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Enhancement of the *in-vitro* dissolution and *in-vivo* oral bioavailability of silymarin from liquid-filled hard gelatin capsules of semisolid dispersion using Gelucire 44/14 as a carrier

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Objective: The purpose of this study was to improve peroral bioavailability of the very poorly water-soluble hepatoprotectant silymarin through formation of semisolid dispersion (SD) system with Gelucire 44/14. *Method:* Binary SD systems were prepared by the solvent-fusion method and confirmed by differential scanning calorimetry (DSC). The solubility and *in vitro* release at pH values of 1.2 and 7.4 were then determined. The pharmacokinetic parameters and relative bioavailability of orally administrated silymarin pure (SP), silymarin-Gelucire 44/14 SD (GL) capsules were assessed and compared to that of Hepaticum® (silybin-cyclodextrin) capsules (CP) as a reference standard (RF) using New Zealand albino rabbits. Results: A linear increase in solubility of silymarin with respect to the weight fraction of the carrier has been observed. *Results:* The solubility of silymarin SD increased ~ 1.5-to-7-fold (relative to pure silymarin) at 1-to-15% of Gelucire 44/14 which in turn dramatically increased the dissolution rate of silymarin-Gelucire SD (91% within 10 min). The DSC study showed complete disappearance of the silymarin endothermic peak confirming formation of silymarin SD. In the bioavailability study, SD of silymarin with Gelucire 44/14 profoundly increased the AUC_{0-12} and C_{max} values (~13-fold relative to RS). *Conclusion:* The solubility and dissolution pattern of silymarin were found to be carrier ratio dependent. Moreover, the *in vitro* solubility and dissolution data established very good correlation with the calculated *in vivo* pharmacokinetic parameters. The ratios between the mean AUC_{0-12} for GL capsules and that of CP capsules was significantly higher (156.2%). However, the T_{max} values of the three formulations remained eventually unchanged.

1. Introduction

Silymarin, a flavonolignan obtained from the seeds of 'milk thistle' (*Silybum marianum*), has been widely used as an excellent hepatoprotective agent. It is a mixture of mainly three flavonolignans, including silybin, silydianin, and silychristin. Silybin has been found the major constituent and the most active moiety (Pepping 1999). However, the hepatoprotective effect of silymarin was negatively affected by its very poor water solubility which in turn decreases its bioavailability after oral administration (Wu et al. 2007; Zhang et al. 2006). Consequently, after oral administration, only 23–47% of oral silymarin is absorbed and undergoes extensive enterohepatic circulation. On the other hand, the estimated T_{max} and $t_{1/2}$ of the orally administered silymarin were 2–4 h and 6 h, respectively (Pepping 1999). Therefore, various formulation approaches have been followed to improve the dissolution rate as well as the bioavailability of silymarin and silybin. The most popular approaches are the incorporation of silymarin into liposomes, self-emulsifying formulations, and the preparation of solid dispersions (SD) based on polyvinylpyrrolidone (PVP) (Yan-yu et al. 2006; El-Samaligy et al. 2006; Wu et al. 2006; Woo et al. 2007; Iosio et al. 2010; Sun et al. 2008). Moreover, complexation of silymarin with β-cyclodextrin or silybin phospholipid for oral administration and recently nanostructured lipid carriers for parenteral delivery was also studied (Voinovich et al. 2009; Yan-yu et al. 2005; Jia et al. 2010).

Lipidic self-emulsifiying formulations and liposome have succeeded in improving the absorption of silymarin; however, these formulations cause many problems in terms of solubility considerations and physicochemical stability. Consequently, SDs were selected for evaluation as an alternative drug delivery system to enable a higher drug loading per unit dose. SD of silymarin or silybin has been focused on water-soluble carrier only (Sun et al. 2008; Voinovich et al. 2009). Solubilization of poorly soluble drugs such as silymarin within the GIT is considered a crucial step in the oral absorption process. The selection of suitable carriers has extensively been reviewed (Ford 1986). The use of water-soluble carriers with no intrinsic solubilising properties such as poly(ethylene glycol) 6000 (PEG 6000) and PVP as well as lipid-based carriers with solubilising properties have been studied as tools to enhance the solubility of poorly water soluble drugs (Barker et al. 2003; Karatas et al. 2005; Barakat 2006; Lukovac et al. 2010). Such carriers enhance the water solubility

of lipophilic drugs upon contact with the aqueous medium leading to formation of either a fine emulsion or micro-emulsion which in turn promote drug absorption (Sachs-Barrable et al. 2007). Moreover, some of these carriers may also have desirable self-emulsifying properties, readily forming fine dispersions of lipid-solubilized drug in the aqueous contents of the GIT thereby creating optimal conditions for improved permeability into the enterocytes.

