Design and Hepatoprotective Evaluation of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin Solid Dispersion and Self-Micro Emulsifying Drug Delivery Systems

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Abstract: Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin are widely used drugs for the treatment of hepatitis C virus, have poor bioavailability due to their low aqueous solubility that limits their dissolution rates. To overcome these limitations solid dispersions (SD_s) and self-microemulsifing drug delivery systems (SMEDDS) were prepared in an attempt to improve their release profile. SD_s were prepared using co-precipitation and melting methods at various drug-polymer ratios. Polyethylene glycol (PEG 6000), polyvinylpyrrolidone (PVP K30 and PVP K17) or sodium desoxycholate were used to prepare SD_s by co-precipitation method. PEG 4000, PEG 6000 or poloxamers (F68 and F127) were used to prepare SD_s by melting method. On the other hand, Ternary phase diagram was constructed using Miglyol® 812 (oil), Tween® 80 (surfactant), Transcutol® HP (co-surfactant) and water to identify the efficient self-microemulsification region. In-vitro release studies were studied for the prepared SD_s and SMEDDS. DDB release from all prepared SD_s did not show any significant improvement when compared to their corresponding commercial product except for the melts prepared by poloxamer F68 used in 1:5 drug to carrier weight ratio. Silymarin release from all SD_s was significantly improved when compared to their corresponding physical mixture, Silymarin powder or its commercial product. Silymarin:Sodium desoxycholate with 1:3 weight ratio, gave the highest drug dissolution behaviour. On the other hand, it was found that the optimal formulation with the best Self-microemulsifying and dissolution behaviour for DDB or Silymarin consisted of 10% Miglyol® 812, 40% Tween® 80, and 50% Transcutol® HP. This formulation showed higher extends of DBB or Silymarin release compared to their powder or commercial products. The optimized formulations of DDB or Silymarin SD_s and SMEDDS were evaluated regarding their hepatoprotective activity against carbon tetrachloride-induced oxidative stress in Albino rats when challenged with commercial products DBB pillules® and Mariagon® capsules. These develobed formulations might be useful in the prevention of used successfully hepatic fibrosis.

1. Introduction:

It has been estimated that there are over 170 million carriers of hepatitis C virus (HCV) worldwide, with an increasing incidence of new infections [1]. Hepatitis C represents a public health problem in Egypt. Different studies done in Egypt showed high prevalence (on different populations in different regions, and different age groups) [2]. Dimethyl 4.4` – dimethoxy – 5,6,5`,6` dimethylene – dioxybiphenyl – 2,2` dicarboxylate [3](DDB) is synthetic analogue of schizandrin C, one of the active components isolated from *Fructus schizandra*, a traditional oriental medicinal plant. This compound (DDB) was shown to protect against liver injury induced by Carbon tetrachloride (CCL_4) [4]. In addition, DDB was used successfully for treatment of cases of chemically induced hepatitis [5 & 6] and has a beneficial effect on liver enzymes and the resulting histopathological changes [7]. However, its oral preparations have been known to have limited bioavailability due to its extremely low solubility in water, a problem that also limits its parenteral dosage forms preparation.

Silymarin, a mixture of three isomeric flavonolignans, was first isolated from milk thistle seeds in 1968. Silymarin consists primarily of three flavonolignans: silybine (Silibinin), silychristin (silichristin) and silidianin [8]. Silybine is the most biologically active component with regard to milk thistle's antioxidant and hepatoprotective properties. A standardized milk thistle extract composed of Silymarin and silybine was developed in Europe and is known commercially as Legalon® [9]. Silymarin therapy decreases complications, hastens recovery, and shortens hospitalization in patients with acute

viral hepatitis [10]. Silymarin prevent hepatic fibrosis through suppression of inflammation and hypoxia in the fibrotic liver. Silymarin was found to be poorly absorbed from the GI tract, with bioavailability estimated as 23-47%. Peak plasma concentrations occur two to four hours after an oral dose. The elimination half-life is approximately six hours. The major problem in the development of an oral solid dosage form of Silymarin is the extremely poor aqueous solubility [11].

Solid dispersion (SD) is defined as a dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by either the melting (fusion), solvent or melting - solvent method [12]. Dispersions obtained through the fusion process are often called melts and those obtained by the solvent method are frequently referred to as co-precipitates or co-evaporates [13,14]. Formulation of solid dispersions is advantageous in enhancing the dissolution of poorly water-soluble drugs and is often accompanied by an increase in their relative bioavailability.

Self-microemulsifying drug delivery systems (SMEDDS) also represent a promising approach for formulating drugs with poor aqueous solubility. They are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, together with hydrophilic cosolvents/cosurfactants these systems upon mild agitation followed by dilution in aqueous media, such as gastrointestinal fluids, these systems can form fine microemulsions [15 , 16].

These microemulsions empty rapidly from the stomach promoting wide distribution of the drug throughout the gastrointestinal tract, thereby minimizing irritation that frequently encountered with extended contact between bulk drug substances and the gut wall. Moreover, these self-microemulsifying formulations provide a large interfacial area for partitioning of the drug between oil and water, as well as a good interface for lipolytic enzymes to digest the oil, there by promoting rapid drug release between various phases of intestinal contents [17]. Furthermore, the break down products that result from the oils enzymatic hydrolysis are surface active products which stabilize the formed microemulsions as well as act as membrane permeation enhancers[18].

Thus, for lipophilic drug compounds that exhibit dissolution rate limiting absorption, the SMEDDS may offer an improvement in the rate and extent of absorption and may result in more reproducible blood time profiles [19].

When comparing the SMEDDS to conventional emulsions; SMEDDS were proved to be superior as they are physically stable formulations and are easily manufactured. Moreover, when compared to microemulsions; SMEDDS showed improved physical stability upon long term storage due to the absence of water content, thus they can be filled directly into soft or hard gelatin capsules for convenient oral delivery [16].

