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 REVIEW

Silymarin Based Complexes - a mini Review

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Abstract. *Silymarin (SIL) is a component extracted from Silybum marianum herb and is studied in medicine due to its protective activities on certain organs (liver, kidney, heart, brain). The review discusses some methods that increase silymarin bioavailability such as development of complexes with cyclodextrins, phospholipids, liposome and nanostructured material (hydroxyapatite-HAP). The interactions between SIL and alpha-lipoic acid, metallic nanoparticles (gold nanoparticles-GNP, and silver nanoparticles – SNP), some carotenoids (β-carotene and lycopene) and curcumin were debated too. Some combined treatments (e.g. SIL + curcumin) highlighted anticancer activity against colon cancer cells (DLD-1, HCT116 and LoVo) and protective effect against chemicals toxicity*.

Key words: Silymarin, complexes, hydroxyapatite, phospholipids, metallic nanoparticles, carotenoid

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1. Introduction

Silymarin (SIL) is a component extracted from Silybum marianum herb also knows under the Milk thistle, Lady's Thistle, Mariana lactea Hill, Marian Thistle, St Mary's Thistle name. The content of silymarin in the plants hydro-alcoholic extract is 70-80% and consists in a mixture of flavonolignans, flavonoid, lipids and sterols. The principal flavonolignas of sylimarin are silibin, isosilibin (A and B), silydianin, and silychristin [1, 2]. The plant has been used since ancient times, 2000 years, in medicine to treat kidney, spleen, liver and gall bladder diseases. Also the nursing mothers use the plant for stimulating milk production, as a bitter tonic, for haemorrhoids and dyspeptic complaints [3].

Silymarin is currently a compound studied in medicine due to its protective activities on certain organs. Figure 1 shows a scheme showing how silymarin can treat certain organs affected by disease through antioxidant, anti-inflammatory, anti-fibrotic, anti-cancer and anti-toxin mechanisms.

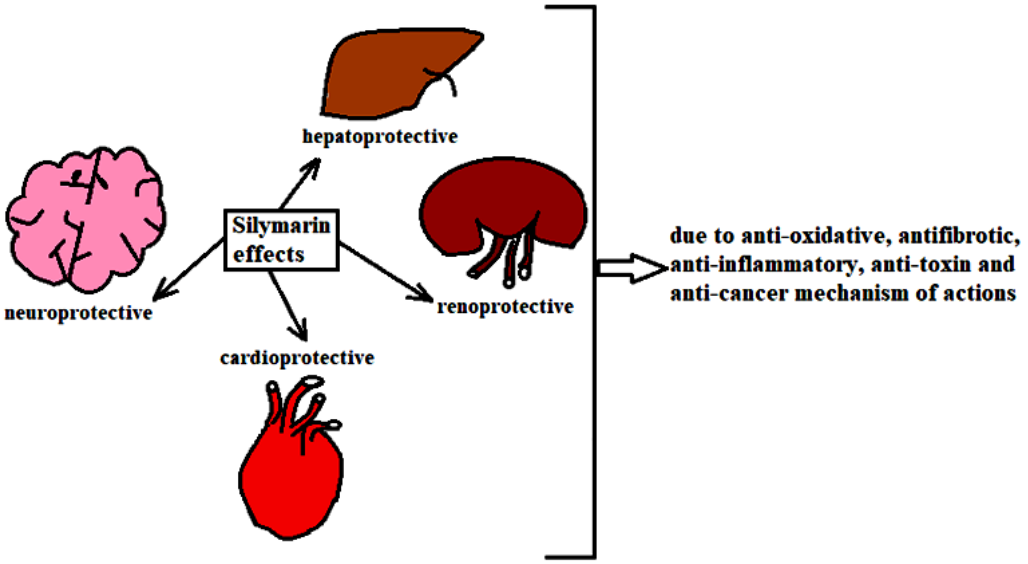

Fig. 1. Silymarin protective effect on certain organs

The ***liver*** is an organ with important role in maintaining the homeostasis and is responsible for metabolic functions and processes such as bile production, energy generation, vitamin storage and metabolism of proteins and lipids. Due to risk factors (fat consumption, alcohol consumption, viral infections, autoimmune diseases) to which the liver is exposed, pressure is exerted that will unbalance its functional nature. This will result in acute diseases in the first phase which if not treated in time, become chronic (hepatitis, cirrhosis, cancer) and ultimately death occurs [4].