Gelucires® are a family of naturally derived carriers used in formulations of semi-solid oral dosage forms to improve the solubility and bioavailability of poorly water-soluble active ingredients (Barker et al. 2003; Sheen et al. 1991; Damian et al. 2000). Gelucire carriers are solid waxy materials of saturated polyglycolized glycerides. The two numbers of their names (e.g. Gelucire 44/14) are corresponding to the approximate melting point and HLB value. Gelucires with high HLB have been reported to increase dissolution rates (Karatas et al. 2005). Gelucire 44/14 is widely used. It combines interesting properties because of its unique composition of surfactants (i.e., mono- and di-) esters of PEG, co-surfactants (mono-) glycerides, and oily phase (di- and tri-) glycerides (Gattefosse 1999). Gelucire appears to promote rapid drug release and bioavailability. Dordunoo et al. (1991) reported increases in the dissolution rate of temazepam and triamterene when formulated in Gelucire compared to PEG dispersions.

Similarly, Damian et al. (2000) showed improved dissolution of a poorly soluble drug, antiviral agent UC-781, when dispersed in Gelucire compared to PEGs (Damain et al. 2000). Improvement of oral absorption and bioavailability of several poor water-soluble drugs such as DMP 323 from dispersion in Gelucire compared to equivalent PEG or PEG/PVP dispersions in dogs has been reported (Pozzi et al. 1991; Shui-Mei 2000; Yüksel et al. 2003; Aungst et al. 1997). The aim of the current study was to enhance silymarin dissolution in GIT and hence improve its bioavailability using novel formulation of silymarin-Gelucire SD.

2. Investigations, results and disscussions

The solubility of silymarin in water at 25 °C was found to be 0.58 mg/ml; therefore, it can be considered as a practically insoluble drug. Silymarin solubility in the presence of different concentration of Gelucire at 37 °C is shown in Fig. 1. It is obvious that there was a reasonable increase in solubility of the drug. Moreover, the increase in solubility was linear with respect to the weight fraction of the carrier; at 1% and 15% of Gelucire the increase in solubility was about 1.47–7.1-fold, respectively, compared to pure silymarin. The increase in solubility in the presence of Gelucire can probably be explained by increased wettability of silymarin and micellar solubilisation. Indeed, Gelucire and other surfactants cause a decrease of the interfacial tension between the drug and the dissolving solution. The same effect of Gelucire was observed for the solubility of piroxicam and 37 °C (Karatas et al. 2005).

Fig. 2 shows the thermograms of silymarin, Gelucire and their corresponding binary systems, PM and SD at 1:3 drug:carrier weight ratios. The DSC thermogram of silymarin showed two characteristic endothermic peaks the first at 158.7 °C, corresponding to the melting of the drug. The second brad peak at 200 °C may be due to phase transformation of the drug (vaporization). For the pure carrier, the main peak at 43.3 °C with a broad shoulder was attributed to the melting transition of the Gelucire. As Gelucire is a mixture of materials, either it is logical to speculate that broad shoulder is due to the melting of minor components of the mixture or to mixed crystals formed between different components (Sutananta et al. 1994).

