PHYTOTHERAPY RESEARCH
Phytother. Res. (2017)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5768

REVIEW

Effects of Silymarin on Diabetes Mellitus Complications: A Review

Aline Maria Stolf, Cibele Campos Cardoso and Alexandra Acco*
Department of Pharmacology, Federal University of Paraná, Curitiba, PR, Brazil

Diabetes mellitus is a common metabolic disorder that is caused by a deficit in the production of (type 1) or response to (type 2) insulin. Diabetes mellitus is characterized by a state of chronic hyperglycemia and such symptoms as weight loss, thirst, polyuria, and blurred vision. These disturbances represent one of the major causes of morbidity and mortality nowadays, despite available treatments, such as insulin, insulin secretagogues, insulin sensitizers, and oral hypoglycemic agents. However, many efforts have been made to discover new drugs for diabetes treatment, including medicinal plant extracts. Silymarin is a powder extract of the seeds from *Silybum marianum*, a plant from the Asteraceae family. The major active ingredients include four isomers: silybin, isosilybin, silychristin, and silydianin. Silymarin is indicated for the treatment of hepatic disorders, such as cirrhosis, chronic hepatitis, and gallstones. Moreover, several studies of other pathologies, including diabetes, sepsis, osteoporosis, arthritis, hypercholesterolemia, cancer, viral infections, and Alzheimer's and Parkinson's diseases, have tested the effects of silymarin and reported promising results. This article reviews data from clinical, *in vivo*, and *in vitro* studies on the use of silymarin, with a focus on the complications of diabetes, including nephropathy, neuropathy, healing delays, oxidative stress, hepatotoxicity, and cardiomyopathy.

Keywords: silymarin; silybin; diabetes; healing; hepatopathy; neuropathy.

INTRODUCTION

Diabetes is a metabolic disturbance that is characterized by chronic hyperglycemia, resulting in a deficiency in insulin secretion (type 1 diabetes mellitus) or a deficiency in the response to insulin (type 2 diabetes mellitus). Characteristic symptoms include weight loss, thirst, polyuria, and blurred vision (Alberti and Zimmet, 1998). In 2015, 415 million people were estimated to be living with diabetes, representing one in every 11 adults, and 5.0 million deaths occurred as a consequence of this disease. The number of cases is expected to increase to 642 million in 2040, representing one in every 10 adults, and $673bn was spent for healthcare for diabetic patients in 2015 (International Diabetes Federation, 2015).

Numerous complications result from diabetes. Despite the available treatments, such as injectable insulin, insulin secretagogues, insulin sensitizers, and oral hypoglycemic agents, the mortality and morbidity associated with diabetes remain high. Moreover, agents that are traditionally used for the treatment of diabetes and diabetes complications can cause severe side effects (Mirhoseini *et al.*, 2013). Therefore, exploring new therapeutic interventions is necessary.

The use of complementary and alternative medicine, including herbal medicine, is increasing, and the action, safety, and efficacy of these products need to be investigated (Mirhoseini *et al.*, 2013). Several medicinal plants are used for the treatment of diabetes, but not all of them have been pharmacologically tested (Baharvand-Ahmadi *et al.*, 2016). Some plants that are being tested for the treatment of diabetes or diabetes complications have antioxidant properties (Nasri *et al.,* 2015; Nasri and Rafieian-Kopaei, 2014).

In this context, the natural compound silymarin has received increasing interest in recent decades. The aim of this article is to review data from *in vitro*, *in vivo*, and clinical studies on the use of silymarin for the treatment or prevention of diabetes complications, including nephropathy, neuropathy, healing delays, oxidative stress, hepatotoxicity, and cardiomyopathy.

SILYMARIN

Silymarin is a standardized extract that is obtained from the seeds of *Silybum marianum* (L.) Gaertn (Fig. 1A), a plant from the Asteraceae family (Ottai and Abdel-Moniem, 2006). The extract consists of approximately 65%–80% silymarin (i.e., a flavonolignan complex). Isolation and the complete characterization of the main constituents of silymarin by high-performance liquid chromatography revealed that the most abundant compounds are silybins A and B (also known as silibinins A and B), followed by isosilybins A and B. Three other flavonolignans (i.e., silychristin, isosilychristin, and silydianin) were also isolated, in addition to the flavonoid taxifolin. The structures of compounds (Fig. 1B)

* Correspondence to: Alexandra Acco, Federal University of Paraná (UFPR), Biological Science Sector, Department of Pharmacology, Centro Politécnico, Cx. P. 19031, Curitiba, Paraná, Brazil.
E-mail: aleacco@ufpr.br

Received 22 July 2016
Revised 16 December 2016
Accepted 17 December 2016

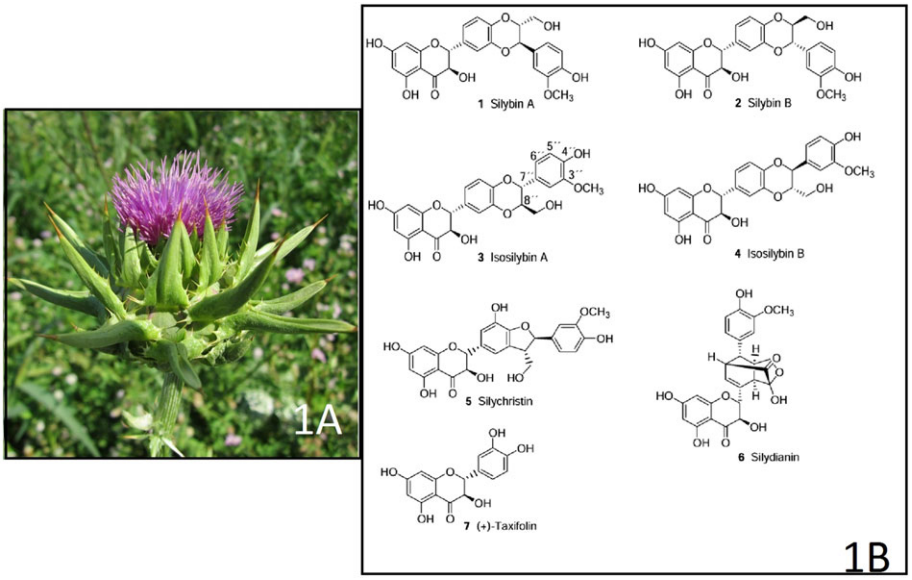

Figure 1. (A) *Silybum marianum* plant (https://pixabay.com, accessed May 2016) and (B) chemical structures of compounds of silymarin (Pferschy-Wenzig *et al.,* 2014) [Fig. 1B was reproduced with kind permission of Dr. Atanas G. Atanasov]. [Colour figure can be viewed at wileyonlinelibrary.com]

were confirmed by two-dimensional nuclear magnetic resonance spectroscopy and circular dichroism spectroscopy (Kim *et al.,* 2003).

The classic indication for silymarin is for the treatment of gallbladder (Kazazis *et al*., 2014) and hepatic diseases, such as cirrhosis, chronic hepatitis (Ferenci *et al.*,1989; Salmi and Sarna, 1982), and non-alcoholic fatty liver disease (NAFLD; Aller *et al*., 2015). The treatment regimen for hepatic cirrhosis is 140 mg three times daily (Ferenci *et al.*, 1989). After oral administration in a single dose, silymarin (Legalon 140™) is well tolerated, even at higher doses (>700 mg, or more than five capsules). An average of 10% of the administered silybin is found in plasma in an unconjugated form; 4 to 6 h after administration, plasma levels are undetectable. Urinary elimination as total silybin represents only 5% in cirrhotic patients (Weyhenmeyer *et al.,* 1992).