In the present, SIL is being extensively studied in liver conditions. Its antioxidant activity allows to inhibite reactive oxigen species producing enzimes which prevent the formation of free radicals and regulating the intracellular content of glutathione. Also it improves the hepatic lipid homeostasis by decreasing de novo lipogenesis via the downregulation of peroxisome proliferator-activated receptor g, acetyl-CoA carboxylase, and fatty acid synthase. Regarding the antifibrotic effect, SIL inhibits the conversion of hepatic stellate cells into myofibroblasts through the inhibition of fibrogenic pathways, downregulates TGF-ß1 mRNA and inhibits NF-kB.

The mechanism by which SIL exerts anti-inlammatory effect is that it can prevent activation of the inflammasomes, it suppresses the release of cytokines and inhibits the nuclear-kappa B transcription factor, which are important in regulating the immune response in inflammatory states. It also restores the pathway known as insulin receptor substrate- 1/PI3K/Akt which can reduce MAFLD-induced insulin resistance and steatosis and activate the farnesyl X receptor, which can diminish hepatic inflammation [5].

In the case of anticancer effect, in-vitro studies show that silymarin inhibited population growth of HepG2 human hepatocellular cancer cells in a dose-dependent manner after treatment with 50 and 75 µg/ml silymarin for 24 h. Also it decreased mitochondrial transmembrane potential through an increase in the level of cytosolic cytochrome c (cyt. c). Treatment with 50 and 75 µg/ml of silymarin for 24 h leads to

apoptosis with loss of cells in the G1 phase [6]. J. K. Mastron and coworkers show that SIL increased the percentage of cells in the G0/G1 phase and decreased the percentage of cells in the S-phase, with concomitant upregulation of retinoblastoma protein, p53, p21Cip1, and p27Kip1 and downregulation of cyclin D1, cyclin E, CDK4, and phospho-Rb [7]. According to literature research, silymarin demonstrates positive effects in most forms of liver disease and the mechanism of action by which it produces these clinical effects is attributed to its action.

Kidneys are organs which control biological mechanisms such as fluid, electrolyte, pH balance, blood pressure, excretion of toxins and waste, vitamin D metabolism, and hormone synthesis [8]. A decline in their functioning, natural or by nephrotoxic medications can cause several damage. Recently, researching studies have shown that silymarin can have renoprotective effect due to its antioxidant, antiinflammatory and anti-apoptotic actions [9]. F. Turgut et al investigate the protective effects of silymarin on ischemia and reperfusion lesions in the kidney tissues of rats. They observed that SIL protects the rat kidney against I / R damage by testing kidney function, by serum and tissue antioxidant levels that presented high values when treated with SIL, and by tissue oxidant levels that were significant smaller if treated with SIL. They conclude that SIL protective effect is associated with its antioxidant properties [10]. Another study conducted by M.A. Ahmed et coworkers investigate the rrotective effect of silymarin against kidney injury induced by carbon tetrachloride in male rats. The results show that SIL decreases the creatinine, urea and MDA and increases albumin and GSH. This study demonstrates the ability of SIL to prevent CCl_4 from lipid peroxidation, has cytoprotection activities, elevated ROS scavengers and reduced lipid peroxidation indicated by MDA level [11]. A similar study investigates the effects of silymarin on valproic acid (VPA)-induced kidney damage in rats. VPA cause oxidative stress and leads to kidney disfunction. SIL administration protects rat kidney against valproic acid induced damage via anti-oxidative effect and improves the biochemical, histological and structural changes [12]. Silymarin attenuates renal dysfunction after contrast-induced nephropathy in mice by the reduction of serum levels of urea, creatinine and cystatin C. Also SIL prevents renal oxidative stress after contrast-induced nephropathy in mice showing a dose dependent decrease in oxidative stress reducing it significantly. This renoprotective role is due to antigenotoxic and antiapoptotic activities [13].

The ***heart*** is an priority organ that pump blood throughout the entire body, controls heart rate and blood pressure. Heart diseases such as hypertension, atherosclerosis, ischemia, vascular dysfunction, cardiotoxicity, cardiomyopathies and heart failure are responsible for many deaths. In this context SIL shows a wide range of mechanisms in preventing the cardiovascular disease by increasing enzymatic antioxidants, mitochondrial enzymes and expression of Nrf2. It Also decreases lipid peroxidation, expressions of NOX4, LDL, total cholesterol, and triglyceride level in the blood, thus preventing cardiac dysfunction and dyslipidemia. Antioxidant activities of silymarin offer protection against oxidative stress-induced hypertension, atherosclerosis and cardiac toxicity, increase the membrane stability and help in tissue regeneration [14]. B. M. Razavi and collaborators evaluated the preventive effects of silymarin against cardiotoxicity induced by chemicals such as metals, oxidative agents, environmental pollutants and anticancer drugs.