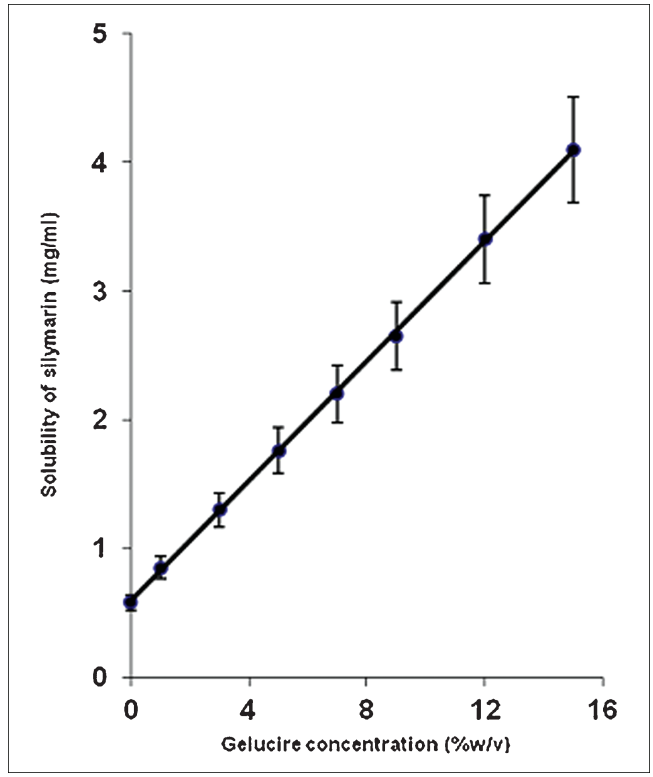

Fig. 1: Phase solubility diagram of silymarin with different concentration of Gelucire in water at 37 °C

The Gelucire thermogram was found to be in agreement with an earlier repot (Barakat 2006). In the thermogram of the PM, the endothermic peak of Gelucire appeared at 39.8 °C. On the other hand, silymarin showed a less intense and broader melting peak at 152.8 °C. The broadening of silymarin peak most likely reflects the partial solubility of silymarin in the melted Gelucire during the run and hence decreasing the strength of intermolecular bonds. In comparison to silymarin-Gelucire PM, the thermogram of their SD, the difference between the two

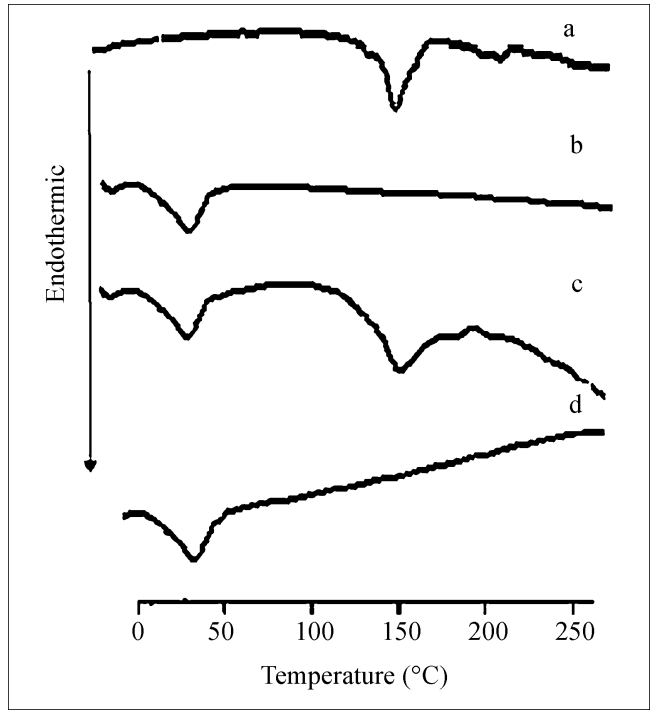

Fig. 2: DSC thermograms of silymarin-Gelucire binary systems

Table 1: Dissolution efficiency at 30 min (Mean ± SD) of silymarin-Gleucire binary systems, physical mixture (PM), solid dispersions (SD) and capsules, silymarin pure (SP), commercial product (CP) and silymarin-Gelucire solid dispersion (GL)

Media	Drug:carrier ratio	Silymarin	Silymarin-Gelucire binary systems		Silymarin capsules		
			PM	SD	SA	CP	GL
pH							
1.2	1:0	14.7 ± 2.54			12.8 ± 0.81	60.17 ± 3.1	
	1:1		18.5 ± 2.82	43.8 ± 4.15			76.5 ± 3.95
	1:3		22.2 ± 3.31	78.7 ± 4.56			
pH	1:0	26.5 ± 1.75			27.2 ± 2.48	79.31 ± 3.6	
7.4	1:1		40.6 ± 3.42	65.1 ± 3.62			
	1:3		51.2 ± 2.77	83.2 ± 2.45			82.02 ± 2.88

