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HEPATOPROTECTIVE FUNCTION AS WELL AS SOLUBILITY AND ORAL BIOAVAILABILITY OF NANO-BASED SILYMARIN: A POTENTIAL REVIEW

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Keywords:

Silymarin, Nanoemulsion, Solid lipid nanoparticles, Nanostructured lipidcarriers, Liposome, Polymeric particles, Bioavailability

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ABSTRACT: Silymarin is a mixture of flavonolignan and polyphenolic bioactive natural products found in the milk thistle plant Silybum marianum. It has anti-oxidant, anti-inflammatory, anti-cancer, and antiviral activities potentially useful in treating several liver disorders, such as chronic liver diseases, cirrhosis and hepatocellular carcinoma. However, the benefits are curtailed by its extremely poor water solubility (<50 µg/mL), low bioavailability and poor intestinal absorption. To solve these problems, nano-based technological strategies appear to be a promising method to potentiate therapeutic action. The purpose of this study is to review the different nanostructured systems available in the literature as delivery strategies to improve the absorption and bioavailability of silymarin.

INTRODUCTION: Humans have widely used plant-based natural products as medicines against various diseases since ancient times. Modern medicines are mainly derived from herbs based on traditional knowledge and practices. Nearly 25% of the major pharmaceutical compounds and their derivatives available today are obtained from natural resources [1, 2]. There are a lot of molecules able to generate health benefits if used in a large range of diseases. Among these molecules, over the centuries, silymarin has had a very important role [3]. There exists a widely held view that silymarin (milk thistle) promotes liver health through antioxidant, anti-inflammatory, anti-proliferative and immunomodulatory effects [4].

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The currently employed standardized milk thistle extracts made from the fruits contain 30–65% silymarin as an active ingredient. Silymarin is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans, *i.e.*, silibin A, silibin B, isosilibin A, isosilibin B, silichrist in, isosilichristin, silidianin and the flavonoid taxifolin, the most effective antioxidant of these molecules [5]. Among these substances, silybin is prevalent, and it has the most important biological effect. It makes up about 70% of the total composition of silymarin in the form of two disaster eoisomeric compounds: silybin A and silybin B [6, 7].

Due to chronic alcohol abuse and modern lifestyle, hepatic disease is a major health concern. The search for new and optimizing known agents for the therapy of hepatic diseases is still of great importance. According to the World Health Organization, alcohol is the third largest risk factor for premature mortality, disability, and loss of health.

Alcoholic liver disease (ALD) is responsible for the majority of alcohol-related deaths [9]. At the same time, non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common liver disease worldwide. The prevalence of NAFLD in the general population of Western countries is 20-30%. About 2-3% of the general population is estimated to have non-alcoholic steatohepatitis (NASH), which may progress to liver cirrhosis and hepato-carcinoma [10]. One indication of the importance of NASH as a cause of the end-stage liver disease is the frequency of NASH as an indication for liver transplantation, currently and over the course of time. NASH is the third most common indication for liver transplantation in the United States, and that the frequency of NASH as an indication is steadily increasing. Hepatic steatosis, which is ubiquitous in noncirrhotic NASH, can dissipate following the development of cirrhosis [11-12]. Infection with hepatitis B and C virus (HBV and HCV, respectively) affects the liver and results in a broad spectrum of disease outcomes.

Infection with HBV can spontaneously resolve and lead to protective immunity, resulting in chronic infection and, in rare cases, cause acute liver failure with a high risk of dying. In contrast to HBV, infection with HCV becomes chronic in most cases [13]. People with chronic hepatitis B and/or C virus infections remain infectious to others. They are at risk of serious liver disease such as liver cirrhosis or hepatocellular cancer (HCC) later in life [14-15]. Antiviral therapy applies nucleoside analogs and interferonα and targets viral replication as well as antiviral immune responses, *i.e.*, activation of T cells and modulation of innate immune cells [16]. Both silymarin and silibinin inhibit HCV infection in cell culture by variably blocking viral entry, viral fusion, viral RNA and protein synthesis, HCV N5SB RNA dependent RNA polymerase activity, and virus transmission [16-20].

Pharmacokinetic parameters of silymarin and the active principle of any silymarin-containing products are always referred to and standardized as silibinin. Unfortunately, silymarin is very poorly soluble in water; that is, 3.2 mg/100 ml (silibinin: 0.4 mg/100 ml). This is due to both its inefficient absorption in the intestine and an elevated metabolism of the first liver passage after its absorption, two mechanisms that decrease haematic concentration and consequently the arrival at the target organ [21].

Hepatoprotection of silymarin reportedly rests on five properties [22]:

• Activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of GSH (glutathione)

• Ability to regulate membrane permeability and to increase membrane stability in the presence of xenobiotic damage

• Capacity to regulate nuclear expression by means of a ‘steroid-like’ effect

• Inhibition of the transformation of stellate hepatocytes into myofibroblasts responsible for the deposition of collagen fibers leading to cirrhosis.

• Also acts on the nucleus where it appears to increase ribosomal protein synthesis by stimulating RNA polymerase I and the transcription of rRNA.