In our study, solid dispersion (SD) and SEDDS were prepared with the intention of improving the solubility, dissolution rate and ultimately bioavailability of the poorly water soluble drugs, DDB and Silymarin. Solid dispersion was prepared by co-precipitation and melting method. Polyethylene glycols exemplified by PEG 6000 and 4000, polyvinylpyrrolidone namely PVP K30 and PVP K17, bile salts represented by sodium desoxycholate, poloxamers exemplified by poloxamer F68 and poloxamer F127 were used in different drug: polymer weight ratios as hydrophilic matrix. SMEDDS were also developed to increase the dissolution rate of DDB and Silymarin. Various types of Self-microemulsifying formulation were prepared using Miglyol® 812, Tween® 80, and Transcutol®HP as oil phase, surfactant and co-surfactant, respectively. In vitro dissolution studies of the prepared solid desperations and SEDDS of DDB and Silymarin were performed and compared to that of pure DDB and pure Silymarin powder to their commercial products. Then selected formulations were subjected to hepatotoxicity studies.

2. Materials and Methods:
2.1. Materials:

Dimethyl-4-4`-dimethoxy-5, 6, 5`, 6` dimethylenedioxy-biphenyl-2, 2,-dicarboxylate (DDB) and Silymarin pure powders were obtained from Arabic Company of Medicinal Plants (Mebaco Co.), Cairo, Egypt. DDB Pilules® (each pillule contains 1.5 mg of DDB) was obtained from Beijing Union Pharmaceutical Factory, China, and imported by AL Ahram Pharmaceuticals and Medical Equipment Company Cairo, Egypt. Mariagon® capsules containing 140 mg of Silymarin were obtained from Alpha Chemical Advanced Pharmaceutical Industries Co. (ACAPI), Cairo, Egypt. Sodium phosphate dibasic anhydrous was obtained from El- Gomhouria Company for Drugs, Cairo, Egypt. Potassium dihydrogen orthophosphate, pure grade of methanol, formalin 10% and ethyl alcohol were obtained from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt. Polyvinylpolypyrrolidone (PVP K30 and PVP K17) and Polyethylene glycols (PEG 4000 and PEG 6000) were obtained from Fluka, Switzerland. Poloxamer F127, poloxamer F68 and Hematoxylin-Eosin stain were obtained from Sigma-Aldrich chemie-GmbH Steinheim, Germany. Sodium

desoxycholate was obtained from Difco laboratories, Michigan, U.S.A. Tween® 80 (polyoxyethylene sorbitan monooleate, HLB=15) was purchased from Merck Schwchardt, Darmstadt, Germany. Transcutol® HP (diiethylene glycol monoethyl ether) was kindly donated by Gattefosse, Saint Priest, France. Miglyol® 812 (medium chain triglycerides) was kindly provided by Sasol, Germany. Carbon tetrachloride (CCL_4) was obtained from Egyptian Company for Chemicals and Pharmaceuticals (ADWIA), Cairo, Egypt.

2.2. Methods:
2.2.1. Preparation of DDB and Silymarin Solid Dispersions:

Both solvent evaporation method (co-precipitation method) and fusion method (melting method) were used to prepare DDB and Silymarin solid dispersions.

2.2.1.1. DDB and Silymarin Solid Dispersions prepared by Co-precipitation method:

Different polymeric carriers were used to prepare DDB or Silymarin co-precipitation solid dispersion namely: polyethylene glycols exemplified by PEG 6000 in weight ratios of 1:1, 1:2 and 1:3 drug to carrier, polyvinylpyrrolidone namely PVP K30 and PVP K17 in weight ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 drug to carrier and bile salts represented by sodium desoxycholate in weight ratio of 1:2 and 1:3 drug to carrier. Each of the aforementioned carriers was dissolved in the least volume of methanol then added to drug methanol solution. The resultant solution was evaporated until dryness using thermostatically controlled magnetic stirrer. The obtained solid masses were kept in desiccators over anhydrous calcium chloride until complete dryness. Finally, the dried masses were pulverized and granules that passed throughout the sieve (USA standard testing sieve set) of 0.63 mm size were clarified for further investigation.

2.2.1.2. Preparation of DDB and Silymarin Solid Dispersions using Melting Method:

Different polymeric carriers were used to prepare DDB and Silymarin solid dispersions using melting method, namely: polyethylene glycols represented by PEG 4000 and PEG 6000 in weight ratio of 1:1, 1:2, and 1:3 drug to carrier and poloxamers exemplified by poloxamer F68 in weight ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 drug to carrier and poloxamer F127 in weight ratio of 1:1, 1:2 and 1:3 drug to carrier. Each of the aforementioned carriers was melted over a heated water bath maintained at 70ºC. Then, the drug was added to the melted mass and stirred well till homogenous matrices were

formed. The obtained masses were kept in desiccators over anhydrous calcium chloride until complete dryness. Finally, the dried masses were pulverized and the granules that passed through sieve (USA standard testing sieve set) of 0.63 mm in diameter were clarified for further investigation.

2.2.1.3. Preparation of DDB and Silymarin Physical Mixtures:

The physical mixtures of DDB and Silymarin with aforementioned carriers were prepared in the same molar ratios utilized previously for comparative purpose.

2.2.2. Construction of Ternary Phase Diagrams:

Ternary phase diagram was constructed with systems comprising of an oily phase, a surfactant and a cosurfactant. Briefly, mixtures of the oil, surfactant, and cosurfactant were accurately weighed at different ratios into well capped vials and were then shaken to ensure complete mixing. Following that, these mixtures were poured into 200 ml of distilled water containing a magnet adjusted to rotate at a speed of 60 rpm. The clarity of the formed aqueous dispersion and the emulsification time, time needed for the system to completely disperse upon dilution, were visually assessed to identify the microemulsifying regions.

2.2.2.1. Preparation of DDB and Silymarin SMEDDS:

After identification of the microemulsifying regions in the constructed phase diagrams, SMEDDS were selected at desired component ratios as presented in table (1) for further studies.

Oil, surfactant, and cosurfactant were accurately weighed into glass vials. Then, either DDB (10 mg/gm of SMEDDS) and Silymarin (70 mg/gm of SMEDDS) was added to the above mixture and the components were gently stirred and vortexed until the added drug was perfectly dissolved. These mixtures were visually analyzed for drug precipitation after storage for 72 hours at ambient temperature.

Table 1: Composition of the investigated SMEDDS.

