JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

MILK THISTLE (*SILYBUM MARIANUM*): A VALUABLE MEDICINAL PLANT WITH SEVERAL THERAPEUTIC PURPOSES

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doi: 10.15414/jmbfs.2020.9.4.836-843

ARTICLE INFO

Received 5. 6. 2019
Revised 19. 9. 2019
Accepted 25. 10. 2019
Published 3. 2. 2020

Regular article

OPEN ACCESS

ABSTRACT

This review provides a systematic and in-depth overview of the promise and potential of milk thistle (*Silybum marianum*) as an interesting alternative nutraceutical preparation for pharmaceutical and medicinal applications. Moreover, it aims to summarize and update the existing evidence of extract of milk thistle in the treatment of various diseases by *in vitro*, *in vivo*, and clinical studies, and special care is paid to the action mechanisms. The main active component of milk thistle, collectively known as silymarin, consists of a mixture of flavonolignans and flavonoid taxifolin. Silymarin acts as a hepatoprotective, anticancer, anti-inflammatory, immunomodulatory, neuroprotective and lactogenic agent. Precise mechanisms of silymarin action still needs investigations and molecular/genetic background of silymarin synthesis is crucial to be elucidated for reinforcement of the therapeutical potential of the plant by breeding.

Keywords: *Silybum marianum*, silymarin, flavonolignans, pharmacological properties

INTRODUCTION

Due to unmatched availability of chemical diversity, natural products from medicinal herbs (as pure compounds or standardized extracts) provide unlimited opportunities for therapeutic usage. Botanical preparations for medicinal purposes contain various types of bioactive compounds **(Sasidharan *et al.*, 2011)**, of which natural polyphenols have attracted increasing attention as potential agents for the prevention and treatment of liver diseases **(Li *et al.*, 2018)**. Among them, flavonolignans (i.e., naturally occurring hybrid molecules biogenetically originating from flavonoids and lignans) are the most common class of compounds present in milk thistle extract **(Divers and Barton, 2018)** that has long been used for hepatoprotection **(Vue and Chen, 2016)**. Besides this, many other health promoting actions of the extract give it the opportunity to be an interesting alternative source for pharmaceutical and medicinal applications.

HABITAT AND BOTANICAL SURVEY

Milk thistle, *Silybum marianum* (L.) Gaertn. (syn. *Carduus marianus* L.; **Smith *et al.*, 2005;** Figure 1) is one of the most important medicinal members of *Asteracae* family (**Qavami *et al.*, 2013**) that originated in the Mediterranean Basin. It grows in warm, dry soil **(Abenavoli *et al.*, 2010)** in many European countries, North Africa, South and North America, Central and Western Asia and southern Australia (**Carrier *et al.*, 2003; Bhattacharya, 2011**). The herb is very competitive with the ability to grow on light soils with regular water deficit (due to strong root system; **Andrzejewska *et al.*, 2011**) and with tends to establish in tall dense patches eliminating other plant species (by shading or by competition for water and nutrients; **Smatana and Macák, 2011; Vereš and Týr, 2012**).

Figure 1 *S. marianum* (according to **Habán *et al.*, 2015**)

In Slovakia, milk thistle growing dominated among the medicinal plants during the years 2013 and 2014 **(Habán *et al.*, 2015)**. In this country, the herb growing is confined to areas of beet, corn and warmer areas of potato production from 200 to 600 above sea level **(Habán, 2004).**
Generally, two species of the genus *Silybum* (*S.*) are known, *S. marianum* with variegated leaves and *S. eburneum* Coss. & Dureu with completely green leaves (**Adzet *et al.*, 1993**). Regarding *S. marianum*, two different varieties have been described, *S. marianum* (L.) Gaertn. var. *purple* **(Althagahafy *et al.*, 2013)** and *S. marianum* var. *albiflora* with white flowers differing from each other in the content of flavonolignans **(Samu *et al.*, 2004)**.
Milk thistle is an annual, rarely a biennial medicinal plant **(Habán *et al.*, 2016)** growing up to 2.0 meters in height. Stem is 40 – 200 cm high, glabrous or slightly downy, and its upper part is erect and branched (**Montemurro *et al.*, 2007**). The primary leaves are large, alternate and glossy, with spiny margins and characteristic white veins (50 - 60 cm in length, and 20 - 30 cm in width; **Gresta *et al.*, 2007**). Flower head have about 5 cm in diameter (**Montemurro *et al.*, 2007**). The fruits are one-seeded (**Zhu *et al.*, 2013**), hard-skinned achenes (Fructus silybi mariani; Figure 2), 6 – 8 mm long, shiny, usually brownish in

