with hepatitis. A double-blind investigation was done on 20 patients with active chronic hepatitis who were treated by 240 mg silipide twice a day for a week, and the γ-glutamyl transpeptidase level reduced significantly when compared with the control group.[6] Another examination assessed 157 patients with viral hepatitis. Twenty-nine patients were treated with silymarin (140 mg, 3 times a day) while 28 received placebo. The results showed significant reduction in bilirubin content and aspartate transaminase and alanine transaminase levels in the group under treatment with silymarin when compared with that of the control group.[33] In contrast, silymarin did not improve diseases in an examination on 151 patients with viral hepatitis.[34]

Another mechanism by which silymarin acts against liver injury is by stimulation of liver tissue regeneration, which is done by increase in protein synthesis. Silibinin, apart from increasing protein synthesis, causes an increase in the formation of ribosomes and in DNA synthesis. However, the increase in protein synthesis is induced only in injured livers and not in healthy livers. Silibinin stimulates protein synthesis in the liver probably by physiological regulation of RNA polymerase I and stimulates the formation of ribosomes.[35]

Silymarin has been shown to inhibit the hepatic cytochrome P450 (CYP) detoxification system. Silibinin is able to inhibit numerous hepatic CYP enzyme activities. This effect might explain the hepatoprotective properties of silymarin against the intoxication due to *Amanita phalloides*. The toxin of *Amanita phalloides* that becomes lethal for hepatocytes is activated by the CYP system. Therefore, inhibition of toxin bioactivation contributes to the limitation of its toxic effects. Furthermore,

silymarin can contribute toward protection against free radicals generated by enzymes of the CYP system.[36]

One of the most effective property of silymarin is its ability to inhibit cellular permeability, which is associated with quantitative and qualitative alterations in membrane lipids.[37] This suggests that silymarin may influence lipoprotein secretion and uptake. In this regard, silymarin and silibinin have been shown to reduce the turnover and synthesis of phospholipids in the rat liver. Moreover, silibinin has been shown to reduce labeled glycerol incorporation into lipids of isolated hepatocytes and also neutralize the inhibition of phospholipid synthesis.[38]

Furthermore, silibinin is able to stimulate phosphatidylcholine synthesis and increase the activity of cholinephosphate cytidylyltransferase in rat liver.[31]

It should be noted that data on the effect of silymarin on triglyceride metabolism in the liver are scanty. Silibinin partly antagonizes the increase in triglyceride and total lipids produced in the liver by carbon tetrachloride. It may also diminish triglyceride synthesis in the liver.[25]

Silymarin is able to provide protection against hepatotoxicity of carbon tetrachloride and lipid peroxidation. Silymarin has also been shown in several experimental models to have hepatoprotection against injury induced by paracetamol, halothane, alloxan, and microcystin (a hepatotoxin).[25,39]

Hypoglycemic Activity

Silymarin has been shown to reduce the plasma levels of cholesterol and low-density lipoprotein in hyperlipidemic animals. It also reduces phospholipid levels, especially those transported in low-density lipoprotein.[25]

Laboratory studies show that silymarin led to improved low-density lipoprotein excretion and reduced cholesterol synthesis in liver cells and also in prevention of complications of high cholesterol and reduction in formation of atherosclerosis plaque in hypercholesterolemia rat and rabbit.[40]

Data obtained from animal models of hepatic injury showed that silymarin was capable of normalizing the increase in plasma lipids following administration of carbon tetrachloride and antagonizing the serum-free fatty acids reduction induced by thioacetamide. In hepatic injury produced by paracetamol, silymarin improved low-density lipoprotein binding to hepatocytes, which is a reliable factor for the reduction of low-density lipoprotein in plasma. However, in an animal model of hepatic injury induced by thioacetamide, silymarin was not able to normalize the reduction in triglycerides in serum.[25]

The results of clinical research have also shown that silymarin can be introduced as a blood cholesterol reducer in hypercholesterolemia patients. The daily use of silymarin in 420 mg dosage on 15 people with hypercholesterolemia led to reduced cholesterol concentration in the gallbladder compared with the control group, implying cholesterol synthesis in the liver.[41] Fourteen patients with type II hyperlipidemia were clinically examined, as silymarin in 420 mg doses led to reduced total blood cholesterol and increased high-density lipoprotein cholesterol in patients.[42]

Renal Diseases

It has been reported that silybin present in the silymarin inhibits renal toxicity resulting from administration of cisplatin in laboratory mice. Moreover, silybin has inhibited renal disorders resulting from the effect of cyclosporine in laboratory mouse.[43]

Recent studies have suggested that silymarin for kidney health might be as important as for liver. Silymarin concentrates in kidney cells and aids in regeneration of the cells by increasing protein and nucleic acid synthesis. It has been suggested that silymarin increases cell replication by 30%, which is related to 2 important components of silymarin: silybin and silychristin. Silymarin has beneficial effects on diabetic nephropathy.[44-46]

It has been shown that silymarin might be effective for the prevention of nephropathy-induced premature death in diabetic patients. A study conducted on 60 diabetic patients with urinary albumin excretion >300 mg/day showed that the silymarin-treated group had 50% decrease in urine albumin–creatinine ratio after 3 months of silymarin consumption. In most cases renal impairments have been attributed to oxidative stress, inflammation, and fibrosis, and silymarin seems to act in these ways.

