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Hepatoprotective herbal Drug, Silymarin from experimental pharmacology to clinical medicine-A review

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ABSTRACT

Silymarin, a flavonolignan from 'milk thistle' (Silybum marianum) plant is used almost exclusively for hepatoprotection and amounts to 180 million US dollars business in Germany alone. In this review we discuss about its safety, efficacy and future uses in liver diseases. The use of silymarin may replace the polyherbal formulations and will avoid the major problems of standardization, quality control and contamination with heavy metals or bacterial toxins. Silymarin consists of four flavonolignan isomers namely- silybin, isosilybin, silydianin and silychristin. Among them, silybin being the most active and commonly used. Silymarin is orally absorbed and is excreted mainly through bile as sulphates and conjugates. Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals. It acts by antioxidative, anti-lipidperoxidative, anti-fibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms. Silymarin has clinical applications in alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxic and drug induced liver diseases and in diabetic patients. Though silymarin does not have antiviral properties against hepatitis virus, it promotes protein synthesis, helps in regenerating liver tissue, controls inflammation, enhances glucuronidation and protects against glutathione depletion. Silymarin may prove to be a useful drug for hepatoprotection in hepatobiliary diseases and in hepatotoxicity due to drugs. The non traditional use of silymarin may make a breakthrough as a new approach to protect other organs in addition to liver. As it is having a good safety profile, better patient tolerability and an effective drug at an affordable price, in near future new derivatives or new combinations of this drug may prove to be useful.

KEY WORDS - Hepatitis, hepatoprotection, hepatotoxicity, herbal drugs- silymarin

INTRODUCTION

Silybum marianum, commonly known as 'milk thistle' (Family: Asteraceae/Compositae) is one of the oldest and thoroughly researched plants in the treatment of liver diseases. The plant itself grows as a stout thistle in rocky soils with large purple flowering heads. The leaves are characterized by milky veins, from which the plant derives its name (1). The extracts of milk thistle is being used as a general medicinal herb from as early as 4th century B.C. and first reported by Theophrastus (2). In the 1st century A.D., Dioskurides used this plant as emetic as well as a general medicinal herb (2). It became a favoured medicine for hepatobiliary diseases in 16th century and the drug was revived again in 1960 in central Europe (1, 2). The active constituents of the plant are obtained from the dried seeds and consist of four flavonolignans which are collectively known as silymarin. Characterization of these active compound by (Wagner et al, 1974) while reviewed its history, properties and the clinical effects by (Flora et al, 1998). The potential benefits of silymarin in the treatment of liver diseases have raised many controversies. The safety and efficacy of this herbal drug has been analyzed by a systematic approach. (Sailer et al, 2001) Silymarin, a single herbal drug formulation which is mostly used in liver diseases amounts to about 180 million US dollars in Germany alone. It is interesting to note that herbal drug sale tripled between 1992 and 1996 in Germany and nearly one third of outpatients attending liver clinics use natural remedies (2). Amongst the flavonoids, which have proven antioxidative, antiviral or anticarcinogenic properties like

glycyrrhizin, phyllanthin, silybin, picroside and baicalein, can serve as primary compounds for further development as hepatoprotective drugs (2). However, herbal formulations and natural products face many major problems like their standardization, and quality control. Moreover, clinical studies using such drugs in liver diseases lack in proper patients selection, randomization, placebo control and also proper use in end points like death, liver transplantation, histological documentations, and enzymatic markers. This review discusses about the safety, efficacy and future uses of silymarin in the treatment of liver diseases from the conducted experimental and clinical trials. A literature search was made through PubMed and scientific papers published in the last 16 yr (1990-2005) were reviewed. Some older references giving historical documentation were also included. New applications of silymarin are highlighted by giving the nontraditional uses of this drug in order to protect other organs in addition to liver.

CHEMISTRY OF SILYMARIN

Silymarin is extracted from the dried seeds of milk thistle plant, where it is present in higher concentrations than in other parts of the plant1. The active principle was first isolated and chemically characterized during 1968-1974 (3). Later the biochemical effects of silymarin on RNA, protein and DNA synthesis was reported. (Sonnenbichler and Zet, 1986).

