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Research Article

Clinical Studies of Silymarin as a Protective Agent Against Liver Damage Caused by Anti-TB Drugs, Methotrexate, and in Cases of Chronic Hepatitis C and Diabetes Mellitus

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ABSTRACT

The liver is the organ in charge of homeostasis and metabolism of sundry substances (endogenous and exogenous, including drugs); but when these are metabolized, they generate more toxic and/or reactive metabolites, that can damage the liver causing cirrhosis, steatosis and/or hepatocarcinoma. Human have been used several medicinal plants (MP) since ancestral times to treat their ailments, diseases and liver disorders, including *Sylibum marianum*. This MP is used in the treatment of jaundice and other biliary diseases, as well as in support therapy for edible mushrooms poisoning and in the treatment of some hepatic diseases. From this medicinal plant, silymarin (SLM, mixture of flavonoids) is obtained, it has an important antioxidant, anti-inflammatory and hepatoprotector effect. The last activity has been demonstrated through several preclinical and in some clinical studies. To date, a few clinical studies describe the hepatoprotective and/or nephroprotective effect of SLM against the damage caused by anti-TB drugs, methotrexate and in cases of type II diabetes mellitus or chronic hepatitis C. Nevertheless, this type of research is more frequent in preclinical trials (using rats or mice) or *in vitro* assay.

Key words: Silymarin, Hepatotoxicity, Silybin, Hepatoprotector, Nephroprotector, Anti-TB drugs, Methotrexate, Diabetes Mellitus.

INTRODUCTION

Human beings have used medicinal plants (MP) since ancestral times to treat several ailments, diseases and alterations. In addition, there are paper that describe the low or scarce toxicity of the MP, and it appears that this cause minimal side effects compared with current allopathic drugs. The use of MP to treat hepatic diseases are no exception; there are reports that *Murraya koenigii, Origanun majorana, Cnidoscolus chayamansa, Silybum marianum* (*S. marianum*), *Phyllanthus niruri, Panus giganteus*, among many others, have a hepatoprotector (HPP) activity.[1-6] Since 23-79 BC, there have been writings that mention that the juice and seeds of *S. marianum* (or milk thistle), were used against snake venom or for melancholy depression, and currently it is widely used to treat some ailments related with the liver.[2,7]

The use of complementary or alternative medicine has been increasing among patients with some chronic diseases, due mainly to access to information about the use of MP, as well as to dissatisfaction with their conventional therapies added to the corresponding deficient medical care system.[8] There are reports that describe the fact that herbal preparations are widely used voluntarily by patients, as is the case with silymarin (SLM, obtained from *S. marianum*) to prevent or alleviate the hepatotoxicity caused by chemotherapy, anti-arthritic and anti-microbial drugs, including anti-TB, EtOH or against sundry hepatotoxic agents such as thioacetamide (TAM) or carbon tetrachloride (CCl_4), among other substances.[5]

On the other hand, Rowe[9] performed an analysis regarding mortality from hepatic diseases, where hepatic cirrhosis held fourth place among the chronic diseases in terms of years of life, only behind coronary, cerebrovascular and chronic pulmonary obstruction diseases, while liver cancer ranked 21. Reports from WHO described that, in 2019, at the global level 296 million people presented hepatitis B and 58 million hepatitis C, and annually approximately 3 million cases of each hepatitis type appear. Today, there are 325 million people with different hepatitis kinds, being B and C hepatitis the most common. Chronic hepatitis C (CHC) is a serious health problem due to the fact that its diagnosis is late and the symptoms are presented in an advanced state of the disease, and in many cases, it causes cirrhosis and/or liver cancer.[10-12]

Yearly, around 2 million people die of non-alcoholic hepatic disease; therefore, it is considered the main cause of premature mortality globally, and is ranked among the 10 main causes of morbidity and mortality. Some factors that contribute to the presence of non-alcoholic hepatic diseases are viral infections (hepatitis B and C), obesity, metabolic syndrome, autoimmune diseases (rheumatoid arthritis), type 2 diabetes mellitus (T2DM), pathological states where several drugs are consumed for prolonged times, such as methotrexate (MTX), the mixture of rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA), the last are first-line drugs used in the treatment of tuberculosis (TB).[3,7,13]

It is important to describe that the INH/RIF/PZA mixture mainly causes hepatotoxicity in more than 16% of cases, reason so that many patients drop out of their treatment; this factor favors the appearance of resistant TB and in few cases, HPP substances such as SLM are used.[14] One of the drugs mainly used

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for the treatment of rheumatoid arthritis and in autoimmune diseases, as well as cancer, is methotrexate (MTX), which also causes elevated hepatotoxicity.[15] It should be mentioned that to date there are several works that describe the HPP effect of SLM against the liver damage generated by stress, CCl_4, EtOH, micotoxins, thioacetamide (TAM), among other substances in animal model.

HPP effect of SLM in murine models against some hepatotoxic substances

Kim *et al.*,[16] evaluated the effect of SLM (100 mg/kg, intragastric -i.g.- route) in Balb/C mice restricted in metabolic cages over 6 hours to induce stress. They determined in the animals' blood, the lipid peroxidation (LOx), the aspartate aminotransferase (AST), alanine aminotransferase (ALT), tumor necrosis factor alpha (TNF-α), interleukin (IL)-1 and IL-6 levels. The results showed that the animals treated with SLM significantly reduced the levels of stress enzymes (AST, ALT) and LOx compared with the stress group. They also found that mice treated with SLM showed increased levels of GSH. Regarding the expression of TNF-α, IL-1, IL-6, these cytokines significantly reduced in the animals' livers treated with SLM. With this result, the authors concluded that SLM has the ability to prevent hepatic lesions caused by stress, due to its antioxidant and anti-inflammatory action.

