REVIEW ARTICLE

Phytomedicine: *Silybum marianum* (Silymarin) as an Effective Hepato-protective Source from Nature

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ABSTRACT

Objective: To review the efficacy and safety of milk thistle for management of hepatic disorders

Methodology: A thorough literature survey was carried out for the article. Following key words (*Silybum marianum*, Liver) were used to search the articles on Google Scholar and PubMed. More than 50 articles published between 2000 and 2018 were taken into consideration.

Result: The review explored the main constituents of milk thistle and its properties to protect liver against various toxins, alcohol effects, and viral attacks. Silymarin is capable of regenerating liver and bring the hepatic markers, Alanine transaminase (ALT), Aspartate transaminase (AST) and others, to normal levels. Reported toxicities of silymarin are few.

Conclusion: *Silybum marianum* has proved its higher efficacy and safety in hepatic disorders, as compared to other therapies and therefore is an agent of choice. Furthermore, its cost effectiveness and ability to be developed as various dosage forms, like emulsion and nanoparticles, undoubtedly ensures its extensive use in future.

Key words: Silybum marianum, Liver

عنوان: نباتاتی دوا(Silymarin) جگر کے امراض سے بچنے کا قدرتی ذریعہ۔

تعارف: اس تحقیق کا مقصد ایک خاص نباتاتی دوا(Silymarin) کے جگر کے امراض کے علاج کے لیئے کرشماتی اثرات کا جائزہ کرنا ہے۔

طریقہ کار: جیسے لیئے گوگل اسکالر پر Key words جگر اور Silybum marianum کو تلاش کرتے ہوئے سال 2000 سے سال 2018 تک اس موضوع پر شائع ہونے والے 50 سے زائد آرٹیکلز کا ادبی سروے کیا گیا۔

نتائج: جائزہ سے ہونے والے انکشافات سے پتا چلا کے (Silymarin) میں پائے جانے والے اجزاء اور اس کی خصوصیات جگر کو کئی مختلف بیماریوں سے محفوظ رکھ سکتا ہے۔ اور اسے دوبارہ تقویت بخشتا ہے۔ اور اسکی قوّتِ مدافعت میں اضافہ کرتا ہے۔

حاصل مطالعہ: (Silymarin) جگر کے امراض کے علاج کے لیئے اپنی نمایاں خصوصیات کی بنا پر دوسری ادویات کے مقابلے میں خود کو بہتر ثابت کرنے کی صلاحیت رکھتا ہے۔ جسکی وجہ سے یہ سب سے مشہور اور سب سے زیادہ استعمال ہونے والی نباتاتی دوا ہے۔ اور اس کی مدد سے کئی دوسری ادویات بنانے میں مدد ملتی ہے۔ مستقبل میں اس کا استعمال بڑھ سکتا ہے۔

INTRODUCTION

Silybum marianum L, commonly known as milk thistle or Marian thistle belongs to botanical family *Asteraceae.*

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It is an annual or biannual specie found all over the globe especially in the Southern Europe and Asia (Figure I). Milk thistle has been famous since the olden days, to cure hepatic and gall bladder ailments such as hepatitis B and C, liver cirrhosis, cancers, jaundice, and alcoholic and non-alcoholic fatty liver disease. In addition to its traditional uses, it has also clinically proven its efficacy to combat certain poisoning conditions which include snake and insect stings, alcohol as well as mushroom Amanita poisoning[1]. Its role also encompasses the stimulation of biliary secretion and production of breast milk[2]. Chinese traditional medicine and Indian herbal practitioners

use *Silybum marianum*[3]. Silymarin is an example of a herbal medicinal preparation[4].