Silymarin is a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin. The structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory

actions. Among the isomers silybin is the major and most active component and represents about 60-70 percent, followed by silychristin (20%), silydianin (10%), and isosilybin (5%) (5) Silipide (IdB 1016) is the silybin - phosphatidylcholine complex which ensures a large increase in the bioavailability of silybin (7).

PHARMACOKINETICS

Silymarin is insoluble in water and typically administered as a sugar coated tablet(8) or as an encapsulated standardized extract (1). The absorption by oral route is as low as 2-3 per cent of the silybin recovered from rat bile in 24 h. About 20-40 per cent of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates in human beings (5). The peak plasma levels after an oral dose are achieved in 4-6 h in experimental animals and in human beings 1, 9-11, and elimination half-life is approximately 6, 5 h, (12). The pharmacokinetic variables (mean SD), after an oral dose of 240 mg silybin in 6 healthy volunteers has been reported to be as follows: Absorption half life 0.17 0.09 h, elimination half life 6.32 3.94 h, maximum plasma concentration 0.34 0.16 µg/ml and time to maximum plasma concentration 1.32 0.45 h (9-12).

Pharmacokinetic studies with silybinphosphatidylcholine complex have shown an increase in the oral bioavailability of silybin in healthy human subjects, probably by a facilitatory role of drug complex on the passage of the drug across the gastrointestinal tract (13). Silybin dihemisuccinate is given in emergency cases with the poisoning of Amanita phalloides (14).

EXPERIMENTAL PHARMACOLOGY

Hepatoprotective activity of silymarin has been demonstrated by various researchers from all over the world against partial hepatectomy models and toxic models in experimental animals by using acetaminophen, carbon tetrachloride, and ethanol, D-galactosamine, and Amanita phalloides toxin.

Hepatectomy: Rats with partial hepatectomy, where 70 per cent of liver is removed, when subjected to silymarin pretreatment showed increased synthesis of DNA, RNA, protein and cholesterol suggesting the regeneration of liver (15,16). Interestingly, the increased protein synthesis was found in damaged livers with partial hepatectomy, but not in the respective controls (17). The mechanism of increased protein synthesis is not known, but probably silymarin initiates a physiologic regulator, so the silybin fits in to a specific binding site on the polymerase, thus stimulating ribosome formation (18). Probably silymarin is able to enter the nucleus and specifically stimulate RNA polymerase I, owing to its structural similarity to steroids. Silymarin has been found to suppress nuclear factor kappa B (NF-κ-B) DNA binding activity and its dependent gene expression5.

Carbon tetrachloride: Among various chemical agents, carbon tetrachloride (CCl_4) has been thoroughly studied for its hepatotoxic properties (19). Various hepatoprotective agents have been studied to observe the beneficial effects against the chemically induced liver injury produced by carbon tetrachloride (20).

Silymarin when compared with various polyherbal formulations in CCl_4 induced hepatotoxicity in rats has led to complete normalization of elevated transaminases levels (21). Mouriel and Mourelle found that silymarin treatment protected completely against harmful increase in the membrane ratios of cholesterol phospholipids and sphingomyelin: phosphatidylcholine in rats with carbon tetrachloride induced cirrhosis (22). Rats with chronic CCl_4 induced liver damage were treated with oral silymarin, 50 mg/ kg administered for 5 days. Collagen content in livers of animals pre-treated with CCl_4 was increased approximately four- fold in comparison to control. It prevented the cirrhotic changes in rats. It also reduced liver collagen content by 55 per cent (23).

Acetaminophen: Acetaminophen is an analgesic and antipyretic agent known to cause centrilobular hepatic necrosis at toxic doses. Silymarin has been studied for its protective action against acetaminophen induced toxicity in animal models. Ramellini & Meldolesi in their in vitro studies on rat hepatocyte showed that silymarin treatment normalized the elevated biochemical parameters of liver and serum, caused by acetaminophen, by its stabilizing action on plasma membrane (24).