Silymarin is a biopharmaceutics Classification System (BCS) class II drug which classified as poorly water-soluble and highly permeable in the intestines [23]. Silymarin's effectiveness as a hepatoprotective drug is discounted by its poor water solubility and low bioavailability after oral administration. In order to overcome the defects of silymarin, many approaches have been searched, such as liposomal delivery system, nanostructured lipid carriers, silybin–phospholipid complex, nano-suspension, nanoparticle, *etc.* [24-27] For improvement of bioavailability, the dissolution velocity and solubility enhancement of silymarin should be the main target [28-29].

TABLE 1: PHYSICOCHEMICAL PROPERTIES OF SILYMARIN

Property	Value
Molecular formula	$C_{25}H_{22}O_{10}$
Molecular weight (g mol^{-1})	482.44
Solubility	49.7 g· ml^{-1} (pH 7.4 PBS)
Melting point	156 ºC
max of absorbance	288 nm
LogP	2.8

Natural drugs possess some unique advantages, such as low toxicity and side effects, low price, and good therapeutic potential.

However, concerns associated with the biocompatibility and toxicity of natural compounds present a greater challenge of using them as medicine. Consequently, many natural compounds are not clearing the clinical trial phases because of these problems [30-31]. Nano-technology can enhance the solubility of poorly water-soluble drugs such as silymarin. Nanotechnology applied for silymarin delivery could be used as a potential approach to enhancing the bioavailability and bioactivity of other poorly water-soluble drugs. Nanotechnology is shown to bridge the barrier of biological and physical sciences by applying nanostructures and nanophases in various fields of science [32].

Nano Based Silymarin Delivery Strategy: There has been an enormous development in the field of delivery systems to provide natural-based active compounds to its target location for the treatment of various diseases [33-34]. Currently, nano-based drug delivery systems facilitate the advanced system of drug delivery.

Solid Lipid Nanoparticle (SLNs): SLNs are a drug carrier system suitable for poorly water-soluble or chemically unstable drugs. SLNs have received increasing attention because SLNs combines many of the advantages of polymeric nanoparticles, fat emulsions, and liposomes [35]. SLNs can improve the bioavailability of poorly water-soluble drugs after oral administration and increase the targeting potential of the drug [36-37]. The preparation methods for SLNs include high-pressure homogenization (HPH), high shear homogenization combined with ultrasound, solvent emulsification/evaporation, and micro emulsion method [38]. To achieve a prolonged drug release from the Silymarin-loaded SLNs, He *et al.* (2007) studied the drug loading mechanism and *the in-vitro* release of the SM-SLNs prepared by the hot and the cold homogenization methods. In vitro release experiments showed that a prolonged release of the cold-SMSLNs can be achieved. Therefore, they further assessed the feasibility of the cold-SM-SLNs to achieve *in-vivo* a prolonged release and specific drug targeting to the liver via the oral route. After oral administration, the concentrations of silybin in plasma and various organs in mice were determined by reversed-phase HPLC (RP-HPLC). In their study, TEC, TIC, and RTEC were used to evaluate the cold-SM-SLNs liver targeting effects. The results showed that the SLNs could improve the oral bioavailability of SM and accumulate the drug in the organs belonging to RES, such as the liver, spleen, and lungs. In particular, the cold-SM-SLNs had the best targeting effect on the liver. Based on the body distribution pattern and disposition kinetics, SLNs may offer an effective approach to improve the oral bioavailability of poorly soluble drugs and to enhance the liver targeting of antihepatotoxic drugs *via* the oral route of administration [39].

Nanosuspension: Nanosuspensions of drugs are nanosized, heterogeneous aqueous dispersions of insoluble drug particles stabilized by surfactants. When a drug molecule has many disadvantages such as the inability to form a salt, large molecular weight and dose, high log P, and melting point that hinder them from developing suitable formulations, the nanosuspension technique is available for that drug molecule [40]. Nanosuspensions can be prepared by high-pressure homogenization (HPH), wet milling, precipitation method, etc. Although the precipitation technology is cost-effective and straightforward in the pharmaceutical industry, the tendency of the pharmaceutical particles to grow, and the difficulty in inhibiting that growth, poses obstacles to their production [41].

Y wang *et al.* (2010) developed two nanosuspension of silybin SN-A and SN-B. Morphological analysis was carried out by TEM, AFM, and SEM, which showed irregular particle shapes produced by lower pressure homogenization and larger particle size compared to that produced by higher pressure homogenization. In conclusion, the obtained results show that nanosuspensions could enhance the in-vitro drug release and intestinal epithelium membrane permeability, improve the in-vivo oral bioavailability, and modify the IV c-t profile. For the BSC class II drugs, nano-suspensions appear to be a promising approach to enhancing oral bioavailability and achieving a sustained plasma concentration [42].

Mesoporous Silica Nanoparticles: Mesoporous silica nanoparticles (MSNs) were first commercially prepared by Mobil in 1992 and were mainly used for petrochemical catalysis. The large surface area and large mesoporous channels allow for high drug loading and maintain drugs in an

amorphous or non-crystalline state within the pores, enhancing solubility and facilitating drug dissolution [43]. MSNs exhibit stability over a wide range of processing and environmental conditions, including extremes of temperature, pH, and humidity [44].