thermograms was quite clear. Complete disappearance of the silymarin endothermic peak was observed for their SD, such result could be attributed to the formation of an amorphous SD. The dissolution test results (n = 6) of silymarin and its binary systems Gelucire in enzyme free simulated gastric fluid (pH 1.2) are shown in Fig. 3. Results of the dissolution efficiencies at 30 min (DE_{30}) in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) are shown in Table 1. AS seen from Fig. 3, silymarin reached 22% drug dissolution within 30 min in the acidic medium. In addition, all the binary systems with Gelucire display better dissolution properties with respect to the drug alone. The dissolution profiles of silymarin from their PM with Gelucire were improved during the first 20 min followed by a slower release rate that nearly equalled that of the untreated drug. The improvement of silymarin dissolution efficiency obtained with the PM can be attributed to the results of local solubilization action of the carrier, operating in the microenvironment on the hydrodynamic layer surrounding the drug particles. The direct contact of these two components at the solid state due to mixing in physical mixture leads to a high concentration of the carrier silymarin surface hence acting as a driving force for silymarin to get into solution by improvement of wetting characteristics and micellar solubilization of the drug (Damian et al. 2000).

On comparing between the dissolution profiles of silymarin powder and silymarin-Gelucire SD in simulated gastric fluid (pH 1.2), it was found that an enhancement in the dissolution of silymarin from SD was achieved even at 1:1 drug:carrier weight ratio. It could also be seen that the rate and extent of the dissolution were a function of the carrier ratio. After 10 min, the percentage of dissolved silymarin was nearly 11%, 50.5% and 90.2% for the pure drug, SD at 1:1 and 1:3 drug:carrier weight ratios, respectively. Thus, there was a 4.55–8.2-fold increase in the silymarin solubility upon dispersion with Gelucire. However, one-way analysis of variance of DE_{30} values (Table 1) indicated that there was a significant difference ($p > 0.05$) between these formulations. Reduction of drug crystal size, enhancement of drug's wettability and dispersibility from the dispersion system, increased dissolution of the drug-hydrophilic carrier, the carrier's solubilization effect, decreased drug's crystallites aggregation, and/or conversion of the drug from crystalline state to the amorphous state, have been proposed as possible mechanisms of increased dissolution rates of solid dispersions (Ford 1986; Damian et al. 2000).

Results of the dissolution efficiencies at 30 min (DE_{30}) in enzyme free simulated gastric fluid (pH 1.2) and in simulated intestinal fluid (pH 7.4) of different silymarin formulation are listed in Table 1. All formulations showed lower DE_{30} in the gastric fluid when compared with the intestinal fluid. This finding is expected as silymarin (with its three components) is a weak acidic drug with pKa = 6.4. Fig. 4 shows the dissolution profiles of silymarin from its formulated capsules which were composed of silymarin pure (SP), SD at 1:3 drug:carrier weight ratio (GL) or commercial product (CP) (Hepaticum® capsules). The extent of the dissolution enhancing effect was found to be dependent on

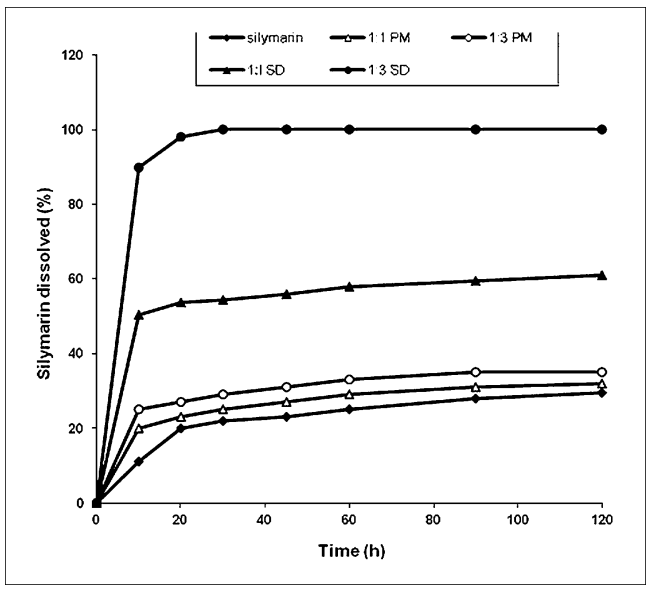

Fig. 3: Dissolution profiles of silymarin-Gelucire binary systems in simulated gastric fluid of pH 1.2