color and with a white silk-like pappus at the apex (**Corchete, 2008**). The weight of achenes is low (28 – 30 g/1000 pieces; **Andrzejewska *et al.*, 2011**) and the early extract of crushed milk thistle fruits is termed as milk thistle extract (**Wagner *et al.*, 1968**).

Figure 2 Fructus silybi mariani **(**according to **Habán *et al*., 2015)**

CHEMICAL COMPOSITION OF MILK THISTLE EXTRACT

The active complex of milk thistle is the lipophilic extract from its seeds (achenes) collectively known as silymarin (**Abenavoli *et al.*, 2010**). Silymarin contains number of structurally related flavonolignans (flavonolignans isomers), the flavonoid, and many other constituents (**Albassam *et al.*, 2017**). The silybins A and B, the isosilybins A and B, silychristin A, isosilychristin, and silydianin are the seven major flavonolignans which together with the flavonoid taxifolin (Figure 3) may be considered as silymarin marker compounds for quantitation analyses (**AbouZid *et al.*, 2016**).

Structurally, flavonolignans have a broad structural diversity in consequence of the C-C or C-O linkage of the C_6C_3 unit to the flavonoid nucleus in different positions, affording dioxane, furan, cyclohexane rings or simple either side chains. In general, these compounds contain several chiral centres, hence they usually occur in the form of stereoisomers in nature (**Scupor *et al.*, 2016**). The flavonolignans are produced by oxidative coupling between a flavonoid (taxifolin) and a phenylpropanoid, usually coniferyl alcohol (**Dewick, 2002**; **Bijak 2017**) followed by coupling of the two radicals (**AbouZid *et al.*, 2017**).

Among the isomers, silybin is the major and most active component and represents about 60 - 70 %, followed by silychristin (20 %), silydianin (10 %), and isosilybin (5 %; **Saller *et al.*, 2001**). Besides them, the chemical composition of milk thistle also include other flavonoids (such as quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodyctiol, chrysoeriol), proteins (25 – 30 %), sugars (arabinose, rhamnose, xylose, glucose), tocopherol, sterols (cholesterol, campesterol, stigmasterol, sitosterol), and lipids (15 – 30 %) in the form of triglycerides (60 % linoleic, 30 % oleic and 9 % palmitic acid; **Abenavoli *et al.*, 2010**). Despite a high nutritional value of *S. marianum* fruit lipids, the oil is considered an unwanted byproduct of silymarin production and has to be removed from the fruits prior to silymarin extraction (**AbouZid *et al.*, 2016**).

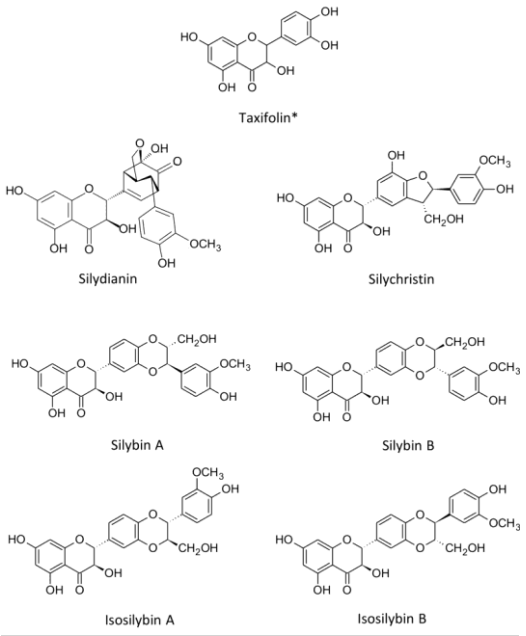

Figure 3 Chemical structure of the silymarin marker compounds (according to **Drouet *et al.*, 2018**)