The HPP effect of the SLM against chronic hepatic fibrosis has been described. Chen *et al.*,[17] performed a study in ICR male rats (n=24), where they caused hepatic fibrosis by administration of TAM (100 mg/kg i.p. route); the rats were randomly distributed into 4 groups (n=6): G1: ISS (i.p.), G2: ISS (i.p.) + SLM, 150 mg/kg (orally), G3: TAM 100 mg/kg (i.p.) + ISS and G4: TAM, 100 mg/kg + SLM, 150 mg/kg. The treatment with SLM did not affect body weight or the liver size, nor did it cause alterations in the biochemical values (triglycerides, cholesterol, bilirrubin, albumin, AST and ALT) respect to the group with hepatic damage caused by TAM. They only noted that SLM reduced the chronic hepatic damage caused by TAM.

In addition, in a murine model using the micotoxin fumonisin B-1 (FB-1) as hepatotoxic agent has been described. Sozmen *et al.*,[18] developed an assay in Balb/C mice (n=15) randomly divided into 6 groups: G1: ISS, G2: SLM (100 mg/kg, i.g), G3 and G4: FB1 (1.5 and 4.5 mg/kg, i.p.), G5: SLM + FB1 (100 mg/kg + 1.5 mg/kg) and G6: SLM + FB1 (100 mg/kg + 4.5 mg/kg). They performed liver histological analysis and the detection of apoptotic cells using the TUNEL assay. The results showed that treatment with SLM significantly reduced the apoptotic cells generated by FB1 and determined the levels of TNF-α and caspase 8; the mice treated with SLM (100 mg/kg) showed a significant reduction ($p < 0.0001$) in these parameters. According to the results obtained, the authors concluded that SLM reduces the hepatotoxic damage caused by FB1 in Balb/C mice.

Another study described the induction of hepatic damage with CCl_4 in Wistar rats (n=10) and these animals were treated with SLM at two doses (50 and 200 mg/kg/day) administered by oral route during 30 days. The results indicated that SLM at 50 mg/kg/day showed a significant reduction in the LOx levels (0.08 nmol/mg) regarding to the group that only received CCl_4 (0.22 nmol/mg); this same trend was presented in the levels of protein oxidates (PO), where SLM significantly reduced the PO values respect to CCl_4 group. Likewise, levels of hyaluronic acid reduced in the group treated with SLM, the levels very similar to those of healthy animals, which were administered with the vehicle (carboxymethylcellulose), demonstrating that SLM administered at 50 mg/kg has the ability to inhibit the fibrogenetic mechanism and the initial progression of hepatic fibrosis.[19]

Similar results were obtained when seed oil of *S. marianum* was tested in the same model (hepatic damage with CCl_4 administered in a single dose on day 22), in male Swiss mice treated with this oil at 10 g/kg during 21 days, administered by i.p. route The results regarding hepatic markers showed that the group treated with CCl_4 (1 mL/kg dissolved in olive oil at 50%) increased serum activity of AST by 67.8 times, ALT by 67.5 times, and gamma glutamyl transferase (GGT) by 2.7 times, with respect to the control group (healthy animals). In the group pretreated with seed oil of *S. marianum* before the administration of CCl_4, there were significant reductions in the values of AST, ALT and GGT being of 42,45%, 50% and 32%, respectively, compared with the CCl_4 group; these results helped show the HPP ability of SLM. SLM also reduced 2.2 levels of LOx regarding the group with hepatic damage caused by CCl_4. Also, the activity of CAT, SOD, GPX, GST and GR was increased respect to the group with hepatic damage, with values very close to those of the healthy group. In the liver histological analysis, SLM reduced the expression of TNF-α compared to the CCl_4 group. The authors concluded that pretreatment with SLM counteracts the hepatic damage caused by CCl_4 due to its antioxidant activity.[20]

On the other hand, it is well known that acetaminophen or paracetamol (analgesics) also cause hepatic and renal damage, since AST and ALT values are increased, as well as levels of blood ureic nitrogen (BUN), serum creatinine (SCr) and serum nitrous oxide (NO). Bektur *et al.*,[21] performed a study in female Swiss mice, where the group with hepatic damage induced with acetaminophen (500 mg/kg, single dose) were treated with SLM (100 mg/kg) during 7 days. The acetaminophen increased values of AST, ALT, BUN, ON and SCr, and the animals treated with SLM presented values of ALT, AST, BUN and SCr very similar to the control group (administered with ISS). At the histological level, SLM reduced necrosis and apoptosis. The authors mentioned that the HPP effect of SLM was due to its antioxidant and anti-inflammatory effects.

Domitrovic *et al.*,[22] used SLM (100 mg/kg) as a positive control to evaluate the HPP effect of myricetrin or myrecetin3-Oα-rhamnoside (at 10, 30 and 100 mg/kg) in Balb/C mice, using CCl_4 (2 mL/kg) as hepatotoxic agent, administered by oral route during 2 days. They performed immunohistochemical analysis, and determined levels of ALT, AST and GSH. The results showed that SLM reduced ALT (1217 vs 2126, U/L), AST (1384 vs 2538, U/L) levels compared with the group treated with CCl_4 and also reduced GSH (40.6 vs 20.6, μmol/g protein) level, as well as reducing the expression of COX-2 and TNF-α, compared with CCl_4 and reduced the proteins present in an inflammatory process, such as TGF-β1 and α-SMA, with regards to the group treated with CCl_4. These results show the hepatoprotector activity of SLM against the hepatic damage caused by CCl_4.