Silibinin is a major component of silymarin and may protect rat cardiomyocytes against isoproterenol-induced apoptosis. In the case of oxidative agents through mechanisms such as decrease of cytochrome c release from mitochondria, increasing the level of Bcl-2 protein, inhibiting the translocation of Bax from cytoplasm to mitochondria and upregulation of SIRT1. It also down-regulated p53 phosphorylation, increased the expression of procaspase-3, inhibited the cleavages of ICAD and PARP, activated tyrosine kinase pathway and finally increased PKC activity and phosphorylated ERK.

Regarding anticancer drugs, it is known that doxorubicin induces cardiotoxicity by increased plasma CPK and LDH activities and NO level. So pretreatment with silymarin reduced the toxic effects of doxorubicin in rat heart tissue except hyperlipidemia and reduced NO level. Another drug, cisplatin, is associated with cardiotoxicity including arrhythmia, cardiomyopathy and congestive heart failure. SIL protected heart against cisplatin-induced myocardial injury by reducing the activity of serum biochemical markers like lactate dehydrogenase (LDH), creatine kinase isoenzyme MB (CK-MB) and cardiac troponin I (cTnI) [15].

Neurodegenerative diseases such as cerebral ischemia, Alzheimer's and Parkinson are the most common brain illnesses which lead to cognitive and memory impairment.

Alzheimer disease is defined by loss of memory, cognitive decline and dementia in which are included pathological manifestation such as extracellular amyloid plaques aggravation, intracellular neurofibrillary tangles, loss of neurons and synapses. Factors associated to Alzheimer risk are diabetes and brain traumatic injury. Parkinson Disease leads to movement disability, motor and cognitive dysfunction followed by reduction in levels of dopamine (DA) in the striatum (STR). It is defined by loss or degeneration of dopaminergic (DAergic) neuronal cell in the midbrain [16]. K. P. Devi et al highlighted in their mini review that silymarin has been used for 2000 years in the treatment of Alzheimer, Parkinson and cerebral ischemia diseases. Their results show that SIL effects include modulation of various antioxidant mechanisms, several kinases involved in cell signaling pathways, inhibition of the inflammatory response generated during neurodegeneration, neurotropic effects, regulation of neurotransmitters and inhibition of apoptosis [17]. Specialized literature shows that the administration of silymarin in Alzheimer disease attenuated the cognitive impairment and the deposition of extracellular amyloid beta fibrils in senile plaques. For example, in an animal model Silymarin improved memory impairment and cognitive abnormalities and this fact is due to reduction of oxidative stress and inflammatory responses. In the case of Parkinson disease, Silymarin attenuated the loss of dopaminergic neurons in substantia nigra pars compacta and motor behavioral abnormalities generated by intrastriatal administration of parkinsonian neurotoxin, 6-hydroxydopamine. It was reported that silymarin inhibit monoamine oxidase-B through the neuroprotective mechanism to counteract the loss of dopamine. Treatment of cerebral ischemia with silymarin improved the brain histochemical changes and the relationships between physical activity and cognitive functions. Silymarin is anti-apoptotic in cerebral ischemia reducing downregulating apoptosis, inducing molecules such as p53, apoptotic protease-activating factor 1 (apaf-1), and caspase-9 [18].

Given the progress in nanotechnology and nanoscience, it has been found that silymarin is a multifunctional compound that by addition to the liver, brain, heart and kidneys, treats also metabolic syndrome and diabetes mellitus 2.

In metabolic syndrome, SIL can modulate glucose homeostasis, prevent damage to pancreatic beta cells and reduce the production of nitric oxide (NO) induced by interleukin IL-1β. The mechanism by which Silymarin has protective effect against diabetes mellitus 2 is that it can reduce the level of interleukin 1β and cyclooxygenase-2 (COX-2), inhibite NF-κB recruitment, reduce intracellular cholesterol esterification by the decrease of acyl CoA enzyme activity, and inhibite the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [19].