A comparative study of andrographolide and silymarin on acetaminophen induced cholestasis has produced the dose dependent cholestatic and anticholestatic effects of these drugs (25). In our laboratory, the hepatoprotective herbal drugs silymarin and andrographolide were compared in experimental toxic models of carbon tetrachloride and paracetamol in mice. The hepatoprotective effects of silymarin were studied on various parameters like macroscopic appearance, microscopic observation, and its mechanism of action. Silymarin when given to mice in a dose of 100 mg/kg i. p for 7 days, lead to a robust growth of liver and the weight of the liver tissue was more than twice that of the carbon tetrachloride treated group. It also reduced and restored the phenobarbitone induced sleeping time in paracetamol as well as carbon tetrachloride models. Further silymarin prevented hepatic cell necrosis in 87.5 per cent of animals when subjected to paracetamol induced hepatotoxicity. However, silymarin gave a small percentage of protection (only 16%) against carbon tetrachloride induced hepatic necrosis. From this study, it has been concluded that silymarin showed histopathological evidence of hepatoprotection by preventing hepatic cell necrosis or by hepatic cell regeneration (26). Silybin dihemisuccinate, a soluble form of flavonoid of silymarin, has protected rats against liver glutathione depletion and lipid peroxidation induced by acute acetaminophen hepatotoxicity and showed potential benefits of silymarin as an antidote (27). With relatively high doses (0.05 mmol/ 1), silymarin has been shown to reduce acetaminophen enhanced CYP 2El mediated cytotoxicity of methotrexate in human hepatocytes (28). In vitro experiments with kidney cells damaged by paracetamol, cisplatin and vincristine have demonstrated that administration of silybin before and after the drug-induced injury can lessen or avoid the toxic effects (29).

Ethanol: Acute and chronic administration of ethanol produces a drastic decrease in the hepatic content of reduced glutathione (GSH); an important biomolecule against

chemically induced cytotoxicity (8). The hepatoprotective activity of silymarin against ethanol-induced damage has been tested in different animals. The administration of ethanol has produced a marked increase in serum alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (γ-GT) levels, with a disturbance in reduced and oxidized glutathione ratio. The group which received silymarin did not show any significant changes in these parameters, showing its protective role against ethanol (30).

Galactosamine: Galactosamine produces liver damage, with histopathological changes resembling human viral hepatitis (8). Galactosamine administration in rats produced cholestasis, due to inhibition of the synthesis of bile acids and also their conjugation with proteins or to damage in the biliary system. Saraswat et al (31) reported the significant anticholestatic effect of silymarin in comparison to andrographolide. The effects of silymarin in normalizing elevated serum transaminases and alkaline phosphatases have been shown in isolated rat hepatocytes, which are inferior to CI-1, a herbal protein from *Cajanus indicus* (32).

Iron: Iron overload is associated with liver damage, characterized by massive iron deposition in hepatic parenchymal cells, leading to fibrosis and eventually to hepatic cirrhosis (33). The oxidative stress due to increased hepatic lipid peroxidation is the major mechanism of iron-induced hepatotoxicity. Pretreatment in rats with silymarin reduced ironinduced increase in lipid peroxidation and levels of serum enzymes, as also noted in Withania somnifera indicating their hepatoprotective action (34).

Amanita phalloides toxin: In mice, silymarin was 100 per cent effective in preventing liver toxicity if given as pretreatment or up to 10 min after Amanita toxin poisoning. Severe liver damage and resultant death was avoided if silymarin was administered with in 24 h (35). In a study with dogs, none died when given silymarin 5-24 h after ingesting an LD50 dose of Amanita phalloides (85 mg/kg) compared with 33 per cent in controls. Liver enzymes and liver biopsies showed significant protective effect of silymarin posttreatment (36).

Silymarin has also been found to protect liver cells from injury caused by ischaemia (37), radiation (38) and viral hepatitis1.

MECHANISM OF ACTION

The preclinical studies using different hepatotoxic substances showed that silymarin has multiple actions as a hepatoprotective agent. The antioxidant property and cell-regenerating functions as a result of increased protein synthesis are considered as most important (39).