System Number	Composition		
	Oil Miglyol® 812	Surfactant Tween® 80	Co-surfactant Transcutol®HP
S_1	10	40	50
S_2	10	50	40
S_3	10	60	30
S_4	10	70	20
S_5	10	80	10

2.2.3. In- Vitro Release Studies of DDB and Silymarin Solid Dispersions and SMEDDS:

The in- vitro release of DDB and Silymarin from the prepared solid dispersions and SMEDDS were performed in 900 ml phosphate buffer of pH 7.4 maintained at 37 ± 0.5^{o}C using USP Dissolution Tester apparatus II (Dissolution apparatus, Varian, Germany). Solid dispersion corresponding to 30 mg of DDB, 140 mg of Silymarin, as well as SMEDDS corresponding to 10 mg of DDB /gm SMEDDS., 70 mg of Silymarin /gm SMEDDS. Each preparation was separately placed in the dissolution vessel and the shaft was adjusted at rotation speed of 50 rpm. Samples of 5 ml were withdrawn at predetermined time intervals, filtered through Millipore® filter membrane of 0.45 µm pore size (Millipore, Massachusetts, USA.) and spectrophotometrically measured for DDB at 280 nm. [20] and for Silymarin at 324 nm. [21] using Shimadzu Spectrophotometer UV-1601 PC, Japan.

The removed samples were replenished with equal volumes of the medium to keep the dissolution volume constant. Cumulative amount of DDB or Silymarin dissolved after one hour was used to compare between different investigated formulations.

2.2.4. Hepatotoxicity test:
2.2.3.1. Design of the experiment:

Forty eight Sprague Dawley albino rats of mixed sex weighing 100 g each were obtained from the Animal House Unit of the National Research Center, Dokki, Giza, Cairo, Egypt. The animals were housed for 7 days before using in this study under constant environmental nutritional conditions according to "the principles of laboratory animals care", (NIH publication85-23, revised1985).Prior to the experiment the rats were fasted overnight with free access of water. Rats were divided into eight groups, each group comprising of six rats.

Group 1: Placebo group which ingested one ml saline daily.

The animals in the   remaining seven groups received 0.25 ml of carbon tetrachloride (CCL_4) in liquid paraffin (1:1 v/v) per 100g body weight intraperitoneally once to induce hepatic damage [22]. The drug doses in the forthcoming groups were calculated according to Paget and Barnes table [23].

Group 2: Received only CCL_4 and blood samples were taken after 3 days according to the method reported by Janakat and Al Merie [22].

Group 3: Received commercial product (Marriagon®). Each rat received the equivalent of 2.52 mg of Silymarin and repeated for 7 days.

Group 4: Received powder of formula 1 composed of 1 gm Silymarin+3 gm sodium desoxycholate (co-precipitates) prepared by solvent method). Each rat received the equivalent of 2.52 mg of Silymarin and repeated for 7 days.

Group 5: Received SMEDDS solution of S1 composed of 70 mg Silymarin dissolved in 10% Miglyol®, 40% Tween® 80 oil and 50% Transcutol® HP. Each rat received the equivalent of 2.52 mg of Silymarin daily and repeated for 7 days.

Group 6: Received the commercial product DDB (pilules®). Each rat received the equivalent of 0.27 mg of DDB and repeated for 7 days.

Group 7: Received powder of formula 2 composed of 1 gm DDB+5 gm poloxamer (F68) prepared by fusion method. Each rat received the equivalent of 0.27 mg of DDB and repeated for 7 days.

Group 8: Received SMEDDS solution of S1 composed of 10 mg DDB dissolved in 10% Miglyol® 812, 40% Tween® 80 oil and 50% Transcutol® HP. Each rat received the equivalent of 0.27 mg of DDB daily and repeated for 7 days.

2.2.3.2. Histopathological investigations:

Histopathological survey was performed aiming at declaration of the changes occurred in liver tissues of rats. This was done for comparative purposes between newly reached preparations from pure materials of Silymarin and DDB to their commercial products. Tissue specimens from liver of treated and control rats were fixed in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hematoxyline and eosin according to Bancroft et al. [24]. The sections were thereafter examined and photographed using a microscope at a magnification power of 200 X. The degree of hepatic injury was estimated using an ordinal scale modified from Palaa and Charbonneau [25] according to table (2).

Table 2: Histological Grading of Liver Injury.

Grade	Description
0	No apparent injury by light microscopy
I	Swelling of hepatocytes
II	Ballooning of hepatocytes
III	Lipid droplets in hepatocytes
IV	Necrosis of hepatocytes

2.2.4. Statistical analysis:

Data obtained were statistically analyzed using Fischer Exact Probability test at P < 0.05 (SPSS 14, 2006).

3. Results and Discussion:

3.1. In-Vitro Release Study of DDB and Silymarin Solid Dispersions

With the aim of improving the dissolution behaviour of DDB and Silymarin, the in-vitro release studies of their different solid dispersions as well as their commercial products were shown in figures (1-9).

It was found that pure DDB powder didn't dissolve in the release conditions adopted in the current investigation. However, about 19.38% of DDB was released from the commercial DDB Pilules[®] after 1 hour. Regarding the prepared physical mixture, co-precipitation and melts of DDB with the investigated carriers, they all showed high extent of drug dissolution when compared to DDB powder but showed less extent of drug dissolution when compared to the commercial DDB Pilules[®] (data not shown) except poloxamer F68 melt (P<0.05). In 1:5 (drug to carrier) ratio, the results revealed comparable drug dissolution when compared to commercial DDB pilules[®] (Figure 1).

It is worth mentioning that pure Silymarin powder and the commercial Silymarin capsules released nearly 39% of their Silymarin content after 1 hour of the dissolution run.

Generally, it was apparent from investigating the dissolution results of the prepared physical mixtures of Silymarin with the investigated carriers that its dissolution was low when compared to that of either pure Silymarin powder or commercial Silymarin capsules(P<0.05) (Figs 2-8). This unexpected observation could be related to the salting out of Silymarin from the dissolution medium due to the preferential dissolution of the water soluble carriers.