Nasr S.S. *et al.* (2019) prepared silymarin-loaded mesoporous silica nanoparticles (MSNs) and to assessed the system's dissolution enhancement ability on the pharmacodynamic performance of silymarin as a hepatoprotective agent. They show that Silymarin-loaded MSNs resulted in an enhancement in silymarin's bioavailability. This was reflected as an enhancement in its hepatoprotective action.

The drug delivery system employed affected none of the major mechanisms of silymarin hepato-protective action - membrane stabilizing, antioxidant capacity, or protein synthesis enhancement; on the contrary, it enhanced them all. This was demonstrated through the favorable values of most physiological and biochemical parameters obtained with the formulation-treated group compared with the free drug–receiving group. The formulation had also shown no fatalities among rats throughout 22 days of oral administration, proving that prolonged oral administration of MSN-based systems is a promising alternative to other nano-systems with an acceptable safety profile [45].

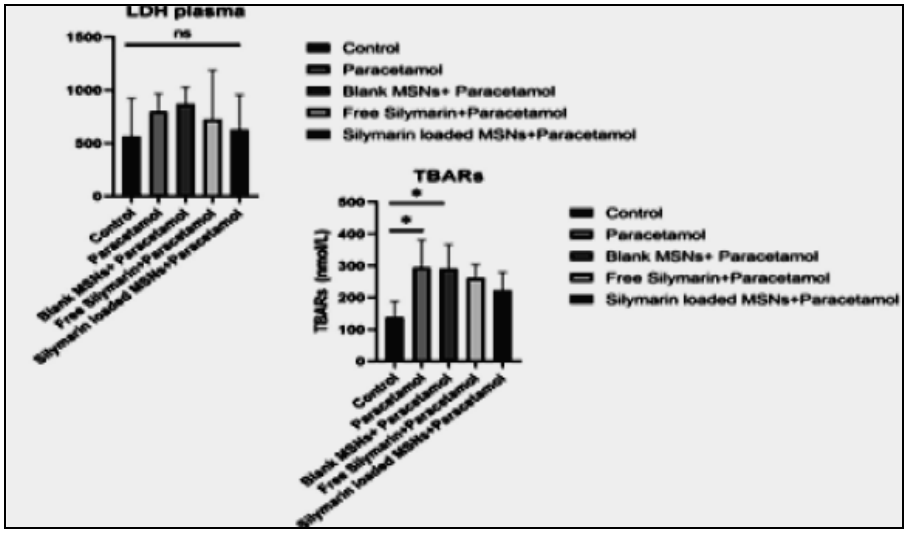

FIG. 1: EFFECT OF DIFFERENT TREATMENTS ON THE LEVEL OF TBARS AND LDH IN RAT'S LIVER HOMOGENATE. Nasr, Sarah & Nasra, Maha & Ali, Heba & Abdallah, Ossama. (2019). mesoporous silica nanoparticles, a safe option for silymarin delivery: preparation, characterization, and *in-vivo* evaluation**.** Drug delivery and translational research. 10.1007/s13346-019-00640-3(45).

Nanoemulsion: Nanoemulsions are the only kinetically stable formulations. The long-term physical stability of nanoemulsions makes them unique from other drug delivery systems and therefore are sometimes referred to as approaching thermo-dynamic stability [46]. Nanoemulsions can be used to enhance the therapeutic efficacy as well as the physical and chemical stability of the many herbal drugs. Nanoemulsions have more solubilization capacity than simple micellar solutions. Due to this property, nanoemulsions offer several advantages over unstable dispersions, such as the rapid onset of action, reduced inter-subject variability in terms of gastrointestinal fluid volume, and longer shelf life [47]. Parveen R *et al.* (2011) prepared silymarin nanoemulsionby titration method. They were used a nanoemulsion approach to increase its release rate and bioavailability. Different process and formulation variables were evaluated and thermodynamic stability studies were carried out to find out the optimized thermodynamically stable and characterized formulation. In that study, an optimized silymarin nanoemulsion was prepared using 5% w/w of sefsol 218 as the oily phase, 35% w/w of S_{mix} (tween 80 as the surfactant, ethyl alcohol as the co-surfactant, 2:1), and 60% w/w of distilled water as an aqueous phase.

The results of the pharmacokinetic study were supported by the estimation of enzyme activity in serum, which again proved that administration of

less than half dose of silymarin in nanoemulsion form produces similar protection against CCl_4 induced toxicity in rats as compared to more than a double dose of silymarin in solution and suspension form. Nanoemulsion approach developed for silymarin will provide better biopharmaceutic properties as compared to the lipid-based system [48].