(i) Antioxidant properties: Free radicals, including the superoxide radical, hydroxyl radical (.OH), hydrogen peroxide (H_2O_2), and lipid peroxide radicals have been implicated in liver diseases (40). These reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to xenobiotics (41). The mechanism of free radical damage include ROS- induced peroxidation of polyunsaturated fatty acid in the cell membrane bilayer, which causes a chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane lipids and proteins. Subsequently cell contents including DNA, RNA, and other cellular components are damaged (42).

The cytoprotective effects of silymarin are mainly attributable to its antioxidant and free radical scavenging properties. Silymarin can also interact directly with cell membrane components to prevent any abnormalities in the content of lipid fraction responsible for maintaining normal fluidity (22).

(ii) Stimulation of protein synthesis: Silymarin can enter inside the nucleus and act on RNA polymerase enzymes resulting in increased ribosomal formation. This in turn hastens protein and DNA synthesis (6). This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver.

(iii) Anti-inflammatory actions: The inhibitory effect on 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis is a pivotal pharmacological property of silymarin. Leukotriene B_4 synthesis was reduced while prostaglandin E_2 synthesis was not affected at higher concentrations of use of silibinin (5). A study which evaluated the action of silibinin in isolated Kuppfer cells indicated a strong inhibitory effect on leukotriene B4 (LTB4) formation with the IC 50 value of 15 moles/1. But no effect was observed on tumour necrosis factor-alpha (TNF-α) formation (43). The NF-κB is a key regulator of inflammatory and immune reactions. Silymarin is found to suppress both NF-κB DNA binding activity and its dependent gene expression induced by okadaic acid in the hepatoma cell line HEP G2. But the NF-κB activation induced by TNF-α was not affected by silymarin, demonstrating a pathway dependent inhibition by silymarin (44). The results of an in vivo study in male BALB/c mice treated with silymarin suggested that parenteral exposure to silymarin results in suppression of T-lymphocytes at low doses and stimulation of inflammatory process at higher doses. So the ability of immune system to bacterial infection will increase at higher doses, and may be an additional therapeutic application of this flavonoid mixture (45).

(iv)Antifibrotic action: Liver fibrosis can result in remodeling of liver architecture leading to hepatic insufficiency, portal hypertension and hepatic encephalopathy. These processes involve complex interplay of cells and mediators (46). In the initial phase there will be proliferation of hepatic parenchymal cells. The conversion of hepatic stellate cells (HSC) into myofibroblast is considered as the central event in fibrogenesis. Silymarin inhibits NF- and also retards HSC activation. It also inhibits protein kinases and other kinases involved in signal transduction and may interact with intracellular signaling pathways (46).

(v) Drug and toxin related liver damage: Drugs in common use can cause toxic effects on the liver which can mimic almost every natural diseases of the liver. About 2 per cent of all causes of jaundice in hospitalized patients are drug induced. About a quarter of cases of fulminant hepatic failure are thought to be drug related. Early detection is needed to identify a drug related hepatic reaction. Severity is greatly increased if the drug is continued after symptoms develop or if the serum liver enzymes continue to rise (19).

Hepatocellular injury due to drugs seems to be the primary

event. This is rarely due to the drug itself and a toxic metabolite is usually responsible. The drug metabolizing enzymes activate chemically stable drugs to produce electrophilic metabolites. These potent agents bind covalently to liver molecules such as proteins and fatty acids which are essential to the life of the hepatocyte and necrosis ensues. This follows exhaustion of intracellular substances such as glutathione which are capable of preferentially conjugating with toxic metabolites. In addition, metabolites with an unpaired electron are produced by oxidative reactions of cytochrome P 450. This free radical can also bind covalently to proteins and to unsaturated fatty acids of cell membranes. This results in lipid peroxidation and membrane damage. The end result is hepatocyte death related to failure to pump calcium from the cytosol and to depressed mitochondrial function. Drugs like paracetamol and halothane produce clear cut zone 3 necrosis of liver (19).

Silymarin has a regulatory action on cellular and mitochon\drial membrane permeability in association with an increase in membrane stability against xenobiotic injury (47). It can prevent the absorption of toxins into the hepatocytes by occupying the binding sites as well as inhibiting many transport proteins at the membrane (48). These actions along with antiperoxidative property make silymarin a suitable candidate for the treatment of iatrogenic and toxic liver diseases.