There are some studies performed in rats and mice with hepatic damage induced by EtOH, where it has been observed that SLM exercises HPP effect, by increasing levels of ALT, AST, CAT, SOD, glutation-S-transferase and gamma-glutamyl; however, the effect was less than observed in the group that received vitamin C.[5,23,24] One additional work performed in obese patients with fatty liver (with steatohepatitis) reported the HPP effect of a mixture of SLM (540.3 mg) plus vitamin E (36 mg), added to a hypocaloric diet compared with a group that only received hypocaloric diet (1520 Kcal, 52% carbohydrates, 25% lipids and 23% proteins) with exercise. The results showed that the group treated with SLM/Vit E/diet improved hepatic function, from which the authors concluded that SLM could be used to reduce hepatic damage caused by some substances, including drugs.[25]

There are many bibliographical reviews on the hepatoprotector activity of several extracts obtained from medicinal plants, natural and synthetic compounds (curcumin, naringenin or N-acetylcysteine, among others), where it was found that SLM is widely used as positive control in the majority of the studies performed with murine models (rats and mice). Generally, it is administered orally, in sundry doses (2.5, 100, 150 and 200 mg/kg). The majority of the studies concluded that the HPP effect of SLM was due mainly to its antioxidant and

anti-inflammatory activity.[3,26] It has also been reported that SLM is an HPP agent against some xenobiotics (EtOH, CCl_4, paracetamol and/or acetaminophen).[5,27]

The present review describes the articles where SLM was used as an HPP agent in clinical studies (blind and double blind) in patients with TB, with arthritis, or with CHC and in pre-clinical models. According to the results found in different studies, SLM significantly reduces the levels of some biochemical parameters (AST, ALT, ALP and bilirubin); it also reduces the degree of LOx and restores levels of superoxide dismutase (SOD), caused by anti-TB drugs.

Experimental

This review includes manuscripts that describe the hepatoprotector effect of SLM performed in clinical and pre-clinical trials with hepatic damage caused by anti-TB drugs, methotrexate, and in cases of chronic viral hepatitis and T2DM. This review was performed consulting some scientific portals: Sciencedirect, Pubmed, Worldwidescience, Springer. The key words used during the information search were: silymarin, hepatoprotector, antituberculosis, methotrexate, rifampicin, isoniazide, pyrazinamide, chronic hepatitis C, type 2 diabetes mellitus, nephroprotector and the combination of these words. The review included a total of 71 articles that met the search criteria.

RESULTS AND DISCUSSION

SLM obtaining and its traditional use

The SLM is obtained from the milk thistle or Santa María thistle (*S. marianum)*, a species from the family *Asteraceae* (Figure 1). It is characterized by having branches with sharp thorns, a milky sap and oval leaves that can reach up to 30 cm. The flowers are bright pink and can measure up to 8 cm in diameter.[5,28] It is an annual or biennial plant native in the Mediterranean regions of Europe; however, it is also distributed in North Africa, the Middle East, and some parts of the USA, and is cultivated in Hungary, China, and some countries of South America such as Argentina, Venezuela and Ecuador. It is traditionally used in the treatment of jaundice or other biliary diseases, as well as in support therapy for mushroom poisoning and the treatment of some hepatic diseases worldwide.[5,29,30] SLM has some activities, such as hepatoprotector, antioxidant, since it reduces the production of oxygen reactive species, LOx and increases levels of exogenous antioxidant enzymes (glutation peroxidase, glutation reductase, SOD, CAT); it is also anticancerigenic, antidiabetic, antifibrotic, anti-inflammatory (it mainly inhibits nuclear transcription factor kB, inhibits protein kinase and inhibits COX-2 and TNF-α), is an estrogenic regulator, antidiabetic, antibacterial, antimycobacterial, antituberculosis, and is used to treat Alzheimer and Parkinson, osteoporosis, among other conditions.[2,7,31-33]

Figure 1: *Silybum marianum* (*Asteraceae* family).

Chemical composition of SLM

Currently, the extracts made from milk thistle fruits contain approximately 80% SLM as active ingredient. It is the name of a complex mixture of polyphenolic molecules that include seven flavolignanes: silybin A and B, isosilybin A and B, silicristin, isosilicristin, silidianine, as well as quercetin, betaine and taxifolin (Figure 2); some authors describe that this last compound is the most activity, but is found in a lower amount.[29,34,35] Other authors describe that silybin A and B are the most abundant constituents with greater antioxidant and HPP activities, which represents approximately 60-70 %, followed by silicristin (20%), silidianine (10%) and isosilybin (5%).[36,37] In Mexico, there are sundry companies that commercialize it as a nutritional supplement, as is the case of Homeostasis de México®, with presentation in capsules of 150 mg, or General Nutrition Center® (GNC) with tablets of 1300 mg.

Clinical studies of the HPP effect of SLM in patients with pulmonary TB

To date, there are few clinical trials that support the use and safety of SLM as HPP agents. Luangchosiri *et al*.,[38] presented the results of a double-blind randomized study in patients with pulmonary TB, who received the normal treatment for TB [INH:RIF:PZA (5:10:25 mg/kg/día), plus ethambutol (Etb, 15 mg/kg/day)], and were divided into two groups: one group received SLM (one tablet of 140 mg/day) and the other group received placebo. The total of patients that met the criteria established in the protocol were 55 people, distributed with n=27 and n=28, respectively. Comparison was carried out of the biochemical parameters (ALT, AST, ALP, albumin, total bilirubin and direct bilirubin). Comparative analysis between both groups showed that the group treated with SLM presented 28% less risk of suffering damage than the placebo group. The authors concluded that SLM reduced the incidence of hepatic damage from anti-TB drugs, since it reduced the levels of SOD.

A double-blind trial performed in China with patients diagnosed with TB, who received their standard anti-TB therapy: group I (183) received SLM (capsules of 200 mg/2 times a day/orally) and group II (187) received Vitamin C as placebo. At the start of the study and every two months, AST, ALT, total bilirubin, GGT and ALP levels in peripheral blood were quantified. 46 and 28 cases of each group (SLM and placebo, respectively), developed hepatic damage. The authors recommend the rational use of hepatoprotector such as SLM, since they clearly observed a scant HPP effect in patents that received anti-TB treatment.[14]

Additional clinical assay performed by Eunyoung Heo *et al*.,[39] included 108 TB patients, who received conventional anti-TB treatment (INH:RIF:PZA) and were divided into two groups: SLM group (45 patients) received a tablet of 140 mg/day and control group (58 patients) received placebo (twice a day) during 4 weeks. The ALT, AST and total bilirubin values in all patients were determined at weeks 2, 4 and 8. The authors did not observe significant changes in the parameter's values determined during the experimental period.