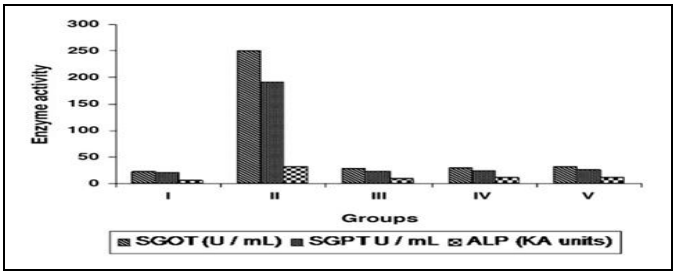

FIG. 2: STATISTICAL GRAPH OF HEPATOPROTECTIVE ACTIVITY OF BULK DRUG SUSPENSION, MARKETED CONVENTIONAL FORMULATION AND OPTIMIZED PREPARED NANO-EMULSION FORMULATION IN TERMS OF ENZYME ACTIVITY (SGOT, SGPT, AND ALP). Parveen R, Baboota S, Ali J, Ahuja A, Vasudev Ss, Ahmad S. Oil based nanocarrier for improved oral delivery of silymarin: *In-vitro* and *in-vivo* studies. Int J Pharm. 2011; 413:245–53 [48].

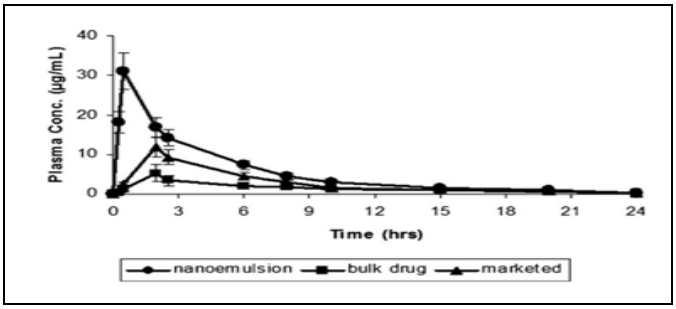

FIG. 3: PLASMA CONCENTRATION PROFILE OF SILYMARIN AFTER ORAL ADMINISTRATION OF DIFFERENT FORMULATIONS TO ADULT WISTAR ALBINO RATS. Data is expressed as mean ± SD (n = 6). Parveen improved oral delivery of silymarin: *In-vitro* and *in-vivo* studies. Int J Pharm. 2011; 413:245–53 [48].

Lipid-polymer Hybrid Nanoparticles (LPNs): Lipid-polymer hybrid nanoparticles possess a shell-core structure consisting of a polymer core and a phospholipid shell.

LPNs were developed from liposomes and polymer nanoparticles [49]. LPNs can be freeze-dried to improve their stability [50].