CLINICAL TRIALS

(i) Alcoholic liver diseases: Long-term alcohol consumption can result in a spectrum of clinical syndromes and pathological changes ranging from fatty liver, fibrosis, and cirrhosis. The only established therapy for this condition is abstinence from alcohol along with supportive measures (49). Animal studies (26,27,30,31,45) and clinical trials (1,2,4,5,8,41,50) suggest that silymarin can be useful in the management of early or progressive liver damage when given for variable periods from 3-6 months. Some trials have produced conflicting results which may be due to heterogeneity of patients, variations in alcohol consumption during study period and the complexity of disease pathogenesis.

A double-blind controlled study by Salmi and Sarna (50) evaluated the effect of silymarin in 106 patients with alcoholic liver disease. All had elevated serum transaminase levels (ALT and AST) and 90 had confirmed histological diagnosis. Alcohol was prohibited during trial. The patients were randomly allocated to either silymarin or placebo group and the duration of the trial were 4 wk. Ninety seven patients completed the study (47 in silymarin group and 50 in placebo treated group). A highly significant decrease in ALT and AST levels was observed with silymarin when compared with placebo. No significant difference was noted between the groups in reduction of serum total and conjugated bilirubin. In the subpopulation examined, bromosulphophtalein (BSP) reduction returned to normal significantly. Among the subgroup with a second histological work-up, normalization of histological changes occurred more often in silymarin group (11 out of 15) than in controls (4 out of 14).

In a Hungarian study of 36 patients, with chronic alcoholic liver disease, a dose of 420 mg/day of silymarin resulted in normalization of serum transaminases (AST, ALT and γ-GT), total bilirubin and an improvement in the histological examination of liver biopsies after 6 months of treatment. In addition, procollagen III peptides were found to be significantly decreased in the silymarin group (51).

In a double blind comparative study in 116 patients with histological proven alcoholic hepatitis, 420 mg/day of silymarin for 3 months was found to significantly alter the course of the disease (52). Both silymarin and placebo groups had similar rates of abstinence, and significant improvement in the score of alcoholic hepatitis and serum aminotransferase activity, irrespective of treatment with silymarin or placebo. One patient in silymarin group and 3 in placebo died of hepatic failure during trial, which was not statistically significant. No side effects of the drug were encountered (52). Human studies have shown that silymarin is generally nontoxic and without side effects when administered to adults in a dose range of 240-900 mg/day in two or three divided doses. At higher doses of more than 1500 mg/day, silymarin may produce a laxative effect which may be due to increased bile flow and secretion. Mild allergic reactions have also been noted, but such situations may not necessitate discontinuing the treatment.

Silymarin has no direct effect on ethanol metabolism and has no role in reducing ethanol levels or the rate at which ethanol is removed from the body (53).

(ii) Liver cirrhosis: In a randomized clinical trial in 170 patients with cirrhosis, 87 patients (alcoholic 46, non-alcoholic 41) were treated with silymarin, 420 mg/day for three times and 83 patients (alcoholic 45, non-alcoholic 38) received placebo (54). The mean observation period was 41 months. Out of 24 dropouts, 14 were in treatment group and 10 in placebo group. In placebo group, 37 patients died and death was related to liver disease in 31 of these and in treatment group 24 died and death was related to liver disease in 18. The survival rate was 58 per cent in silymarin group and 39 per cent in placebo. Subgroup analysis indicated that the treatment was effective in patients with alcoholic cirrhosis and in patients initially rated as Child A.

Pares et al studied the effect of silymarin in alcoholics with liver cirrhosis with respect to their survival, clinical and laboratory changes (55). This randomized double blind multicenter study comparing 450 mg/day in three divided doses (n=103) with placebo (n=97), enrolled 200 alcoholics with histologically or laparoscopically proven liver cirrhosis. The primary outcome was time to death and the secondary outcome was progression of liver failure. Survival was similar in patients receiving silymarin or placebo and was not influenced by the gender, the persistence of alcohol intake, the severity of liver dysfunction or by the presence of alcoholic hepatitis in the liver biopsy.