On the other hand, upon increasing the carrier weight ratio in the prepared coprecipitates formulae, a simultaneous increase in the extent of Silymarin dissolved was detected (Figs 2-8). This finding is probably attributed to the crystallization of drug molecules in very minute crystals in the polymeric matrices [26], which results in rapid dissolution of the embedded drug. The co-precipitates of Silymarin with, polyethylene glycol 6000 (Fig. 2), polyvinyl pyrrolidone K30 (Fig.3) and sodium desoxycholate (Fig. 4) polyvinyl pyrrolidone K 17 (Fig.5), prepared at 1:3 (drug to carrier) weight ratios, released nearly 62.27%, 62.29%, 74.82% and 50.95% of their drug content after 1 hour respectively. It is worth mentioning that a twofold increment in the extent of Silymarin dissolution was calculated for sodium desoxycholate coprecipitates when compared to the commercial Marriagon[®] capsules and pure Silymarin.

Regarding the prepared melts of Silymarin, all carriers showed higher extent of drug dissolution (P<0.05), even when employed at low carrier ratio (Figs.2-8). Moreover, it was observed that increasing the weight ratio of the carrier caused a concurrent increase in the extent of Silymarin dissolved when polyethylene glycol 6000 (Fig. 2), polyethylene glycol 4000 (Fig. 6) and poloxamer F127 (Fig. 8) were employed as carriers (P<0.05). Controversy, there was no appreciated increase in drug dissolution when the weight ratio of poloxamer F68 (Fig.7) to Silymarin was increasing from 2:1 to 3:1 in the prepared melts (P<0.05).

Therefore solid dispersion of DDB with poloxamer F68 in weight ratio of 1:5 (drug to carrier) prepared by melting method (Fig.1) as well as Silymarin- sodium desoxycholate coprecipitates in weight ratio of 1:3 (Fig.9) were selected for further investigation.

3.2. Construction of Ternary Phase Diagrams:

Ternary phase diagram composed of Miglyol[®] 812, Tween[®] 80, and Transcutol[®]HP was constructed to identify microemulsifying regions and to select suitable concentration of each component required for SMEDDS formation.

Tested oil implicated in SMEDDS in the present investigation was Miglyol[®] 812, a medium chain triglyceride derived from coconut oil. It is reported that medium chain triglycerides improve the intestinal absorption of drugs [27, 19, 28]. And have been extensively used in SMEDDS due to their high fluidity at ambient temperature, better solubility properties, and superior self-emulsification ability compared to long chain triglycerides [29, 30]. Additionally, medium chain triglycerides showed better chemical stability due to the lack of double bonds that can catalyze the oxidation of the incorporated drugs of drug substance [31].

Tween[®] 80 was selected to prepare the SMEDDS as non-ionic SAA which tends to form stable microemulsions unaffected by pH and ionic strength changes [31]. Furthermore, Tween[®] 80 was proven to enhance the intestinal permeability of drugs [32].

Transcutol[®] HP (diethylene glycol monoethyl ether) was used in the present investigation as it has previously shown to possess high solubilization power for DDB and is known to enhance the permeability of drugs [33]

It is quite obvious from figure 10 that there are five distinct regions in the phase diagram.

Oil, surfactant, and co-surfactant mixtures present in region (a) are considered efficient SEDDS as they emulsify in nearly 2 minutes or less forming an emulsion with milky appearance. However, the ternary mixtures present in region (b) represent SEDDS that take more than 2 minutes to completely emulsify forming a milky emulsion. This long emulsification time is attributed to the presence of high surfactant concentration above 50% w/w that led to the formation of gel like masses that take long time to dissolve when added to aqueous media.

On the other hand, ternary mixtures present in region (c) represent efficient SMEDDS as they formed clear microemulsions when added to aqueous media in less than 2 minutes. Ternary mixtures present in region (d) represent SMEDDS that took long time to emulsify. Both region (c) and (d) had high ratio of Transcutol® HP that enabled the microemulsion region to be reached where Transcutol® HP molecules penetrate into the surfactant film at the oil globule interface by positioning themselves towards the aqueous phase, causing the contraction of the polyoxyethylene part of the surfactant molecules, which leads to stabilization of the interfacial surfactant film [34]. However, mixtures present in region (e) did not form any emulsion and the oil droplets were visualized on the surface due to the presence of high oil concentration associated with low concentrations of surfactant and co-surfactant.

3.3. Preparation of DDB and Silymarin SMEDDS:

The mixtures present in region (c) and (d) were loaded with 10 mg DDB or 70 mg Silymarin for further investigation. It is worth noting that none of the drug loaded systems showed any signs of drug precipitation after storage for 72 hours at ambient temperature.

3.4. In-Vitro Release Studies of DDB and Silymarin from the Prepared SMEDDS:

In-vitro release studies are often performed to predict how a delivery system might work in ideal situations, which might give some indication of its performance in vivo [35]. Hence drug release investigations were performed for the prepared SMEDDS containing either DDB or Silymarin in comparison to their commercial products in phosphate buffer of pH 7.4 for one hour. The results were graphically illustrated in Figures (11) and (12).

It was observed from DDB release profiles presented in Figure (11) that all of the investigated SMEDDS showed more than 85% of DDB released after one hour. This could be resorted to the complete solubility of DDB in the tested SMEDDS that formed minute oil droplets in the dissolution medium and gave higher extent of drug release [36]. On the other hand, the commercial DDB showed lower extent of drug dissolution which was expected due to its poor aqueous solubility.

It was remarkable that the higher the ratio of Transcutol® HP used, the higher was the extent of DDB released as exemplified by system S1 which contained 50% Transcutol® HP. On the contrary, the higher the ratios of Tween® 80 used, the lower was the extent of DDB released as illustrated in system S5 that contained 80% Tween® 80. The first could be attributed to the creation of void spaces among surfactant molecules owing to the high ratio of Transcutol® HP which facilitated the diffusion of DDB into the aqueous medium [37]. However, the surfactant, at high concentration, formed transparent viscous liquid crystalline gel at the surfactant-water interface [38, 39] that delayed DDB diffusion into the release media [40].