A clinical trial performed in TB patients (n=35), who received their respective anti-TB treatment (INH:RIF:PZA:Etb, 5:10:20:15 mg/kg respectively) and SLM as HPP agent.[40] In this study, group I was placebo and group II, three tablets/day of SLM (140 mg, with the commercial name Livergol®) was administered by oral route. After two weeks of treatment, no significant differences were observed; the AST, ALP and total bilirubin values between both groups were similar, and the

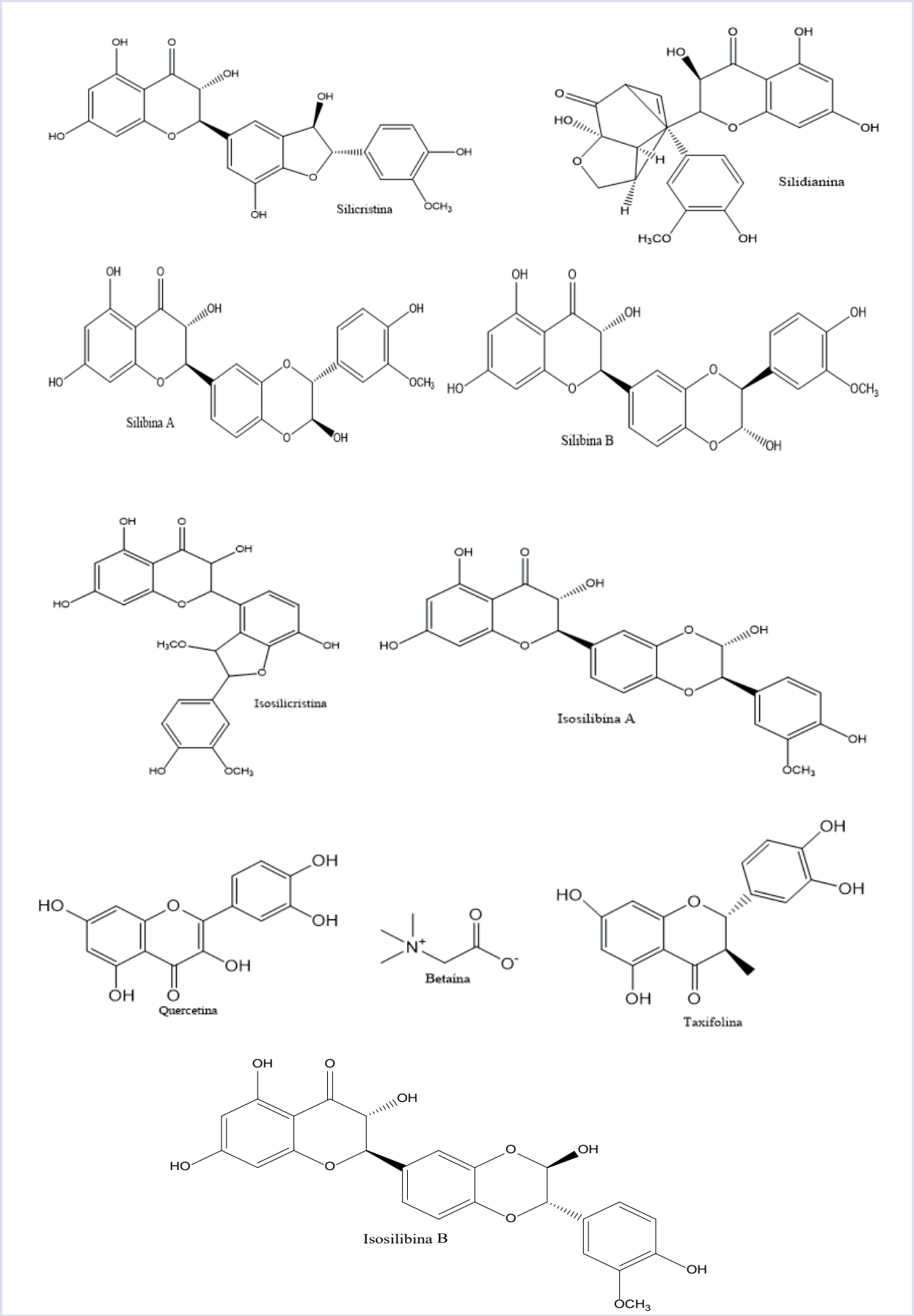

Figure 2: Flavolignans and flavonoid present in the silymarin obtained from *Silybum marianum*.

group that received SLM showed a slight reduction in nauseas, vomit, diarrhea and general ill feeling compared with the placebo group. The authors concluded that SLM did not reduce the hepatic damage caused by anti-TB drugs.

In a second clinical trial performed by the same author, in patients recently diagnosed with TB with respective treatment (INH:RIF:PZA:Etb, 5:10:20:15 mg/kg).[41] They evaluated the HPP effect of SLM (group I); in this study, three tablets/day of SLM (140 mg) was administered daily by oral route, and group II received placebo, each group had 27 patients. As in the previous study, in this case no difference in AST, ALP and total bilirubin values was observed between both groups. The group that received SLM only showed a slight reduction in nauseas, vomit, diarrhea and general ill feeling compared with the placebo group. However, they did not report the duration of the study. The authors concluded that SLM does not reduce hepatic damage caused by anti-TB drugs.