In addition to label indications, many studies have reported the beneficial effects of silymarin for the treatment of several diseases (Fig. 2), including sepsis (Kang *et al*., 2004; Toklu *et al*., 2008), burns (Toklu *et al*., 2007), osteoporosis (Kim *et al.,* 2013; Mohd Fozi *et al*., 2013), arthritis (Gupta *et al*., 2000), hypercholesterolemia (Krecman *et al.,* 1998; Skottova and Krecman, 1998), cancer (Singh et al., 2008; Bosch-Barrera and Menendez, 2015; Scavo *et al.,* 2015; Yurtcu *et al.,* 2015), viral infections (Lani *et al.,* 2015), diabetes (Malekinejad *et al.*, 2012; Soto *et al.*, 2014; Zhang *et al.*, 2014; Ebrahimpour Koujan *et al.*, 2015), Alzheimer's disease (Duan *et al.,* 2015; Kumar *et al.,* 2015), and Parkinson's disease (Haddadi *et al.,* 2014; Lee *et al.,* 2015). Silymarin treatment likewise attenuated the severity of radiotherapy-induced mucositis in a clinical trial (Elyasi *et al.*, 2016). Concerning diabetes, studies have also indicated that silymarin may be used to treat or attenuate specific diabetes complications, as discussed in the following sections and shown in Table 1.

Several articles have reported the findings of *in vitro*, *in vivo*, and clinical studies of silymarin and its constituent compounds and found that its main beneficial action involves antioxidant effects in different organs and diseases. Different mechanistic explanations for the antioxidant effects of silymarin have been described, including (i) preventing free radical formation by inhibiting specific reactive oxygen species (ROS)-producing enzymes or improving the integrity of mitochondria in conditions of stress, (ii) decreasing inflammatory responses by inhibiting nuclear factor κB (NF-κB)-dependent pathways, and (iii) maintaining an optimal redox balance in the cell by activating a range of antioxidant

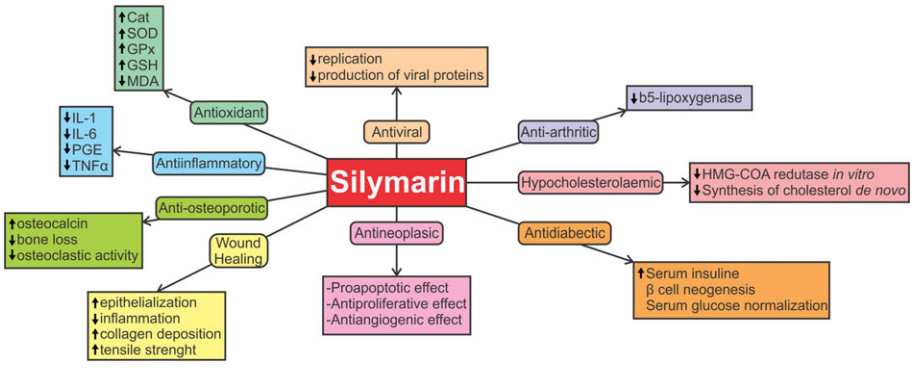

Figure 2. Schematic representation of off-label effects of silymarin reported in the literature. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. Posological regimen of silymarin or silybin applied experimentally for the treatment of diabetes complications

Drug	Species	Dose, via, and treatment period	Complication	Reference
Silymarin	Rats	100 mg/kg, ip, 2 months	Neuropathy	Baluchnejadmojarad *et al*. (2010)
Silybin	Rats	100 mg/kg, vo, 20 days	Neuropathy	Di Cesare et al. (2012)
Silybin	Rats	10 or 30 mg/kg, vo, 22 weeks	Retinopathy	Zhang *et al.* (2014)
Silymarin	Rats	30 mg/kg, t, 5, 50 or 25 days	Healing	Sharifi *et al.* (2012)
Silymarin	Rats	6 mg/mL/rat or 12 mg/mL/rat, t, 10, 20 or 30 days	Healing	Oryan *et al.* (2012)
Silymarin	Rats	70 mg/kg, vo, 14 days	Metabolic disorders	Vengerovskii *et al.* (2007)
Silymarin	Human	200 mg/day, 4 months	Metabolic disorders	Huseini *et al*. (2006)
Silymarin	Mice	25, 50 or 100 mg/kg, ip, 3 or 4 weeks	Cardiomyopahy	Taghiabadi *et al.* (2012)
Silymarin	Rats	120 mg/kg, ip, 10 days	Cardiomyopahy	Tuorkey *et al.* (2015)
Silybin	Mice	20 mg/kg, ip, 4 weeks	Cardiomyopathy and hepatopathy	Salamone (2012b)
Silybin and vitamin E	Rats	47 + 15 mg/rat, vo, 7, 14, 30 or 60 days	Hepatopathy	Grattagliano *et al.* (2013)
Silymarin	Rats	50 mg/kg, vo, 28 days	Hepatopathy	Malekinejad *et al.* (2012)
Silymarin	Gerbil	100 mg/kg, vo, 7 weeks	Hepatopathy	Bouderba *et al.* (2014)
Silybin-beta-cyclodextrin	Humans	135 mg/d, vo ,6 months	Hepatopathy	Lirussi *et al.* (2002)
Silymarin	Mice	20 mg/kg, ip, 4 weeks	Nonalcoholic steatohepatitis (NASH)	Salamone *et al*. (2012a)
Silymarin	Mice	100 mg/kg, vo, 7 days	Nephropathy	Tan *et al*. (2015)
Silymarin	Rats	60 or 120 mg/Kg, 60 days	Nephropathy	Sheela *et al.* (2013)
Silymarin	Rats	200 mg/kg, vo, 9 weeks	Nephropathy	Soto *et al.* (2010)
Silymarin	Rats	100 mg/kg, vo, 4 weeks	Nephropathy	Vessal *et al.* (2010)
Silymarin	Humans	420 mg/d, vo, 3 months	Nephropathy	Fallahzadeh *et al.* (2012)

"vo" means oral via, "t" means topical via, "ip" means intraperitonial via.

enzymes and non-enzymatic antioxidants, mainly via the activation of nuclear factor-erythroid two-related factor (Nrf2; Surai, 2015; Tan *et al.*, 2015; Zhao *et al*., 2015; Fallahzadeh *et al.*, 2012; Salamone *et al*., 2012a; Soto *et al.*, 2010; Vengerovskii *et al.,* 2007). Growing evidence indicates that the Nrf2 antioxidant response plays an important role in cellular defense by activating a wide variety of genes that are involved in early antioxidant defense reactions (Surai, 2015), inflammatory processes, apoptotic processes, metabolism, detoxification, and cellular proliferation (Morales-Gonzáles *et al*., 2015). Data have shown that silymarin improves liver regeneration (Morales-Gonzáles *et al*., 2015) and lung injury (Zhao et al., 2015) by mediating the Nrf2 signaling pathway. Nrf2 is a key regulator of oxidative stress, which is involved in the pathogenesis of diabetes, and then, the therapeutic potential of silymarin for the treatment of diabetes complications has become increasingly evident.

NEPHROPATHY

Diabetic nephropathy involves impairment of the glomerular filtration barrier as a consequence of high blood glucose levels, inflammation, and oxidative stress (Faulkner *et al.*, 2015). High blood pressure also represents an important risk factor for disease onset. Diabetic nephropathy is the primary cause of end-stage renal failure worldwide (Conserva *et al.*, 2016).

Evidence indicates that silymarin may produce beneficial effects in renal diseases. In a model of renal ischemia–reperfusion injury, mice were treated with 100 mg/kg silymarin for 7 days before the induction of ischemia. Compared with the control group, silymarin-treated animals had low levels of serum creatinine and urea. The treatment also attenuated damage in renal tubule cells and the number of apoptotic cells. Renal myeloperoxidase, tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and IL-6 levels also decreased. The expression of renal CD68 in silymarin-treated mice was lower, whereas Bcl-2 expression was higher (Tan *et al.*, 2015).