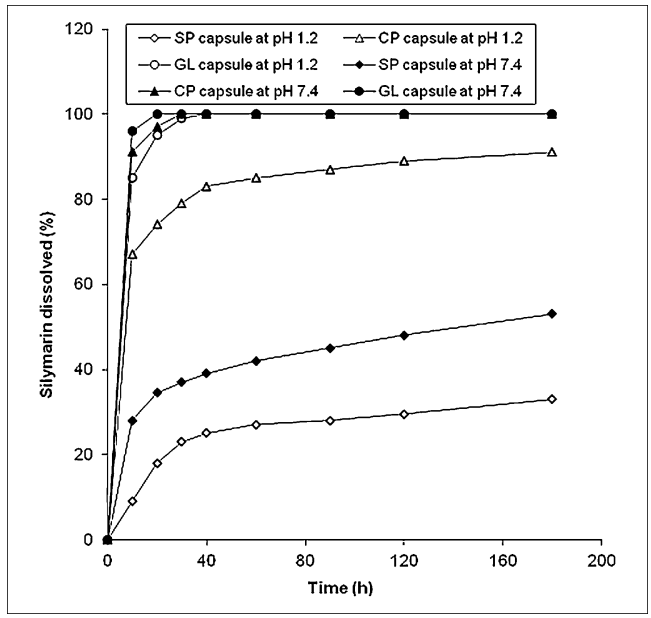

Fig. 4: Dissolution profiles silymarin capsules (SP), silymarin-Gelucire capsules (GL) and silymarin commercial product capsules (CP) in simulated gastric (pH 1.2) and simulated intestinal (pH 7.4) fluids

Table 2: Pharmacokinetic parameters of silybin after oral administration after oral administration of silymarin capsules (SP), silymarin-Gelucire capsules (GL) and silymarin commercial product capsules (CP)

Parameters	SP capsules	CP capsule	GL capsules
C_{max} (μg/ml)	250.62 ± 85.44	1462.10[a] ± 285.6	1523.96[a] ± 350.2
T_{max} (h)	1.20 ± 0.29	1.13 ± 0.3	1.01 ± 0.29
AUC_{0-12} (g.h/ml)	587.53 ± 43.65	4891.7[a] ± 301	7643.01[a,b] ± 447.3

[a] Significantly different from the silymarin capsules
[b] Significantly different from the commercial product capsule

the form of silymarin. As seen from Fig. 4, silymarin reached 79 and 100% drug dissolution within 30 min in the acidic medium from CP and GL capsules, respectively. However, only 23% of the drug dissolved from SP capsules during the same period in the same medium. It was reported that Hepaticum® capsules (CP) have a markedly enhanced dissolution by an advanced formulation that combines micronized silymarin, enriched silybin and silybin- ß-cyclodextrin inclusion complex. However, one-way analysis of variance of DE_{30} values (Table 1) indicated that there was a significant difference ($p > 0.05$) between CP and GL capsules in the acidic media. In contrast, the statistical analysis showed that the difference between these two formulations in the intestinal medium (pH 7.4) was not significant.

The pharmacokinetic parameters such as the peak concentration (C_{max}), the time to reach peak concentration (T_{max}) and area under the concentration-time curve (AUC_{0-12}) were determined and tabulated in Table 2. The pharmacokinetic parameters are reported as mean ± S.E., C_{max} and T_{max} were determined directly from the observed silybin plasma concentration-time data which was shown in Fig. 5. The silybin plasma profiles after administration of different formulations of silymarin capsules; silymarin pure (SP), SD at 1:3 drug:carrier weight ratio (GL) or commercial product (CP), are shown in Fig. 5. SP capsules showed the lowest silybin plasma level with AUC_{0-12} of only 587.5 μg h/ml and C_{max} of 250.62 μg/ml. Compared to SP capsules, GL and CP profiles showed double peaks of maximum concentrations, characteristic of enterohepatic circulation (Wu 2006). Furthermore, SD of silymarin using Gelucire led to dramatic increase in the AUC_{0-12} and C_{max} values to 13-folds and 6.1-folds, respectively. This was attributed to the profound enhancement of the solubility and dissolution associated with the SD of silymarin and Gelucire.

Interestingly, comparison of the AUC_{0-12} and C_{max} in case of GL capsules with the CP capsules, these parameters showed improvements. The relative bioavailability of GL to cyclodextrin CP was 156.2%. In addition, a C_{max} of GL capsule was higher than of the CP capsules but the difference was found to be insignificant. However, there was no significant difference between the T_{max} of the three formulations. Therefore, it was rational to suggest that alternative mechanisms other than improved dissolution may contribute to improvement of the bioavailability of silymarin. It was reported that he primary mechanism by which lipid excipients improve the bioavailability of orally administered therapeutics is through improved dissolution rates in the GI environment. Since Gelucires have desirable self-emulsifying properties because their unique composition of surfactants, co-surfactants and oily phase (Gattefosse 1999). Therefore, it creates optimal conditions for improved drug permeability. This may be another reasonable explanation for the improvement effect in the oral bioavailability of silymarin.