BIOAVAILABILITY OF SILYMARIN

Silymarin is found in the entire plant but it is concentrated in the fruit and seeds (**Abenavoli *et al.*, 2010**). Commercial fruit extracts from *S. marianum* typically contain 70 – 80 % silymarin (**AbouZid *et al.*, 2016**) which represents 1.5 - 3 % of the fruit's dry weight (**Bijak, 2017**). In natural milk thistle achenes, the content of silymarin complex is about 0.2 – 0.6 % (**Habán *et al.*, 2009**) depending on the variety (**Habán *et al.*, 2010**). The standardized extract obtained from the dried seeds of milk thistle contains approximately 60 % silymarin (**Saller *et al.*, 2001**). The absorption of silymarin from gastrointestinal tract in human is about 20 – 50 % (**Abenavoli *et al.*, 2010**). The low bioavailability could be attributed to its poor water solubility (**Cavaretta, 2015**), degeneration by gastric fluid **(Blumenthal *et al.*, 2000**) or poor enteral absorption **(Comoglio *et al.*, 1995**). For this reason, silymarin is usually administered to body in the form of encapsulation, sugar coated tablets, self-microemulsifying drug delivery system (SMEDDS) or beta cyclodextrin inclusion complex (**Ghosh *et al.*, 2010**). In addition to this, absorption of silymarin decreases with age and at the age of 60 years it can decrease to only 10 % (**Corchete, 2008**).

All pharmacokinetic parameters of silymarin are referred to, and standardized as silybin, i.e., to the primary and most active component of the silymarin complex (**Abenavoli *et al.*, 2010; Javed *et al.*, 2011**). According to **Saller *et al.*** (**2001**), bioavailability of silybin depends also on other factors, such as (i) the content of accompanying substances with a solubilising character (such as other flavonoids, phenolderivates, aminoacids, proteins, tocopherol, fat, cholesterol and others found in the extract) and (ii) the concentration of the extract itself.

After absorption, about 80 % of the silybin dose is excreted in the bile (**Abenavoli *et al.*, 2010**) in the form of sulfates and glucuronide conjugates (**Saller *et al.*, 2001**) and approximately 10 % enters the enterohepatic circulation (**Abenavoli *et al.*, 2010**). In the liver cells, silybin undergoes both phase I and phase II of biotransformation (**Javed *et al.*, 2011**). By CYP2C8, it is metabolized into o-demethylated silybin (major metabolite) and mono- and dihydroxy- silybin (minor) metabolites (**Jančová *et al.*, 2007**). During phase II, it undergoes multiple conjugation reactions resulting in the formation of silybin monoglucuronide, silybin diglucuronide, silybin monosulfate, and silybin diglucuronide sulfate (**Wu *et al.*, 2008**; **Javed *et al.*, 2011**). Recent metabolic studies illustrate that silybin B is more efficiently glucuronidated compared to silybin A suggesting a stereoselectivity in their metabolism (**Wen *et al.*, 2008**).

The peak plasma concentrations after an oral application of silymarin is achieved between 1.5 – 4 h (**Corchete, 2008**). The tissue distribution of silybin in mice was studied by **Zhao and Agarwal (1999)** who have found peak levels of free silybin in liver, lung, stomach, pancreas (at 0.5 h), skin and prostate (at 1 h) in the animals after their oral exposure to silybin at the dose of 50 $mg.kg^{-1}$ body weight (BW). In bile, silybin concentrations reached approximately 100 times those found in serum (10^{-5} to 10^{-4} $mol.L^{-1}$ of silybin in bile) with peak concentrations reached within 2 – 9 h (**Saller *et al.*, 2001**).

Besides biliary excretion, about 5 % of silymarin is excreted in the urine as total silymarin (with a renal clearance of approximately 30 mL/min; **Corchete, 2008**) and its remaining unchanged and not absorbed part is also excreted via faeces (**Saller *et al.*, 2001**). For total silybin, an elimination half-life of approximately 6 h was estimated (**Weyhenmeyer *et al.*, 1992**). Pharmacokinetics analysis performed by **Wen *et al.* (2008)** have revealed that after oral administration, silymarin flavonolignans were rapidly eliminated from human plasma with short half-lives (1 – 3 h, 3 – 6 h, and 3 – 5 h for the free, conjugated, and total silymarin flavonolignans, respectively). Conjugated silychristin exhibited a relatively longer half-life (~ 8 h) than the other flavonolignans and free silychristin and silydianin were not detectable or at very low concentrations.