Regarding the prepared SMEDDS, containing Silymarin it is obvious that only system S1 had higher extent of drug release in comparison to Marigon® capsules (Fig 12). This may be due to the presence of large amount of Transcutol® HP in system S1 (50 %) w/w in which Silymarin is extremely soluble. unexpectedly, the rest of the prepared SMEDDS had lower extent of Silymarin release compared to both the raw material and commercial product. This might be attributed to the precipitation of Silymarin due to lowering the solvent capacity of the surfactant and or the co-surfactant as a result of SMEDDS dilution [41].

Conclusively, system S1 composed of 10 % Miglyol 812®, 50% Transcutol HP® and 40 % Tween 80® was further investigated in the histopathology study as it showed higher extent of dissolution when loaded with either DDB or Silymarin.

3.5. Liver toxicity Studies:

The gross appearance of liver specimens of the control group (group 1) was normal regarding their size and colour. Liver histological examination showed normal hepatic lobules as shown in figure 13, with grade (0). These results were in complete agreement with those reported by Dass et al. [42].

3.5.1. Carbon Tetrachloride Hepatitis Induction in Rats:

Rats liver specimens belonging to group (2) treated with CCL_4 were examined after 3 days. They showed necrobiotic changes of hepatocytes including vascular degeneration, nuclear pyknosis and necrosis. Narrowing of hepatic sinusoids and hyperplasia of Kupffer cells were also noticed (Fig. 14). The hepatic injury appeared as grade IV.This result was in accordance with Johnson et al. [43] and Recknagel et

al. [44] who mentioned that CCL_4 is one of the most commonly used hepatotoxic agents in experimental study of liver diseases. Furthermore, mentioned that CCL_4 is biotrasformed by cytochrom P-450 in liver to produce highly reactive trichloromethyl free radical. This radical, in presence of oxygen generated by metabolic leakage from mitochondria, cause lipid peroxidation of membrane lipids which led to loss of integrity of cell membranes and damage of hepatic tissue. Moreover, De Groot and Noll [45] and Azri et al. [46] reported that the changes in structures of the endoplasmic reticulum and other membrane cause loss of metabolic enzyme activation, reduction of protein synthesis and loss glucose-6-phosphatase activation which over all leads to liver damage.

3.5.2. Different Silymarin formulae for Treatment of Induced Liver Hepatitis in Rats:

Liver specimens of rats, belonging to group (3) which were exposed to CCL_4 followed by treatment with the commercial Silymarin capsules (Mariagon®) for 7 days, showed swelling of hepatocytes and narrowing of sinusoids. Moreover, focal areas of coagulative necrosis were also seen. The liver specimens appeared as grade (III) as clearly demonstrated in figure 15.

Liver specimens of rats belonging to group (4), exposed to CCL_4 and then treated by Silymarin co-precipitate prepared according to formula 1 using Silymarin and sodium-desoxycholate in 1:3 weight ratio respectively, revealed mild swelling of hepatocytes. The latter appeared granular with vesiculated nuclei. The hepatic sinusoids contained mononuclear cells mainly lymphocytes and macrophages. The liver specimens appeared as grade (I) as clearly demonstrated in figure 16.

Liver specimens of rats belonging to group (5), exposed to CCL_4 then treated by Silymarin loaded SMEDDS prepared according to system S1 for 7 days, showed necrobiotic changes of hepatocytes, with disorganisation of hepatic cords. The hepatocytes appeared foamy with focal necrotic areas. Accordingly, the liver specimens appeared to be Grade (IV) as shown Figure (17).

Therefore, histopathological studies showed that CCL_4 caused necrosis and fibrosis of liver tissue and administration of Silymarin after CCL_4 treatment, showed hepatocytes regeneration that varied in its grade according to the type of formula administrated. Silymarin co-precipitate prepared according to formula (1) was proved to be more effective than commercial capsules as it improves liver tissue injury to be appeared as grade (I). This is probably substantiated by the increase in drug dissolution from this formula when compared to commercial capsules. Therefore, it is rational to state that the improvement in drug dissolution led to a simultaneous increase in the proportion of drug absorbed which may have contributed to the observed enhancement in hepatocytes regeneration. Further studies are required to confirm the above mentioned mechanism and also establish other mechanisms involved in the observed hepato-regenerative effect.

3.5.3. Different DDB formulae for Treatment of Induced Liver Hepatitis in Rats:

Liver specimens of group (6) treated with DDB commercial product for 7 days showed mild swelling of hepatocytes and narrowing of sinusoids as depicted in figure 18. The liver specimens appeared as grade (II).

Regarding, liver specimens of rats of group (7), treated by DDB melt prepared according to formula 2 composed of DDB and poloxamer F68 in 1:5 weight ratio for 7 days, showed swelling of hepatocytes. Moreover, the hepatic sinusoids were infiltrated with mononuclear cells mainly lymphocytes and macrophages. The liver specimens appeared to be grade (II) as clearly demonstrated in Figure (19).

Interestingly, liver specimens of rats of group (8) treated by DDB loaded in SMEDDS prepared according to system S1 and composed of 10 % Miglyol 812®, 40% Tween 80®, 50% Transcutol® HP in 1:4:5 weight ratios respectively, for 7 days, showed mild swelling of hepatocytes with prominent central situated nuclei. In addition, narrowing of hepatic sinusoids was observed. The liver specimens appeared to be grade (I) as shown in figure 20.

This improvement was attributed to the increase in drug dissolution from this formula and supported by previous study that had been shown that the ingredients present in the SMEDDS play a vital role in improving the solubility and absorption of the DDB [33].
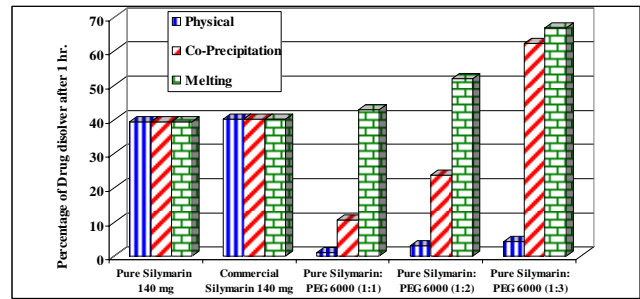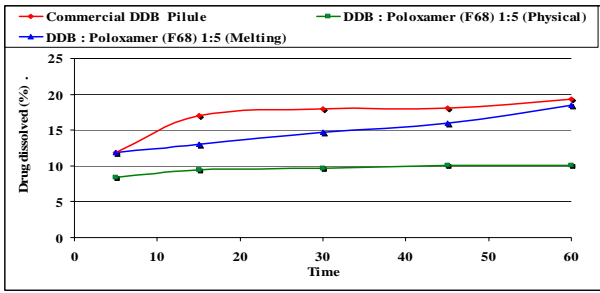
Fig. (1): Percentage Dissolution of DDB from its Solid Dispersions with poloxamer at 1:5 Weight Ratios Prepared by Physical Mixture and melting methods Compared with its Value from Commercial DDB Pilule.