In conclusion, SD of silymarin with Gelucire 44/14 had a pronounced effect on the drug solubility, *in vitro* dissolution rates and bioavailability. Further, the relative bioavailability of silymarin SD with Gelucire 44/14 test formulation was superior to that of reference standard (commercial product; Hepaticum®) that contains micronized silymarin and silybin-cyclodextrin complex. Interestingly, the *in vitro* solubility and dissolution have shown very good correlation to the *in vivo* results. The relative bioavailability data calculated as the ratios of mean AUC_{0-12} for GL capsules relative to that of CP capsules was significantly high (156.2%). However, the T_{max} values of the three formulations remained eventually unchanged.

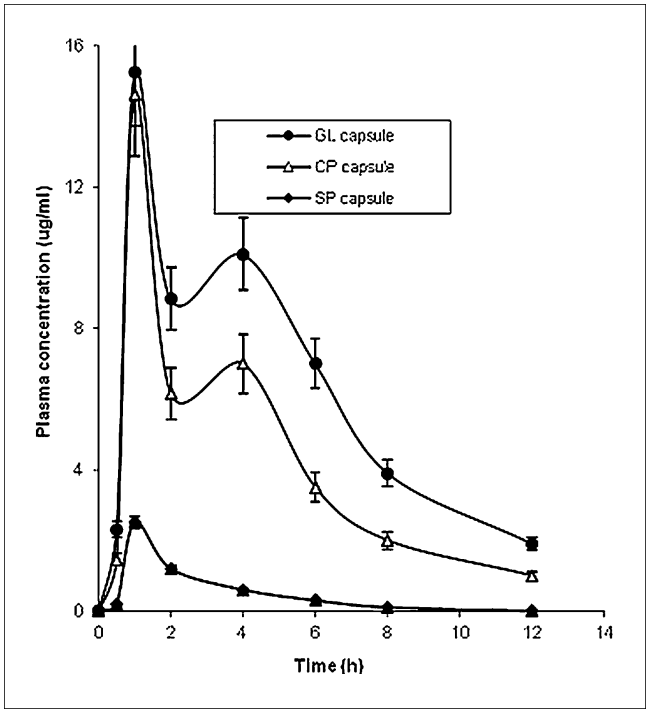

Fig. 5: The mean (±S.E.) plasma silybin concentration versus time profiles in rabbits after oral administration of profiles silymarin capsules (SP), silymarin-Gelucire capsules (GL) and silymarin commercial product capsules (CP)

3. Experimental

3.1. Materials

Silymarin and Hepaticum® capsules were kindly provided by Medical Union Pharmaceuticals Co. (MUP Co., Egypt), Abu-Sultan-Ismailia, Egypt. Gelucire 44/14 (Gelucire) and silybin were purchased from Sigma Chemical Co. (ST. Louis, MO, USA). All other reagents and chemicals were of analytical grade.

3.2. Solubility study of silymarin

Solubility study of silymarin in the presence of Gelucire was performed according to Higuchi and Connors (1965). Briefly, an excess amount of drug was placed in test tubes containing different concentrations (0%, 1%, 3%, 5%, 7%, 9%, 12 % and 15% w/v) of Gelucire in water, capped and shaken in a water bath at 37 ± 0.5 °C for 48 h. After equilibrium attainment, the resulting suspensions were centrifuged and the supernatant fluids filtered through a 0.45 μm filter. The filtrates were diluted and analyzed for their silymarin concentration spectrophotometrically (UV spectrophotometer 2 Genus PC,

USA) at 287 nm using drug-free samples as control. The experiment was performed in triplicate.

3.3. Preparation of silymarin-Gelucire binary systems

3.3.1. Preparation of physical mixtures (PM)

PM of silymarin with Gelucire at 1:1 and 1:3 w/w drug:carrier ratios were prepared by mixing the drug and the carrier thoroughly for few minutes until a homogenous mixture was formed.