Effective daily doses of silymarin in adult human ranges from 240 mg to 600 mg (**Saller *et al.*, 2008**). According to **Hawke *et al.* (2010)**, low bioavailability of silybin A and silybin B associated with customary doses of silymarin may be overcome with doses above 700 mg. Non-toxicity of silymarin without any side effects has been observed in adults receiving daily oral dose 900 mg of silymarin in two or three divided doses (**Abenavoli *et al.*, 2010**). However, its excessive intake may cause adverse drug reactions (ADRs) where gastrointestinal effects are more common among them (**Karimi *et al.*, 2011**). Indeed, at higher doses of more than 1500 mg/day, laxative effect of silymarin with an increased bile flow and secretion may appear (**Abenavoli *et al.*, 2010**).

Very low acute toxicity of silymarin was also confirmed in toxicological studies conducted on several animal species. No mortality or any signs of adverse effects were observed in mice (20 $g.kg^{-1}$ BW) and dogs (1 $g.kg^{-1}$ BW) perorally administered with silymarin (**Corchete, 2008**). The 50 % lethal dose (LD_{50}) values after intravenous injection were estimated to be 400 $mg.kg^{-1}$ BW in mice, 385 $mg.kg^{-1}$ BW in rats and 140 $mg.kg^{-1}$ BW in rabbits and dogs (**Fraschini *et al.*, 2002**).

HEALTH BENEFICIAL EFFECTS OF SILYMARIN AND THEIR MOLECULAR MECHANISMS

Hepatoprotective activity

The primary site of silymarin action in *Mammalia* is liver (**Abenavoli *et al.*, 2010**). Generally, silymarin is most well-known for its antioxidant and chemoprotective effects on this organ (**Post-White *et al.*, 2007; Trappoliere *et al.*, 2009; Loguercio *et al.*, 2011; Loguercio *et al.*, 2012**) and it is frequently prescribed and self-prescribed as an additional and alternative hepatoprotective drug (**Testino *et al.*, 2013**). Since it is efficient in restoration of liver function and regeneration of liver cells, silymarin has been widely used to remedy various liver disorders (**Pradhan *et al.*, 2006; Dixit el al., 2007; Govind *et al.*, 2008; Tůmova *et al.* 2010**), such as alcoholic liver disease, nonalcoholic fatty liver disease, viral hepatitis, drug-induced liver injury and mushroom poisoning (**Abenavoli *et al.*, 2018**), steatohepatitis (**Milosevic *et al.*, 2014**), and cirrhosis (**Parés *et al.*, 1998**).

Regarding liver function as detoxification organ, it was shown that silymarin and silymarin-derived compounds preserve the liver against its damage induced by adverse effect of various xenobiotics, such as carbon tetrachloride (**Lettéron *et al.*, 1990**), phalloidin (**Vo *et al.*, 2016**), alpha-amanitin (**Karimi *et al.*, 2011**), acetaminophen (**Papackova *et al.*, 2018**), ethanol (**Brandon-Warner *et al.*, 2012**) and the chemotherapy drug - doxorubicin (**Patel *et al.*, 2010**). The underlying molecular mechanism of silymarin hepatoprotective action is attributed mainly to its ability to directly scavenge free radicals produced during hepatic metabolism of the above mentioned toxic substances (**Trouillas *et al.*, 2008**). Besides this, antioxidant activity of silymarin is mediated by: (i) preventing free radical formation (via inhibition of specific enzymes responsible for free radical production), (ii) maintaining the electron-transport chain integrity of mitochondria in stress conditions and optimal redox status of the cell by activating a range of antioxidant enzymes and non-enzymatic antioxidants (mainly via transcription factors, including nuclear factor erythroid 2-related factor 2; Nrf2 and nuclear factor kappa B; NF-κB), (iii) activating an array of vitagenes, responsible for the synthesis of protective molecules (including e.g., HSP, thioredoxin, sirtuins) and providing additional protection in stress conditions (**Surai, 2015**), and by (iv) increasing cellular glutathione content and inhibiting lipid peroxidation (**Esmaeil *et al.*, 2017**).