Animal models of diabetes have provided evidence of the beneficial effects of silymarin against diabetic nephropathy. Rats with diabetic nephropathy that was induced by streptozotocin and nicotinamide received 60 and 120 mg/kg silymarin for 60 days. Silymarin-treated animals exhibited reductions of blood glucose, glycosylated hemoglobin, urine albumin and volume, serum creatinine, and uric acid. The histopathological evaluation revealed preservation of the tubular epithelium and a reduction of intertubular hemorrhage, mainly at a higher dose (Sheela *et al.*, 2013). In similar studies, silymarin treatment reduced oxidative stress in renal tissue through the recovery of antioxidant enzymes (Soto *et al.*, 2010; Vessal *et al.*, 2010). Data results from our laboratory corroborated these data, in which we found a reduction of oxidative stress (i.e., decreases in lipoperoxide levels and increases in catalase activity) in homogenates of renal tissue from rats with streptozotocin-induced diabetes that were treated with silymarin (104.1 mg/kg) for 10 days (Stolf, 2016).

A randomized, double-blind, placebo-controlled study was performed in patients with type 2 diabetes mellitus with persistent macroalbuminuria. The patients underwent treatment for 6 months with a renin-angiotensin system inhibitor plus an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker at the highest dose that is approved by the US

Food and Drug Administration. Among these patients, 60 were divided equally into placebo-treated and silymarin-treated groups. The silymarin-treated group received three daily doses of 140 mg silymarin for 3 months. Silymarin-treated patients presented a better urinary albumin-creatinine ratio and lower levels of urinary TNF-α and urinary and serum malondialdehyde (Fallahzadeh *et al.*, 2012).

NEUROPATHY

Approximately, 50% of diabetic patients develop neuropathy. This condition can be either symptomatic or asymptomatic. Symptoms can include numbness, prickling, pain, and allodynia. The mechanisms of diabetic neuropathy involve ischemia, impaired growth factor support, and oxidative stress. Severe cases can lead to toe, foot, and leg amputation (Gordois *et al.*, 2003; Baluchnejadmojarad *et al.*, 2010).

Some studies have reported the potential beneficial effects of silymarin in *in vitro* and *in vivo* models of neuropathy. Di Cesare *et al.* (2013) evaluated the effects of treatment with silybin and α-tocopherol in the neuronal-derived SH-SY5Y cell line and primary cultures of rat cortical astrocytes that were challenged with oxaliplatin, an antineoplasic agent that can induce neurotoxicity. Such toxicity is caused by oxidative damage in nervous system tissues, leading to neuropathic pain. The treatment protected the cells against the generation of superoxide anions, malondialdehyde, carbonylated proteins, and DNA oxidation. The treatment did not suppress the apoptotic effect of oxaliplatin when tested in neoplastic cells. A similar study was performed in rats. Oxaliplatin was administered intraperitoneally daily for 21 days at a dose of 2.4 mg/kg, leading to neuropathic pain and increases in oxidative stress parameters in the sciatic nerve, spinal cord, and plasma. Treatment with 100 mg/kg silybin and α-tocopherol reduced pain and improved motor coordination (Di Cesare et al., 2012).

In rats with streptozotocin-induced diabetes, daily treatment with 100 mg/kg silymarin for 2 months reduced the deficit in sciatic motor nerve conduction velocity and reduced hyperalgesia. The activity of superoxide dismutase was restored, and malondialdehyde levels decreased (Baluchnejadmojarad *et al.*, 2010). The authors concluded that silymarin had a potential beneficial effect in the treatment and prevention of diabetic neuropathy. However, these findings have not yet been confirmed in clinical trials.

RETINOPATHY

Retinopathy is one of the most common complications associated with diabetes and one of the major causes of adult blindness (International Diabetes Federation, 2015). The mechanisms that are involved in this condition include damaged retinal vasculature, neurodegeneration, and inflammation (Jonsson *et al.*, 2016; Vindeirinho *et al.*, 2016). Ocular imaging techniques are very important for diagnosis and the evaluation of treatment efficacy (Tan *et al.*, 2016).

Lin *et al.* (2013) evaluated the effects of silybin in age-related macular degeneration *in vitro* and *in vivo.* Retinal pigmented epithelial cells were subjected to hypoxic conditions. The treatment increased the expression of proline hydroxylase-2 and inhibited hypoxia-inducible factor 1α (HIF-1α) and vascular endothelial growth factor, molecules that are related to angiogenesis. In rats, silybin prevented retinal edema and neovascularization. In another experiment with Sprague Dawley rats, diabetes was induced by streptozotocin and a high-fat diet. Silybin was administered for 22 weeks (Table 1). This treatment prevented the obliteration of retinal capillaries and reduced leukostasis and retinal intercellular adhesion molecule-1 (ICAM-1) in experimental diabetes (Zhang *et al.*, 2014). The authors concluded that silybin prevented vascular retinal damage, which at least partially occurred as a consequence of a reduction of ICAM molecules and leukostasis. We found no clinical trials on silymarin for the treatment of retinopathy.

IMPAIRED HEALING

Neovascularization is essential to wound healing (King *et al.*, 2014). Because of this, another consequence of microvascular deficiency in diabetic patients is impaired healing. Reports have indicated that silymarin and related compounds can improve healing. An *in vitro* study evaluated the efficacy of silybin against the toxicity of sulfur mustard, a vesicant agent that can cause serious skin injuries. HaCat cells (i.e., an aneuploid immortal keratinocyte cell line from adult human skin) were treated with silybin-bis-succinate, a water-soluble prodrug of silybin. The treatment reduced cytotoxicity, apoptosis, and IL-6 and IL-8 production, with a reduction of inflammation. These results may suggest the potential use of silybin for the treatment of skin injuries (Balszuweit *et al.*, 2013). Gadad *et al.* (2013) developed sterile lyophilized wafers that were impregnated with silymarin and investigated their *in vitro* actions. They used a model of cell migration and microvascular endothelial cells from the adult dermis (HMVECad). In an environment with high glucose concentrations that simulated cellular conditions associated with diabetes, the use of the silymarin-containing wafers improved endothelial cell migration. The results indicated that silymarin at least partially prevented the reduction of angiogenesis in diabetes, improved endothelial cell migration, and consequently promoted better healing in these patients.

The topical application of 30 mg/kg silymarin in Wistar rats reduced inflammation and increased epithelialization in excision wounds, with no differences in the percentage of wound contraction, collagen deposition, or hydroxyproline levels (Sharifi *et al.*, 2012). However, another study with Wistar rats reported differences in collagen and hydroxyproline levels. Oryan *et al.* (2012) investigated the effects of topical application of silymarin on incisional wounds. Treatment with 6 or 12 mg/mL in rats for 10, 20, or 30 days increased tissue healing, collagen and glycosaminoglycan deposition, and tensile strength. Differences were observed between the 6 and 12 mg/mg doses. The lower dose decreased the number of lymphocytes and increased the number of fibrocytes. The higher dose increased the

number of lymphocytes and macrophages and increased the number of fibrocytes. The topical application of 10% and 20% silybin accelerated the time of healing and increased the levels of stromelysine 1 (i.e., an enzyme involved in the remodeling process), *N*-acetyl glucosamine, *N*-acetyl galactosamine, and hydroxyproline (i.e., components of the extracellular matrix) with 10, 20, or 30 days of treatment. These effects occurred in a dose-dependent and time-dependent manner. The results indicated an improvement in the remodeling phase and augmentation of the index of collagen and glycosaminoglycan content that was induced by silybin in rats (Tabandeh *et al.,* 2013). We found no clinical trials concerning would healing in patients who are treated with silymarin or its constituent compounds.

METABOLIC DISORDERS

Diabetes involves disturbances in the metabolism of carbohydrates, fat, and protein, resulting from alterations in insulin secretion or action. In severe forms, ketoacidosis may occur, leading to stupor, coma, and death. Mitochondrial mutations are also related to diabetes (Alberti and Zimmet, 1998). Evidence shows that silymarin can reverse metabolic damage and improve mitochondrial respiratory activity. Streptozotocin-induced diabetes in Wistar rats was treated intragastrically with 70 mg/kg silymarin for 14 days. The treated animals exhibited lower levels of blood glucose and cholesterol, a reduction of the lipoperoxidation index, and normalized oxidative phosphorylation disturbances in liver mitochondria (Vengerovskii *et al.,* 2007). Similarly, Detaille *et al.* (2008) evaluated the activity of silymarin in isolated hepatocytes. After isolation, the cells were perfused with Krebs–bicarbonate-calcium in the presence or absence of silybin. This compound decreased hepatic glycolysis by inhibiting pyruvate kinase and reducing dihydroxyacetone phosphorylation. Silybin also reduced the levels of ROS that were produced within the electron transport chain. ROS production was fully abrogated by 100 μM silybin.