The results of some recent trials are not in favour of silymarin use. Lucena et al conducted a doubleblind placebo controlled clinical trial in 60 patients with alcoholic cirrhosis, with silymarin MZ-80 (a pharmaceutical preparation) at a dose of 150 mg t.i.d. for 6 months (56). The parameters studied were

erythrocyte total glutathione content, malondialdehyde (MDA) and serum amino terminal peptidase of procollagen type III (PIIINP). Silymarin produced a small increase in glutathione and a decrease in lipid peroxidation in peripheral blood cells. But there were no concurrent changes on laboratory indices of pathology.

(iii) Mushroom poisoning: The most remarkable use of silymarin is in the treatment of Amanita phalloides (Death cap) poisoning, a toxic mushroom widespread in Europe and North America. Amanita phalloides possess two extremely powerful hepatotoxins, amanitin and phalloidin (LD 50 of amanitin is 0.1 mg/ kg body weight) 1. Benzyl penicillin (3, 00,000 to 10, 00,000 U/kg/day) along with silybin (20-50 mg/ kg/day, iv) is shown to be effective against amanitin poisoning along with other supportive measures.

There are no controlled trials available in the treatment of mushroom poisoning, other than a few case studies or individual case reports. Carducci et al presented a report of a family of four poisoned by Amanita mushroom (amatoxin), admitted to a hospital in Naples with severe liver damage (14). Although they were treated with standard therapy, the clinical picture worsened till the third day when it was decided to add silybin hemisuccinate by intravenous route to the therapy. After silybin administration, the patients showed a favourable course with a rapid reduction of clinical picture. All patients were discharged on day 10-13. Subsequent investigations after 2 months revealed no morphological alterations in hepatobiliopancreatic echography. The investigators suggested that silybin may play a significant role in protecting hepatic tissue not yet injured by the toxins.

The results of a 20 yr retrospective study from clinical data of 2108 patients hospitalized in North America and Europe with amatoxin poisoning due to 35 species of mushrooms and Chi square statistical comparison of survivors and dead versus treated individuals supported silybin use either alone or in combination (57).

(iv)Viral hepatitis: Even though silymarin does not affect viral replication it may have beneficial role in viral hepatitis by its inhibitory action on inflammatory and cytotoxic cascade of events induced by the viral infection (5). Also, it can improve the regeneration process and normalize the liver enzymes by its action on protein synthesis (15).

Lirussi and Okolicsanyi compared silymarin and ursodeoxycholic acid in a rather heterogeneous population of patients with active cirrhosis, the majority of whom were HCV positive (58). In this subgroup, ursodeoxycholic acid was reported to show no effect. The mean changes in blood chemistry values assessed in this trial were similar with the two compounds.

A silybin-phosphatidylcholine complex, IdB 1016 (240 mg twice daily) was studied in a short term placebo-controlled pilot study in 20 patients with chronic active hepatitis. Of the various biochemical parameters evaluated, AST levels were significantly reduced in silymarin group, with no consistent differences in the other liver function tests (59).

(v) Toxic and iatrogenic liver diseases: Clinical trials in toxic liver disease are sparse and of poor quality, but few reported that the combination of silymarin with potentially hepatotoxic drugs may prevent or reduce adverse reactions. The pharmacological rationale for the use of silymarin relates to its antioxidant action, selective inhibition of leukotriene formation by Kupffer cells as well as its antiapoptotic action (5).

Tacrine is an anticholinesterase drug; used for the treatment of Alzheimer's disease. But in many patients at therapeutic doses it causes an increase in liver transaminases values, preventing an effective usage. Allain et al conducted a 12 wk randomized double- blind placebo-controlled study on outpatients with mild-to-moderate dementia of Alzheimer's type (60). The objective was to evaluate the ability of silymarin to antagonize or prevent the hepatotoxic effects of tacrine. Silymarin 420 mg/ day was given first and tacrine was added at 40 mg/ day for 6 wk, and then increased to 80 mg/day. No significant difference was observed for serum ALT between the two groups. Side effects especially gastrointestinal disorders were much less frequent in the silymarin group. They recommended coadministration of silymarin with tacrine to improve tolerability in the initial phases of treatment. Some beneficial effects were also noted when combined with anti-psychotics (61).