Today there is only one work that describes the HPP effect of silibinin (a constituent of SLM) in patients with TB (patients between 18 and 60 years old) with recent diagnosis, and were divided into two groups. Group I: 277 patients received their respective anti-TB treatment (INH:RIF:PZA:Etb) plus three silibinin capsules (silybin-phospholipids complex, 35 mg) per day during 8 weeks, and group II (299 patients) was the placebo group that received only anti-TB drugs. The HPP effect was determined by quantifying of ALT, AST, total bilirubin, direct bilirubin, alkaline phosphatase, total proteins and albumin values at 2, 4 and 8 weeks. The results showed that there was no statistically significant difference on the quantified parameters between both groups.[42]

In 2017 and 2019, de Avelar *et al.*[13] and Tao *et al.*[43] performed two reviews of the articles that describe the HPP effect of SLM in patients; in these reviews only four articles[14,38,39,42] were performed in patients with anti-TB treatment.

Clinical studies on the HPP effect of SLM against hepatic damage caused by methotrexate

Hagag *et al.*,[44] evaluated the protector effect of SLM in a clinical trial on the hepatic toxicity caused by MTX in patients (children) with acute lymphoblastic leukemia. The patients were separated into two groups (n=40), group I received SLM (420 mg/day) for one week after MTX administration, and the group II received only MTX. After treatment, the hepatic profile (serum bilirrubin, total proteins, serum alkaline phosphatase, ALT and AST), as well as their respective renal profiles (ureic nitrogen in blood and serum cystatin-C) were quantified. According to the results obtained, the group treated with SLM/MTX showed significantly lower levels of ALT, AST and alkaline phosphatase after chemotherapy with MTX, compared with the MTX group. In addition, the authors describe that after MTX treatment, they observed a statistically significant reduction in urea and serum cystatin-C levels in Group I (SLM) compared with Group II. The authors conclude that SLM at a dose of 420 mg/day administered for one week after each dose of MTX improves some hepatic and renal functions in children with acute lymphoblastic leukemia that received chemotherapy protocols based on MTX.

Clinical studies of the HPP effect of SLM on patients with chronic hepatitis C

Salmond *et al.*,[45] carried out a randomized, double-blind assay (n=118) over 48 weeks (24 weeks with treatment/24 weeks of follow-up), evaluating the effect of SLM and a mixture of SLM with thirteen other antioxidants (SOX or Hep573) in patients with CHC. The patients were divided into three groups: (I) placebo, (II) SLM, and (III) SLM plus SOX. At the end of treatment, the authors determined levels of ALT, isoprostanes (oxidative stress), liver damage and quality of life. The results showed that the mixture of SLM plus SOX antioxidants induced normalization of levels of ALT significantly, respect to groups I and II. Regarding the study parameters (isoprostanes and liver damage) there was no significant improvement among the 3 groups, and through test, the participants mentioned improvement regarding quality of life. The authors concluded that treatment with natural compounds (SOX) or SLM helps normalize levels of ALT and slightly improves the quality of life of patients with chronic CHC.

In other clinical trial (double-blinded), Fried *et al.*,[46] described the HPP effect of SLM in patients with CHC treated with interferon. Patients were divided randomly into 3 groups: group I and II received SLM at 420 and 700 mg, respectively, whose commercial name is Legalon 140 and the third group was placebo (gelatin capsules); these treatments were administered 3 times/day during 24 weeks. The results were analyzed taking into account serum levels of ALT, observing that there was no significant difference among the 3 groups (placebo vs SLM at 420 and 700 mg). With these results, the authors concluded that SLM at the doses tested did not significantly reduce serum ALT levels with respect to placebo group.

Likewise, the evaluation of SLM was performed in patients with CHC in Egypt, where 177 patients met inclusion criteria and were randomly grouped into two groups, who received 127 mg SLM (n=88) and placebo (multivitamins and low concentrations of minerals, n=89); the study lasted 30 days and, at the end, ALT level was determined and along with markers of hepatic fibrosis (quantification of hyaluronic acid and glycoprotein of human cartilage). The results showed that levels of ALT did not show difference between experimental groups, as with the hepatic fibrosis markers, after 12 months of treatment. The authors concluded that SLM did not show effect on hepatitis C virus in patients treated during this period; however, they mention that perhaps a higher dose of SLM given for a longer time can help determine if this substance helps prevent complications caused by this virus.[47]

An additional study was performed in two groups of 33 patients each, diagnosed with hepatitis C, which evaluated the HPP effect of SLM (3 capsules of 500 mg/day) and spirulin (3 capsules of 140 mg/day), administered orally during 6 months. 29 patients from the SLM group and 30 patients from the spirulin group finished the treatment. Both substances favored levels of ALT; however, they suggest that an additional study be performed with more patients because the results were not conclusive.[48]

Clinical studies on the HPP effect of SLM in patients with type 2 diabetes mellitus

There is only one study performed in patients with T2DM, who received SLM orally for 4 months. This trial was performed considering that SLM is an antioxidant agent beneficial for patients with T2DM, in whom permanent oxidative stress can damage pancreatic β cells, causing anomalies that aggravate the condition. The trial was randomized, double-blind, during 4 months, and 51 patients with T2DM were divided into two groups: group I (n= 25) received conventional therapy plus SLM (tablet of 200 mg, three times/day) orally, and the second group (26 patients) received their therapy plus placebo. The results were based on a comparison of the following clinical parameters: glucosylated hemoglobin (HbA1c), fasting plasma glucose (FPG), insulin, total cholesterol, low-density lipoproteins (LD), high-density lipoproteins (HDL) and triglycerides, AST, ALT, at the start and finish of the study. The patients that received SLM showed reductions in the levels of the aforementioned parameters, mainly with significant reduction in HbA1c and GPA, LDL, triglyceride, AST and ALT levels. From these findings, the authors concluded that co-treatment with

SLM in those patients with T2DM over 4 months has a beneficial effect, since the glycemic profile improved.[49]