Liver gluconeogenesis and glycogenolysis were evaluated in a perfusion system, in which silybin treatment reduced glucose-6-phosphate hydrolysis, leading to the inhibition of gluconeogenesis and glycogenolysis. These effects were attributed to inhibition of the enzyme glucose-6-phosphatase in rat liver microsomes (Vengerovskii *et al.,* 2007).

Ligeret *et al.* (2008) evaluated hepatic alterations after cold preservation (i.e., a necessary condition for organ transplants). The assays involved liver perfusion after 24 h with silybin added to the cold preservation solution. Compared with the control group, silybin restored bile secretion and glutathione (GSH) production, reduced lipoperoxidation (LPO) and superoxide production, and increased the respiratory control ratio. The results suggested that silybin may be useful for improving the outcome of liver transplantation (Ligeret *et al.,* 2008). These data are relevant because some patients develop diabetes after liver transplantation. The preoperative risks for developing this condition include advanced age, alcoholic hepatitis, ascites, hepatic coma, and esophageal varices (Liu *et al.,* 2016).

Although only a few clinical studies of silymarin have been conducted, the results have reflected the findings of laboratory studies (Costa Pereira *et al*., 2016), including with regard to glycemia and lipid profiles. A clinical trial that included patients with type 2 diabetes mellitus (n = 25) who received silymarin (200 mg/day) for 4 months reported significant decreases in fasting glycemia, total cholesterol, low-density lipoprotein, and triglycerides compared with placebo and compared with the respective indices at the beginning of the study. The authors concluded that silymarin treatment has a beneficial effect on improving the glycemic profile (Huseini *et al*., 2006).

HEPATOPATHY

Other complications of diabetes include liver diseases that are mainly caused by insulin resistance and oxidative stress (Bouderba *et al*., 2014). Two hepatic complications are largely present in patients with diabetes: (i) concomitant autoimmune hepatitis (Matsumoto *et al*., 2016) and (ii) NAFLD (Forlani *et al*., 2016). A study of 118 diabetic patients found a prevalence of 24.5% of concomitant autoimmune hepatitis in diabetic patients (Matsumoto *et al*., 2016). NAFLD is a public health concern, the major treatment for which remains lifestyle changes, including weight reduction, the prevention of weight gain, a healthy diet, and physical activity (Zelber-Sagi *et al*., 2016).

Oxidative stress is involved in the pathogenesis of NAFLD and non-alcoholic steatohepatitis (NASH), and treatment with antioxidant substances, including silymarin and silibinin, can contribute to the control of these imbalances in lipid metabolism (Salomone *et al.,* 2016; Salamone *et al*., 2012a). Treatment with silibinin (20 mg/kg) reduced the activation of NF-κB in a model of obese mice with NASH and counteracted the progression of liver injury through anti-inflammatory actions (Salamone *et al*., 2012a). These data have translational importance because patients with type 2 diabetes mellitus also commonly have NAFLD, with a very high rate of NASH (Tilg et al., 2016). In another model of liver steatosis, Wistar rats were treated with a complex that contained silymarin and vitamin E. The treated animals presented less hepatic lipid infiltration, lower serum transaminase levels, and improvements in parameters of nitrosative and oxidative stress, including reductions of malondialdehyde and thiobarbituric acid, nitrosothiols, nitrotyrosine, and proinflammatory keratins in the liver and blood. Mitochondrial oxidative phosphorylation complexes were evaluated in the liver, heart, and skeletal muscles. The treatment exerted a protective effect on respiratory chain proteins, which were more expressive in the liver (Grattagliano *et al.,* 2013). Notably, however, silymarin (47 mg) was combined with vitamin E (15 mg) in the study by Grattagliano *et al.* (2013), thus hindering definitive conclusions concerning the antioxidant effects of silymarin *per se*. Cytochrome P450 (CYP) 3A2 and oxidative stress were also evaluated in the liver in rats with streptozotocin-induced diabetes. Treatment with 50 mg/kg silymarin for 28 days abrogated oxidative stress, reflected by reductions of malondialdehyde and nitric oxide levels and an increase in glutathione

peroxidase and total thiol molecules. In diabetic animals, CYP3A2 was upregulated, and silymarin treatment restored these levels to normal (Malekinejad *et al.*, 2012).

The sand rat (*Psammomys obesus*) is a rodent species of the Gerbillidae class. In the natural environment, its diet is based on vegetables. When these animals receive a high-fat diet, they develop diabetes, obesity, and metabolic syndrome. For this reason, this species is frequently used as an animal model of human diabetes (Berdja *et al.,* 2016; Bolton *et al.,* 2012; Scherzer *et al.,* 2011). Bouderba *et al.* (2014) induced diabetes in *P. obesus* with a high-calorie diet for 14 weeks. Diabetic animals presented hepatic steatosis, hepatic and plasma oxidative stress, hyperinsulinemia, hyperglycemia, and dyslipidemia. Daily treatment with 100 mg/kg silybin beginning on week 7 reduced oxidative stress, hepatic steatosis, triglycerides, and insulin resistance. In mice with streptozotocin-induced diabetes, silymarin treatment restored the activity of hepatic catalase (Stolf, 2016).

Silybin-β-cyclodextrin (IβI/S) is a formulation of silybin with improved solubility and absorption. A double-blind, randomized study of IβI/S vs. placebo was performed in patients with diabetes and chronic liver disease who received 6 months of treatment. The treated group presented reductions of blood glucose, triglycerides, and malondialdehyde. The conclusion of this study was that silymarin may be useful in conditions with augmented mitochondrial ROS formation because of its antioxidant properties (Lirussi *et al.,* 2002). Thus, regarding hepatic benefits, silymarin acts through its antioxidant and anti-inflammatory properties and stimulates liver regeneration (Bahmani *et al*., 2015; Vargas-Mendoza *et al*., 2014).

CARDIOMYOPATHY

Metabolic alterations in diabetic patients lead to functional and structural changes in the myocardium. Diabetic cardiomyopathy is associated with high rates of mortality (Hayat *et al.,* 2004). This form of cardiomyopathy is related to insulin resistance, hyperinsulinemia, and alterations in mitochondria and the endoplasmic reticulum and occurs independently of hypertension and coronary artery disease (Jia *et al.,* 2016).

In an *in vitro* model of hypoxia/reoxygenation, neonatal rat cardiomyocytes were treated with 2,3-dehydrosilybin, a minor component of silymarin. Treated cells had a lower index of cell death and lower activity of lactate dehydrogenase. Treated cells also exhibited decreases in ROS, reactive nitrogen species, hydrogen peroxide, and the formation of protein carbonyls (i.e., a marker of protein oxidative modification). Furthermore, the treatment regulated the activity and phosphorylation of protein kinase Cε, a component of cell survival and mitogenesis (Gabrielova *et al.,* 2015). The treatment of H9c2 embryonic rat heart cells with silybin reduced the hypertrophic response that was induced by phenylephrine by inhibiting oxidative stress (Anestopoulos *et al.,* 2013).

Acrolein is a highly reactive aldehyde that is able to induce apoptosis, oxidative stress, and inflammation, thus leading to neurodegenerative and cardiac diseases. A protective effect was demonstrated in mice that were treated with silymarin. Lower malondialdehyde levels and the restoration of GSH, superoxide dismutase (SOD), and catalase levels were observed in the heart in treated animals, indicating a reduction of oxidative stress. Serum cardiac troponin and creatine kinase-MB, indicators of cardiac lesions, were also reduced. The Bax/Bcl-2 ratio, cytosolic cytochrome *c* content, and cleaved caspase-3 were also reduced, indicating protection against apoptosis (Taghiabadi *et al.,* 2012). Another study that treated db/db mice with 20 mg/kg silybin, i.p., daily for 4 weeks found that silybin exerted antioxidant and anti-inflammatory actions by lowering the levels of isoprostanes, 8-deoxyguanosine, nitrites/nitrates, and TNF-α in the heart and liver. Silybin treatment also restored GSH levels in both tissues (Salamone *et al.*, 2012b). These authors concluded that silybin improved both myocardial and hepatic injury.