Many of the therapeutic agents used for differe\nt indications like rifampicin, isoniazid (INH), phenothiazines can cause liver injury, especially when administered for a long period. The combination of silymarin with such potentially hepatotoxic drugs may prevent such adverse reactions.

(vi) Liver related mortality: As per the analyzed studies, the major causes of liver related deaths were upper gastrointestinal bleeding (UGB), hepatic failure, or primary liver cell carcinoma (62). The overall liver related mortality as reported in the trials was 10 per cent with silymarin and 17.3 per cent with placebo showing a significant reduction in mortality. Bleeding of oesophageal varices constitute one of the most serious complications of cirrhosis. The number of patients having one or more episodes of UGB without fatal outcomes was similar in silymarin and placebo group (55).

Hepatocellular carcinoma (HCC) has been reported as a cause of death in a study, but other systematic studies on this aspect are lacking (53). The recent data show that the incidence of HCC was lower in the silymarin treated patients (50).

(vii) In diabetic patients: Diabetes mellitus in insulin resistant patients with cirrhosis leads to a progressive impairment in insulin secretion together with the development of hepatic insulin resistance leading to fasting hyperglycaemia and a diabetic glucose tolerance profile (63). The reduction in lipid peroxidation produced by silymarin can improve metabolic control and reduce requirements for endogenous insulin in such patients.

Velussi et al conducted a trial in 60 cirrhotic diabetic patients who were being treated with silymarin. Patients were randomly assigned to receive silymarin 600 mg/day or no silymarin for 12 months, with both the groups receiving standard therapy. The baseline features were similar in both the groups. Silymarin treatment produced significant reduction in daily and fasting blood glucose, daily glucosuria, glycosylated haemoglobin values, malondialdehyde values and

a drop in insulin requirement and fasting insulinaemia. In contrast, the status of untreated patients declined during the trial. The authors concluded that silymarin may reduce the lipoperoxidation of cell membrane and insulin resistance, by significantly decreasing endogenous insulin overproduction and the need for exogenous insulin administration.

ADVERSE EFECTS

Silymarin is reported to have a very good safety profile5. Both animal and human studies showed that silymarin is non toxic even when given at high doses (>1500 mg/day). However, a laxative effect is noted at these doses may be due to increased bile secretion and bile flow (65).

Most commonly noted adverse effects were related to gastrointestinal tract like bloating, dyspepsia, nausea, irregular stool and diarrhoea. It was observed in 2 to 10 per cent of patients in clinical trials, which were similar to placebo66. It also produced pruritus, headache, exanthema, malaise, asthenia, and vertigo.

Some serious adverse events were reported in three patients. A 57 yr old lady developed serious symptoms of gastroenteritis associated with collapse while the other two reported cases were allergic in nature after ingestion of herbal tea containing silymarin (66, 67).

DRUG INTERACTIONS

In vitro studies showed that silymarin in higher concentrations has an inhibitory effect on both phase I and phase II drug metabolizing enzymes68. The CYP3A4, CYP2D6 and CYP2C9 are the major enzymes inhibited by this flavonolignan. But the concentrations that obtained in plasma at pharmacological doses are comparatively very less about 0.5 moles compared to that needed for the inhibition of cytochrome enzymes about 10 moles (69). Recent reports suggest that silymarin is a potent inhibitor of hepatic UDP glucuronosyltransferase1A1 (UGT1A1), but its clinical significance is not known (66). However, clinicians should take appropriate precautions while prescribing co-administered drugs which are metabolized by similar mechanisms (68,69). Enhanced glucuronidation is an important phase II liver detoxification pathway. Glucuronic acid is conjugated with toxins to facilitate their elimination from the body via bile. Silymarin may similarly facilitate the bilirubin conjugation with glucuronic acid or inhibit γ glucuronidase enzyme from the toxic pathogenic intestinal bacteria. This may be of help in patients with jaundice1. Silymarin and related flavonolignans displayed inhibition of catalytic activities of cytochrome P450 isoenzymes in vitro in concentrations greatly exceeding physiologically reachable ones and due to low solubility of silybin, it is virtually impossible to reach such toxic concentrations in vivo. So these findings imply that no adverse effects of silymarin in terms of drug interactions should be expected (69-71).