The potential effects of silymarin in diabetic nephropathy have been shown in a few studies. It is also effective in preventing proteinuria in type 2 diabetes mellitus with overt nephropathy. The effect of silymarin has mostly been related to its antioxidant and anti-inflammatory effects.[6,47] In this regard, adding silymarin to renin–angiotensin system inhibitors in type 2 diabetic patients having proteinuria with overt nephropathy could reduce malondialdehyde, urinary excretion of albumin, and tumor necrosis factor-α.[46,48]

Nervous System

It has been reported that alcohol consumption may lead to reduced learning ability in rats' offspring. However, this effect was prevented when it was co-administered with silymarin. Inflammation of the nerves is the main cause of extensive nerve cell damage. Moreover, silymarin can inhibit brain damage caused by blockages in brain vessels.[49] The influence of silymarin has been proved in the improvement of nerve conduction of nerve fibers in patients with diabetes.[50]

Diabetes mellitus has been shown to reduce learning, memory, and cognitive skills, and administration of silymarin at 100 mg/kg dose enhanced the ability to store data in memory and to recall it in diabetic rats. However, it does not improve short-term spatial memory in diabetic rats. This beneficial effects of silymarin has been attributed to attenuation of lipid peroxidation in hippocampus tissue.[51]

Endocrine Gland

Results gained through study on animals suggest that silymarin protects pancreas from chemical compounds including alloxan

and cyclosporine. Sixty patients affected by alcoholic liver cirrhosis with insulin resistance diabetes were treated with silymarin. The outcome showed significant reduction in fasting blood glucose, daily mean blood glucose level, glycosuria, and insulin demand during 6 months.[52]

Hematologic Effects

One of the main properties of silymarin is antioxidant effects on blood compounds. Oxidation of blood compounds was found to be important in intensifying cardiovascular and other chronic diseases. Based on laboratory reports, silymarin helps prevent hemolysis of red blood cells induced by copper and administration of hydrogen peroxide and other oxygen-derived free radical producing materials.[53]

Immune System

Laboratory data indicate that silymarin has no effect on nonstimulated neutrophils and chemotactic and phagocytic activities. However, silymarin inhibits myeloperoxidase release when neutrophils are evoked. Incubation of neutrophils with silymarin results in retardation of the function of leukocyte mobility inhibitors.

Treatment with silymarin led to increase in lectin as the stimulator of lymphoblast deformation in a double-blind, placebo-controlled trial on 40 patients with alcoholic liver cirrhosis. Besides, the number of $T8^{+}$ cells dropped and lymphocytotoxicity suppressed significantly compared with that of the control group.[54]

Anticancer Effects

Laboratory data show that silymarin and especially silybin produce chemopreventive effects on epidermal cancer cells, prostate, as well as animal breast cancer.[55] Silymarin showed cytoprotective effects on cancer cells of prostate and human breasts encountered with carcinogenic agents. Pre-inoculation of cancer cells with silybin before exposure to silybin led to increased *Adriamycin* effect in the prevention of cell growth.[56]

It should be noted that because of the strong antioxidant effects of *Silybum marianum*, there is a concern that this plant may produce interactions in fixing lymphocytotoxicity function of chemotherapy medicines acting through biochemical peroxidative paths. However, silybin can strength cytotoxic cisplatin and doxorubicin synergestic interactions, and there is no evidence about its interaction with their cytotoxicity effect.[57]

Data from animal studies emphasize that silymarin can prevent carcinogenesis in epithelial tumors of different rat models. For example, feeding rats with silymarin could protect them against chemicals and UVB effects causing cancer.[58]

Stimulating effects of silymarin on liver DNA is exerted on noncancerous cells. Silymarin in a study on hepatoma-bearing rats did not stimulate tumor growth.[59] Among studies in human subjects, there is a report of a man with hepatocellular carcinoma through biopsy proved to be unable to have surgery and then his disease improved by taking daily 450 mg silymarin.

Osteoporosis

Taxifolin found in *Silybum marianum* has considerable estrogenic properties. Additionally, flavonoid compounds in silymarin can affect metaphysis of femur without any connection with B estrogenic receptors through exerting any estrogenic agonist effect on the uterus.[60]

Psoriasis

Silymarin has been used traditionally to treat psoriasis. It may act to improve psoriasis by removing unwanted metabolites from body cells especially liver and also inhibiting cAMP cycle and the synthesis of leukotrienes. There is a potential of enhanced cAMP cycle and increased synthesis of leukotrienes in patients with psoriasis that its inhibition by silymarin possibly leads to improvements.[61]

Side Effects and Toxicities

Toxicity and Contraindications. Types of allergic reactions can occur in people who are sensitive to natural products. An allergic reaction to *Silybum marianum* has also been reported. There is a report of an adverse reaction to *Silybum marianum* in an English woman who seemingly had a capsule containing several plant extracts. However, it is not obvious that to which plant in the capsule the reaction was produced. Also a case of anaphylaxis in a patient with a known allergy to the kiwi fruit has been reported.[62]