Silymarin afforded cardiac protection against hyperglycemia-induced apoptosis in cardiomyocytes. In rats with alloxan-induced diabetes that was treated with 120 mg/kg silymarin, immunohistochemistry of the heart showed lower immunoreactivity of caspase 3, a pro-apoptotic protein, and augmented Bcl-2, an anti-apoptotic protein, in treated animals. The ratio of DNA cardiac fragmentation was also reduced in treated animals, indicating protection against apoptosis. The treatment also reduced the plasma levels of cholesterol, triglycerides, glycemia, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), and the AST/ALT ratio. Plasma insulin levels were restored after treatment, reflecting the restoration of pancreatic cells (Tuorkey *et al.,* 2015). We found no clinical trials that evaluated the effects of silymarin in cardiomyopathy.

FINAL CONSIDERATIONS

The studies reviewed herein provide evidence that treatment with silymarin or its constituent compounds are capable of attenuating common diabetic complications in several organs. The data were obtained from *in vitro* investigations, animal models, and clinical studies. However, data are still lacking concerning the safety of silymarin and its constituent compounds in diabetic patients, mainly with prolonged treatment. Cheng *et al.* (2014) warned about this problem because their study showed that silymarin treatment led to insulin resistance in rats. A recent meta-analysis evaluated five randomized controlled trials that included 270 diabetic patients who were treated with silymarin. Silymarin reduced fasting blood glucose but had no effect on lipid profiles. Few data are available on the effects of silymarin on diabetic complications in randomized controlled trials (Voroneanu *et al.,* 2016) or its pharmacokinetics profile. Regarding drug interactions, silymarin may interact with metronidazole (Izzo *et al.,* 2016) and ribavirin (Liao et al., 2016), lowering the plasma concentrations of both drugs and thus compromising its pharmacokinetics and therapeutic efficacy. Silymarin appears to be a promising drug for controlling diabetes complications, but further investigations of its pharmacokinetics, safety, and possible adverse effects in this particular patient population are necessary and should be encouraged.

Acknowledgements

We are thankful to CNPq and CAPES for the scholarship given to authors.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med: A Journal Of The British Diabetic Association* 1998;**15**(7):539–53. doi: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S. PubMed PMID: 9686693.

Aller R, Izaola O, Gómez S, Tafur C, González G, Berroa E, Mora N, González JM, De Luis DA. Effect of silymarin plus vitamin E in patients with non-alcoholic fatty liver disease. A randomized clinical pilot study. *Eur Rev Med Pharmacol Sci* 2015;**19**(16):3118–3124. PMID: 26367736.

Anestopoulos I, Kavo A, Tentes I, Kortsaris A, Panayiotidis M, Lazou A, et al. Silibinin protects H9c2 cardiac cells from oxidative stress and inhibits phenylephrine-induced hypertrophy: potential mechanisms. *J Nutr Biochem* 2013;**24**(3):586–94. doi: 10.1016/j.jnutbio.2012.02.009. PubMed PMID: 22818713.

Baharvand-Ahmadi B, Bahmani M, Tajeddini P, Naghdi N, Rafieian-Kopaei M. An ethno-medicinal study of medicinal plants used for the treatment of diabetes. *J Nephrol*. 2016;**5**(1):44–50. doi: 10.15171/jnp.2016.08. PubMed PMID: 27047810; PubMed Central PMCID: PMC4790187.

Bahmani M, Shirzad H, Rafieian S, Rafieian-Kopaei M. *Silybum marianum*: Beyond hepatoprotection. *J Evid Based Complementary Altern Med* 2015;**20**(4):292–301. doi: 10.1177/2156587215571116. PubMed PMID: 25686616.

Balszuweit F, John H, Schmidt A, Kehe K, Thiermann H, Steinritz D. Silibinin as a potential therapeutic for sulfur mustard injuries. *Chem Biol Interact* 2013;**206**(3):496–504. doi: 10.1016/j.cbi.2013.06.010. PubMed PMID: 23810508.

Baluchnejadmojarad T, Roghani M, Khastehkhodaie Z. Chronic treatment of silymarin improves hyperalgesia and motor nerve conduction velocity in diabetic neuropathic rat. *Phytother Res: PTR.* 2010;**24**(8):1120–5. doi: 10.1002/ptr.3078. PubMed PMID: 19960427.

Berdja S, Smail L, Saka B, Neggazi S, Haffaf el M, Benazzoug Y, et al. Glucotoxicity induced oxidative stress and inflammation in vivo and in vitro in *Psammomys obesus*: Involvement of Aqueous Extract of *Brassica rapa* rapifera. *Evid Based Complementary Altern Med: ECAM* 2016;**2016**:3689208. doi: 10.1155/2016/3689208. PubMed PMID: 27047569; PubMed Central PMCID: PMC4800080.

Bolton K, Segal D, Walder K. The small leucine-rich proteoglycan, biglycan, is highly expressed in adipose tissue of *Psammomys obesus* and is associated with obesity and type 2 diabetes. *Biologics: Targets & Therapy* 2012;**6**:67–72. doi: 10.2147/BTT.S27925. PubMed PMID: 22532774; PubMed Central PMCID: PMC3333821.

Bosch-Barrera J, Menendez JA. Silibinin and STAT3: a natural way of targeting transcription factors for cancer therapy. *Cancer Treat Rev* 2015;**41**(6):540–6. doi: 10.1016/j.ctrv.2015.04.008. PubMed PMID: 25944486.

Bouderba S, Sanchez-Martin C, Villanueva GR, Detaille D, Koceir EA. Beneficial effects of silibinin against the progression of metabolic syndrome, increased oxidative stress, and liver steatosis in *Psammomys obesus*, a relevant animal model of human obesity and diabetes. *J Diabetes* 2014;**6**(2):184–92. doi: 10.1111/1753-0407.12083. PubMed PMID: 23953934.

Cheng KC, Asakawa A, Li YX, Chung HH, Amitani H, Ueki T, et al. Silymarin induces insulin resistance through an increase of phosphatase and tensin homolog in Wistar rats. *PLoS One* 2014;**9**(1):e84550. doi: 10.1371/journal.pone.0084550. PubMed PMID: 24404172; PubMed Central PMCID: PMC3880317.

Conserva F, Gesualdo L, Papale M. A systems biology overview on human diabetic nephropathy: from genetic susceptibility to post-transcriptional and post-translational modifications. *J Diabet Res* 2016;**2016**:7934504. doi: 10.1155/2016/7934504. PubMed PMID: 26798653; PubMed Central PMCID: PMC4698547.

Costa Pereira TM, Pimenta FS, Porto ML, Baldo MP, Campagnaro BP, Gava AL, Meyrelles SS, Vasquez EC. Coadjuvants in the diabetic complications: nutraceuticals and drugs with pleiotropic effects. *Int J Mol Sci*. 2016;**17**(8):1273. doi:10.3390/ijms17081273. PubMed PMID: 27527163. PMCID: PMC5000671.

Detaille D, Sanchez C, Sanz N, Lopez-Novoa JM, Leverve X, El-Mir MY. Interrelation between the inhibition of glycolytic flux by silibinin and the lowering of mitochondrial ROS production in perifused rat hepatocytes. *Life Sci* 2008;**82**(21–22):1070–6. doi: 10.1016/j.lfs.2008.03.007. PubMed PMID: 18448125.

Di Cesare Mannelli L, Zanardelli M, Failli P, Ghelardini C. Oxaliplatin-induced oxidative stress in nervous system-derived cellular models: could it correlate with in vivo neuropathy? *Free Radic Biol Med* 2013;**61**:143–50. doi: 10.1016/j.freeradbiomed.2013.03.019. PubMed PMID: 23548635.