In addition to its antioxidant properties, silymarin exerts its hepatoprotective activity through antiviral, anti-inflammatory, and immunomodulatory actions in liver and immune cells (**Polyak *et al.*, 2007; Morishima *et al.*, 2010; Wagoner *et al.*, 2010**). Recent studies have shown that silymarin is an effective antiviral treatment for hepatitis C virus (HCV; **Wagoner *et al.*, 2010**). In the study by **Dehmlow *et al.* (1996)**, silybin-induced selective inhibition of leukotriene formation by Kupffer cells responsible for the hepatoprotective properties of silybin was reported.

Moreover, it has been found that silymarin forms a complex impeding the entrance of toxins into the interior of liver cells, metabolically stimulates hepatic cells (**Morales-González *et al.*, 2013**), increases protein synthesis in hepatocytes by stimulating RNA polymerase I activity (**Vargas-Mendoza *et al.*, 2014**), and acts as an iron chelator (**Borsari *et al.*, 2001**). Taking into account this consideration, **Pietrangelo *et al.* (1995)** have reported appreciably decreased iron-induced hepatotoxicity (manifesting by dramatic accumulation of malondialdehyde-protein adducts into iron-filled periportal hepatocytes) in iron overloaded rats after their treatment with silybin.

Anticancer activity

Both *in vivo* and *in vitro* studies have revealed that silymarin has anticancer potential against various types of cancer **(Post-White *et al.* 2007; Won *et al.*, 2018**). Through interference with the expression of cell cycle regulators and proteins involved in apoptosis, silymarin and silybin modulate imbalance between cell survival and apoptosis (**Zi *et al.*, 2000; Tyagi *et al.*, 2002**). The study with oral cancer cell lines (HSC-4, YD15 and Ca9.22) have provided an evidence for silymarin-induced apoptosis in the cells via caspase-8 and death receptor 5 activation (**Won *et al.*, 2018**). Moreover, the anticancer efficacy of silybin is exerted through its ability to affect cancer cell proliferation and metabolism, inflammation, and angiogenesis (**Deep and Agarwal, 2010**). Proliferative inhibition effects of silymarin on tumor cells were observed in various organs, such as prostate (**Zi *et al.*, 2000; Tyagi *et al.*, 2002; Davis-Searles *et al.*, 2005**), ovaries (**Fan *et al.*, 2014; Kayedpoor *et al.*, 2017**), breast (**Rastegar *et al.*, 2013; Hajighasemlou *et al.*, 2014**), lung (**Sharma *et al.*, 2003**), skin (**Dhanalakshni *et al.*, 2004; Vaid *et al.*, 2010**) and bladder (**Tyagi *et al.* 2003; Tyagi *et al.* 2004; Zhu *et al.*, 2016; Sun *et al.*, 2017**). **Zi *et al.* (2000)** have found the antiproliferative action of silybin on androgen-independent prostate cancer PC-3 cells via increased accumulation of insulin-like growth factor-binding protein (IGFBP-3) and inhibition of insulin-like growth factor I (IGF-I)-induced insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation. In human breast carcinoma cells MDA-MB 468, the inhibitory impact of silymarin on the cell growth and proliferation were associated with a G1 arrest in cell cycle progression concomitant with an induction of up to 19-fold in the protein expression of cyclin-dependent kinase (CDK) inhibitor Cip1/p2 (**Zi *et al.*, 1998**). **Scambia *et al.* (1996**) purposed that tumor cell growth inhibition by silybin may be due to its interaction with nuclear type II estrogen binding sites (EBS II). The findings by **Dhanalakshmi *et al*. (2004)** suggest that silybin affords strong protection against UV-induced damage in epidermis in SKH-1 hairless mice. This protective effect is attributed to decreased thymine dimer positive cells inhibition and an up-regulation of p53-p21/Cip1 cascade, possibly resulting in an inhibition of both cell proliferation and apoptosis. Apoptosis of human bladder transitional-cell papilloma RT4 cells due to silybin-induced significant decreased (up to elimination) of survivin protein levels (a member of the inhibitor of apoptosis protein gene family) was observed in the research by **Tyagi *et al.* (2003)**.