3.3.2. Preparation of silymarin SDs

SDs were prepared by the solvent-fusion method. Known masses of Gelucire were dispensed into glass vials and placed in a thermostatically controlled water bath, adjusted at approximately 55 °C. The required amount of silymarin to prepare SDs at 1:1 and 1:3 drug: carrier weight ratios was dissolved in ethyl alcohol and warmed to 40 °C. The molecular solution was added gradually to the molten vehicle with continuous stirring. The obtained solution was evaporated under vacuum in a rotary evaporator (Heidolph-Eleksto GmbH + CoKG, Germany) at 50 °C. The molten mixture was allowed to cool and solidify.

3.3.3. Preparation of the capsule formulations

Liquid-filled hard gelatin capsules were prepared for the 1:3 (w/w) SD as described before to the point of evaporation. The SD prepared mixture was volumetrically filled into the bodies of the hard gelatin capsules using a plastic injector, and then allowed to cool for 2 h before being capped and stored until use.

3.3.4. Differential scanning calorimetry (DSC)

DSC thermograms of silymarin, Gelucire, and their binary systems were obtained using a DSC instrument (DSC-50 Shimadzu, Japan). Samples of 5 mg were heated in sealed aluminum pans over a temperature range of 30–250 °C with a rate of 10 °C/min under a stream of nitrogen.

3.3.5. Dissolution study

The dissolution was carried out using USP rotating paddles dissolution apparatus (Model DT-06, Hanson Research, USA), the dissolution media were 900 ml of enzyme-free simulated gastric (pH 1.2) or intestinal (pH 7.4) fluids. Temperature was maintained at 37 ± 0.5 °C with stirring speed of 100 rpm. Samples containing 70 mg of silymarin or its equivalent in the test SDs or PM with Gelucire were tested. Samples of 5 ml at predetermined time intervals for 120 min were withdrawn and quantitatively analyzed for their drug content. The withdrawn samples were instantaneously replaced with equal volume of preheated dissolution media. The dissolution efficiency parameter at 30 min (DE_{30}) was calculated from the area under the % dissolved silymarin versus time curve applying the trapezoidal rule and expressed as a percentage of the area of the rectangle described by one hundred % dissolution in the same time (Khan 1975). The dissolution tests of capsules containing 140 mg of silymarin (i.e.; equal to that of the commercial product), pure silymarin (SP), silymarin-Gelucire SD at 1:3 (w/w) drug: carrier (GL) and silymarin commercial product (CP) were also performed.

3.4. Bioavailability study

3.4.1. HPLC assay technique

Since silybin is the major component of silymarin, pharmacokinetic and bioavailability study of silymarin is often carried out through quantitative determination of silybin in plasma (Yan-yu et al. 2006). The plasma concentrations of silybin were quantitatively determined employing HPLC assay procedures using variable wave length UV at 288 nm. The mobile phase consisted of a mixture of distilled water:acetonitril (70%: 30%). The mobile phase was filtered under vacuum using a 0.45 μm filter, degassed using a sonicator, and adjusted to pH 4.0 using phosphoric acid. The flow rate of the mobile phase was maintained at 1 ml/min throughout the analysis procedures using C18 reversed-phase column (4.6 × 250 mm) maintained at 40 °C. The samples were pretreated and the concentration of silybin was quantitatively determined according to the procedures described earlier (Yan-yu et al. 2006).

3.4.2. Pharmacokinetics study

Eighteen adult male New Zealand albino rabbits weighing 2 ± 0.21 kg were obtained from the animal facilities of the National Research Center, Giza, Egypt. Animals were treated through the standard regulations of International institutional guidelines and guidelines set forth in the Guide for the Care and Use of Laboratory Animals, NIH Publication No. 96–03, 1996. Animals were randomly classified into three groups and fasted for 24 h before the study. The animals were housed in cages under proper environmental conditions and had free access to water. Capsules full of pure silymarin (SP) or silymarin-Gelucire SD (1:3 w/w drug: carrier) (GL) equivalent to 280 mg kg^{-1} of silymarin were orally administered to group A and group B of rabbits, respectively. Group C received the same equivalent amount of silymarin from the commercial product (CP). After oral administration, 1 ml blood samples were collected from the ear vein at 0.5, 1, 2, 4, 6, 8 and 12 h. The plasma obtained after centrifugation (10 min, 4000 rpm) was immediately stored at − 20 °C until analyzed. Peak concentration (C_{max}) and peak times (T_{max}) were derived directly from the experimental points. The other pharmacokinetic parameters were computed by software program 3p97.

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