Because SIL has poor solubility in water, poor bioavailability resulting from the instability in gastric environment and poor intestinal absorption, it was necesary to improve them by interactions with various chemicals to enhance the treatment of various conditions in the biomedical field. It is necessary for silymarin to be investigated in more detail in order to elucidate the mechanism of action and target organs. So, in this sense the enhancement with other chemicals may produce sinergical effect and open new research area [20, 21].

2. Methods to increase silymarin bioavailability

To increase bioavailability and solubility of silymarin were developed complexes with cyclodextrins and phospholipids, microemulsions, nanoemulsions, liposomes, polymer nanocarriers, solid-lipid nanoparticles, nanostructured lipid carriers, and polymer-based nanocarriers [22, 23]. In the following we will discuss some of these complexes.

2.1. Silymarin loaded liposomes

Liposomes are vesicles prepared with phospholipids. Liposomes structure is similar to cellular membrane, and it can be seen in figure 2 a representative scheme of liposome vesicle in which is encapsulated the drug.

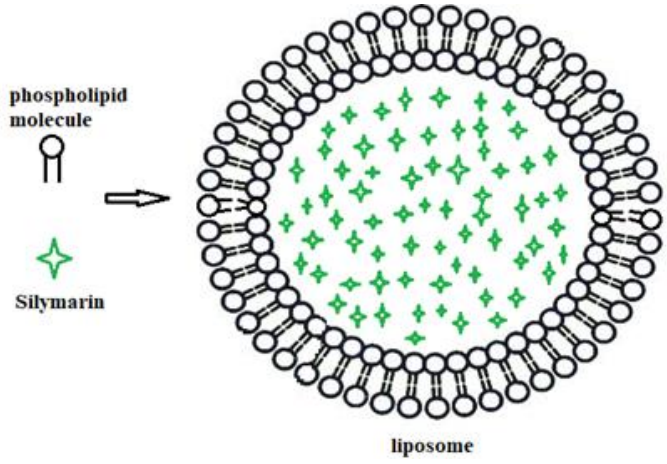

Fig. 2. Schematic representation of the structure of a liposome encapsulating silymarin

Liposomes carriers are capable to encapsulate hydrophilic and lipophilic drugs, resulting in controlled release properties, cell affinity, and tissue compatibility, reduced drug toxicity and improved drug stability [23, 24]. M. Elmowafy et al prepared silymarin loaded liposomes formulations consisting in hydrogenated soy phosphatidylcholine and cholesterol with or without distearoylphosphoethanolamine-(polyethyleneglycol)-2000 and various amounts of b-sitosterol b-D-glucoside (Sito-G) as the hepatic targeting moiety. The results show that increasing the amount of Sito-G in the liposomes decreased drug encapsulation efficiencies from 70% to 60%. They prepared ten formulations (non-PEGylated and PEGylated) most of them stable during 2 months at 4°C and room temperature. They use HepG2 cells and demonstrate that Sito-G containing liposomes are potential drug carriers for silymarin to hepatic cells. Also, maximizing the HepG2 cell uptake of silymarin is dependent on liposome composition [25]. H. Maheshwari et al study the efficacy of silymarin loaded liposomes in in vivo animal models of gastroprotection and hepatoprotection and showed a superior hepatoprotection of 55.6 % in comparison to plain silymarin and plain liposomes (33.08 % and 24.2 %, respectively). In the case of gastroprotection the liposomal formulation of silymarin reduced the ulcerogenicity ($p < 0.05$) [26]. M. S. El-Samaligy et al investigate the bioavailability of silymarin using a buccal liposomal delivery system using a lipid mixture of soybean lecithin and cholesterol in a 9:1 optimized molar ratio. At this molar ratio it produced an optimum hydrophobicity which decreased the formation of transient hydrophilic holes. In this way the liposome can be stable in the presence of other surfactants. If they increased the molar ratio of cholesterol, the silymarin release also increased. In another study also conducted by M. S. El-Samaligy et al, they add to this basic liposome stearylamine as a positively charge that was capable to enhance the encapsulation efficiency, dicetylphosphate as negatively charge and non-ionic surfactants (Tween 20 or Tween 80). This formula, lecithin/CHOL/SA/Tween 20 can supply the need of silymarin to children who suffer from liver diseases. The optimal molar ratio of this formula was 9:1:1:0.5 and showed promising drug encapsulation efficiency of 69.22 ± 0.6% and contributed to enhancing bioavailability by increasing drug permeation. [23, 27, 28].