Figure 1: Silybum marianum

The fruit of *Silybum marianum* contains lipids, carbohydrates, proteins, phytosterols, and tocopherols[5]. Silymarin is a mixture (lipophilic extract) from the seeds and fruits of *Silybum marianum* which contains silibinin/silybin (50-60%), isosilybin/ isosilibinin (5%), silychristin (20%), and silydianin (10%). Silimonin and isosilychristin are also present in minor quantities. Among these isomers flavonolignans, derivatives of flavanone, silibinin is in a huge amount and is highly active (Figure 2)[6].
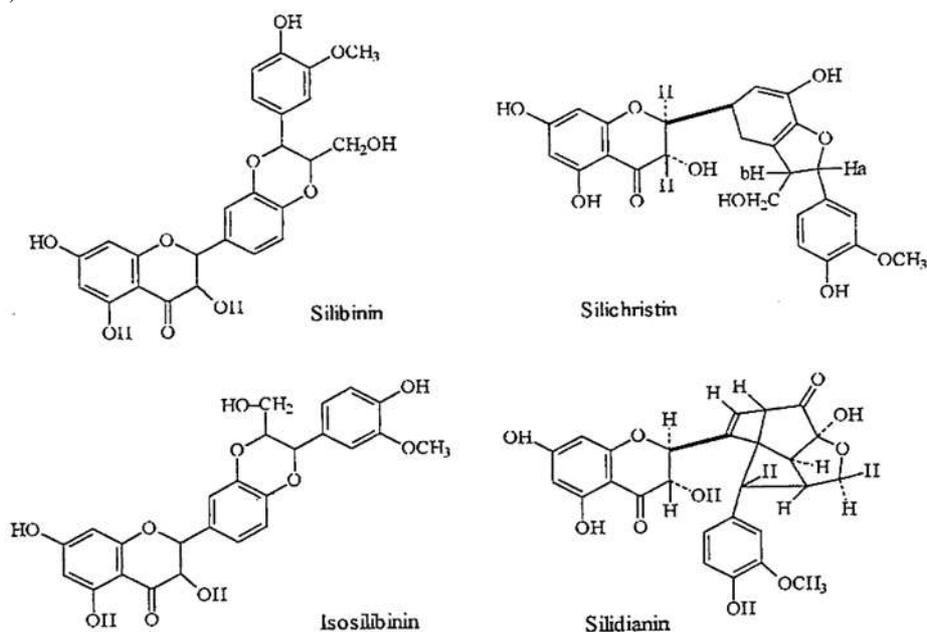

Figure 2: Chemical constituents of milk thistle

The pharmacokinetics of silymarin indicates 20-50% absorption after an oral dose, and 80% excretion in bile. Most studies conducted on silibinin, the main active constituent, confirm its half-life of 6 hours. Following an oral dose, silibinin is excreted 3-8% in urine, 20-40% is metabolized as sulphate and glucuronide derivatives and is found in bile juice. The rest is excreted unchanged in faeces. Peak concentration of silibinin is achieved within 2-9 hours[7].

Silymarin possesses excellent tolerance and has no major adverse drug reactions[8,9]. Due to these attributes several companies have started to market silymarin as a nutritional supplement in USA and Europe. In several studies on laboratory animals, silymarin has been found nontoxic even at higher doses[10]. The various aspects of its pharmacological actions and diverse health benefits have led us to select silymarin as the drug of choice for this study.

METHODOLOGY

More than 50 different articles, published between 2000 and 2018, were studied to pool up the information on *Silybum marianum*. Silymarin was studied for its pharmacological actions on liver, the articles describing actions on silymarin on other organs are excluded from the study. The reported toxicology and drug-drug interaction was also evaluated.

DISCUSSION

Mechanism of Action: Silymarin works in various ways i.e. as an antioxidant, an anti-inflammatory agent, an anti-apoptotic agent, produces anti-fibrotic effect, influenced by controlling the permeability of

hepatocytes membrane, to forbid the entry of toxic agents. It also promotes regeneration of liver cells to heal damage. Liver is the most important homeostatic organ and the main site of detoxification of drugs. Liver damage, a major cause of death all over the world, is associated with certain environmental toxins, pharmaceutical agents, alcohol, and viral attacks[11] (Figure 3).
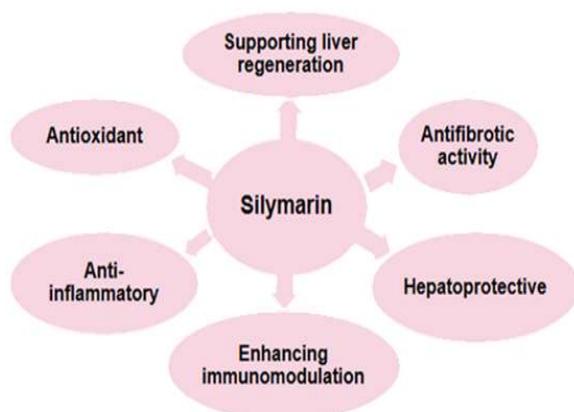