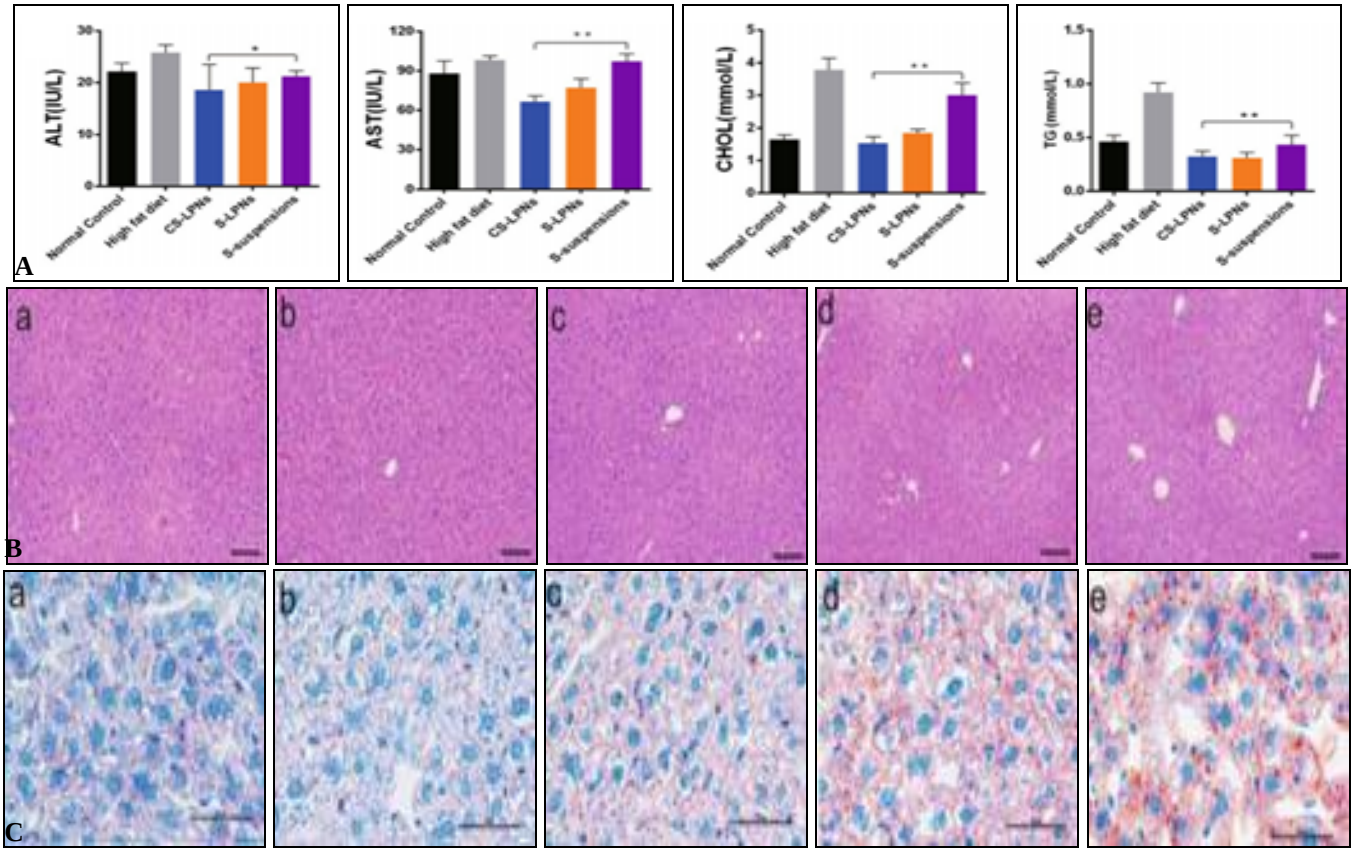

FIG: 4: (A) EFFECTS OF CHITOSAN-MODIFIED SILYMARIN-LOADED LIPID-POLYMER HYBRID NANOPARTICLES (CS-LPNS) ON ALANINE TRANSAMINASE (ALT), ASPARTATE TRANSAMINASE (AST), CHOLESTEROL (CHOL), AND TRIGLYCERIDE (TG) LEVELS IN SERUM. (B) HEMATOXYLIN AND EOSIN STAINING RESULTS (SCALE BAR, 100 MM). (A) NORMAL DIET, (B) CHITOSAN-MODIFIED SILYMARIN LOADED-LPNS (CS-LPNS), (C) SILYMARIN-LOADED LPNS (S-LPNS), (D) SILYMARIN SUSPENSION (S-SUSPENSION), (E) HIGH-FAT DIET (HFD). (C) OIL RED O STAINING RESULTS (SCALE BAR, 50 MM). (A) NORMAL DIET, (B) CS-LPNS, (C) S-LPNS, (D) S-SUSPENSION, (E) HFD. Liang, J., Liu, Y., Liu, J. et al. Chitosan-functionalized lipid-polymer hybrid nanoparticles for oral delivery of silymarin and enhanced lipid-lowering effect in NAFLD. J Nanobiotechnol 16, 64 (2018) [51].

Liang *et al.* (2018) prepared chitosan-modified, silymarin-loaded LPNs (CS-LPNs) to enhance the oral bioavailability of silymarin and improve its lipid-lowering efficacy for NAFLD treatment.

In that study, the rational development and utilization of CS-LPNs as effective therapeutics for NAFLD were investigated. CS-LPNs enhanced the uptake of the nanocarriers by fat-emulsion-treated HepG2 cells and Caco-2 cells.

This suggests that improved uptake of the nano-particles could be achieved *in-vivo*, which may increase the oral bioavailability of silymarin.

Using atransgenic mouse model of NAFLD, they confirmed that CS-LPNs inhibit lipid accumulation in the mouse liver and enhance the therapeutic efficacy of silymarin. These findings indicate that CS-LPNs may be a new treatment option for NAFLD [51].

Liposomes: Liposomes are a delivery system that can improve the therapeutic activity and safety of drugs.

They consist of lipid bilayers with an inside water phase that can encapsulate both water-soluble and lipophilic drugs [52].

Liposomes have also been employed for improving the solubility and bioavailability of some poorly soluble drugs.

Liposomes can be prepared using a wide range of methods, such as thin-film dispersion (TFD) [53], reversed-phase evaporation (RPE) [54], alcohol injection [55], and spray-freeze-drying [56].

N. Kumar *et al*. (2014) developed silymarin liposomes to improve oral bioavailability of silybin besides targeting hepatocytes, and immune cells. They were found CTC50 (concentration at which 50% cells die) value of silymarin 151.2 mg/ml in Chang Liver cells.

The dose of paracetamol to cause hepatotoxicity to Chang liver cells was 50 mM. The liposomal formulation of silymarin was found to be one and half-fold more active than silymarin in increasing percentage viability.

