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Molecular Complexation of Silymarin and Its Biological Effect

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ABSTRACT

Silymarin is the extract of Silybum marianum, or milk thistle, and its major active compound is silybin, which has a remarkable biological effect. It is used in different liver disorders, particularly chronic liver diseases, cirrhosis and hepatocellular carcinoma, because of its antioxidant, anti-inflammatory and antifibrotic power. Indeed, the anti-oxidant and anti-inflammatory effect of silymarin is oriented towards the reduction of virus-related liver damages antiviral effect associated with its intravenous administration in hepatitis C virus infection. With respect to alcohol abuse, silymarin is able to increase cellular vitality and to reduce both lipid peroxidation and cellular necrosis. Furthermore, silymarin/silybin use has important biological effects in non-alcoholic fatty liver disease. These substances antagonize the progression of non-alcoholic fatty liver disease, by intervening in various therapeutic targets: oxidative stress, insulin resistance, liver fat accumulation and mitochondrial dysfunction. Silymarin is also used in liver cirrhosis and hepatocellular carcinoma that represent common end stages of different hepatopathies by modulating different molecular patterns.

Keywords: Bioavailability, inclusion complexes, silymarin, silybin, antioxidants

I.INTRODUCTION

Silymarin (482.43) is not a good water soluble hepatoprotective agent. The oral absorption of silymarin is only about 23 – 47%, leading to low bioavailability of the drug, which limits its use. It is a mixture of mainly three flavanolignans, namely, silybin, silydianin, and silicristine, with silybin being most active. silymarin has been used medicinally to treat liver disorders, including acute and chronic liver hepatitis, toxin –/drug-induced hepatitis and cirrhosis, and alcoholic liver diseases. It is also reported to be effective in certain cancers.[1–5] The poor aqueous solubility of the drug may lead to dissolution-related bioavailability problems. Many approaches such as solubilization with surfactant systems, formation of water soluble complexes, and use of pro-drugs and soluble salt formation have been reported for improving the solubility and dissolution, and in turn the bioavailability of the drug.[6–8] Cyclodextrin and its derivatives play an important role in the formulation development due to their effect on solubility, dissolution rate, chemical stability, and absorption of a drug.[9]

Nagarsenkar et al. reported faster dissolution and better bioavailability of ketorolac solid dispersion with HP-β-CD.[10] Reddy et al. reported enhanced solubility and dissolution rate of Celecoxib by complexation with β-Cyclodextrin.[11]

The objective of the present study is to investigate the possibility of improving the solubility and dissolution rate of silymarin by complexation with β-cyclodextrin and also to compare the different complexation methods with respect to their dissolution study. In addition, the physiochemical characteristics of solid inclusion complexes were also investigated. Silymarin is a hepatoprotective agent, having poor water solubility and oral absorption of about 23 – 47%, leading to low bioavailability of the drug. The aim of the present study is to improve the solubility and dissolution rate and in turn the hepatoprotective activity of the drug, by formulating its inclusion complex with beta (β)-cyclodextrin, using different methods. The phase solubility analysis indicates the formation of 1:1 molar inclusion complex of the drug with beta cyclodextrin. Apparent stability constant for Silymarin (Kc) was 722 K-1 with β-cyclodextrin complex. The inclusion complexes were prepared by four different methods, namely, physical mixing, kneading, co-precipitation, and solvent evaporation. The prepared complexes were characterized using differential scanning colorimetry, scanning electron microscopy, and x-ray diffractometry. The inclusion complex prepared by the co-precipitation methods exhibits an overall best result, with respect to the formulation of sustained release formulations.