Acute Toxicity. In animals, silymarin was found to have no significant side effect even when administered at high doses. Because of the stimulatory effects of the plant on the liver and gallbladder, some experts believe that a mild laxative effect may occur during earlier days of consumption. However, in a randomized, controlled trial the side effects of this herb hardly dominated the placebo. In a study of several thousand patients, very low incidences of adverse effects were found, which mainly were limited to mild gastrointestinal disorders.[63]

Chronic Toxicity. Long-term use of this herb is safe with no incidence of abnormality. There is no report alluding to adverse herb reactions during the course of a disease or in patients with organ-specific disorder.[64]

Drug Interactions With Other Herbal or Chemical Medicines. Silybum marianum can lower demand for insulin in diabetic patients with alcohol-related liver cirrhosis. However, there is no study about the effect of the herb on glucose metabolism change in patients without liver disease.[65]

In a double-blind trial on 6 women patients who were chronically using psychoactive drugs, the amount of liver enzymes including alanine transaminase and aspartate

transaminase were increased. Taking 400 mg silymarin twice daily for 90 days led to decreased lipoperoxidase and liver damage when compared with the control group.[66]

Silymarin can reduce adverse effect of chemical medicines such as anticancer drugs including paclitaxel, cisplatin, methothexate, fluorouracil, and blood lipid reducing drugs including clofibrate, lovastatin, pravastatin, and psychoactive drugs like haloperidol, tacrine, and some other medicines including nitrous oxide, acetaminophen, metronidazole, and cyclosporine.[67]

Consumption During Breastfeeding and Pregnancy

Silybum marianum consumption during pregnancy, breastfeeding, and also for children is permitted. Although the side effects caused by plants in long term have not been proven during pregnancy, during breastfeeding, and also for children, this belief is considered based on long-term historical use as a food.

Consumption of this drug is suggested for the treatment of pruritus associated with bile duct obstruction in pregnant women. In addition, use of this herb in pregnant women leads to inhibited liver damage. Administration is essential to pregnant women poisoned with *Amanita* mushroom.[68]

Dose

The doses of plant recommended here is the amount of commonly administered doses of the herb rather than an applicable document. However, doses are only determined for silymarin alone and should be adjusted when combined with others. Furthermore, they vary based on the type and severity of the condition of treatment and even the patient's individual characteristics.

In adults, reports of herb specialists suggest a range of doses. The doses used in the literature ranged from 280 to 800 mg daily silymarin. Most studies have applied concentrated standard product containing 70% to 80% silymarin. Standardized plant extract dose is defined as 100 to 200 mg substance taken orally twice a day with meals.[64]

In several studies, taking a 100 mg dose of Silybin coadministered with phosphatidylcholine, 3 times daily, has been introduced due to its higher reabsorption. In Europe, silybin was injected intravenously at 20 to 50 mg/kg of body weight dosage 3 or 4 times daily for the treatment of acute hepatotoxic induced by *Amanita* poison.[64]

Tea is not a desirable method of administration because silymarin cannot dissolve in water well. But they can be used as herbal tea if seeds are crushed and fried. The usual dose is described as 12 to 15 g fried and crushed grains, 3 times a day with meals. The dose of tincture is 3 to 6 mL, 3 times daily with meal.[9,69,70]

Available standardized products should contain at least 70% silymarin.[3]

Discussion

As already mentioned, silymarin or *Silybum marianum* has been considered as a key for all diseases. It acts with various mechanism; however, it is a very potent antioxidant and it seems that the main mechanism of action of this plant is activity as a free radical scavenger and prevention of lipid peroxidation. *Silybum marianum* has been shown to have some additional mechanism by which it is able to protect liver, specifically. It increases the cellular glutathione in the liver, regulates membrane permeability of liver cells, and increases their membrane stability in the presence of xenobiotic damages, regulates nuclear expression by means of a steroid-like effect, and inhibits the transformation of stellate hepatocytes into myofibroblasts, the one that is responsible for the deposition of collagen fibers leading to cirrhosis.[71] How it acts in other organs, other than acting as an antioxidant, is not clear. Silymarin (and especially silybin) is a powerful antioxidant and free radical scavenger that restores a lot of internal antioxidants and prevents oxidative stress.[72]

Oxidative stress has been shown to be implicated in a wide range of pathological conditions such as chronic inflammation and the generation of damage, particularly during ischemia/reperfusion,[73,74] neurological disorders,[75,76] diabetes,[77,78] atherosclerosis,[79,80] cardiovascular diseases,[81,82] cancer,[83,84] gastrointestinal,[85,86] and infectious,[86-88] diseases or wound complication.[89,90] These conditions involve many changes such as alterations in redox stat.[91-94] Antioxidants possess the ability to counteract these conditions.[64,66,92]

Various experimental and clinical investigations have demonstrated promising results for the treatment and prevention of life-threatening diseases with plants antioxidants.[95-104] These agents are also effective in inhibition of toxic agents–induced complications.[105-110] Therefore, *Silybum marianum* or silymarin, which is a powerful antioxidant and free radical scavenger,[4] should mainly act in this way in the prevention of diseases and pathologic conditions.