Figure 3: Actions of silymarin

Many researches have established the role of silymarin in hepato-protection. Machicao and Sonnenbichler in 1997 reported silymarin stimulates RNA polymerase to enhance rRNA production which, in turn, increases the synthesis of plasma membrane proteins to ensure its stability[11]. The antioxidant nature of silymarin contributes to its membrane stabilizing action. The interaction between flavonoid components of silymarin and polar ends (head) of phospholipids of hepatocytes' membrane prevent lipid peroxidation[12]. Liver protection is due to inhibition of synthesis of leukotriene B due to silibinin found in silymarin[13].

The anti-inflammatory action of silymarin is central in treatment of viral hepatitis and liver cirrhosis. This control of inflammation is brought by inhibiting the intrahepatic Nuclear Factor kappa B (NF-kb), which decreases Tumor Necrosis Factor-alpha (TNF-á), Interferon (IFN-g), Interleukin-2 and inducible Nitric Oxide[14]. On exposure of alcohol, carbon tetrachloride, and many other toxins, liver stellate cells rapidly turn into myofibroblasts and cause fibrogenesis, accumulation of collagen in liver. Silymarin increases level of á-SMA (smooth muscle actin) marker for detection of myofibroblasts, and acts as an anti-fibrotic agent to cure liver toxicities[15-17]. Oxidative stress is one of the prominent causes of liver impairment. Brandon Warner et al in 2010 stated silibinin as an inhibitor of Cytochrome P-450 dependent reactive oxygen species generation in liver carcinoma cells in

vitro[18]. *Silybum marianum* protects gall bladder and hinders cholestasis via inhibiting cAMP-phosphodiesterase[19].

Silymarin in Liver Fibrosis and Cirrhosis: Liver injury may cause fibrosis, especially in case of chronic hepatic injuries, which in turn becomes a reason for liver cirrhosis and hepatocellular carcinoma (HCC). Liver cirrhosis alone caused approximately one million deaths worldwide in 2010[20,21].

Literature reports the cirrhosis activity by CCl4 induced mechanism in rats. The dose of 50 mg/kg of silymarin was given orally at the last day of 4th, 8th, and 12th weeks respectively. Histopathology of liver was assessed. Anti-fibrotic effects of silymarin were related to stimulation of liver stellate cells (through expression of TGF-â) and mast cells stabilization[22]. In a thiocetamide (100 mg/kg, intra peritoneal)-induced liver cirrhotic condition, mice at the Institute of Cancer Research (ICR) were cured with 150mg/kg silymarin ($P < 0.05$). Silymarin amended the hepatic lesions and caused down-regulation of hepatic MMP-2, MMP-13, TIMP-1, TIMP-2, AP-1, KLF6, TGF-â1, á-SMA and COL-á1[23]. To find the anti-fibrotic effects a comparative study for 8 weeks was conducted on nilotinib (10 and 20 mg/kg) and silymarin (100mg/kg). Cirrhosis was induced by CCl4 in Wistar rats. Nilotinib 20 mg/kg reduced hepatic fibrosis by 68% and collagen formation by 49%. Silymarin decreased fibrosis and collagen formation by 47% and 18% respectively[24].

In a rat model, liver fibrosis was produced by N-nitrosodimethylamine (DMN) and oral Silibinin was induced therapeutically which reduced levels of AST, ALT, and ALP. Oxidative stress reduction and decline in collagen formation was also observed post silibinin treatment which suggests the use of silibinin in hepatic fibrosis[25]. In another study, silymarin proved its anti-fibrotic potential in vivo via changing the expression of genes associated with mitochondrial ETC (electron transport chain) and arrangement of cytoskeleton[26].

An oral dose of silymarin (20 and 100 mg/kg) was given to rats which were then given CCl4 (2 ml/kg). Silymarin inhibited CCl4 initiated inflammation and fibrogenesis in dose-dependent manner by reducing the gene expression of chemokine MCP-1, cytokine TGF-beta. The effect was also found on human stellate cells which was indicative of hepato-protective role of silymarin[27]. In many rat and mice models, silymarin exhibited anti-inflammatory properties in treatment of liver ailments such as cholestatic liver injury[28].