Phase solubility analysis for silymarin

Phase solubility studies were performed to determine the stoichiometric proportions of silymarin with β-cyclodextrin.[12,13] The data was used to determined the stability constant of the complexes. For this, the stock solution of 0.01 M β-cyclodextrin was prepared using distilled water. These stock solutions were diluted with distilled water to give molar solutions in the range of 0.002 to 0.01 M β-cyclodextrin. Five ml of each molar solution was filled in screw capped vials and the excess quantity of the drug was added to each vial separately.[7,14] The vials were shaken at an ambient temperature, for 48 hours, using a laboratory shaker (Remi). The supernatant solutions were collected carefully and filtered using Whatman filter paper (No. 41). The concentration of the drug in filtered solutions was determined using a UV visible spectrophotometer. No changes in λ max of the drug were found after complexation with cyclodextrin, hence absorbance of the resultant solutions were recorded at 286 nm, which was λ max of the drug. From the slope and intercept value (S0) of the phase solubility curve, a stability constant (Kc) was determined.

$$KC = Slope/[S0 (1 – Slope)]$$

Preparation of physical mixture and inclusion complexes

Physical mixture method

The required molar (1:1) quantities of the drug and cyclodextrin were weighted accurately and mixed together thoroughly in a mortar, with vigorous trituration, for about three hours. These mixtures were then passed through sieve No. 44, and finally stored in airtight containers till further use.[7,15,16]

Kneading method

The required quantities of the drug (Silymarin) and β-cyclodextrin were weighed accurately in a ratio of 1:1. A homogenous paste of cyclodextrin was prepared in a mortar by adding water : Methanol mixture (1 : 1) in small quantities. Silymarin powder was then added to this paste in portions, with continuous kneading, for three hours. An appropriate quantity of water : Methanol mixture (1:1) was added further to maintain suitable consistency of the paste. This paste was dried in a hot air oven at 45°–50° for 24 hours. The dried complexes were then powdered and passed through sieve No. 44 and stored in airtight containers till further use.[15]

Co-precipitation method

Quantities of drug and cyclodextrin, in the required molar ratio (1:1), were dissolved in methanol

: Water, respectively. The solution of the drug was added dropwise into cyclodextrin solution. The contents were continuously stirred for 6 hours and finally were dried at 45°– 50° for 48 hours, collected, and stored in airtight containers till further use.[16]

Solvent evaporation method

In this technique, silymarin along with solubilizsing additives such as acetone were dissolved at 25°C temperature. Next, the required moles of β-cyclodextrin in hot distilled water were added dropwise into this solution, with continuous stirring, for one hour. The complexes formed were filtered and dried under a vacuum. Then the prepared solid mass was stored in a desiccator under vacuum to a constant weight. The dried products were removed, pulverized, and passed through sieve No. 100 and finally stored in a closed airtight container.

Characterization of silymarin inclusion complexes

Drug content estimation

The quantities of inclusion complex equivalent to 70 mg of silymarin were dissolved in water: Methanol mixture (1:1). Appropriate dilutions were made and the drug content of each complex was calculated from UV absorbance recorded at λ max 286 nm.

Scanning electron microscopy

The morphology of the inclusion complexes by physical mixture, kneading method, and co-precipitation method was studied using a scanning electron microscope (JSM-5610 LV Jeol, Japan). The samples were coated with platinum to provide a conductive layer for observing images at 15 kV.

IR spectrum analysis

Infra-red (IR) spectra of the drug and inclusion complexes were recorded using the KBr method using Fourier Transform Infrared Spectrophotometer (FT IR-8400 S). A baseline correction was made using dried potassium bromide, and then the spectra of the dried mixtures of drug and inclusion complexes with potassium bromide were recorded.

Differential scanning calorimetric analysis

This scanning was performed using DSC model (Perkin Elmer). The samples were placed in a closed platinum crucible and DSC thermograms were recorded at a heating rate of 10°/minute in the range of 20° to 310°C.[16]

X-ray diffraction study

The X-ray diffraction pattern of the selected inclusion complex was compared with that of pure silymarin. This was performed by measuring 2θ in the range of 4° to 50° with reproducibility of ±0°–001° on an X-ray diffractometer (Phillips).