Synergic HPP effect of SLM in murine models

As SLM is a natural product, many authors have reported that natural products with antioxidant activity are effective to prevent oxidative stress, due to possible interactions or synergism.[26] Rassol *et al.*,[50] evaluated the hepatoprotector effect of combined SLM/Glycyrrhiza glabra (glycyrrhizin, GLN) at different doses, where hepatic damage was induced with CCl_4, in male albino Wistar rats (n=10). The ethanol extract of SLM/GLN (solubilized in dimethyl sulfoxide) was evaluated separately at doses of 200/50 mg/kg and 100/25 mg/kg administered by oral route during six weeks. The combined doses (SLM/GLN) showed good protector effect against oxidative stress in the liver, the best effect was observed at high doses. This mixture reduced serum ALT (41.32 IU/L), AST (37.19 IU/L), ALP (129.86 IU/L) levels with respect to CCl_4 group (hepatotoxic group), where serum levels of ALT were 94.83 IU/L, AST of 73.21 IU/L and 157.96 IU/L for ALP. Likewise, they determined the activity of antioxidant enzymes SOD, CAT, GSH and LOx, and in this case found that the combination SLM + GLN at the highest dose presented the better hepatoprotector effect, by increasing levels of SOD (67.68 µg/mg tissue), CAT (45.65 µg/mg protein), GSH (7.91 µg/mg protein) and TBARS (46.56 nmol/g tissue) with regards to the group treated with CCl_4, which showed lower enzyme levels [54.59 µg/mg tissue (SOD), 20.26 µg/mg protein (CAT), 2.99 µg/mg protein (GSH) and 80.51 nmol/g tissue (TBARS)]. The authors concluded that the SLM/GLN had the ability to eliminate reactive oxygen species (ROS), which protects against the oxidative damage caused by CCl_4 in the hepatic lesion induced artificially, and that this recovery was produced after six weeks of treatment.

Kim *et al.*,[51] reported the synergic effect of the aloe vera and SLM mixture called "ACTIValoe N-931 complex", administering the dose of 85 mg/kg (35 and 50 mg/kg of aloe vera and SLM), 170 mg/kg (70 and 100 mg/kg, of aloe vera and SLM) and 340 mg/kg (200 and 140 mg/kg of aloe vera and SLM) in a chronic model (8 weeks), where hepatic damage was induced by CCl_4 in male Sprague-Dawley rats. The hepatoprotector ability of the mixture was determined by ALT, AST, LOx values, and the content of hepatic glutation. The results showed that the dose of 85 mg/kg reduced the levels of ALT (1142.2 UI/L) and AST (468.4 UI/L), with regards to the CCl_4 group, which presented elevated values (2112.7 and 1086.7 UI/L, respectively). On the other hand, doses of 170 and 340 mg/kg significantly reduced levels of MDA, with protein values of 0.39 and 0.40 nmol/mg, respectively. The authors concluded that the hepatoprotector effect of this mixture could be attributed to the reduction of oxidative stress, and suggested that this mixture could be used to prevent hepatocellular damage and hepatic fibrosis.

Another work describes the hepatoprotector activity of the combination of SLM/quercetin; this mixture was evaluated in a model of hepatic necrosis induced by TAM in male albino mice (n=6). The treatments were evaluated separately: SLM (100 mg/kg/day) and quercetin (100 mg/kg/day) and in two combined doses: SLM (50 mg/kg/day) + quercetin (50 mg/kg/day) and SLM (100 mg/kg/day) + quercetin (100 mg/kg/day). When determining the biochemical parameters of ALT, AST, ALP and total bilirrubin, the group treated with TAM showed elevated values (514.07 U/L, 859.66 U/L, 1095.83 U/L, 65.78 mg/dL, respectively), and the group treated with SLM/quercetin (at 100 mg/kg each) presented significant reduction in these parameters: ALT (78.30.45 U/L), AST (152.04 U/L), ALP (214 U/L) and total bilirrubin (18.45 mg/dL). These values were very similar to the group of healthy animals. The authors stressed that these two treatments have hepatoprotector effect, suggesting that the combination of SLM (100 mg/kg) + quercetin (100 mg/kg) is the one that showed the best HPP effect, by reducing levels of enzyme markers, which indicates a stabilization of the plasma membrane translated into a reduction of the hepatic damage caused by TAM.[52]

The combination of SLM with dry grape extract (DGE) has been evaluated on a model of hepatic fibrosis induced by TAM (100 mg/kg i.p route.) administered during 6 weeks. The hepatoprotector effect of this combination was evaluated in male Wistar rats (150-200 g). The animals were divided into 7 distinct groups (n=8) and treatments were administered as follows: (1) control (ISS *vía* i.p route), (2) negative control, groups 3 to 5 were orally administered SLM (50 mg/kg), DGE (100 mg/kg) and DGE (200 mg/kg), group 6 received the combination of SLM (500 mg/kg) and DGE (100 mg/kg) and group 7 was treated with a combination of SLM (50 mg/kg) and DGE (200 mg/kg). From the biochemical parameters evaluated at the end of the study (ALT, AST, ALP, total bilirubin and total proteins), it was observed that the different treatments or in combination had the ability to reduce levels of hepatic enzymes compared with the group treated only with TAM (100 mg/kg). However, the authors stressed that the combination of SLM (50 mg/kg) and DGE (200 mg/kg) reduced the levels of hepatic enzymes to values similar to healthy controls (ISS), concluding that this combination is the one that has the best hepatoprotector effect in this model.[53]

Hepatoprotector effect *in vitro* of SLM

Elmorsy *et al.*,[54] determined the HPP effect *in vitro*, using the cellular line of hepatocarcinoma (HepG2). They found that SLM showed anticarcinogenic, antimetastatic and apoptotic effect, since it reduced the levels of proteins CXCR-4 and Slit-2/Robo-1 at low doses. In a study where hepatic damage was induced with a pretreatment of 48 hrs with PZA or INH or RIF at different doses (0.1, 1, 10 and 100 mM) and later, each of these groups was co-treated for 24 hrs more with RIF (0.1 or 0.5 mM), INH (10 or 70 mM) or with PZA (10 or 84 mM). They found that RIF, INH and PZA reduced the production of ATP in the mitochondria of HepG2 cells at different times, showing an IC_{50} = 0.5, 57 and 57 mM for RFI, INH and PZA, respectively, at 48 hrs. However, this study did not report the effect of SLM. On the other hand, SLM at different concentrations (50, 75, 100 and 200 µg/mL) inhibited the growth of HepG2 cells in dependent dose form, the 80% inhibition at 200 µg/mL being greater. In addition, SLM at 50 and 57 µg/mL induced morphological changes and favored apoptosis in this line in 22 and 50%, respectively.[55]