2.2. Complexes with Cyclodextrins

To improve the solubility and dissolution rate of silymarin, a formulation with (β)-cyclodextrin was prepared. Cyclodextrins (CD) are naturally occurring cyclic polysaccharides and are obtained from starch by enzymatic reaction. They have conical cavities which are capable to incorporating a great variety of biomolecules via non-covalent hydrophobic interactions, hydrogen or van der Waals bonds to form inclusion complexes [29, 30]. A. Ghosh et al prepare and evaluate silymarin β-cyclodextrin molecular inclusion complexes using physical mixture method, kneading method, co-precipitation method and solvent evaporation method. The obtained complexes by co-precipitation method had drug content 100%. For the other complexes obtained by physical mixture method and solvent evaporation method the drug content was 97%. The drug content obtained by kneading method was 91%. On the other hand, the quickest rate of dissolution is shown by kneading method (T90 = 47 min) followed by physical mixture (T90 = 62 min), then solvent evaporation with (T90 = 92 min) and finally co-precipitation (T90 = 94 min). They conclude that all inclusion complexes show increase

in dissolution against the drug alone [31]. T. F. Kellici and coworkers prepared a lyophilized silibin−2-hydroxypropyl-β-cyclodextrin (SLB-HP-β-CD) complex and evaluated the antiproliferative activity comparatively to silibinin in MCF-7 human cancer cells. The interaction of silibin with 2-hydroxypropyl - β-cyclodextrin conducted to an increase in silibin solubility compared to pure silibin. Results showed a pronounced solubilization effect at pH 6.8. Dissolution rate was studied at pH 2.0, 4.5, and 6.8. The results show that dissolution of SLB from its SLB−HP-β-CD lyophilized product is rapid reaching 70−80% of dissolved SLB within 1 h at pH 4.5 and 6.8. while at pH 2.0, dissolution is slower reaching 50% of SLB dissolved within 3 h. MCF-7 cells was exposed to free silibin and SLB−HP-β-CD complex and the results show a decreased proliferation in a dose-dependent manner [32].

2.3. Complexes with Phospholipids

Phospholipids are amphiphilic molecules with solubility in aqueous and oily mediums [33, 34]. They contain a hydrophilic head and a hydrophobic tail as can be seen in figure 3, and are the major constituent of cell membrane.

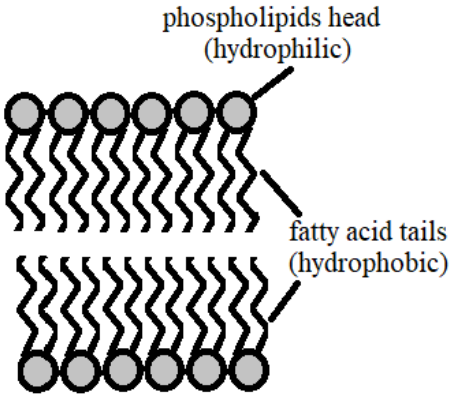

Fig. 3. Representative scheme of phospholipid in cell membrane

Two types of phospholipids are distinguished: *glycerophospholipids* such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, cardiolipin and derivatives of glycerol and *sphingophospholipids* (which do not contain glycerol) such as sphingomyelin [35]. The role of phospholipids in cell membrane organization was studied through Langmuir Blodgett Technique (LBT) which can offer important models for investigation of the existing molecular forces at fluid interfaces. M. Tomoaia Cotisel and coworkers carry out thermodynamic and structural research on supramolecular structures of molecular and colloidal self-association, self-assembled and supramolecular structures of organic molecules, measure the surface potential of biomolecules, the transfer of LB structures on various solid supports, the interactions with drugs and offers essential information about biological process at fluid interfaces [36-45].

Phospholipids have inherent hepatoprotective activity and are capable to form complexes with silymarin for improving its bioavailability and the therapeutic efficacy. A representative scheme can be seen in figure 4 in which plant extract in this case, silymarin

with phospholipids, forms phytosome. The term phytosome are patented by an italian pharmaceutical and nutraceutical company, Indena.