Di Cesare Mannelli L, Zanardelli M, Failli P, Ghelardini C. Oxaliplatin-induced neuropathy: oxidative stress as pathological mechanism. Protective effect of silibinin. *J Pain: Official Journal Of The American Pain Society* 2012;**13**(3):276–84. doi: 10.1016/j.jpain.2011.11.009. PubMed PMID: 22325298.

Duan S, Guan X, Lin R, Liu X, Yan Y, Lin R, et al. Silibinin inhibits acetylcholinesterase activity and amyloid beta peptide aggregation: a dual-target drug for the treatment of Alzheimer's disease. *Neurobiol Aging* 2015;**36**(5):1792–807. doi: 10.1016/j.neurobiolaging.2015.02.002. PubMed PMID: 25771396.

Ebrahimpour Koujan S, Gargari BP, Mobasseri M, Valizadeh H, Asghari-Jafarabadi M. Effects of *Silybum marianum* (L.) Gaertn. (silymarin) extract supplementation on antioxidant status and hs-CRP in patients with type 2 diabetes mellitus: a randomized, triple-blind, placebo-controlled clinical trial. *Phytomedicine: International Journal Of Phytotherapy And Phytopharmacology.* 2015;**22**(2):290–6. doi: 10.1016/j.phymed.2014.12.010. PubMed PMID: 25765835.

Elyasi S, Hosseini S, Niazi Moghadam MR, Aledavood SA, Karimi G. Effect of oral silymarin administration on prevention of radiotherapy induced mucositis: a randomized, double-blinded, placebo-controlled clinical trial. *Phytother Res: PTR.* 2016;**30**(11):1879–85. doi: 10.1002/ptr.5704. PubMed PMID: 27555604.

Fallahzadeh MK, Dormanesh B, Sagheb MM, Roozbeh J, Vessal G, Pakfetrat M, 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: The Official Journal Of The National Kidney Foundation* 2012;**60**(6):896–903. doi: 10.1053/j.ajkd.2012.06.005. PubMed PMID: 22770926.

Faulkner J, Pye C, Al-Shabrawey M, Elmarakby AA. Inhibition of 12/15-lipoxygenase reduces renal inflammation and injury in streptozotocin-induced diabetic mice. *J Diabet Metab*. 2015;**6**(6). doi: 10.4172/2155-6156.1000555. PubMed PMID: 26823989; PubMed Central PMCID: PMC4727755.

Ferenci P, Dragosics B, Dittrich H, Frank H, Benda L, Lochs H, et al. Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. *J Hepatol* 1989;**9**(1):105–13. PubMed PMID: 2671116.

Forlani G, Giorda C, Manti R, Mazzella N, De Cosmo S, Rossi MC, et al. The Burden of NAFLD and its characteristics in a nationwide population with type 2 diabetes. *J Diabet Res* 2016;**2016**:2931985. doi: 10.1155/2016/2931985. PubMed PMID: 27123461; PubMed Central PMCID: PMC4829721.

Gabrielova E, Kren V, Jaburek M, Modriansky M. Silymarin component 2,3-dehydrosilybin attenuates cardiomyocyte damage following hypoxia/reoxygenation by limiting oxidative stress. *Physiol Res / Academia Scientiarum Bohemoslovaca* 2015;**64**(1):79–91. PubMed PMID: 25194130.

Gadad PC, Matthews KH, Knott RM. Silymarin released from sterile wafers restores glucose impaired endothelial cell

migration. *Int J Pharm* 2013;**457**(1):40–9. doi: 10.1016/j.ijpharm.2013.09.006. PubMed PMID: 24055598.

Gordois A, Scuffham P, Shearer A, Oglesby A, Tobian JA. The health care costs of diabetic peripheral neuropathy in the US. *Diabetes Care* 2003;**26**(6):1790–5. PubMed PMID: 12766111.

Grattagliano I, Diogo CV, Mastrodonato M, de Bari O, Persichella M, Wang DQ, et al. A silybin-phospholipids complex counteracts rat fatty liver degeneration and mitochondrial oxidative changes. *World J Gastroenterol* 2013;**19**(20):3007–17. doi: 10.3748/wjg.v19.i20.3007. PubMed PMID: 23716980; PubMed Central PMCID: PMC3662940.

Gupta OP, Sing S, Bani S, Sharma N, Malhotra S, Gupta BD, et al. Anti-inflammatory and anti-arthritic activities of silymarin acting through inhibition of 5-lipoxygenase. *Phytomedicine: International Journal Of Phytotherapy And Phytopharmacology.* 2000;**7**(1):21–4. doi: 10.1016/S0944-7113(00)80017-3. PubMed PMID: 10782486.

Haddadi R, Nayebi AM, Farajniya S, Brooshghalan SE, Sharifi H. Silymarin improved 6-OHDA-induced motor impairment in hemi-parkisonian rats: behavioral and molecular study. *Daru: Journal Of Faculty Of Pharmacy, Tehran University Of Medical Sciences*. 2014;**22**:38. doi: 10.1186/2008-2231-22-38. PubMed PMID: 24726284; PubMed Central PMCID: PMC4001109.

Hayat SA, Patel B, Khattar RS, Malik RA. Diabetic cardiomyopathy: mechanisms, diagnosis and treatment. *Clin Sci* 2004;**107**(6):539–57. doi: 10.1042/CS20040057. PubMed PMID: 15341511.

Huseini HF, Larijani B, Heshmat R, Fakhrzadeh H, Radjabipour B, Toliat T, Raza M. The efficacy of *Silybum marianum* (L.) Gaertn. (Silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial. *Phytother Res: PTR.* 2006;**20**:1036–1039. doi: 10.1002/ptr1036. PubMed PMID: 17072885.

International Diabetes Federation, 2015. URL www.idf.org, IDF Diabetes Atlas, accessed in 21/11/2016.

Izzo AA, Hoon-Kim S, Radhakrishnan R, Williamson EM. A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. *Phytother Res: PTR.* 2016;**30**(5):691–700. doi: 10.1002/ptr.5591. PubMed PMID: 26887532.

Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol* 2016;**12**(3):144–53. doi: 10.1038/nrendo.2015.216. PubMed PMID: 26678809; PubMed Central PMCID: PMC4753054.

Jonsson KB, Frydkjaer-Olsen U, Grauslund J. Vascular changes and neurodegeneration in the early stages of diabetic retinopathy: which comes first? *Ophthalmic Res* 2016. doi: 10.1159/000444498. PubMed PMID: 27035578.

Kang JS, Jeon YJ, Park SK, Yang KH, Kim HM. Protection against lipopolysaccharide-induced sepsis and inhibition of interleukin-1beta and prostaglandin E2 synthesis by silymarin. *Biochem Pharmacol* 2004;**67**(1):175–81. PubMed PMID: 14667940.

Kazazis CE, Evangelopoulos AA, Kollas A, Vallianou NG. The therapeutic potential of milk thistle in diabetes. *Rev Diabet Stud*. 2014;**11**:167–174. doi: 10.1900/RDS.2014.11.167. PubMed PMID: 25396404. PMCID: PMC4310066

Kim JL, Kim YH, Kang MK, Gong JH, Han SJ, Kang YH. Antiosteoclastic activity of milk thistle extract after ovariectomy to suppress estrogen deficiency-induced osteoporosis. *Biomed Res Int* 2013;**2013**:919374. doi: 10.1155/2013/919374. PubMed PMID: 23781510; PubMed Central PMCID: PMC3678416.

Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME. Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*). *Org Biomol Chem* 2003;**1**(10):1684–9. PubMed PMID: 12926355.

King A, Balaji S, Keswani SG, Crombleholme TM. The role of stem cells in wound angiogenesis. *Adv Wound Care* 2014;**3**(10):614–25. doi: 10.1089/wound.2013.0497. PubMed PMID: 25300298; PubMed Central PMCID: PMC4183912.