Moreover, it was found that silymarin can down-regulate gene products involved in the proliferation of tumor cells [cyclin D1, epidermal growth factor receptor (EGFR), cyclooxygenase-2 (COX-2), transforming growth factor β (TGF-β), insulin-like growth factor 1 receptor (IGF-IR)], invasion [matrix metallopeptidase 9 (MMP-9)], angiogenesis [vascular endothelial growth factor (VEGF)] and metastasis (adhesion molecules). The anti-inflammatory effects of silymarin are mediated through suppression of NF-κB-regulated gene products, including COX-2, lipooxygenase (LOX), inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF) and interleukin-1 (IL-1; **Agarwal *et al.*, 2006**).

Anti-inflammatory and immunomodulatory activity

Several studies have reported the beneficial effect of silymarin in different experimental models of acute and chronic inflammation, e.g., in rats with formalin-induced paw edema (**Alhadidi *et al.*, 2009**), allergic airway inflammation, atopic dermatitis, and allergic rhinitis (**Bakhshaee *et al.*, 2011; Choi *et al.*, 2012; Mady *et al.*, 2016**).

The main anti-inflammatory properties of the primary extract of milk thistle include suppression of 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis (**Saller *et al.*, 2001**), and multiple pro-inflammatory mRNAs and signaling pathways, such as NF-κB, forkhead box O (FOXO; **Lovelace *et al.*, 2015**), COX-2, LOX, iNOS, TNF and IL-1 (**Agarwal *et al.*, 2006**). The findings by **Li *et al*. (2016)** have demonstrated that silymarin is able to attenuate airway inflammation induced by cigarette smoke extract in human bronchial epithelial cells via suppression of extracellular signal-regulated kinase/p38 mitogen-activated protein kinase (ERK/p38 MAPK) pathway activity and autophagy.

There is growing evidence that silymarin has also some immunomodulatory effects (**Farsam *et al.*, 2011; Riahi-Zanjani *et al*., 2015; Rahnama *et al*., 2015**). In a dose-dependent manner, silymarin-induced increased proliferation of both mitogen and alloantigen stimulated lymphocytes with enhanced secretion of interferon gamma (IFNγ), IL-4, and IL-10 in the cells was revealed in the study of **Wilasrusmee *et al*. (2002)**. As an immunomodulatory agent, stimulation of both cellular and humoral immune functions in cyclophosphamide-induced immunosuppressive BALB/c mice was observed after their intraperitoneal injection with low dose (50 $mg.kg^{-1}$ BW) of silymarin for 14 consecutive days (**Karimi *et al.*, 2018**). In detail, the authors found that silymarin significantly increased the number of peripheral neutrophils and the spleen cells. However, high dose of silymarin (150 $mg.kg^{-1}$ BW) has no immunomodulatory effect in these mice, most probably due to silymarin´s scavenging activity of ROS which are necessary for activation and proliferation of the lymphocytes (**Victor *et al.*, 2004; Griffiths *et al.*, 2005**). The findings by **Zhao and Li (2015)** have shown that silymarin increases serum acid phosphatase activity (as one of the reliable indicators of macrophage activation), lysozyme and nitric oxide (NO) contents, macrophage phagocytosis and immune indexes of thymus and spleen in immunosuppressive mice leading to improved non-specific immune functions and resistance to infectious agents. In healthy rainbow trout treated with silymarin (at a dose of 100, 400 a 800 $mg.kg^{-1}$ BW), lysozyme and complement activities, as well as total protein and globulin levels were significantly higher compared to control group (**Ahmadi *et al.*, 2012**). Expression of usually suppressed major histocompatibility complex class I (MHCI) molecules (involved in the regulation of the immune response) in human neuronal and neuroblastoma cell lines after silymarin treatment was demonstrated in the study of **Sakai *et al*. (2001)**.

Neuroprotective potential

Silymarin has been shown to be putative neuroprotective agent against many neurological diseases including Alzheimer's and Parkinson's diseases, cerebral ischemia (**Borah *et al.*, 2013**) and obsessive-compulsive disorder (OCD; **Lu *et al.*, 2010; Yin *et al.*, 2011**). Its neuroprotective actions have been documented in *in vivo* (**Baluchnejadmojarad *et al.*, 2010**) and *in vitro* models **(Yin et al., 2011**). Inhibition of monoamine oxidase activity (MAO; catalyzing the oxidative deamination of monoamines; **Yin *et al.*, 2011),** suppression of oxidative stress and the inflammatory responses generated during neurodegeneration, modulation of several kinases involved in cell signaling pathways, neurotropic effects, alteration of cellular apoptosis, and estrogen receptor machineries (**Borah *et al.*,**

2013; Pandima Devi *et al.*, 2017; Ullah and Khall, 2018) are main mechanisms that are responsible for neuroprotection by silymarin.