Fig. (2): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical, co- precipitation and melting using PEG 6000.
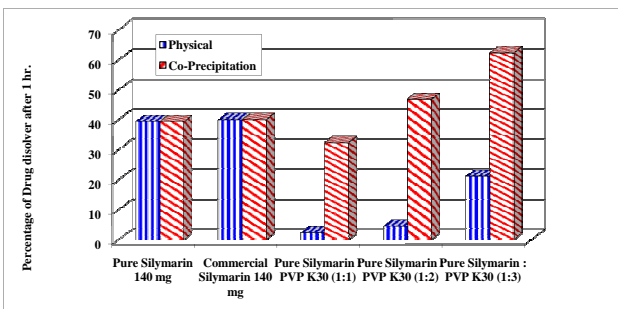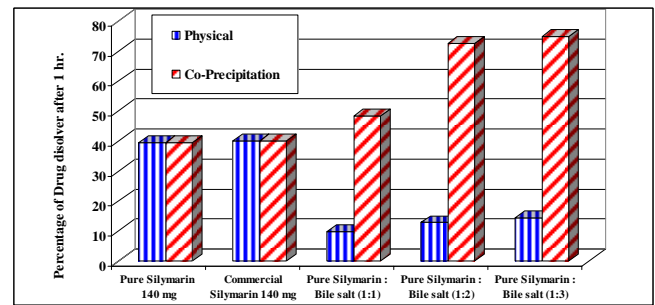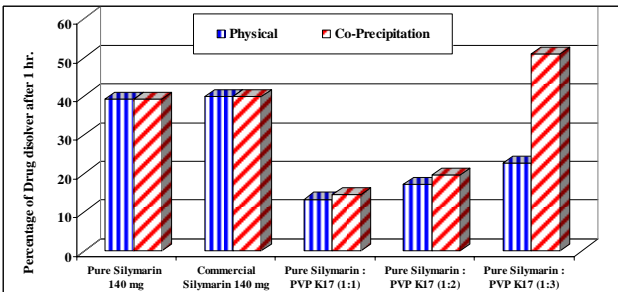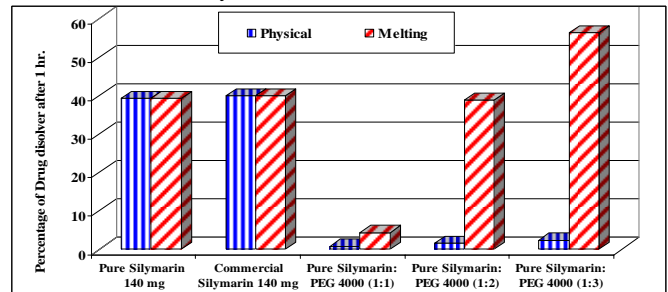

Fig. (3): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and co- precipitation using PVP K30.

Fig. (4): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and co- precipitation using bile salt (sodium desoxycholat).

Fig. (5): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and co- precipitation using PVP K17.

Fig. (6): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and melting using PEG 4000.
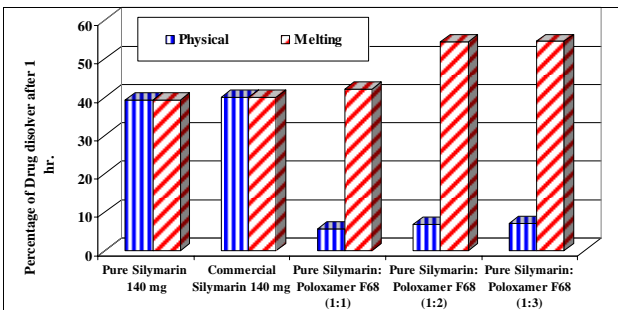
Fig. (7): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and melting using poloxamer F 68.
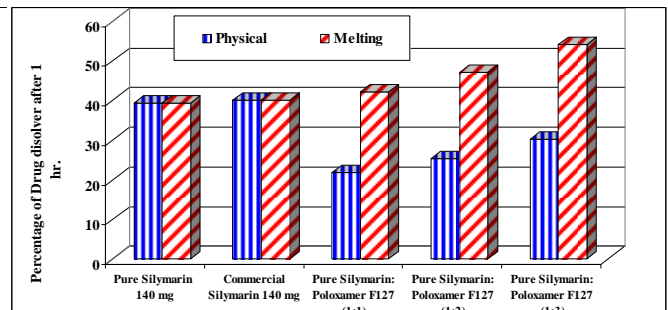

Fig. (8): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and melting using poloxamer F 127.
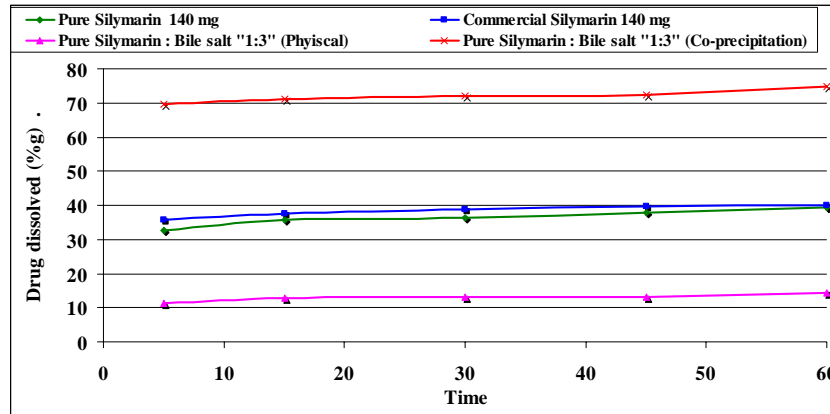
Fig. (9) Percentage Dissolution of Silymarin from its Solid Dispersions with Sodium Desoxycholate as Bile Salt at 1:3 Weight Ratios Prepared by Physical Mixture and coprecipitation Compared with its Value from Commercial Silymarin (Marriagon) in Phosphate Buffer of pH 7.4 at 37 ± 0.5 ºC.