Silymarin in Non-alcoholic Fatty Liver Disease: Non-alcoholic fatty liver disease (NAFLD) is regarded

as the most common hepatic disease globally. The rate of prevalence of NAFLD is 30% in the West and majority of people develop NASH (non-alcoholic steatohepatitis) which leads to cirrhosis and hepatocellular carcinoma[29,30].

In a research, Otsuka Long-Evans Tokishima Fatty (OLETF) rats were given 390 mg/kg/day silymarin to treat NAFLD. In eight weeks, remarkable improvement occurred with reduction in symptoms of fibrosis and decline in profibrogenic elements[31,32]. In mice experiencing streptozotoin and high fat diet triggered nonalcoholic steatohepatitis (NASH), high doses of silymarin at 500-1000 mg/kg showed reduction in steatosis[33]. Four-week treatment with 20 mg/kg intra peritoneal silibinin in mice produced satisfactory cure in NASH[32]. In a 12-week open label comparative study, silymarin (70 mg three times a day) was compared with vitamin E (400 IU/day) in 71 patients, for treatment of NAFD/NASH. At the end of the study, there was significant decline in serum AST and ALT levels in both the groups. The mean AST levels returned to normal 74.6% in silymarin treated group and 56.30% in vitamin E treated group[34].

In a 24-week comparative randomized study in 2009, Hashemi et al gave 280 mg bid silymarin against placebo in 50 patients. ALT and AST levels reduced to normalization (<40) in 18% and 20% patients respectively (placebo group) and in 52% and 62% patients respectively (silymarin treated group)[35]. Few similar studies were conducted by other researchers and exhibited the efficacy of silymarin in treatment of NAFD/NASH. In two different studies (8 and 12 weeks), silymarin was detected as more effective treatment in comparison to placebo[36,37].

In a study, three different therapies i.e. 140 mg/day silymarin, pioglitazone 15 mg/day and metformin 500 mg/day were compared for the treatment of NAFD patients. Each treatment was given to 22 patients for a period of 8 weeks. The results proved that silymarin and pioglitazone are superior to metformin[38]. Another relevant study on NAFD showed that silymarin reduced release of IL-1 â, hindered the association of NLRP3 inflammatory complex, declined the accumulation of á-tubulin. MEC and Sirtuin 2 were affected by silibinin which stopped the NLRP3 inflammasome activation in mice with NASH[39]. In 2017, Wah et al evaluated the efficacy of silymarin in a double blind RCT (randomized control trial). At the doses of 700 mg thrice a day, silymarin reduced NAFLD by more than 30% as compared to placebo. Liver cirrhosis and stiffness were largely reduced[40].

Silymarin in Hepatitis: Viral hepatitis is a worldwide health issue demanding attention. Hepatitis B and C have become major causes of death. Various new approaches of treatment, which could inhibit viral replication or boost up immunity against viral hepatitis, have been introduced[41]. These treatments are not accessible to all due to their high cost. In such a scenario, silymarin, the herbal drug, is a better alternative. In 2003, Wei et al conducted a meta-analysis to determine the therapeutic efficacy of silymarin alone and in concurrence with lamivudine and interferon (anti-viral) for treating HBV chronic hepatitis. Comparable results were obtained with silymarin and the other antivirals used in diminishing aspartate aminotransferase (AST) and ALT levels. The negative conversion rate of serum HBsAg and HBeAg were found (relative risk 1.50 and 1.80 respectively) at 95% confidence interval 0.18–12.35 and 0.43–7.60 respectively[42].

Since the development of HBV vaccination in the 1980s, virus C chronic hepatitis has now become the major cause of hepatopathy[43]. In a study, Yang et al determined the effect of silibinin, given per OS and high dose of I.V. injection, on HCV-RNA serum level[44]. In another similar study, it was evaluated that I.V. administration of silibinin interferes with the lifecycle of HCV to inhibit its replication for the treatment of hepatitis C. Silibinin can inhibit RNA polymerase function in HCV[45]. Ferenci et al, studied that in patients unresponsive to peg-interferon therapy I.V. administration of silibinin, via blocking HCV polymerase enzymatic activity, at concentration of 75 ìM and 100 ìM reduced viral replication in one to four weeks[46].