FUTURE DIRECTIONS

(i) Antiviral properties: It is viewed that herbal drugs which have antiviral, immunomodulatory and antiinflammatory effects on hepatocyte may prove to be useful in chronic hepatitis. Though, silymarin does not have antiviral properties, phyllanthin (from Phyllanthus amarus) and glycyrrhizin (from Glycyrrhiza glabra) have been shown to possess antiviral properties against hepatitis virus B and C respectively (72, 73). It may be possible in future to combine the hepatoprotective herbal drugs (like silymarin + glycyrrhizin or phyllanthin) to achieve the desired antiviral, immunomodulatory and anti inflammatory activities.

(ii) Hepatocellular carcinoma (HCC): One of the most concerning areas of hepatitis C virus (HCV) is the risk for HCC69. Interestingly the incidence of HCC was lower in the silymarin treated population.

(iii) Co-infection with HIV: HIV and HCV coinfections appear to progress more rapidly and lead to the increased risk for liver disease especially more rapid progression to hepatic cirrhosis (72). Silymarin alone or in the combination may be useful to arrest the disease progression.

(iv)Tissue phase of malaria: Silymarin, in experimental studies, has shown significant hepatoprotective activity in Plasmodium berghei induced hepatic changes in Mastomys natalensis (74). In future, hepatoprotective herbal drugs may be useful in tissue phase of malaria.

(v) While silymarin appears to have few side effects, it is not known whether it exerts any drug interaction with interferon, ribavirin, lamivudine, or other conventional treatment for hepatitis B or C (75).

(vi) Chemoprotective and anticancer agents: Silymarin as a chemoprotective and anticancer agent is becoming increasingly apparent. It inhibits carcinogenic action of many chemicals. Silybin has significantly decreased the incidence of urinary bladder neoplasm by nitrosamine and skin carcinogenesis by benzyl peroxide or 12-O-tetradecanoylphorbol-13-acetate. Various mitogenic, signaling and cell cycle regulators were modulated by silybin and probably act at the receptor level itself (20).

(vii) Adjuvant therapy of cancer: Silymarin has protected liver in a case of promyelocytic leukemia receiving 6-mercaptopurine and methotrexate. The liver toxicity was successfully treated by 800 mg of silymarin and conjunction therapy was without any adverse effects70. The drug may be of help in cisplatin induced nephrotoxicity, doxorubicin induced apoptotic death(76) and better compliance with HIV medications (77).

(viii) Neuroprotective and neurotropic activity: Silymarin may be useful in the treatment and prevention of some neurodegenerative and neurotoxic process partly due to antioxidant activity and may be due to some unknown mechanism. Silymarin inhibits TNF-α and reduced production of inducible nitric oxide syntnase which cause microglia activation (78). Further silymarin may be of use in protecting primary hippocampal neurone against oxidative stress induced apoptosis and neuromodulatory action against persistent viral infections leading to encephalitis (70).

(ix) Miscellaneous activities: Silymarin may be useful in end stage diabetic nephropathy. However more studies are required for its beneficial properties in human diabetic mellitus. It may have protective effect against toxic drugs like amiodarone, doxorubicin and other anthracycline cardiotoxic drugs (70). It may protect against UV irradiation as a preventive against photocarcinogens and may inhibit P-glycoprotein (P-gp) function and increase the susceptibility to

the treatment of cancer and bacterial infections by acting on membrane efflux proteins like P-g p (77). It may have anti-atherosclerotic activity by antioxidative protection of cholesterol transporting lipoproteins. New derivatives of silybin are expected to meet the various challenges (70).

CONCLUSION

Silymarin is a favoured drug for different liver diseases because of its oral effectiveness, good safety profile, availability in India and most importantly at an affordable price. It has established efficacy in the restoration of liver function and regeneration of liver cells. It may prove superior to polyherbal formulations for its better standardization, quality control and free from contamination from heavy metals and microbial toxins. Silymarin may make a breakthrough as a new approach to protect other organs in addition to liver.

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