Silymarin´s anti-inflammatory activities related to neuroprotection are mainly exerted by inhibition of microglia activation. This finding was confirmed by the results of **Wang *et al.* (2002)** which displayed that silymarin significantly inhibited NF-κB activation and the production of inflammatory mediators (TNF-α and NO) in lipopolysaccharide-activated mesencephalic mixed neuron-glia cultures. Additionally, reduced damage to dopaminergic neurons due to impact of silymarin was observed in the study. Besides the preserving of the neurons, silymarin also diminishes the cell apoptosis in *substantia nigra* and thus maintains striatal dopaminergic levels (**Abushouk *et al.*, 2017**). In BALB/c mice, intraperitoneal application of silymarin at the dose 250 $mg.kg^{-1}$ BW for 5 days resulted in increased 5-hydroxytryptamine (5-HT; serotonin) levels in the cortex and increased dopamine and norepinephrine levels in the cerebellum (**Osuchowski *et al.*, 2004**). Considering this, the application of silymarin has been shown to be effective in the treatment of anxiety disorder known as OCD in which a person has uncontrollable, reoccurring thoughts (obsessions) and behaviors (compulsions). The positive effects of milk thistle extract on OCD have been reported in adult humans receiving the doses of 600 mg/day for 8 weeks (**Sayyah *et al.*, 2010**) and 300 mg twice a day for 3 months (**Behl *et al.*, 2010**).

Lactogenic effect

A potential lactogenic impact of silymarin on milk production has been proved in humans and animal models (cows and rats; **Peila *et al.*, 2015**). However, the biochemical mechanisms that lead to these effects have not yet been exactly established (**Capasso *et al.*, 2009**). It was reported that extract from *S. marianum* fruits significantly increases circulation of prolactin levels in female rats (**Capasso *et al.*, 2009; Capasso *et al.*, 2014**) and swine (**Farmer *et al.*, 2014**). The increased prolactin levels could involve, at least in part, dopamine D2 receptor. Addition of effective dose (1 $mg.kg^{-1}$ BW) of a selective dopamine D2 agonist bromocriptine (**Capasso *et al.*, 2009**), inhibiting prolactin secretion (**Gragnoli *et al.*, 2016**) was able to reduce the hyperprolactinemia induced by silymarin BIO-C® suggesting an involvement of the dopamine D2 receptor in the silymarin action (**Capasso *et al.*, 2009**).

FUTURE DIRECTIONS IN THE ENHANCEMENT OF THE THERAPEUTIC POTENTIAL OF MILK THISTLE

Despite broad therapeutic application and strong medicinal potential of *S. marianum*, precise molecular and genetic data are still lacking. Understanding the genetic variability and population genetics of medicinal species is inevitable for optimal design of breeding programs and selection of genotypes suitable for breeding (**Russell *et al.*, 1997**). *S. marianum* is a diploid species with 2n = 34 chromosomes. The karyotype is composed of 6 pairs of metacentric, 10 pairs of submetacentric and 1 pair of acrocentric chromosomes (**Asghari-Zakaria *et al.*, 2008**). Current knowledge suggests that optimal design of breeding programmes may improve desirable properties of *S. marianum*. Milk thistle is considered to be drought resistant (**Karkanis *et al.*, 2011**) nevertheless, diverse genotypes respond differentially to drought stress. Selection of drought tolerant genotypes may be used in breeding programs to develop cultivars with enhanced drought tolerance (**Alemardan *et al.*, 2013**). In milk thistle improvement, other approaches concern breeding for biotic and abiotic stress tolerant cultivars, development of cultivars with simultaneous flowering, reduced thorns and achene shedding (**Alemardan *et al.*, 2013**). Though, prominent interest of breeding programs in the context of medicinal plants is focused on the modification of the secondary metabolite profile aimed to increase the content of beneficial compounds and thus the therapeutic potential of a plant (**Gomez-Galera *et al.*, 2007**). Wild populations originating from diverse regions comprise a gene pool which may serve for the improvement of milk thistle crop (**Shokrpour *et al.*, 2008**). According to several recent studies, mutational breeding is another useful method applicable in plant improvement (**Katar *et al.*, 2013; El-Garhy *et al.*, 2016**).