Region (a) = SEDDS that forms milky emulsion when added to aqueous media in < than 2 minutes.

Region (b) = SEDDS that forms milky when added to aqueous media in > than 2 minutes.

Region (c) = Efficient SMEDDS that forms transparent microemulsion when added aqueous media in < than 2 minutes.

Region (d) = SMEDDS that forms transparent microemulsion when added to aqueous media in > than 2 minutes.

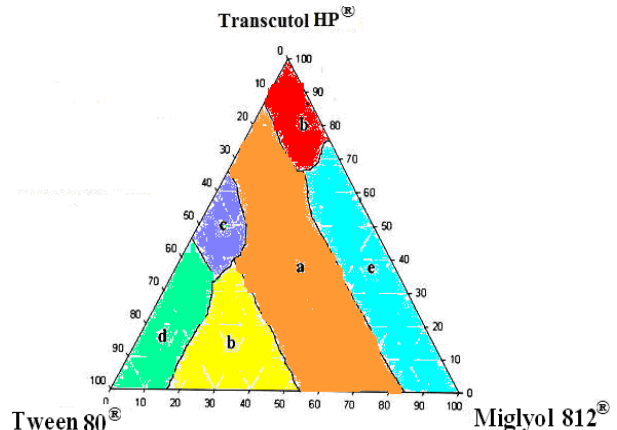Region (e) = No emulsion is formed, oil droplets float on surface.

Fig. (10): Ternary phase diagram composed of Miglyol812®: Tween 80®: Transcutol HP® showing the SEDDS and SMEDDS.
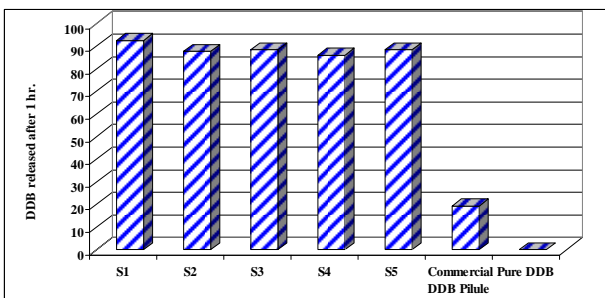

Fig.11.Percent of DDB Released from SMEDDS Composed of Different Weight Ratios of Miglyol812®, Tween 80® and TranscutolHP®.
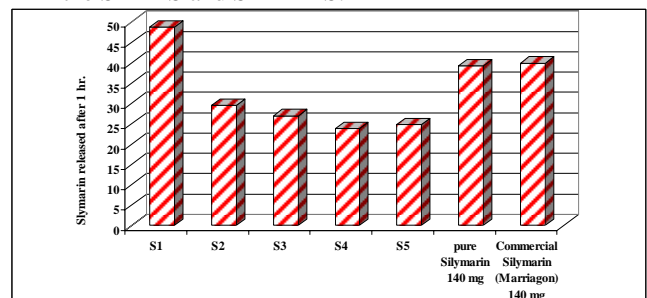

Fig.12.Percent of Silymarin Released from SMEDDS Composed of Different Weight Ratios of Miglyol812®, Tween 80® and TranscutolHP®.
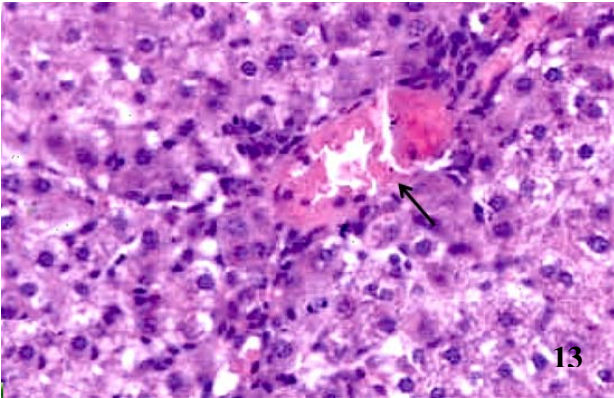
Figure 13: Liver of rats from control group (Group 1) showing normal histological structure of it is portal triads (H&E X200). Grade 0.
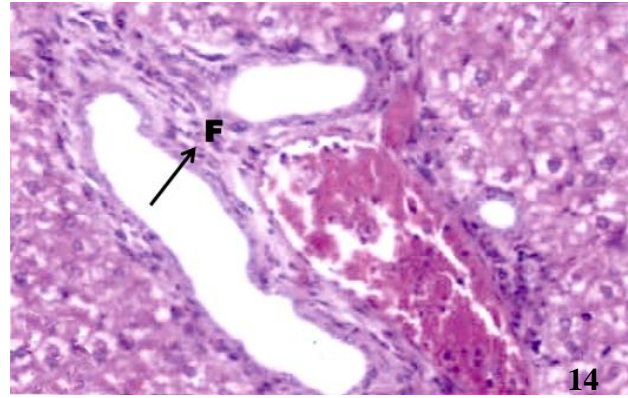

Figure 14: Liver of rats exposed to CCL$_4$ group (Group 2) and examined after 3days showing proliferation of fibrous connective tissue (F), and hyperplasia of bile duct (H&E X200). Garde IV.
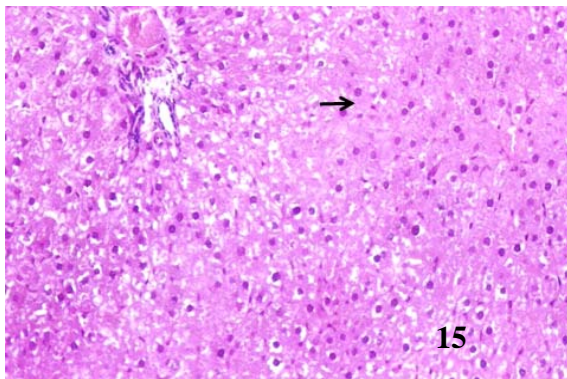