Laboratory data associated with rats have shown that 2-week remedy with silybin would protect them from cyclosporine-induced lipid peroxidation.[18] Silybin prevents low-density lipoprotein from peroxidation in vitro and protects human leukocytes against hydrogen peroxide, preventing DNA damage.[66] Silymarin also has antioxidant effects in human platelets and acts as antioxidant in human liver and pulmonary microsomes and consequently provide protective action against induced lipid peroxidation with chemicals.[66]

Animal studies have shown that increased chronic iron overload leads to oxidation stress and liver damage and that silymarin can inhibit this toxicity by its antioxidant activity.[111]

Human studies have demonstrated that silymarin may enhance superoxide dismutase in red blood cells and lymphocytes level in patients with alcoholic cirrhosis, thus increasing antioxidant effects.[26]

Author Contributions

All the authors wrote the first draft of the article. MRK revised and edited the last version.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was done with support of Research Deputy of Shahrekord University of Medical Sciences, Shahrekord, Iran.

Ethical Approval

This study is exempt from oversight by human subjects research protection as there were no human subjects involved.

References

1. Sewell RDE, Rafieian-Kopaei M. The history and ups and downs of herbal medicine usage. *J HerbMed Pharmacol*. 2014;3(1):1-3.
2. Natural Medicines Comprehensive Database. Milk thistle monograph. Stockton, CA: Therapeutic Research Faculty. http://naturaldatabase.therapeuticresearch.com. Published 2012. Accessed November 12, 2012.
3. Jayaraj R, Deb U, Bhaskar AS, Prasad GB, Rao PV. Hepatoprotective efficacy of certain flavonoids against microcystin induced toxicity in mice. *Environ Toxicol*. 2007;22:472-479.
4. Wu CH, Huang SM, Yen GC. Silymarin: a novel antioxidant with antiglycation and antiinflammatory properties in vitro and in vivo. *Antioxid Redox Signal*. 2011;14:353-366.
5. Brodniewicz T, Grynkiewicz G. Plant phenolics as drug leads—what is missing? *Acta Pol Pharm*. 2012;69:1203-1217.
6. Rafieian-Kopaie M, Nasri H. Silymarin and diabetic neuropathy. *J Renal Inj Prev*. 2012;1(1):3-6.
7. Šimánek V, Křen V, Ulrichová J, Vičar J, Cvak L. Silymarin: what is in the name? *Hepatology*. 2000;32:442-443.
8. Kroll DJ, Shaw HS, Oberlies NH. Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies. *Integr Cancer Ther*. 2007;6:110-119.
9. Braun L, Cohen M. *Herbs & Natural Supplements: An Evidence-Based Guide*. 3rd ed. London, England: Churchill Livingstone; 2010.
10. Murata N, Murakami K, Ozawa Y, et al. Silymarin attenuated the amyloid β plaque burden and improved behavioral abnormalities in an Alzheimer's disease mouse model. *Biosci Biotechnol Biochem*. 2010;74:2299-2306.
11. Lu P, Mamiya T, Lu LL, et al. Silibinin prevents amyloid β peptide-induced memory impairment and oxidative stress in mice. *Br J Pharmacol*. 2009;157:1270-1277.
12. Yang Z, Zhuang L, Lu Y, Xu Q, Chen X. Effects and tolerance of silymarin (milk thistle) in chronic hepatitis C virus infection patients: a meta-analysis of randomized controlled trials. *Biomed Res Int*. 2014;2014:941085. doi:10.1155/2014/941085.
13. Yin F, Liu J, Ji X, Wang Y, Zidichouski J, Zhang J. Silibinin: a novel inhibitor of Aβ aggregation. *Neurochem Int*. 2011;58:399-403.
14. Muriel P, Mourelle M. Prevention by silymarin of membrane alterations in acute CCl4 liver damage. *J Appl Toxicol*. 1990;10:275-279.
15. Heidarian E, Rafieian-Kopaei M. Effect of silymarin on liver phoshpatidate phosphohydrolase in hyperlipidemic rats. *Biosci Res*. 2012;9(2):59-67.
16. Lettéron P, Labbe G, Degott C, et al. Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice. *Biochem Pharmacol*. 1990;39:2027-2034.
17. Ramakrishnan G, Elinos-Báez CM, Jagan S, et al. Silymarin downregulates COX-2 expression and attenuates hyperlipidemia during NDEA-induced rat hepatocellular carcinoma. *Mol Cell Biochem*. 2008;313:53-61.
18. Desplaces A, Choppin J, Vogel G, Trost W. The effects of silymarin on experimental phalloidine poisoning. *Arzneimittelforschung*. 1975;25:89-96.
19. Muriel P, Garciapina T, Perez-Alvarez V, Mourelle M. Silymarin protects against liver damage. *J Appl Toxicol*. 1992;12:439-442.
20. Wang M, LaGrange L, Tao J, Reyes E. Hepatoprotective properties of Silybum marianum herbal prepararion on ethanol-induced liver damage. *Fitoterapia*. 1996;67:166-171.
21. Valenzuela A, Lagos C, Schmidt K, Videla LA. Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat. *Biochem Pharmacol*. 1985;34:2209-2212.
22. Nasri H, Sahinfard N, Rafieian M, Rafieian S, Shirzad M, Rafieian-Kopaei M. Effects of *Allium sativum* on liver enzymes and atherosclerotic risk factors. *J HerbMed Pharmacol*. 2013;2(2):23-28.
23. European Scientific Cooperative on Phytotherapy. *ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products*. 2nd edition supplement. New York, NY: Thieme; 2009:222-248.
24. Salmi HA, Sarna S. Effects of silymarin on chemical, functional, and morphological alterations of the liver: a double-blind controlled study. *Scand J Gastroenterol*. 1982;17:517-521.
25. Skottova N, Kreeman V. Silymarin as a potential hypocholesterolaemic drug. *Physiol Res*. 1998;47:1-7.
26. Feher J, Deak G, Muzes G, et al. Liver-protective action of silymarin therapy in chronic alcoholic liver diseases. *Orv Hetil*. 1989;130:2723-2727.
27. Benda L, Dittrich H, Ferenzi P, Frank H, Wewalka F. The influence of therapy with silymarin on the survival rate of patients with liver cirrhosis. *Wien Klin Wochenschr*. 1980;92:678-683.
28. Bunout D, Hirsch S, Petermann M, et al. Controlled study of the effect of silymarin on alcoholic liver disease. *Rev Med Chil*. 1992;120:1370-1375.
29. Lirussi F, Okolicsany L. Cytoprotevtion in the nineties: experience with ursodeoxycholic acid and silymarin in chronic liver disease. *Acta Physiol Hung*. 1992;80:363-367.
30. Platt D, Schnorr B. Biochemische und elektronenoptische untersuchungen zur frage der beeinflussbarkeit der aethanolschadigung der rattenleber durch silymarin. *Arzneimittelforschung*. 1971;21:1206-1208.
31. Schriewer H, Weinhold F. The influence of silybin from Silybum marianum (L.) Gaertn. on in vitro phosphatidyl choline biosynthesis in rat livers. *Arzneimittelforschung*. 1973;29:791-792.
32. Kabiri N, Ahangar-Darabi M, Setorki M, Rafieian-Kopaei M. The effect of silymarin on liver injury induced by thioacetamide in rats. *J HerbMed Pharmacol*. 2013;2(2):29-33.
33. Magliuo E, Gagliardi B, Fiori GP. Results of a double in the treatment of acute viral hepatitis, carried out at two medical centres. *Med Klin*. 1978;73:1060-1065.