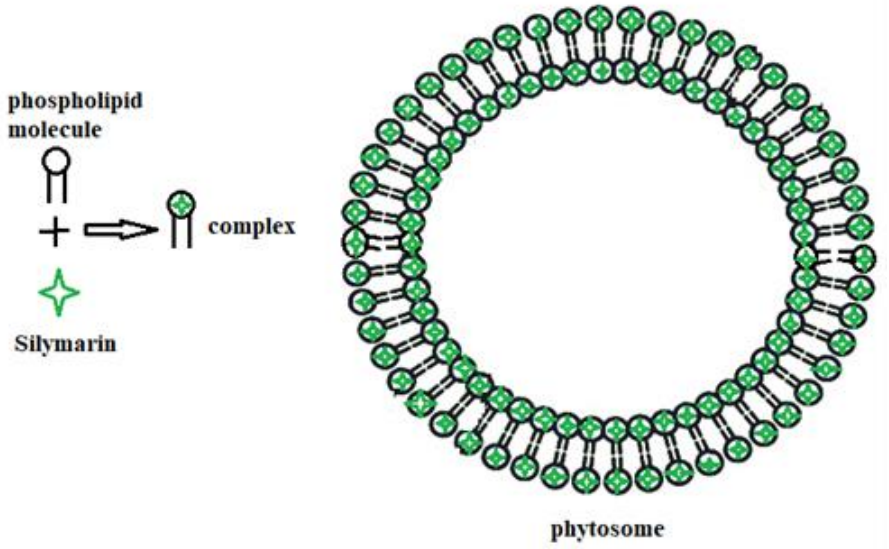

Fig. 4. Schematic representation of the structure of phytosome compound from silymarin and phospholipid

J. Khan et al show in their review details of clinical studies performed on selected phytosomes including silibin phytosome. This phytosome has been studied in 37 patients with chronic hepatitis C and Batts–Ludwig fibrosis stage and 78% of subjects showed a significant decrease in serum ferritin level [46]. A study conducted by A. Di Sario and the group evaluates the hepatoprotective and the antifibrotic effects of a new silibin–phosphatidylcholine–Vitamin E complex in rat liver fibrosis induced by dimethylnitrosamine (DMN) administration and by bile duct ligation (BDL). Test results from aminotransferase values and necroinflammatory score show that treatment with complex was able to prevent the dimethylnitrosamine-induced loss in body and liver weight, as well as to reduce the degree of liver injury. It also reduced the hepatic stellate cells activation and proliferation, and collagen deposition and procollagen mRNA expression in the bile duct ligation model [47]. Another study focused on the effects of a silymarin-phospholipid complex in reducing the toxic effects of aflatoxin B1 (AFB1) in broiler chickens. This study concludes that treatment with silymarin phytososme decreases the toxic severity of AFB1on serum ALT concentration, liver histology, feed intake, BW gain in broilers [48]. A study of silibin-phytosome in prostate cancer patients was carried out by a research group from Colorado, USA. Thirteen prostate cancer patients were participating in this trial. Of course they provided written consent according to federal and institutional guidelines. The daily dose of silibin-phytosome was escalated from 2.5 g to 20 g. The results show that silibin phytosome can be administred in high doses with acceptable toxicity. At 15 and 20 g of daily silibin-phytosome it was observed a grade 2 hyperbilirubinemia noted in 10 of 24 courses (41.67%) with a dose reduction to 13 g daily. The conclusion was that the most significant toxicity observed was

asymptomatic liver abnormalities, unconjugated hyperbilirubinemia with chronic administration [49].

2.4. Complexes with nanostructured materials (hydroxyapatite)

Hydroxyapatite (HAP) is the major constituent of bone and teeth and is essential in the balance development of human skeleton. The biocompatibility, bioactivity, bioaffinity, osteoconductivity and osseointegration property make her applicable in the biomedical field such as orthopedic and dental surgery. The HAP composition consists in calcium and phosphorus but it can be improved with elements that are released in the natural developing bone, such as: Sr, Mg, Zn, Si. The substituted elements can lead to beneficial effects on morphology, structure and mechanical properties. HAP can be used in bone tissue engineering, implant coating, dental materials and drug carriers [50-67].

Some researchers from China prepared amorphous calcium phosphate (ACP) nanospheres and hydroxyapatite (HAP) nanorods to use for silibin loading and release. They found that amount of loaded silibin by amorphous CaP nanospheres and HAP nanorods increases with increasing the initial concentration of silibin (0–6.0 mg mL^{-1}), and respectively reaches to 900 and 825 mg g^{-1} at a silibin initial concentration of 6.0 mg mL^{-1}. Silibin contains hydroxyl and carbonyl groups in structure and this can lead to electrostatic attraction with amorphous CaP and HAP and different loading amount. The as-prepared delivery systems with drug release were immersed into the simulated intestinal fluid (SIF) and simulated gastric fluid (SGF). The results show in both cases of ACP/silibin and HAP/silibin a rapid drug release at the early stage (before 120 min), and a slow and sustained release of silibin in a period of about 1000 min in both solution of SIF and SGF. Also the drug delivery in SIF is faster than in SGF, 68% and 60% after 1000 min in the case of ACP/silibin respectively 85% and 64% after 1000 min in the case of HAP/silibin [68].