On the other hand, SLM induced apoptosis in HepG2, by decreasing the CXCR-4 protein level in a dose-dependent manner and decreasing the level of Slit-2/Robo-1 protein at low doses and it was increase at high doses. With these results, the anticancer, antimetastatic and apoptotic effect on HepG2 was confirmed by altering the Slit-2/Robo-1 pathway. In this study they do not describe the effect of anti-TB drugs on this cell line.[56] Smrati *et al.*,[57] reported that INH at 6.5, 13, 26 and 52 mM caused an increase in ROS, SOD, CAT, and Glucose-6-Phosphate dehydrogenase in HepG2; also, increased Bcl-2/Bax content in this line cel.

Nephroprotector effect of SLM in preclinical and clinical studies

The nephroprotector activity of SLM on male rats with renal toxicity induced with diclofenac sodium (75 mg/kg) administered during 1 month was reported. SLM (250 mg/kg) was administered during one month by oral route. In this assay, the author describe that SLM reduced the creatinine, urea and uric acid levels respect to diclofenac group, these values were very similar to health group, due to the fact that SLM has protective effect against the renal toxicity induced with diclofenac.[58]

Naik *et al.*,[59] also evaluated the nephroprotector activity of SLM in albino Wistar rats (n=6) with renal damage induced with MTX administered in a single dose on the 3rd day from initiating the experiment by i.p. route, and the group pretreated with SLM (50 mg/kg) for 6 days showed

a reduction in urea (54.99 mg/dL), creatinine (1.32 mg/dL), AST (90.33 U/L), ALT (85 U/L) values, as well as the total proteins (31.45±1.40 mg/dL), sodium (208.04±1.40 meq/L) and potassium (3.10±0.03 meq/L) levels were reduced compared to the group with renal damage (induced with MTX), where the values obtained were 64.87 mg/dL, 2.22 mg/dL, 143.00 U/L, 134.00 U/L, 40.34 mg/dL, 246.40 meq/L and 6.40 meq/L, respectively. The authors concluded that SLM shows a significant nephroprotector effect against toxicity induced by MTX in rats.

Gopi *et al.*,[60] performed a study in Kyoto Wistar rats (n=24), with hepatic and nephrotoxic damage induced with acetaminophen (500 mg/kg v.o.); in this case, SLM (25 mg/kg) was used as a positive control. The results showed a significant reduction in the parameters evaluated (triglycerides, total cholesterol, BUN, SCr and AST) in the SLM group. As well as, Shahbazi *et al.*,[61] carried out a review on the nephroprotector effect of SLM against nephrotoxic drugs, mentioning that about 60% of the acute renal lesions in hospitals are caused by drugs, this being one of the main causes of morbi- and mortality. Likewise, the authors stressed the biological activity of SLM against drugs used in chemotherapy of the cancer (cisplatin, doxorubicin), aminoglycoside antibiotics and cyclosporine (immunosupressor), which have the ability to generate nephrotoxicity, concluding that quite possibly the nephroprotector ability of SLM is related with the antioxidant and anti-inflammatory activity of its active compounds, mainly silybin (major compound). A study in pretreated female Wistar rats (n=12) with silybin (200 mg/kg) in a model where renal damage was induced with cisplatin (5 mg/kg), showed reduction in creatinine clearance, proteinuria and partial alteration of the proximal tube and protection of the kidney from tubular necrosis, compared with the group treated only with cisplatin.[62]

To date only one clinical trial describes the nephroprotector effect of SLM in patients on hemodialysis caused by chronic inflammatory diseases (atherosclerosis) and treated with MTX. Roozbeh *et al.*, 2011[63] evaluated the effect of SLM and vitamin E administered separately or in combination in patients with hemodialysis, determining levels of hemoglobin, red blood cell count (RBC) and markers of oxidative stress (MDA, glutation peroxidase). The patients (n=80) were separated into 4 groups randomly: Group I received SLM (140 mg/3 times a day, oral route), group II received vitamin E (400 UI/day, v.o.), group III was treated with the mixture SLM/vitamin E (140 mg/3 times a day) and group IV was control. Administration lasted 21 days, and it was observed that the combination SLM/Vitamin E significantly reduced levels of MDA compared with the MTX group; likewise, the three groups showed significant increase in levels of RCB and GPx compared with the MTX group. These authors concluded that it is necessary to perform studies with larger sample sizes and longer duration to investigate the effect of SLM on cardiovascular results and the requirement for erythropoietin.

Hepatoprotector effect of SLM in murine model with hepatic damage induced by anti-TB drugs

There are several studies on the hepatoprotector effect of SLM in murine models, where it is widely used as positive control in search that determine the HPP effect of different substances. Jahan *et al.*,[64] determined that SLM (50 mg/kg/day) protects against the hepatic damage caused by INH (50 mg/kg/day), in a study performed on rabbits (n=7), to which they gave it orally for 91 days. They found that the group treated with SLM significantly reduced levels of serum bilirrubin (0.25 mg/dL) and increased the level of ALT (37.03 U/L) level compared with the group that received INH, which presented 0.46 mg/dL of serum bilirubin and 22.15 UI/L of serum ALT.