Characterization of the flavonolignan pathway and knowledge of gene expression is important for the comprehension of silymarin dynamics and for the development of strategies to increase its production. However, in milk thistle, only one full length chalcone synthase (CHS) cDNA a two partial CHS genes have been cloned and sequenced to date (**Sanjari *et al.*, 2015**). Chalcone synthase (CHS) is an allosteric enzyme which plays a key role in the biosynthesis of flavonolignans in many plants (**Spribille and Forkman, 1982; Dao *et al.*, 2011; Sanjari *et al.*, 2015**). Three genes of the CHS family identified in *S. marianum* (SmCHS1, SmCHS2 a SmCHS3) are suggested to be involved in silymarin biosynthesis (**Sanjari *et al.*, 2015; El-Garhy *et al.*, 2016**). The transcripts were detected in flower heads, petals, leaves and various parts of the stem (**Sanjari *et al.*, 2015**). Recently, other four cDNA fragments of genes putatively involved in flavonolignan biosynthesis in milk thistle were isolated: chalcone isomerase, flavanone 3-hydroxylase, flavonol 3-hydroxylase and cinnamyl alcohol dehydrogenase (**Torres and Corchete, 2016**).

With the aim to increase the therapeutic potential, research activities are often focused on the effect of diverse elicitors on the production of bioactive compounds in *S. marianum* (**Sánchez-Sampedro *et al.*, 2005; Katar *et al.*, 2013; El-Garhy *et al.*, 2016**). According to some authors, elicitation is an effective strategy for stress tolerance stimulation, activation of signalling pathways in intracellular defense mechanisms and for specific gene expression regulation, leading to the accumulation of important metabolites in the organism (e.g., silymarin in *S. marianum*; **Jimenez Garcia *et al.*, 2013; El-Garhy *et al.*, 2016**). The work by **Sánchez-Sampedro *et al.* (2005)** confirmed that in the cell culture of *S. marianum*, the activity of CHS and silymarin content increased under the action of methyljasmonate. **Torres and Corchete (2016)** demonstrated that chalcone isomerase, flavanone 3-hydroxylase, flavonol 3-hydroxylase and cinnamyl alcohol dehydrogenase were up-regulated by methyljasmonate or cyclodextrins, although with different level of up-regulation. In another study, gamma irradiation was shown to positively affect the expression of CHS2 and CHS3 genes. The combination of this factor with salinity induced the expression of all three CHS genes involved in silybin biosynthesis. The authors have also shown that gamma irradiation applied on seeds of *S. marianum* elevated the expression of CHS1, CHS2 a CHS3 in leaves and positively correlated with the increased silybin A and B content. Results of the study revealed the highest expression of CHS1, CHS2 and CHS3 genes under the 4 000 ppm/100 GY gamma irradiation treatment (**El Garhy *et al.*, 2016**). Gamma irradiation was proposed to represent a suitable tool to broaden genetic variability and to select stable mutant genotype with higher silymarin amount (**Soliman *et al.*, 2018**).

CONCLUSION

Laboratory and clinical researches suggest that silymarin, a complex of active components from *S. marianum* has beneficial effects on human health. Silymarin flavonolignan and flavonoid compounds have been isolated in quantities for biological studies. This allowed determination of hepatoprotective, anticancer, anti-inflammatory, immunomodulatory, neuroprotective and lactogenic activities of pure compounds. Extracts of milk thistle are safe and well tolerated, with minimal toxic or adverse effects. *S. marianum* as prominent source of silymarin starts to attract attention not only of biomedicine researchers but also of plant geneticists and breeders. The cognition of molecular pathways and genes responsible for silymarin production is important for further improvement of the crop and thus strengthening its medicinal properties.

Acknowledgments: This research was supported by European Community under project No. 26220220180: Building Research Centre “AgroBioTech”.

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