Dissolution study of silymarin and its inclusion complexes

Dissolution of inclusion complexes (equivalent to 70 mg of silymarin) was studied using the USP XXII eight station dissolution apparatus (Electrolab TDT-08L). The dissolution was carried out in 900 ml of Phosphate buffer, pH 6.8, at a speed of 75 rpm. Aliquots of 10 mL were withdrawn periodically and replaced with 10 mL of fresh dissolution medium. The concentrations of the drug in the samples were determined by measuring their absorbance at 286 nm, using Shimadzu 1700 UV-visible spectrophotometer. Cumulative percent of the drug released was determined at every point of time. The pure drug was used as a control. The T90 (time required for 90% dissolution of drug) of various solid dispersions were calculated.[17,18]

Phase solubility analysis of silymarin

The phase solubility study was done to determine the stoichiometric proportion of silymarin with complexing agent β-cyclodextrin. The solubility analysis indicated the formation of a 1 : 1 molar inclusion complex of the drug with β-cyclodextrin. The apparent stability constant for silymarin (Kc) was 722 K–1 with the β-cyclodextrin complex.

Characterization of silymarin inclusion complexes

All the inclusion complexes prepared using different methods, such as, physical mixture, kneading method, co-precipitation, and solvent evaporation method were found to be slightly brown, free-flowing powders.

Estimation of drug content

Inclusion complexes prepared by co-precipitation showed nearly 100% drug content. The drug content of the inclusion complex prepared by kneading, physical mixture, and solvent evaporation shows slightly less drug content as compared to that prepared by using co-precipitation.

Scanning electron microscopy

Scanning electron microscopy (SEM) of the physical mixture method, inclusion complex by kneading method, and co-precipitation method were studied. Pure drug particles in the physical mixture method were very small in size with reduced effective surface area, due to agglomeration. They remained dispersed and physically adsorbed on the surface of β-cyclodextrin. The kneaded system is of poor crystal structure, lacks distinct crystal faces, and has numerous cracks and fissures. This may also have contributed to faster dissolution compared to the co-precipitation system.

X-ray diffraction study

The inclusion complex of the drug prepared with β-cyclodextrin, using the co-precipitation method, which showed a good result overall, was characterized further by an XRD study The X-ray diffraction patterns of pure silymarin, as well as the silymarin–β-Cyclodextrin complex obtained by using the co-precipitation method are represented in Figure 6. The peak position (angle of diffraction) is an indication of the amorphous nature of the sample. The diffractogram of pure silymarin shows some intense peaks, which are indicative of crystallinity. However, in case of silymarin complexed with β-Cyclodextrin diffractogram, it attributes to a new solid phase with low crystallinity, indicating inclusion complex formation (more water soluble). A reduced number of signals, of markedly low intensity, are noticeable in the complex, indicating the greater amorphous nature of the inclusion complex compared to the free molecules.

Biological effects of Silymarin

Silymarin is the extract of Silybum marianum, or milk thistle, and its major active compound is silybin, which has a remarkable biological effect. It is used in different liver disorders, particularly chronic liver diseases, cirrhosis and hepatocellular carcinoma, because of its antioxidant, anti-inflammatory and antifibrotic power. Indeed, the anti-oxidant and anti-inflammatory effect of silymarin is oriented towards the reduction of virus-related liver damages through inflammatory cascade softening and immune system modulation. It also has a direct antiviral effect associated with its intravenous administration in hepatitis C virus infection. With respect to alcohol abuse, silymarin is able to increase cellular vitality and to reduce both lipid peroxidation and cellular necrosis. Furthermore, silymarin/silybin use has important biological effects in non-alcoholic fatty liver disease. These substances antagonize the progression of non-alcoholic fatty liver disease, by intervening in various therapeutic targets: oxidative stress, insulin resistance, liver fat accumulation and mitochondrial dysfunction. Silymarin is also used in liver cirrhosis and hepatocellular carcinoma that represent common end stages of different hepatopathies by modulating different molecular patterns. Therefore, the aim of this review is to examine scientific studies concerning the effects derived from silymarin/silybin use in chronic liver diseases, cirrhosis and hepatocellular carcinoma.