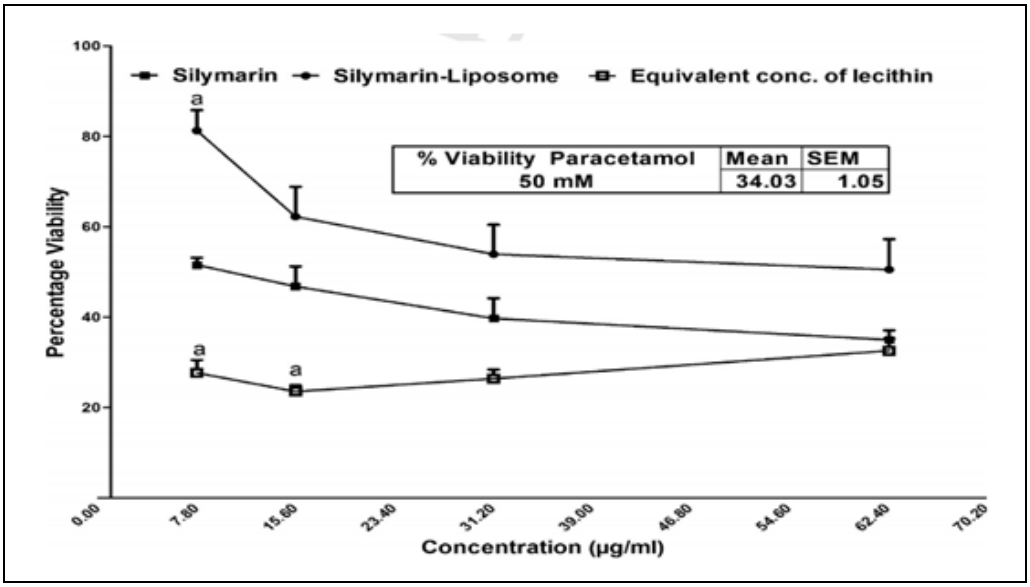

FIG. 5: *IN-VITRO* HEPATOPROTECTION IN PARACETAMOL-INDUCED TOXICITY AGAINST CHANG LIVER CELLS. ALL THE VALUES ARE MEAN ± SEM OF THREE TESTS IN TRIPLICATE, A P < 0.05 COMPARED TO SILYMARIN [57]. Kumar N, Rai A, Reddy ND, et al. Silymarin liposomes improves oral bioavailability of silybin besides targeting hepatocytes, and immune cells. Pharmacol Rep. 2014; 66(5): 788-798 [57].

The paracetamol challenge in control animals raised the AST level about two folds compared to sham animals. Silymarin-liposomes treatment was significantly effective in preventing the paracetamol-induced rise in the AST level, as it brought the levels back to normal, unlike the silymarinper se treatment. Silymarin and its liposome pre-treatment for 7 days significantly protected the liver against the paracetamol-induced rise in the levels of ALT, total bilirubin, and direct bilirubin parameters.

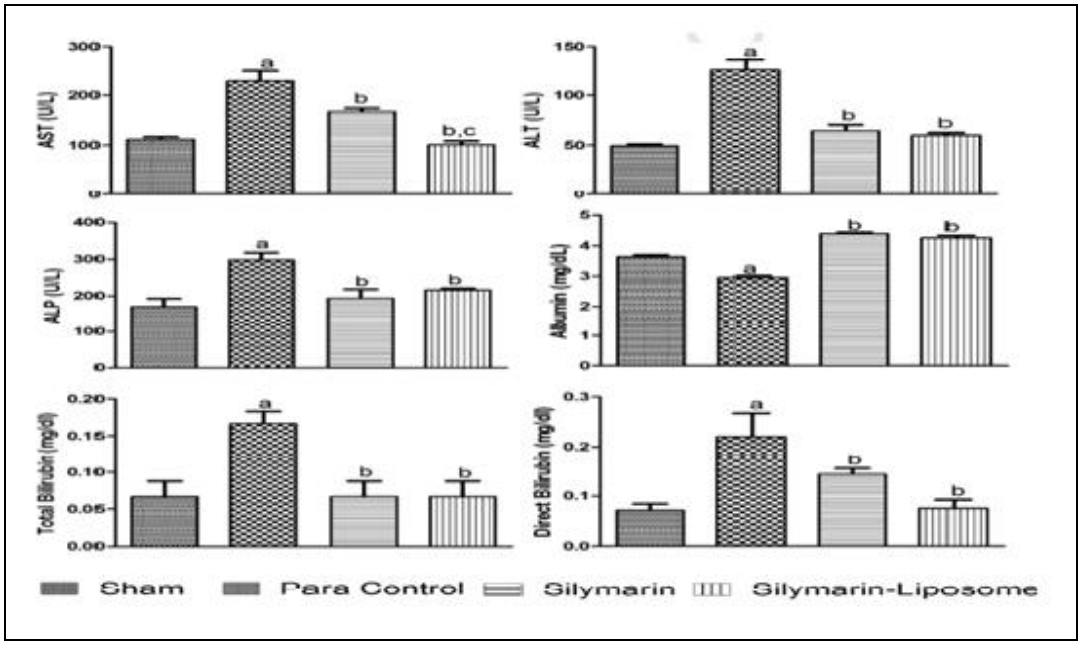

FIG. 6: LFT IN PARACETAMOL-INDUCED TOXICITY IN WISTAR RATS. All the values are mean ± SEM of six animals where (a) P < 0.05 compared to sham, (b) P < 0.05 compared to para control, (c) P < 0.05 compared to silymarin. Kumar N, Rai A, Reddy ND, et al. Silymarin liposomes improves oral bioavailability of silybin besides targeting hepatocytes, and immune cells. Pharmacol Rep. 2014; 66(5): 788-798 [57].