Another study investigates in vitro release and bioavailability of silibin from porous calcium phosphate microparticles. The drug release behavior from calcium phosphate carrier was conducted at pH 1.2 and pH 7.4 showing a faster drug release for higher pH values. The bioavailability test of silibin-loaded calcium phosphate carrier and free silibin was conducted in beagle dogs and the results showed a prolonged 72-h release in vitro and a higher C max (418.5 ± 23.7 ng $mL^{-1)}$ with 167.5% oral relative bioavailability [69].

Hydroxyapatite and silymarin have antimicrobial activity against Pseudomonas sp, Staphylococcus aureus, Streptococcus mutants, Enterococcus faecalis and Candida albicans and promote bone formation around the implant in osteoporotic rats. Also HAP + SIL increases implant osseointegration and improves trabecular microarchitecture [70, 71].

3. Silymarin interaction with α-lipoic acid

SIL and α-lipoic acid are antioxidants with hepatoprotective effects. A. M. Abdulrazzaq and coworkers investigate the combination of SIL + α-lipoic acid + ascorbic acid against acetaminophen (APAP)-induced toxicity in rats as a model [72].

α-lipoic acid is a co factor for oxidative decarboxylation found in in a number of multi-enzyme complexes. It is used in prevention and treatment of diabetic polyneuropathy, cataract formation, radiation injury or heavy metal intoxications, hepatic

disorders, imbalance of redox status such as ischemia-reperfusion, hypertension [29, 73]. The in vivo results show that SIL + α-lipoic acid + ascorbic acid prevents elevation of superoxide dismutase and oxidized glutathione serum levels indicating a diminished burden of oxidative stress, also reduced serum levels of liver enzymes. The combination is able to reduce ALT and AST values in the APAP injured animals, can enhance the restoration of normal liver functions compared to treatment with either compound alone. They conclude that this combination inhibited the occurrence of hepatic injury induced by APAP and show their capacity in maintaining SOD, GSH and MDA levels to levels approximately equivalent to that of the control group [72].

4. Silymarin interaction with carotenoids

Carotenoids contribute to health benefits by protecting the body against atherosclerosis, age-related macular degeneration, cardiovascular diseases and ulcers, osteoporosis, against cancer, leukemia. Also help the immune system, and play a role in cell membrane stability, photosynthesis and cellular differentiation [74-80].

A research group from Romania investigated the molecular structure and monolayer properties of some carotenoids, also the thermodynamic approach on specific interactions in lipid and carotenoid nanofilms, adsorption kinetics at the oil/water interface and the collapse mechanism of some carotenoids by Langmuir Blodgett Technique [81-100].

β-carotene and lycopene are important natural pigments derived from an acyclic structure $C_{40}H_{56}$. They are found in abundance in vegetables and fruits and both show benefic effect on the human body. Due to antioxidant properties, lycopene action can stimulate the SaOs2 cells proliferation, inhibited MC3T3 cell proliferation and inhibited the phosphorylation of extracellular signal-regulated kinase. From a prevention point of view, lycopene is useful against oxidative damage of DNA, lipids, proteins and can protect against the development of gastric cancer [79, 101, 102]. β-carotene acts as a pro-oxidant in cancer prevention. For example, in colon adenocarcinoma cells such as LS-174 and WiDr. In lung cancer cells (A549), β-carotene increases the oxidative stress of 8-oxo-dG marker. In gastric cancer cells (AGS), β-carotene increases the level of caspase 3, ROS, cytochrome c and Bax. In leukemia cell, (HL-60), β-carotene inhibits cancer cell growth by increasing the production of ROS activing the NF-kB [103-106].

Silymarin and carotenoid combination is not enough studied. So we will discuss only the **β-carotene and silymarin** and **lycopene and silymarin**. Also this research needs more attention.

A research from Turkey conducted by E. Yurtku et al, demonstrated the protective effects of in vitro applications of **β-carotene and silymarin** on DNA damage induced by L-arginine in lymphocyte cultures of healthy individuals. The obtained results show no significant change in the treatment with L-arginine + β-carotene + silymarin in comparison to L-arginine + BC or L-arginine + silymarin. This can be due to the action on the same or related radicals [107]. In the case of **lycopene and silymarin**, L. Garavaglia evaluates the effect of this combination on milk production and oxidative status in periparturient dairy cows. They conclude that synergy effects contribute to the mitigation of the negative effects on metabolic adaptation to the lactation [108].