Krecman V, Skottova N, Walterova D, Ulrichova J, Simanek V. Silymarin inhibits the development of diet-induced hypercholesterolemia in rats. *Planta Med* 1998;**64**(2):138–42. PubMed PMID: 9525106.

Kumar J, Park KC, Awasthi A, Prasad B. Silymarin extends lifespan and reduces proteotoxicity in *C. elegans* Alzheimer's model. *CNS Neurol Disord Drug Targets* 2015;**14**(2):295–302. PubMed PMID: 25613505.

Lani R, Hassandarvish P, Chiam CW, Moghaddam E, Chu JJ, Rausalu K, et al. Antiviral activity of silymarin against chikungunya virus. *Sci Rep* 2015;**5**:11421. doi: 10.1038/srep11421. PubMed PMID: 26078201; PubMed Central PMCID: PMC4468427.

Lee Y, Park HR, Chun HJ, Lee J. Silibinin prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease via mitochondrial stabilization. *J Neurosci Res* 2015;**93**(5):755–65. doi: 10.1002/jnr.23544. PubMed PMID: 25677261.

Liao S, Jin X, Li J, Zhang T, Zhang W, Shi W, et al. Effects of silymarin, glycyrrhizin, and oxymatrine on the pharmacokinetics of ribavirin and its major metabolite in rats. *Phytother Res: PTR* 2016;**30**(4):618–26. doi: 10.1002/ptr.5567. PubMed PMID: 26800424.

Ligeret H, Brault A, Vallerand D, Haddad Y, Haddad PS. Antioxidant and mitochondrial protective effects of silibinin in cold preservation-warm reperfusion liver injury. *J Ethnopharmacol* 2008;**115**(3):507–14. doi: 10.1016/j.jep.2007.10.024. PubMed PMID: 18061382.

Lin CH, Li CH, Liao PL, Tse LS, Huang WK, Cheng HW, et al. Silibinin inhibits VEGF secretion and age-related macular degeneration in a hypoxia-dependent manner through the PI-3 kinase/Akt/mTOR pathway. *Br J Pharmacol* 2013;**168**(4):920–31. doi: 10.1111/j.1476-5381.2012.02227.x. PubMed PMID: 23004355; PubMed Central PMCID: PMC3631380.

Lirussi F, Beccarello A, Zanette G, De Monte A, Donadon V, Velussi M, et al. Silybin-beta-cyclodextrin in the treatment of patients with diabetes mellitus and alcoholic liver disease. Efficacy study of a new preparation of an anti-oxidant agent. *Diabetes Nutr Metab* 2002;**15**(4):222–31. PubMed PMID: 12416659.

Liu FC, Lin JR, Chen HP, Tsai YF, Yu HP. Prevalence, predictive factors, and survival outcome of new-onset diabetes after liver transplantation: a population-based cohort study. *Medicine* 2016;**95**(25):e3829. doi: 10.1097/MD.0000000000003829. PubMed PMID: 27336869.

Malekinejad H, Rezabakhsh A, Rahmani F, Hobbenaghi R. Silymarin regulates the cytochrome P450 3A2 and glutathione peroxides in the liver of streptozotocin-induced diabetic rats. *Phytomedicine: International Journal Of Phytotherapy And Phytopharmacology* 2012;**19**(7):583–90. doi: 10.1016/j.phymed.2012.02.009. PubMed PMID: 22445624.

Matsumoto N, Ogawa M, Matsuoka S, Moriyama M. Prevalence and risk factors of diabetes mellitus in patients with autoimmune hepatitis. *Intern Med* 2016;**55**(8):879–85. doi: 10.2169/internalmedicine.55.4957. PubMed PMID: 27086799.

Mirhoseini M, Baradaran A, Rafieian-Kopaei M. 2013. Medicinal plants, diabetes mellitus and urgent needs. *J Herb med Pharmacol* **2**(2): 53–54 .eISSN: 2345-5004

Mohd Fozi NF, Mazlan M, Shuid AN, Isa Naina M. Milk thistle: a future potential anti-osteoporotic and fracture healing agent. *Curr Drug Targets* 2013;**14**(14):1659–66. PubMed PMID: 24093748.

Morales-González JA, Madrigal-Santillán E, Morales-González A, Bautista M, Gayosso-Islas E, Sánchez-Moreno C. What is known regarding the participation of factor Nrf-2 in liver regeneration? *Cells*. 2015; **4**:169–177. doi:10.3390/cells4020169. PubMed PMID: 26010752. PubMed PMCID: PMC4493454.

Nasri H, Rafieian-Kopaei M. Protective effects of herbal antioxidants on diabetic kidney disease. *J Res Med Sci: The Official Journal Of Isfahan University Of Medical Sciences* 2014;**19**(1):82–3. PubMed PMID: 24672573; PubMed Central PMCID: PMC3963332.

Nasri H, Shirzad H, Baradaran A, Rafieian-Kopaei M. Antioxidant plants and diabetes mellitus. *J Res Med Sci: The Official Journal Of Isfahan University Of Medical Sciences* 2015;**20**(5):491–502. PubMed PMID: 26487879; PubMed Central PMCID: PMC4590205.

Oryan A, Tabatabaei Naeini A, Moshiri A, Mohammadalipour A, Tabandeh MR. Modulation of cutaneous wound healing by silymarin in rats. *J Wound Care*. 2012;**21**(9):457–64. doi: 10.12968/jowc.2012.21.9.457. PubMed PMID: 22990400.

Ottai MES, Abdel-Moniem ASH. Genetic parameter variations among Milk Thistle, *Silybum marianum* varieties and varietal sensitivity to infestation with seed-head weevil, *Larinus latus* Herbst. *Int J Agric Biol* 2006;**8**(6):862–866.

Pferschy-Wenzig EM, Atanasov AG, Malainer C, Noha SM, Kunert O, Schuster D, et al. Identification of isosilybin a from milk thistle seeds as an agonist of peroxisome proliferator-activated receptor gamma. *J Nat Prod* 2014;**77**(4):842–7. doi: 10.1021/np400943b. PubMed PMID: 24597776; PubMed Central PMCID: PMC4003856.

Salamone F, Galvano F, Cappello F, Mangiameli A, Barbagallo I, Li Volti G. Silibinin modulates lipid homeostasis and inhibits nuclear factor kappa B activation in experimental nonalcoholic steatohepatitis. *Transl Res* 2012a;**159**:477–486. doi: 10.1016/j.trsl.2011.12.003. Pubmed PMID: 22633099.

Salamone F, Galvano F, Marino Gammazza A, Paternostro C, Tibullo D, Bucchieri F, et al. Silibinin improves hepatic and myocardial injury in mice with nonalcoholic steatohepatitis. *Dig Liver Dis: Official Journal Of The Italian Society Of Gastroenterology And The Italian Association For The Study Of The Liver* 2012b;**44**(4):334–42. doi: 10.1016/j.dld.2011.11.010. PubMed PMID: 22197629.

Salmi HA, Sarna S. Effect of silymarin on chemical, functional, and morphological alterations of the liver. A double-blind controlled study. *Scand J Gastroenterol* 1982;**17**(4):517–21. PubMed PMID: 6753109.

Salomone F, Godos J, Zelber-Sagi S. 2016. Natural antioxidants for non-alcoholic fatty liver disease: molecular targets and clinical perspectives. *Liver Int* **36**(1): 5–20. doi: 10.1111/liv.12975.

Scavo MP, Gentile E, Wolfram J, Gu J, Barone M, Evangelopoulos M, et al. Multistage vector delivery of sulindac and silymarin for prevention of colon cancer. *Colloids Surf B Biointerfaces* 2015;**136**:694–703. doi: 10.1016/j.colsurfb.2015.10.005. PubMed PMID: 26513752.

Scherzer P, Katalan S, Got G, Pizov G, Londono I, Gal-Moscovici A, et al. *Psammomys obesus*, a particularly important animal model for the study of the human diabetic nephropathy. *Anat Cell Biol* 2011;**44**(3):176–85. doi: 10.5115/acb.2011.44.3.176. PubMed PMID: 22025969; PubMed Central PMCID: PMC3195821.