Nanostructured Lipid Carriers (NLCs): Lipid-based nanocarriers, such as nanostructured lipid carriers, a successful example of nanoformulations applied to overcome the limitations of natural compounds. Nanostructured lipid carriers (NLCs) offer many advantages, such as long-term stability, increased bioavailability of encapsulated active ingredient, possibility to obtain a controlled or targeted release, versatility in encapsulating both lipophilic and hydrophilic drugs, and high efficiency of encapsulation [58]. Nanostructured lipid carriers (NLCs) are derived from solid lipid nanoparticles (SLNs) by incorporating liquid glycerides-containing oils into the solid core of SLNs [59-60]. Shangguan *et al.* (2014) were developed SM-loaded NLCs (SM-NLCs) using a high-pressure homogenization method. They were evaluated the oral bioavailability of SM-NLCs in beagle dogs and compared them with marketed SM capsules (Legalon) and fast-release SM solid dispersion pellets. *In-vitro*, the lipolysis test demonstrated the fast digestion of NLC, which was also validated by the rapid absorption after oral administration. The bioavailability of SMNLCs was significantly enhanced by 2.54- and 3.10-fold that of marketed Legalon and solid dispersion pellets. The enhanced bioavailability was reasonably ascribed to facilitated absorption by lipid-based drug delivery systems [61].

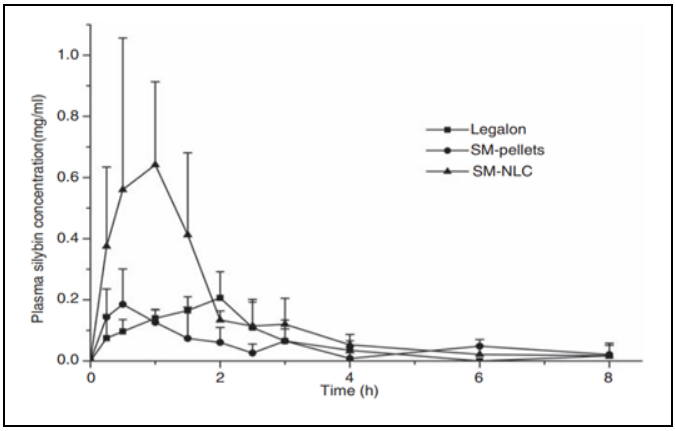

FIG. 7: MEAN PLASMA CONCENTRATION-TIME PROFILES OF SILYMARIN AFTER ORAL ADMINISTRATION OF A SINGLE 8 MG/KG DOSE OF LEGALON CAPSULE, SM-PELLETS, AND SM-NLC IN BEAGLE DOGS. Shangguan M, Lu Y, Qi J, et al. Binary lipids-based nanostructured lipid carriers for improved oral bioavailability of silymarin. J Biomater Appl. 2014; 28(6): 887-896 [61].

Polyamidoamine (PAMAM) Dendrimers: Dendrimers are a new class of artificial macro-molecular compounds, were first synthesized by Tomalia et al. in the mid-1980s [62]. Polyamidoamine (PAMAM) is one of the most studieddendrimers with a well-defined spherical structure and nanometer-scale size. It possesses empty internal cavities and many surface functional end groups, which are responsible for high solubility and reactivity.

PAMAM has demonstrated its potential use as a drug delivery system [63-64]. PAMAM has been reported as a solubility enhancer of drugs and shown the potential in controlled drug delivery [65-67]. X. Huang *et al.* (2011) studied the effect of polyamidoamine (PAMAM) dendrimers on the solubility of silybin. The influence of dendrimer concentration on the solubility of silybin was measured at 37 °C.

They were observed that the extremely low water solubility of silybin was significantly improved by PAMAM dendrimers ($P < 0.05$). The solubility of silybin in the dendrimer solutions increased approximately linearly with an increase of dendrimer concentration. These results may be due to the increase in the number of surface amines and internal cavities, which could improve the interaction or encapsulation with silybin molecules [68].

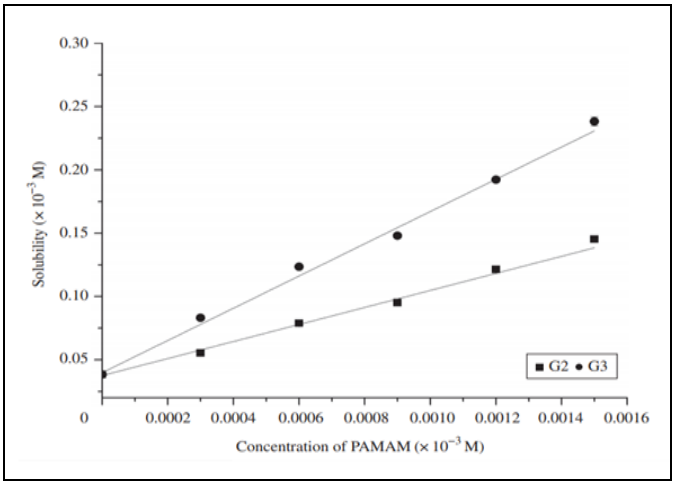

FIG. 8: SOLUBILITY OF SILYBIN IN THE PRESENCE OF INCREASING CONCENTRATION OF PAMAM DENDRIMERS. Huang X, Wu Z, Gao W, Chen Q, Yu B. Polyamidoamine dendrimers as potential drug carriers for enhanced aqueous solubility and oral bioavailability of silybin. Drug Dev Ind Pharm. 2011; 37(4): 419-427 [68].