34. Bode JC, Schmidt U, Durr HK. Silymarin for the treatment of acute viral hepatitis? Report of a controlled trial. *Med Klin*. 1977;72:513-518.
35. Sonnenbichler J, Zetl I. Biochemical effects of the flavonolignane silibinin on RNA, protein and DNA synthesis in rat livers. In: Cody V, Middleton E, Harborne JB, eds. *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationship*. New York, NY: Alan R Liss; 1986:319-331.
36. Baer-Dubowska W, Szafer H, Krajkakuzniak V. Inhibition of murine hepatic cytochrome P450 activities by natural and synthetic phenolic compounds. *Xenobiotica*. 1998;28:735-743.
37. Muriel P, Mourelle M. The role of membrane composition in ATPase activities of cirrhotic rat liver: effect of silymarin. *J Appl Toxicol*. 1990;10:281-284.
38. Videla LA, Valenzuela A. Alcohol ingestion, liver glutathione and lipoperoxidation: metabolic interrelations and pathological implications. *Life Sci*. 1982;31:2395-2407.
39. Nasri H, Rafieian-Kopaei M. Herbal medicine and diabetic kidney disease. *J Nephropharmacol*. 2013;2(1):1-2.
40. Skottova N, Krecman V. Dietary silymarin improves removal of low density lipoproteins by the perfused rat liver. *Acta Univ Palacki Olomuv Fac Med*. 1998;141:39-40.
41. Nassuato G, Iemmolo RM, Strazzabosco M, et al. Effect of silibinin on biliary lipid composition experimental and clinical study. *J Hepatol*. 1992;12:290-295.
42. Somogyi A, Ecsedi GG, Blazovics A, Miskolczi K, Gergely P, Feher J. Short term treatment of type II hyperlipoproteinaemia with silymarin. *Acta Med Hung*. 1989;46:289-295.
43. Zima T, Kamenikova L, Janebova M, Buchar E, Crkovska T, Tesar V. The effects of silibinin on experimental cyclosporine nephrotoxicity. *Ren Fail*. 1998;20:471-479.
44. Brantley SJ, Oberlies NH, Kroll DJ, Paine MF. Two flavonolignans from milk thistle (*Silybum marianum*) inhibit CYP2C9-mediated warfarin metabolism at clinically achievable concentrations. *J Pharmacol Exp Ther*. 2010;332: 1081-1087.
45. Vessal G, Akmali M, Najafi P, Moein MR, Sagheb MM. Silymarin and milk thistle extract may prevent the progression of diabetic nephropathy in streptozotocin-induced diabetic rats. *Ren Fail*. 2010;32:733-739.
46. Soto C, Pérez J, García V, Uría E, Vadillo M, Raya L. Effect of silymarin on kidneys of rats suffering from alloxan-induced diabetes mellitus. *Phytomedicine*. 2010;17:1090-1094.
47. Meyers CM, Briggs JP. Silymarin for diabetic nephropathy: the challenges of botanical product research. *Am J Kidney Dis*. 2012;60:887-889.
48. Fallahzadeh MK, Dormanesh B, Sagheb MM, et al. Effect of addition of silymarin to renin-angiotensin system inhibitors on proteinuria in type 2 diabetic patients with overt nephropathy: a randomized, double-blind, placebo-controlled trial. *Am J Kidney Dis*. 2012;60:896-903.
49. Wang MJ, Lin WW, Chen HL, et al. Silymarin protects dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting microglial activation. *Eur J Neurosci*. 2002;16: 2103-2112.
50. Zang JQ, Mao XM, Zhon YP. Effects of silybin on red blood cell sorbitol and nerve conduction velocity in diabetic patients. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 1993;13:725-726.
51. Roghani M, Khalili M, Baluchnejadmojarad T, Ahmadi M. Protective effect of silymarin on learning and memory deficiency in streptozotocin-diabetic rats. *J Gorgan Univ Med Sci*. 2013; 15:35-41.
52. Soto CP, Perez BL, Favari LP, Reyes JL. Prevention of alloxan-induced diabetes mellitus in the rat by silymarin. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol*. 1998;119:125-129.
53. Zou CG, Agar NS, Jones GL. Oxidative insult to human red blood cells induced by free radical initiator AAPH and its inhibition by a commercial antioxidant mixture. *Life Sci*. 2001;69:75-86.
54. Lang I, Nekam K, Gonzalez-Cabello R, Muzes G, Gergely P, Feher J. Hepatoprotective and immunological effects of antioxidant drugs. *Tokai J Exp Clin Med*. 1990;15:123-127.
55. Zi X, Mukhtar H, Agarwal R. Novel cancer chemopreventive effects of a flavonoid antioxidant silymarin: inhibition of mRNA expression of an endogenous tumor promoter TNF alpha. *Biochem Biophys Res Commun*. 1997;239:334-339.
56. Rafieian-Kopaei M, Baradaran A, Rafieian M. Oxidative stress and the paradoxical effects of antioxidants. *J Res Med Sci*. 2013;18:629.
57. Scambia G, De Vincenzo R, Ranellletti FO, et al. Antiprolifeative effect of silybin on gunaecological malignancies: synergism with cisplatin and doxorubicin. *Eur J Cancer*. 1996;32:877-882.
58. Chatterjee ML, Agarwal R, Mukhtar H. Ultraviolet B radiation-induced DNA lesions in mouse epidermis: an assessment using a novel 32P-posylabelling technique. *Biomed Biophys Res Commun*. 1996;229:590-595.
59. Sonnenbichler J, Goldberg M, Hane L, Madubunyi I, Vogl S, Zetl I. Stimulatory effects of silibin on the DNA synthesis in partially hepatectomized rat livers: non-response in hepatoma and other malignant cell lines. *Biochem Pharmacol*. 1986;35:538-541.
60. Nasri H, Rafieian-Kopaei M. Medicinal plants and antioxidants: why they are not always beneficial? *Iranian J Public Health*. 2014;43:255-257.
61. Koch HP, Bachner J, Loffler E. Silymarin: potent inhibitor of cyclic AMP phosphodiester. *Methods Find Exp Clin Pharmacol*. 1985;7:409-413.
62. Adverse Reactions Advisory Committee. An adverse reaction to the herbal medication milk thistle (*Silybum marianum*). *Med J Aust*. 1999;170:218-219.
63. Bahmani M, Zargaran A, Rafieian-Kopaei M. Identification of medicinal plants of Urmia for treatment of gastrointestinal disorders. *Rev Bras Farmacogna*. 2014;24:468-480.
64. Kafash-Farkhad N, Asadi-Samani M, Rafieian-Kopaei M. A review on phytochemistry and pharmacological effects of *Prangos ferulacea* (L.) Lindl. *Life Sci J*. 2013;10(8 suppl): 360-367.
65. Baradaran A, Nasri H, Rafieian-Kopaei M. Oxidative stress and hypertension: possibility of hypertension therapy with antioxidants. *J Res Med Sci*. 2014;19:358-367.
66. Nasri H, Rafieian-Kopaei M. Protective effects of herbal antioxidants on diabetic kidney disease. *J Res Med Sci*. 2014;19: 82-83.