5. Silymarin interaction with metallic nanoparticles

Metallic nanoparticles such as gold nanoparticles (GNPs) and silver nanoparticles (SNPs) are the most commonly used nanomaterials in a wide range of applications such as medical field, environmental protection, food and textile industry, cosmetics, catalysts, electronics. Many studies use the silver nanoparticle due to their antimicrobial activity against pathogens which is a major problem in present. Silver nanoparticles were used in nanoceramic composites (hydroxyapatite, forsterite) as inovative materials for ortophaedic implants or dental implants [109-111]. Specialized literature shows that SNPs can be functionalized with essential amino acids, antibiotics and anesthetics to maximize the biological activities in biomedical applications [112-118]. Regarding ***SNPs and silymarin*** literature highlighted the use of silymarin in the preparation of silver nanoparticle which lead to spherical nanoparticles in a size range from 1 to 25 nm [119, 120].

Gold nanoparticles are also of particular interest for science, nanotechnology and medical applications [121]. This is due to its ability to bind to biomolecules such as amino acids [122-128], anesthetics [129], chemotherapeutic drugs [130, 131], and proteins [132-135]. ***GNPs and silymarin*** was observed to improve liver function and reduced cholestasis. After administration they decreased the aspartate aminotransferase level in the serum and interfered with glutathione depletion in hepatocytes and stimulated monocyte macrophage function [136, 137].

Research on the conjugation of gold and silver metal nanoparticles with silymarin needs to be improved through new in vitro and in vivo experiments. In this way, important aspects of the interactions between the GNPs + SIL and SNPs + SIL will be identified, as well as the mechanism that intervenes on the organism. It is an elite job that involves time and patience.

6. Silymarin interaction with curcumin

Curcumin (CCM) is an extract from *Curcuma Longa* and is used in food industry. Since ancient times, this extract has been used in traditional Chinese, Indian and Ayurvedic medicine. It has begun to be intensively studied due to its biological activity, namely anti-oxidant, anti-inflammatory, anti-cancer, anti-viral, anti-septic, anti-parasitic, analgesic. Due to its ability to protect the liver, the synergistic effect with silymarin is highlighted in the literature [138, 139].

The combined treatment with silymarin and curcumin was investigated on colon cancer cell lines DLD-1, HCT116 and LoVo showing inhibition of cell proliferation and increased apoptosis. In another study, treatment with this combination of gamma-induced nephrotoxicity in rats showed a decrease in advanced oxidation protein products, an increase in GSH, and a decrease in malondialdehyde. Additive effects were shown on the level of interleukin 18, tumor necrosis factor alpha, C-reactive protein, Bax, factor-related apoptosis and the activity of Caspase-3 [140, 141].

The interactions between silymarin and the various biomolecules or compounds discussed in this review managed to improve silymarin solubility and bioavailability. Silymarin has also led to the development of complexes with biomedical applications and beneficial protective effects on the body. This aspect is desirable and still requires efforts to fully elucidate the mechanisms of action. Medicine has evolved a lot. Through these revolutionary methods it has been shown that the treatment with free silymarin and in

combination can have a potential protective effect on dysfunctional organs. As a potential nanocarrier for silymarin, forsterite or gypsum may improve the activity and silymarin properties [142-150].

7. Conclusions

The importance of silymarin in human health has been discussed. The study of the literature has demonstrated the hepatoprotective, renoprotective, neuroprotective and cardiovascular effects but also other potential benefits on other organs.

Silymarin is known to have low solubility and bioavailability, which is why complexes have been developed to improve its qualities by encapsulating it in liposomes, forming complexes with phospholipids, cyclodextrins and hydroxyapatite. Its interactions with alpha lipoic acid, curcumin and gold and silver nanoparticles have also been highlighted.

Silymarin has been used since ancient times, and it is now extensively studied for its biological activities such as anticancer, anti-oxidant, anti-inflammatory, anti-diabetic, anti-lipemic, anti-osteoporotic, anti-viral, anti-arthritic.

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Notations and/or **Abbreviations**

SIL – silymarin, CCM – curcumin, HAP – hydroxyapatite, GNPs – gold nanoparticles, SNPs – silver nanoparticles

R E F E R E N C E S

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