The oral bioavailability of silybin from the silybin–G2PAMAM complex was assessed in rats and compared to that of silybin suspension. The results indicated that silybin suspension was rapidly absorbed through the rat gastrointestinal tract with a C_{max} of 134.2 ng/mL at a T_{max} of 10 min. The administration of silybin–G2 PAMAM mixture achieved a C_{max} of 182.4 ng/mL at a T_{max} of 15 min, and the whole blood concentration of silybin declined more slowly than that following suspension of silybin [68].

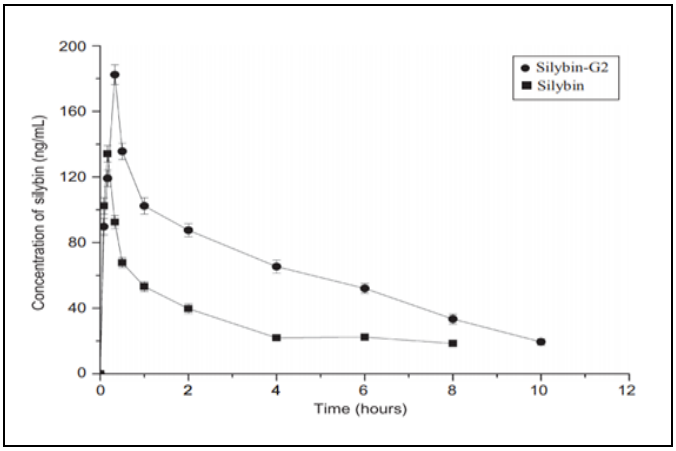

FIG. 9: RAT PLASMA SILYBIN CONCENTRATIONS VERSUS TIME PLOT AFTER A SINGLE ORAL DOSE OF 12 MG/KG EQUIVALENT SILYBIN–DENDRIMER COMPLEX OR SILYBIN (N = 12). Huang X, Wu Z, Gao W, Chen Q, Yu B. Polyamidoamine dendrimers as potential drug carriers for enhanced aqueous solubility and oral bioavailability of silybin. Drug Dev Ind Pharm. 2011; 37(4): 419-427 [68].

Inclusion Complex with Beta (β)-cyclodextrin: Beta-cyclodextrins are cyclic oligosaccharides that havebeen widely used in pharmaceutical applications. They are comprising a variable number of D-(+)-glucose molecules, linked together by α-(1,4)-type bonds [69]. Due to the effect on solubility, dissolution rate, chemical stability, and absorption of a drug cyclodextrin and its derivatives play an important role in the formulation development [70]. Ghosh A. *et al. (*2011) investigated the possibility of improving the solubility and dissolution rate of silymarin by complexation with β-cyclodextrin. They studied the phase solubility to determine the stoichiometric proportion of silymarin with complexing agent β-cyclodextrin. The apparent stability constant for silymarin (Kc) was 722 K–1 with the β-cyclodextrin complex. They concluded that the phase solubility data suggest a 1:1 complex formation with β-cyclodextrin. All inclusion complexes showed an increase in dissolution than in the drug alone. The inclusion complex of the drug prepared by the co-precipitation method shows the best results overall, in terms of drug content and dissolution profile, for preparation of sustained-release formulations [71].

Silymarin Phosphatidylcholine Complex: Phosphatidylcholine (PC) is the most abundant phospholipid of all mammalian cells and subcellular organelles. PC comprises 40–50% of

total cellular phospholipids, although different cell types, individual organelles and even the two leaflets of organelle membranes contain distinct phospholipid compositions [72]. Changes in the hepatic PC have been linked to the development of non-alcoholic fatty liver disease (NAFLD) in humans [73], as well as in liver failure [74], impaired liver regeneration [75] and the severity of alcoholic fatty liver disease [76].

X. Yanyu *et al.* (2006) prepared a silybin–phospholipid complex to make oral bioavailability of silybin increase and to study its physicochemical properties and to compare the pharmacokinetic characteristics and bioavailability after oral administration of silybin–phospholipid complex and silybin-N-methylglucamine in rats.

They studied the solubility and found that higher solubility in water or n-octanol for phospholipids complex than that of the physical mixture. Drugs and phospholipids of phospholipids complex in vitro had not changed characteristics of themselves, but the combination changed their characteristics greatly *in-vivo*, such as a remarkable enhancement of GItract absorption [77].

CONCLUDING REMARKS: The appearance of increasingly advanced and performing Silymarin-based formulations has logically followed step by step the evolution of the nanotechnologies and nanosystems applied to the delivery of poorly water-insoluble drugs and active principle ingredients.

The present article reviewed almost all the nano-based technology applied for silymarin delivery, including Solid Lipid Nanoparticle, nano-suspensions, Mesoporous silica nanoparticles, nanoemulsion, Lipid-polymer hybrid nanoparticles, Liposomes, Nanostructured lipid carriers, Poly-amidoamine (PAMAM) dendrimers, Inclusion complex with beta (β)-cyclodextrin and phos-phatidylcholine complex. Each of these techniques has corresponding advantages and disadvantages. We hope that this review could represent a useful reference for a broad and updated overview of the most efficient and relevant nano-based techno-logies aimed ultimately at improving the therapeutic efficiency of Silymarin.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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