Scientific Evidence

Scientific evidence, achieved so far, allows us to understand the mechanisms of action through which silybin carries out its activity by interacting with various tissues. In this regard, the action of silybin manifests in the modulation of inflammation and apoptosis, which, together with its antioxidant power, represent the key points that led to using it in different pathologies. Silybin acts through the turning-off of pro-inflammatory signals, derived from nuclear factor--B (NF--B) activation, involved in the induction of the synthesis of cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF). Furthermore, silybin induces apoptosis through the modulation of cytoplasmatic levels of bcl-2-like protein 4 (Bax) and B-cell lymphoma 2 (Bcl-2) proteins, cytochrome c release and caspase-3 and 9 activation. The anti-oxidant activity is due to its capacity to act as both free radical scavenging and lipid peroxidation inhibitors, as demonstrated in vitro and in vivo. Silymarin is also a modulator of estrogen signaling, insulin sensitizer, regulator of intracellular transport of drugs, anticarcinogen, antidiabetic through signal regulation of peroxisome proliferator-activated receptor γ (PPAR-γ), antifibrotic and choleretic.

The great number of actions carried out by silymarin explains the reason why a lot of scientific studies have been performed in order to understand its efficacy in various pathologies. In rheumatic diseases, such as rheumatoid arthritis, silymarin acts as an anti-inflammatory by inhibiting migration and activation of neutrophil in the articulations. In different oncological diseases, such as prostate cancer, cervical cancer, hepatocellular carcinoma (HCC), bladder cancer and lung cancer, silymarin reduces cell vitality and runaway cell replication.

Because of its detoxifying power, its hydrosoluble endovenous formulation, it is used as an anti-hepatotoxic drug in poisonings due to acetaminophen, arsenic, carbon tetrachloride, butyrophenones, phenothiazines and Amanita phalloides toxins. In hypercholesterolemia, silymarin/silybin inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, reducing cholesterol synthesis. Lastly, in neurological and psychiatric diseases, this molecule acts through the turning-off of inflammatory signals, which underlies the degeneration of dopaminergic neurons in Parkinson's disease, and it improves the clinical picture ascribable to obsessive-compulsive disorder. Of note, the role of herbal products in chronic liver disease, which currently represents one of the most important health problems in about 10% of the world population, is the most studied topic in the scientific community. Indeed, in chronic liver diseases, silymarin acts through different mechanisms and complex biological interactions able to produce benefits in various pathologies, some of which are systemic and can involve the liver. Researchers have studied for a long time the biological effects that natural products such as silymarin have on pathologies such as viral hepatitis, alcoholic liver disease (ALD), metabolic hepatitis, as well as on the common end stages of hepatopathies, that is, cirrhosis and HCC, on which silymarin carries out an important biological action.

II.CONCLUSION

Through the analysis of literature, it has been demonstrated that silymarin has an effect that allows its use in all of the most frequent causes of liver damage. Indeed, silymarin has three important activities: anti-inflammatory, antioxidant and pro-apoptotic, which represent the "functional triad" that allows for antagonizing the onset and the progression of mechanisms of damage that are responsible for the progression of hepatitis to cirrhosis and HCC. However, it is clear that, also in the end stages of liver pathologies, silymarin can act by limiting de-novo fibrogenesis and antagonizing procarcinogenic mechanisms that cause HCC. Nevertheless, the treatment with silymarin/silybin in routine clinical practice is strongly limited, since it is necessary to obtain scientific data deriving from well-structured trials based on large populations of patients, and to achieve a standardization of methods used for evaluating the therapeutic efficacy, especially in an NAFLD context, that is particularly promising.

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