Figure 15: Liver of rats exposed to CCL$_4$ (Group 3) then treated with Silymarin (commercial product) for 7 days, showed swelling of hepatocytes and narrowing of sinusoids (H&E X200). Garde III.
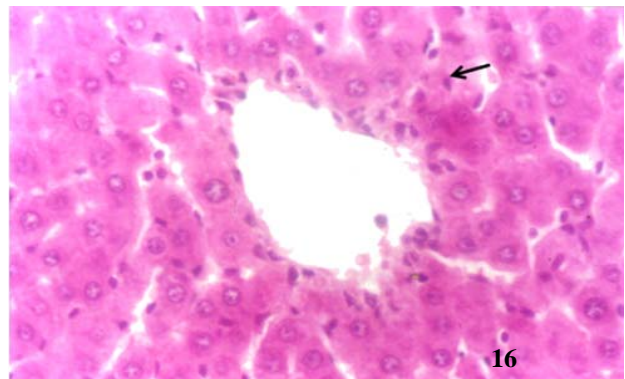

Figure 16: Liver of rats exposed to CCL4 (Group 4) then treated by Silymarin with Sodium desoxycholate prepared by co- precipitates methods (formula 1) for 7 days showing hepatocytes with eosinophilic granules, (arrow) (H&E X200) .Garde I.
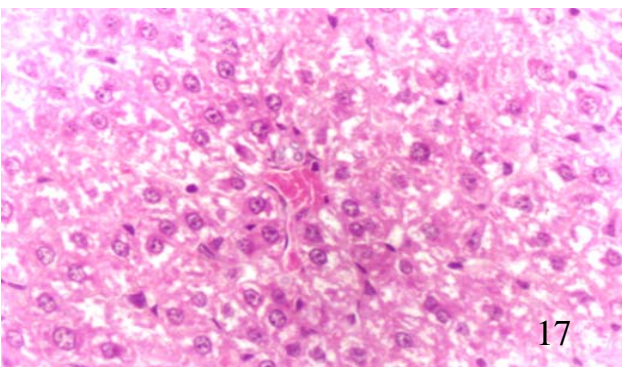

Figure 17: Liver of rats exposed to CCL4 (Group 5) then treated with Silymarin S1 for 7 days showing disorganization of the hepatic cords (H&E X200). Garde IV.
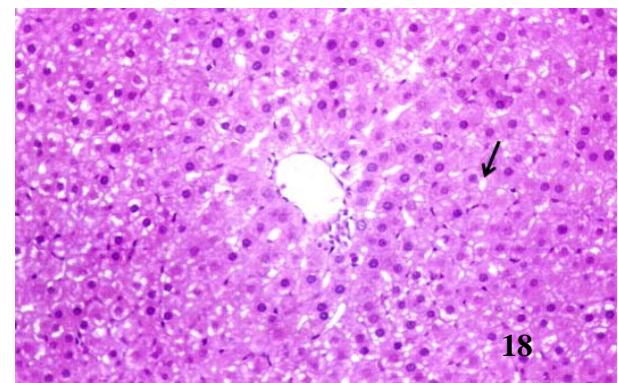

Figure 18: Liver of rats exposed to CCL4 (group 6) then treated with DDB, commercial product for 7 days, showed mild swelling of hepatocytes and narrowing of sinusoids (H&E X200). Garde II.
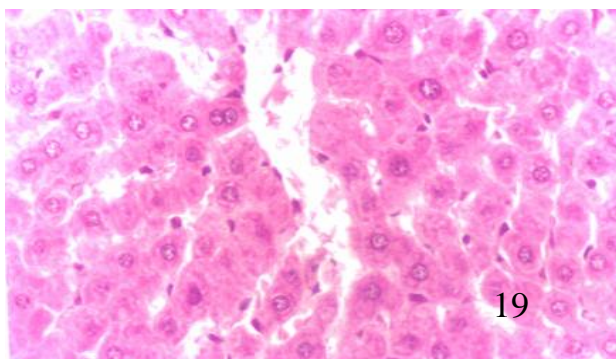
Figure 19: Liver of rats exposed to CCL4 (Group 7) then treated by DDB with poloxamer (F68) prepared by fusion method (formula 2) for 7 days showing swelling of with narrowing of hepatic sinusoids (H&E X200). Garde II.
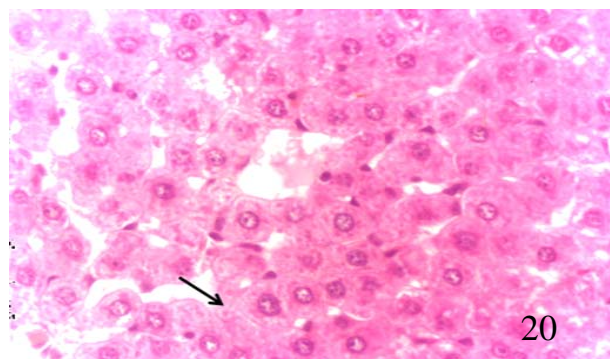

Figure 20: Liver of rats exposed to CCL4 (Group 8) then treated by DDB S1 showing swelled hepatocytes with central vesiculated nuclei (H&E X200). Garde I.

4. Conclusion:

This paper demonstrated that the poorly soluble drugs, Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin when prepared into solid dispersions (SD$_s$) and or self-microemulsifing drug delivery systems (SMEDDS) showed improved in their release behaviour thus avoiding the dissolution step which is the rate limiting step in the absorption process. The optimal formulation of DDB SD$_s$ was prepared by melting method using poloxamer F68 used in 1:5 drug to carrier weight ratio and that for Silymarin was obtained by co-precipitation technique using Sodium desoxycholate in 1:3 weight ratio respectively. The DDB and Silymarin SMEDDS consisting of 10% Miglyol® 812, 40% Tween® 80, and 50% Transcutol® HP exhibited potential in vivo hepatoprotective activity against carbon tetrachloride-induced oxidative stress in Albino rats when challenged with commercial products DDB pillules® and Mariagon® capsules.These develobed formulations might be useful in the prevention of used successfully hepatic fibrosis.

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