67. Baradaran A, Rafieian-Kopaei M. Re: Protective role of silymarin and deferoxamine against iron dextran-induced renal iron deposition in male rats. *Int J Prev Med*. 2014;5:245-246.
68. Schleufe P, Seidel C. Amanita poisoning during pregnancy. *Anasthesiol Intensivmed Notfallmed Schmerzther*. 2003;38:716-718.
69. Rahimian G, Sanei MH, Shirzad H, et al. Virulence factors of *Helicobacter pylori* vacA increase markedly gastric mucosal TGF-β1 mRNA expression in gastritis patients. *Microb Pathog*. 2014;67-68:1-7.
70. Ottariano SG. *Medicinal Herbal Therapy: A Pharmacist's View*. Portsmouth, NH: Nicoln Fields; 1999.
71. Fraschini F, Demartini G, Esposti D. Pharmacology of Silymarin. *Clin Drug Invest*. 2002;22(1):1-7.
72. Becker-Scheibe M, Mengs M, Schaefer M, Bulitta M, Hoffman W. Topical use of a silymarin-based preparation to prevent radiodermatitis: results of a prospective study in breast cancer patients. *Strahlenther Onkol*. 2011;187:485-491.
73. Rabiei Z, Rafieian-Kopaei M, Heidarian E, Saghaei E, Mokhtari S. Effects of *Zizyphus jujube* extract on memory and learning impairment induced by bilateral electric lesions of the nucleus Basalis of Meynert in rat. *Neurochem Res*. 2014;39:353-360.
74. Baradaran A, Rabiei Z, Rafieian M, Shirzad H. A review study on medicinal plants affecting amnesia through cholinergic system. *J HerbMed Plarmacol*. 2012;1(1):3-9.
75. Rafieian-Kopaei M, Gray AM, Spencer PS, Sewell RD. Contrasting actions of acute or chronic paroxetine and fluvoxamine on morphine withdrawal-induced place conditioning. *Eur J Pharmacol*. 1995;275:185-189.
76. Roohafza H, Sarrafzadegan N, Sadeghi M, Rafieian-Kopaei M, Sajjadi F, Khosravi-Boroujeni H. The association between stress levels and food consumption among Iranian population. *Arch Iran Med*. 2013;16:145-148.
77. Baradaran A, Madihi Y, Merrikhi A, Rafieian-Kopaei M, Nasri H. Serum lipoprotein (a) in diabetic patients with various renal function not yet on dialysis. *Pak J Med Sci*. 2013;29(1 suppl): 354-357.
78. Behradmanesh S, Horestani MK, Baradaran A, Nasri H. Association of serum uric acid with proteinuria in type 2 diabetic patients. *J Res Med Sci*. 2013;18:44-46.
79. Madihi Y, Merrikhi A, Baradaran A, et al. Bioactive components and the effect of hydroalcoholic extract of *Vaccinium myrtillus* on postprandial atherosclerosis risk factors in rabbits. *Pak J Med Sci*. 2013;29(1 suppl):384-389.
80. Setorki M, Nazari B, Asgary S, Azadbakht L, Rafieian-Kopaei M. Antiatherosclerotic effects of verjuice on hypocholesterolemic rabbits. *Afr J Pharm Pharmacol*. 2011;5:1038-1045.
81. Khosravi-Boroujeni H, Sarrafzadegan N, Mohammadifard N, et al. White rice consumption and CVD risk factors among Iranian population. *J Health Popul Nutr*. 2013;31:252-261.
82. Sadeghi M, Khosravi-Boroujeni H, Sarrafzadegan N, et al. Cheese consumption in relation to cardiovascular risk factors among Iranian adults—IHHP study. *Nutr Res Pract*. 2014;8: 336-341.
83. Shirzad H, Shahrani M, Rafieian-Kopaei M. Comparison of morphine and tramadol effects on phagocytic activity of mice peritoneal phagocytes in vivo. *Int Immunopharmacol*. 2009;9:968-970.
84. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. *J Med Food*. 2011;14: 969-974.
85. Kiani MA, Khodadad A, Mohammadi S, et al. Effect of peppermint on pediatrics' pain under endoscopic examination of the large bowel. *J HerbMed Pharmacol*. 2013;2(2):41-44.
86. Bagheri N, Taghikhani A, Rahimian G, et al. Association between virulence factors of *Helicobacter pylori* and gastric mucosal interleukin-18 mRNA expression in dyspeptic patients. *Microb Pathog*. 2013;65:7-13.
87. Rafiean-Kopaei M, Baradaran A, Maghsoudi AR, Ghobadi SH, Nasri H. *Helicobacter pylori* infection and serum homocysteine in hemodialysis patient. *Life Sci J*. 2012;9:3696-3702.
88. Bagheri N, Rahimian GH, Salimzadeh L, et al. Association of the virulence factors of *Helicobacter pylori* and gastric mucosal interleukin-17/23 mRNA expression in dyspeptic patients. *EXCLI J*. 2013;12:5-14.
89. Asadi SY, Parsaei P, Karimi M, et al. Effect of green tea (*Camellia sinensis*) extract on healing process of surgical wounds in rat. *Int J Surg*. 2013;11:332-337. doi:10.1016/j.ijsu.2013.02.014.
90. Parsaei P, Karimi M, Asadi SY, Rafieian-Kopaei M. Bioactive components and preventive effect of green tea (*Camellia sinensis*) extract on postlaparotomy intra-abdominal adhesion in rats. *Int J Surg*. 2013;11:811-815. doi:10.1016/j.ijsu.2013.08.014.
91. Nasri H., Rafieian-Kopaei M. Tubular kidney protection by antioxidants. *Iranian J Public Health*. 2013;42:1194-1196.
92. Baradaran A, Nasri H, Nematbakhsh M, Rafieian-Kopaei M. Antioxidant activity and preventive effect of aqueous leaf extract of Aloe Vera on gentamicin-induced nephrotoxicity in male Wistar rats. *Clin Ter*. 2014;165:7-11.
93. Nasri H, Tavakoli M, Ahmadi A, Baradaran A, Nematbakhsh M, Rafieian-Kopaei M. Ameliorative effect of melatonin against contrast media induced renal tubular cell injury. *Pak J Med Sci*. 2014; 30:261-265.
94. Rafieian-Kopaei M, Nasri H. The ameliorative effect of *Zingiber officinale* in diabetic nephropathy. *Iran Red Crescent Med J*. 2014;16:e11324.
95. Rafieian-Kopaei M, Shahinfard N, Rouhi-Boroujeni H, Gharipour M, Darvishzadeh-Boroujeni P. Effects of *Ferulago angulata* extract on serum lipids and lipid peroxidation. *Evid Based Complement Alternat Med*. 2014;2014:680856. doi:10.1155/2014/ 680856.
96. Asgary S, Sahebkar A, Afshani MR, Keshvari M, Haghjooyjavanmard S, Rafieian-Kopaei M. Clinical evaluation of blood pressure lowering, endothelial function improving, hypolipidemic and anti-inflammatory effects of pomegranate juice in hypertensive subjects. *Phytother Res*. 2013;28:193-199. doi: 10.1002/ptr.4977.
97. Rahnama S, Rabiei Z, Alibabaei Z, Mokhtari S, Rafieian-kopaei M, Deris F. Anti-amnesic activity of Citrus aurantium flowers extract against scopolamine-induced memory impairments in rats. *Neurol Sci*. 2014:1-8.
98. Sharafati R, Sharafati F, Rafieian-Kopaei M. Biological characterization of Iranian walnut (*Juglans regia*) leaves. *Turk J Biol*. 2011;35:635-639.