Another study was performed on male Wistar rats with hepatic damage induced with antiTB drugs: the INH:RIF (50:100 mg/kg) were administered by intraperitoneal route and PZA (350 mg/kg) was administered orally, and positive control was SLM (200 mg/kg/oral route). The period of co-treatment was 14 days, and at the end of the period, albumin, total protein, total, bilirubin, ALT, AST and ALP levels were measured. The results showed that ALT, AST and ALP levels in positive groups that received SLM were similar to the healthy group, and were lower than the group with hepatic damage ($p < 0.001$). In addition, at the histological level, SLM reduced steatosis and necrosis in liver.[65] Panchabhai *et al.*,[66] described the hepatoprotector effect of *Tinospora cordifolia* (100 mg/kg) and *Phyllanthus emblica* (300 mg/kg), which were administered by oral route during 90 days in male Wistar rats, with hepatic damage induced with INH:RIF:PZA and SLM (50 mg/kg) was used as positive control. The SLM significantly protected against necrosis caused by anti-TB drugs, the protective effect was similar to that shown by the extracts.

In other study, the extract EtOH of *Moringa oleifera* (plant widely used in traditional medicine) and SLM (200 mg/kg) as positive control was evaluated. The hepatic damage was induced with INH:RIF:PZA (7.5:10:35 mg/kg) administered during 45 days in male Wistar rats. In this study, biochemical parameters (AST, ALP, ALT and bilirrubin), as well as LOx levels were quantified. The results showed that all the treatments (extract and SLM) reduced the levels of the biochemical parameters. Both sample, also reduced LOx with respect to the anti-TB group.[67]

Jaswal *et al.*,[68] evaluated the therapeutic potential of thymoquinone (TQ) on female Sprague Dawley rats with hepatic damage induced with anti-TB drugs (RIF:INH:Etb), administered for 8 weeks. TQ was administered at 10, 20, 40 mg/kg and SLM at 40 mg/kg was positive control. The results showed that TQ at high doses (40 mg/kg) and SLM reduced the levels of AST, ALT, ALP and albumin. The SLM showed the best HPP effect. Another study reported the evaluation of an ethanolic extract of *Ziziphus oenoplia*, where hepatotoxicity was induced with INH:RIF (50:50 mg/kg). The extract (150 and 300 mg/kg) and SLM (100 mg/kg, as positive control) were administered by oral route during 21 days. The results showed that the extract and SLM reduced the AST, ALT, ALP and bilirubin levels, where SLM showed better HPP effect.[69]

Toxicity and adverse effects of SLM

Some acute toxicity studies have been performed in animals (mice, rats, rabbits and dogs), in which SLM (in solution with ISS) was administered by intravenous infusion. The DL_{50} value for mice was 400 mg/kg, 385 mg/kg for rats, and 140 mg/kg for rabbits and dogs, although these values could vary according to the speed of infusion.[70] Likewise, SLM has a good safety profile at high doses (>1500 mg/day) in animals and in people, demonstrating a low toxicity. However, some slight adverse effects have been found, which are limited to gastrointestinal disorders, specifically a laxative effect probably due to the increased secretion of bile and the stimulating effects of SLM on the liver. It is worth mentioning that SLM shows low solubility, low bioavailability and poor absorption (20-50%) by oral route, with rapid biliary and renal elimination.[31,37,71] Recently, a review was published that describes the different formulations (nanoemulsion, liposomes, nanoparticles and others) prepared based on SLM to improve absorption and bioavailability.[31]

CONCLUSION

SLM is a flavolignanes and flavonoids mixture that is obtained from *Silybum marianum*; this mixture has sundry biological activities (antioxidant, anti-inflammatory, hepatoprotector, nephroprotector, among others). The hepatoprotector activity has been investigated mainly in murine models or *in vitro* (HepG2) assay. To date, few clinical studies have been performed on hepatoprotective effect of SLM patients with TB and their respective treatment (INH:RIF:PZA), only six articles and two reviews describe this effect. The authors of these

clinical studies conclude that the HPP effect of SLM is marginal. In the case of chronic hepatitis C, only four clinical studies describe the HPP effect of SLM, and in this case, the majority of authors concluded that SLM had a slight beneficial effect. In the case of patients with T2DM, who received SLM, a single article reported that this mixture of flavonoids shown a beneficial effect on patients that received their respective treatments and were co-treated with SLM over 4 months. One single clinical research article reported that SLM showed a good HPP effect in pediatric patients with acute lymphoblastic leukemia, who received chemotherapy with MTX. Regarding the nephroprotector effect of SLM in patients with renal damage from atherosclerosis and treated with MTX, there is only one clinical study. In that case, they reported that the mixture of SLM/vitamin E showed marginal HPP effect.

The majority of clinical studies were performed with commercial SLM; however, they have not taken into account that solubility and absorption are very low, and the speed of elimination is elevated. Currently, there are already formulations (nanoemulsion, liposomes, nanoparticles) of SLM that favor its bioavailability, but few preclinical investigations have been performed with these formulations.

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CONFLICTS OF INTERESTS

The author read and approved the final version of the manuscript and declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

MAJA was responsible for searching, storing, analyzing and writing the manuscript.

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GRAPHICAL ABSTRACT

Silybum marianum

Polyphenolic mixture

(silybin A and B, isosilybin A and B, silicristin, isosilicristin, silidianine, quercetin, betaine, taxifolin)

DESCRIPTION

The administration of the antituberculous drugs (RIF/INH/PZA) and methotrexate for long time, causes hepatotoxicity and nephrotoxicity mainly. Tuberculosis, Diabetes mellitus T2 or chronic hepatitis C are disease that require long treatment and damage the liver.

Silymarin is widely used as a hepatoprotective agent in *in vivo* research, especially in research carried out on mice and rats.

Few clinical studies have been conducted in patients with tuberculosis where silymarin is administered as a hepatoprotective agent, although the results are not very promising.

The use of silymarin as a hepatoprotective agent against the use of methotrexate is very scarce, there are almost no clinical studies.

There are few clinical studies conducted in patients with type 2 diabetes mellitus or chronic hepatitis C, who receive silymarin as a hepatoprotective agent, in these patients the effect is promising.

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