A case study of a 44 years old female infected with HCV genotype-1, showed that a combination of 1200 mg/day silibinin, 1200 mg/day ribavirin, and 6000 IU/day vitamin D is effective in treatment of HCV hepatitis. The therapy was administered for 238 days[47]. In an important recent development, nano particles of silibinin have been designed. As compare to conventional drugs, these nano particles have greater bioavailability on oral administration in rodents, therefore better distribution and higher serum concentration results. In in-vitro studies, these nanoparticles diminished HCV infection in human liver cells[48]. An Egyptian researcher proved in a randomized study that silymarin in a higher dose (1050 mg/day for 12 weeks) was greater in efficacy than a usual dose of 420 mg/day for 12 weeks. The factors improved at higher doses were serum albumin level and hepatic blood flow[49].

Silymarin in Hepatocellular Carcinoma:

Hepatocellular carcinoma (HCC) has become a major reason of death all over the world[50], causing approximately 0.75 million deaths every year[51]. HCC is a consequence of hepatic inflammation, viral hepatitis, NASH, and contact with toxins like alcohol[52]. The scanty availability of treatments has urged the healthcare professionals to explore herbal products for therapeutic values[53-56].

Mastron et al in 2015 proved that silymarin/silibinin reduce oxidative stress and induce arrest at various stages of cell cycle to treat hepatic cancers[57]. Bosch-Barrera et al in 2017 studied that silymarin reduced toxicity of anti-cancer drugs through inhibition of signal transducers[58]. Hepatocellular carcinoma was induced by N-nitrosodiethylamine. The treatment with silymarin considerably attenuated the changes in levels of ALT, AST, alpha fetoprotein, and decreased MDA-DNA adduct formation, hence proved itself as a likely chemotherapeutic agent[59]. In another study, N-nitrosodiethylamine (NDEA), 10 mg/kg for 12 weeks, was used to induce HCC. The parameters assessed were ALT, AST, MDA, GSH, GR, and others. The group of mice treated with silymarin showed a reduction in MDA and enhancement in GS, GR, and SOD levels. Histopathology was improved and AST and ALT were brought to normal[60]. Nano emulsion of silymarin was developed and optimized. Studies against HCC (Hepatocellular carcinoma) showed that nano emulsion enhanced ROS intensity and declined cell viability $(p<0.05)$[61].

Toxicity Studies of Silymarin: The toxicity studies, after I.V. infusion of silymarin, indicated LD_{50} of 400, 385, and 140 mg/kg in mice, rats, and rabbits respectively whereas at slow rate of I.V. infusion, LD_{50} reached 2 g/kg. After an oral dose, LD_{50} was 10g/kg[62].

As stated in 'Milk Thistle report NTP TR 565', silymarin has great safety margin[63,64]. For two years, 50 rats and mice of both genders were given 1.25%, 2.5%, and 5% of milk thistle extract. No occurrence of toxicity and carcinogenicity was observed. When determining the toxicity of silymarin in humans, no more than 2.4% and 1% occurrence of adverse effects were reported, in blinded and open clinical studies respectively. The main adverse event was a laxative effect. Other effects comprised nausea, vomiting, dyspepsia, urticaria, inflammation like pruritus and arthralgia. In all published studies of silymarin toxicity, only one serious adverse reaction is reported which led to collapse in a 57 years old female[65].

Drug-Drug Interaction Studies on Silymarin : In a 2-week study on Chinese volunteers, the administration of silymarin (140 mg tid) and talinolol (a substrate of multidrug resistant P-glycoprotein) was done concurrently. It was observed that plasma AUC of talinolol was increased by 36% by silymarin[66]. In a simulated study, the interaction of silibinin with warfarin (CYP2C9 substrate) and midazolam (CYP3A substrate) were studied. At higher dose (1,650 mg/day), silymarin was predicted to enhance midazolam and warfarin AUC approximately by 5% and 4% respectively. A clinical study suggested 9% and 13% increase in plasma AUC of midazolam and warfarin at concurrent use of high doses of silymarin[67].

CONCLUSION

The review concluded that silymarin is highly effective in treatment of various liver ailments and is free from any major toxicity. Silymarin because of its low cost, easy availability, and greater therapeutic value, has become a drug of choice. Exploration of other herbal drugs is suggested to counter the side effects of antibiotics and the emerging resistance against them.

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