99. Nasri H, Baradaran A, Ardalan MR, Mardani S, Momeni A, Rafieian-Kopaei M. Bright renoprotective properties of metformin: beyond blood glucose regulatory effects. *Iran J Kidney Dis*. 2013; 7:423-428.
100. Rafieian-Kopaei M, Behradmanesh S, Kheiri S, Nasri H. Association of serum uric acid with level of blood pressure in type 2 diabetic patients. *Iran J Kidney Dis*. 2014;8: 152-154.
101. Taghikhani M, Nasri H, Asgari A, et al. The renal toxicity of hydroalcoholic extract of *Stachys lavandulifolia* Vahl in Wistar rats. *Life Sci J*. 2012;9:3025-3031.
102. Bahmani M., Rafieian-Kopaei M. Medicinal plants and secondary metabolites for leech control. *Asian Pac J Trop Dis*. 2014;4: 315-316.
103. Amirmohammadi M, Khajoenia S, Bahmani M, Rafieian-Kopaei M, Eftekhari Z, Qorbani M. In vivo evaluation of antiparasitic effects of *Artemisia abrotanum* and *Salvia officinalis* extracts on *Syphacia obvelata*, *Aspiculoris tetrapetra* and *Hymenolepis nana* parasites. *Asian Pac J Trop Dis*. 2014;4(suppl 1): 250-254.
104. Bahmani M, Zargaran A, Rafieian-Kopaei M, Saki M. Ethnobotanical study of medicinal plants used in the management of diabetes mellitus in the Urmia, Northwest Iran. *Asian Pac J Trop Med*. 2014;7(suppl 1):348-354.
105. Hosseini-Asl K, Rafieian-Kopaei M. Can patients with active duodenal ulcer fast Ramadan? *Am J Gastroenterol*. 2002;97: 2471-2472.
106. Taghikhani A, Afrough H, Ansari-Samani R, Shahinfard N, Rafieian-Kopaei M. Assessing the toxic effects of hydroalcoholic extract of *Stachys lavandulifolia* Vahl on rat's liver. *Bratisl Lek Listy*. 2014;115:121-124.
107. Heidarian E, Rafieian-Kopaei M. Protective effect of artichoke (*Cynara scolymus*) leaf extract against lead toxicity in rat. *Pharm Biol*. 2013;51:1104-1109.
108. Baradaran A, Nasri H, Rafieian-Kopaei M. Comment on: Antioxidative stress activity of *Stachys lavandulifolia* aqueous extract in humans. *Cell J*. 2013;15:272-273.
109. Baradaran A, Madihi Y, Merrikhi A, et al. Nephrotoxicity of hydroalcoholic extract of *Teucrium polium* in Wistar rats. *Pak J Med Sci*. 2013;29(1 suppl):329-333.
110. Ghaed F, Rafieian-Kopaei M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. *J Res Med Sci*. 2012;17:621-625.
111. Jefferson WN, Padiilla-Banks G, Newbold RR. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. *J Chromatogr*. 2002;777:179-189.