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

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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 need investigations and molecular/genetic background of silymarin synthesis is crucial to be elucidated for reinforcement of the therapeutic 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 (Sasilharam 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 Asteraceae 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; Andrzewská 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 Mucák, 2011; Veres and Týr, 2012). 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. chineum Coss. & Durieu 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 marianii; Figure 2), 6 – 8 mm long, shiny, usually brownish in

Figure 1 S. marianum (according to Habán et al., 2015)
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 (acenhes) 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 C6-C6 unit to the flavonoid nucleus in different positions, allowing 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, eriodictyol, 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 humans is about 20 – 30 % (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, phenolderviates, aminoucids, 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 glucurconid 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 (Jancová et al., 2007). During phase II, it undergoes multiple conjugation reactions resulting in the formation of silybin monogluconuride, silybin diglucuride, silybin monosulfate, and silybin diglucuride sulfate (Wu et al., 2008; Javed et al., 2011). Recent metabolitic 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 (10−4 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 (LD50) 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 (Frascini 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; Trappolieri 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 et al., 2007; Govind et al., 2008; Tumova et al., 2010), such as alcoholic liver disease, nonalcoholic fatty liver disease, viral drug-induced hepatic 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 (Lettieron et al., 1990), phalloidin (Vaidy et al., 2004), Bakhshaee et al., 2007; Won et al., 2001). Silymarin exerts its hepatoprotective effects by: (i) preventing free radical formation (via inhibition of specific enzymes responsible for free radical production), (ii) maintaining the electron-transfer chain integrity of mitochondria in stress conditions and optimal redox status of the cell by activating 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-kB), (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 (Surali, 2015), and by (iv) increasing cellular glutathione content and inhibiting lipid peroxidation (Esmaeiel et al., 2017).

In addition to its antioxidant properties, silymarin exerts its hepatoprotective activity through antiviral, anti-inflammatory, and immunomodulatory activities in liver and immune cells (Polyk et al., 2007; Morishima et al., 2010; Wagoner et al., 2013). Recent studies have shown that silymarin is an effective antiviral treatment for hepatitis C virus (HCV; Wagoner et al., 2010). In the study by Dehloul 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 toxicity (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 apoptosis. Activation of caspase-3 and caspase-9 (mediated by FasL) in human breast cancer 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/p21 (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 (E2). The findings by Dhanalakshmi et al. (2004) suggest that silybin affords strong protective effects against UV-induced damage in epidermis in SKH:1 hairless mice. This protective effect is attributed to decreased thymine dimer positive cells 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 R:4 cells due to silybin-induced significant decreased levels of survivin protein (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 metalloproteinase 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-mediated gene products, including COX-2, lipoxygenase (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 (Alhaddidi et al., 2009), allergic airway inflammation, atopic dermatitis, and allergic rhinitis (Bakhshae 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-oxypentiygen 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; Risahi-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 Wilursumee 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, immunosuppression of BALB/c mice with cyclophosphamide (an immunosuppressive agent) may promote survival of 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 phosphate 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 (MHC1) 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, and Parkinson’s disease (Borah et al., 2013) and obsessive-compulsive disorder (OCD; Lu et al., 2010; Yin et al., 2011). Its neuroprotective actions have been documented in vivo (Baluchnejadmorad 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 anti-inflammatory activity (mediated by transcription factors, including NFκB), and modulation of several kinases involved in cell signaling pathways, neurotrophic effects, alteration of cellular apoptosis, and estrogen receptor machineries (Borah et al., 2013).
2013; Pandima Devi et al., 2017; Ulah and Khali, 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 mesenchephalic mixed neuron-glial 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 positively affected the cell apoptosis induced by 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 (Gomez et al., 2014). Considering the high levels of S. marianum genes, the isolation 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 (Ssuya et al., 2010) and 300 mg twice a day for 3 months (Beltl et al., 2010).

Lactogenetic effect

A potential lactogenetic 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 involucrate, in at least 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 BOC-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 = 54 chromosomes. The karyotype is composed of 6 pairs of metacentric, 10 pairs of submetacentric and 1 pair of acrocentric chromosomes (Agharzi-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 useful in breeding programs to develop cultivars with enhanced drought tolerance (Alemdar 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 achenes shedding (Alemdar et al., 2013). Though, prominent interest of breeding programs in the context of medicinal plants is focused on the modification of the secondary metabolic 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 (Shokpour 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 at silymarin significant 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 (Sreiben and Forkman, 1982; Dao et al., 2011; Sanjari et al., 2015). Three genes of the CHS family identified in S. marianum (SmCHS1, SmCHS2 and SmCHS3) are suggested to be involved in silymarin synthesis (Wang et al., 2002) which displayed that silymarin significant 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 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 CHS2 and CHS3 genes. According to the authors, 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 (Gomez 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 research suggests 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 biochemical studies. This allowed determination of hepatoprotective, antiinflammatory, 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.

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Silybum marianum (L.) Gaertn. have been widely studied due to their therapeutic potential in various medical conditions. The extraction of flavonolignans from milk thistle has been a subject of numerous investigations. The role of milk thistle extract in breast carcinoma cell line T47D has been studied, showing its potential in cancer treatment. The anti-inflammatory and anti-fibrogenic effects of silychristin isomer and variation of flavonolignan levels in milk thistle has been observed.


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