Sharifi R, Rastegar H, Kamalinejad M, Dehpour AR, Tavangar SM, Paknejad M, et al. Effect of topical application of silymarin (*Silybum marianum*) on excision wound healing in albino rats. *Acta Med Iran* 2012;**50**(9):583–8. PubMed PMID: 23165806.

Sheela N, Jose MA, Sathyamurthy D, Kumar BN. Effect of silymarin on streptozotocin-nicotinamide-induced type 2 diabetic nephropathy in rats. *Iran J Kidney Dis* 2013;**7**(2):117–23. PubMed PMID: 23485535.

Singh RP, Gu M, Agarwal R. Silibinin inhibits colorectal cancer growth by inhibiting tumor cell proliferation and angiogenesis. *Cancer Res* 2008;**68**(6):2043–50. doi: 10.1158/0008-5472.CAN-07-6247. PubMed PMID: 18339887.

Skottova N, Krecman V. Silymarin as a potential hypocholesterolaemic drug. *Physiol Res / Academia Scientiarum Bohemoslovaca* 1998;**47**(1):1–7. PubMed PMID: 9708694.

Soto C, Perez J, Garcia V, Uria E, Vadillo M, Raya L. Effect of silymarin on kidneys of rats suffering from alloxan-induced diabetes mellitus. *Phytomedicine: International Journal Of Phytotherapy And Phytopharmacology* 2010;**17**(14):1090–4. doi: 10.1016/j.phymed.2010.04.011. PubMed PMID: 20579862.

Soto C, Raya L, Perez J, Gonzalez I, Perez S. Silymarin induces expression of pancreatic Nkx6.1 transcription factor and beta-cells neogenesis in a pancreatectomy model. *Molecules* 2014;**19**(4):4654–68. doi: 10.3390/molecules19044654. PubMed PMID: 24739928.

Stolf AM. 2016. Efeitos da silimarina sobre a angiogênese e estresse oxidativo em camundongos normoglicêmicos e diabéticos. Thesis of Pharmacology Doctorate Degree. UFPR. Curitiba, PR, Brazil. 2016.

Surai, PF. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. Antioxidants. 2015;**4**:204–247. doi:10.3390/antiox4010204. PubMed PMID: 26785346. PubMed PMCID: PMC4665566.

Tabandeh MR, Oryan A, Mohhammad-Alipour A, Tabatabaei-Naieni A. Silibinin regulates matrix metalloproteinase 3 (stromelysine1) gene expression, hexoseamines and collagen production during rat skin wound healing. *Phytother Res: PTR* 2013;**27**(8):1149–53. doi: 10.1002/ptr.4839. PubMed PMID: 22976003.

Taghiabadi E, Imenshahidi M, Abnous K, Mosafa F, Sankian M, Memar B, et al. Protective effect of silymarin against acrolein-induced cardiotoxicity in mice. *Evid Based Complementary Altern Med: eCAM* 2012;**2012**:352091. doi: 10.1155/2012/352091. PubMed PMID: 23320028; PubMed Central PMCID: PMC3535759.

Tan CS, Chew MC, Lim LW, Sadda SR. Advances in retinal imaging for diabetic retinopathy and diabetic macular edema. *Indian J Ophthalmol*. 2016;**64**(1):76–83. doi: 10.4103/0301-4738.178145. PubMed PMID: 26953028; PubMed Central PMCID: PMC4821126.

Tan J, Hu J, He Y, Cui F. Protective role of silymarin in a mouse model of renal ischemia–reperfusion injury. *Diagn Pathol* 2015;**10**:198. doi: 10.1186/s13000-015-0436-4. PubMed PMID: 26521234; PubMed Central PMCID: PMC4628269.

Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* 2016. doi: 10.1038/nrgastro.2016.147. PubMed PMID: 27729660.

Toklu HZ, Tunali Akbay T, Velioglu-Ogunc A, Ercan F, Gedik N, Keyer-Uysal M, et al. Silymarin, the antioxidant component of *Silybum marianum*, prevents sepsis-induced acute lung and brain injury. *J Surg Res* 2008;**145**(2):214–22. doi: 10.1016/j.jss.2007.03.072. PubMed PMID: 17950327.

Toklu HZ, Tunali-Akbay T, Erkanli G, Yuksel M, Ercan F, Sener G. Silymarin, the antioxidant component of *Silybum marianum*, protects against burn-induced oxidative skin injury. *Burns Journal Of The International Society For Burn Injuries* 2007;**33**(7):908–16. doi: 10.1016/j.burns.2006.10.407. PubMed PMID: 17521818.

Tuorkey MJ, El-Desouki NI, Kamel RA. Cytoprotective effect of silymarin against diabetes-induced cardiomyocyte apoptosis in diabetic rats. *Biomed Environ Sci: BES* 2015;**28**(1):36–43. doi: 10.3967/bes2015.004. PubMed PMID: 25566861.

Vargas-Mendoza N, Madrigal-Santillán E, Morales-González Á, Esquivel-Soto J, Esquivel-Chirino C, García-Luna, González-Rubio M, Gayosso-de-Lucio JA, Morales-González JA. Hepatoprotective effect of silymarin. *World J Hepatol*. 2014;**6**(3):144–149. doi: https://doi.org/10.4254/wjh.v6.i3.144. PubMed PMID: 24672644. PMCID: PMC3959115.

Vengerovskii AI, Khazanov VA, Eskina KA, Vasilyev KY. Effects of silymarin (hepatoprotector) and succinic acid (bioenergy regulator) on metabolic disorders in experimental diabetes mellitus. *Bull Exp Biol Med* 2007;**144**(1):53–6. PubMed PMID: 18256751.

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**(6):733–9. doi: 10.3109/0886022X.2010.486488. PubMed PMID: 20540643.

Vindeirinho J, Santiago AR, Cavadas C, Ambrosio AF, Santos PF. The Adenosinergic system in diabetic retinopathy. *J Diabet Res* 2016;**2016**:4270301. doi: 10.1155/2016/4270301. PubMed PMID: 27034960; PubMed Central PMCID: PMC4789509.

Voroneanu L, Nistor I, Dumea R, Apetrii M, Covic A. Silymarin in type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *J Diabet Res*. 2016; Article ID 5147468. doi: 10.1155/2016/5147468. PubMed PMID: 27340676; PubMed Central PMCID: PMC4908257.

Weyhenmeyer R, Mascher H, Birkmayer J. Study on dose-linearity of the pharmacokinetics of silibinin diastereomers using a new stereospecific assay. *Int J Clin Pharmacol Ther Toxicol* 1992;**30**(4):134–8. PubMed PMID: 1572758.

Yurtcu E, Darcansoy Iseri O, Iffet Sahin F. Effects of silymarin and silymarin-doxorubicin applications on telomerase activity of human hepatocellular carcinoma cell line HepG2. *J Buon: Official Journal Of The Balkan Union Of Oncology* 2015;**20**(2):555–61. PubMed PMID: 26011349.

Zelber-Sagi S, Godos J, Salomone F. Lifestyle changes for the treatment of nonalcoholic fatty liver disease: a review of observational studies and intervention trials. *Therap Adv Gastroenterol* 2016;**9**(3):392–407. doi: 10.1177/1756283X16638830. PubMed PMID: 27134667; PubMed Central PMCID: PMC4830109.

Zhang HT, Shi K, Baskota A, Zhou FL, Chen YX, Tian HM. Silybin reduces obliterated retinal capillaries in experimental diabetic retinopathy in rats. *Eur J Pharmacol* 2014;**740**:233–9. doi: 10.1016/j.ejphar.2014.07.033. PubMed PMID: 25066112.

Zhao F, Shi D, Li T, Li L, Zhao M. Silymarin attenuates paraquat-induced lung injury via Nrf2-mediated pathway in vivo and in vitro. *Clin Exp Pharmacol Physiol* 2015;**42**:988–998. doi: 10.1111/1440-1681.12448. PubMed PMID: 26173462.

