PREPARATION, CHARACTERIZATION AND EVALUATION OF FAST-DISSOLVING SILIBININ-ENRICHED Silymarin (SES)

Khaled A. Khaled

Department of Pharmaceutics, Faculty of Pharmacy, Al-Minia University, Al-Minia, Egypt

The hepato-protective activity of silymarin is well demonstrated. However, silymarin is not a single component, but a mixture of silydianin, silychristin and silibinin. Silibinin is the least soluble and the most active component of silymarin. Accordingly, as silibinin content of silymarin increases, its activity is expected to increase. These were the objectives of the study i.e to prepare silibinin enriched silymarin (SES) and enhance its dissolution by preparing its adsorbates and co-adsorbates with Florite. Silibinin enriched silymarin was prepared by extracting the water soluble components of silymarin using water. The silibinin content of the SES was evaluated using HPLC method of assay. The fast dissolving SES systems were characterized using differential scanning calormetry, x-ray diffractometry and infra-red spectroscopy. The obtained results indicated the compatibility of Florite with SES. The biological activity of SES systems was evaluated in rats using paracetamol as a hepatotoxic agent and compared to that of silibinin alone. The results showed that SES-co-adsorbate was more efficient in lowering the serum level of the specific liver enzymes (ALT and AST) than silibinin alone.

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INTRODUCTION

The World Health Organization (WHO) considers chronic hepatitis a major global health problem. Both hepatitis B (HBV) and hepatitis C (HCV) infections can lead to chronic hepatitis. Egypt has the highest country-wide prevalence of hepatitis C in the world. Even under the most optimistic scenario of zero contemporary transmission, HCV infection in Egypt will remain above 5% of the population for the next 50 years. The main treatment for both HBV and HCV infections has been interferon IFN-α. However, the high cost of interferon may affect the extent of its use in Egypt. Therefore, there is a real need for effective therapeutic agents with low cost and incidence of side effects.

Silybum marianum (milk thistle) is one of the oldest herbal medicines. It contains the active flavonoid component silymarin. The liver-protective activity of silymarin has been demonstrated in numerous experimental models of toxic liver damage, including damage from carbon tetrachloride, galactosamine, thioacetamide, ethanol, paracetamol and the poisonous substances of the death cap fungus, phalloidin and amanitin. Further, pretreatment with silymarin inhibit alcohol-induced liver damage. Silymarin has also been found to protect liver cells from ischemic injury, radiation, iron toxicity and viral hepatitis. Silymarin protective mechanisms include: antioxidation, antilipid peroxidation and protection against glutathione depletion induced by iron. The acute, sub-acute and chronic toxicity of silymarin in humans is very low.

Silymarin is not a single component, but a mixture of silydianin (10%), silychristin (20%) and silibinin (50-60%). The later is the most biologically active. Silibinin is available in Germany as a parenteral preparation for intravenous injection, silibinin hemisuccinate sodium salt, for the treatment of poisoning due to the ingestion of Amanita phalloides. Silibinin exerts strong anticancer activity against human HCC cell.

Two problems are facing the clinical use of silymarin. The first one is the low aqueous solubility of its most active component, silibinin. Consequently, improvement of silymarin aqueous solubility or dissolution rate may be a valuable mean to increase its efficacy as dissolution of the drug could be the rate limiting step for its absorption. The second problem is the lack of standardization of silymarin (the definite contents of silymarin from its various components). The second problem is mainly due to that silymarin components are structural analogs with identical molecular weight. Therefore, clinical pharmacological studies have great difficulty to establish dose-response relationship. In brief, silymarin should be treated as a drug not as a food supplement.

Silymarin is a slightly acidic drug with pKa of 6.4. The solubility of
silymarin components at 37°C and pH 3 was reported to be 20.6, 156.8 and 645.5 mg/l for silibinin, silydianin and silychristin, respectively. The low solubility of silibinin compared to the other components of silymarin was utilized to prepare the silibinin-enriched silymarin (SES).

The objectives of the current study were to maximize the biological activity of silymarin by increasing its silibinin content (preparation of silibinin enriched silymarin (SES)) and enhancing SES dissolution by preparing its adsorbate and co-adsorbate (ad-solubilizate). In this study, porous silica (Florite®) was used to prepare the adsorbates employing solvent deposition method. The dissolution of the drug from the adsorbates was studied at different pH values. The biological activity of fast dissolving SES was compared to that of silibinin alone.

**EXPERIMENTAL**

**Materials**
- Silibinin (Sigma-Aldrich, Inc., St. Louis, MO., USA).
- Silymarin was a gift from Sedico Pharmaceutical Co., Egypt.
- Florite R (Tokuyama Soda, Tokyo, Japan). Florite was used after drying in vacuum oven at 120°C for 3 hrs.
- Methanol HPLC grade (Lab-Scan, Dublin, Ireland).
- Biomérieux transaminases-kits (bioMerieux®, sa, France).
- All other materials were reagent grade and used as received.

**Equipment**
- UV-visible double beam spectrophotometer, Spectronic, Genesys, Milton Roy, USA.
- USP II dissolution apparatus (Model DT-06, Hanson Research, CA., USA).
- Perkin Elmer Instruments, Norwalk, USA.
- Shimadzu model DSC-50 (Shimadzu, Japan).
- Shimadzu IR-470 spectrophotometer (Shimadzu, Japan).
- Philips 1710 diffractometer (Endohoven, Netherlands).

**Methods**

**Preparation of SES**
A weighed amount of 2 g of silymarin was shaken with 500 ml of water in a conical flask at 50 rpm for 12 hrs. The flask contents were filtered and the insoluble material was dried in a hot air oven at 60°C for 12 hrs. The dried material was re-extracted with water using the same procedure. The final dried material was pulverized and sieved using 100 mesh (150 µm) sieve.

**High performance liquid chromatography (HPLC) of SES**
The Perkin Elmer HPLC station was used. The station consists of dual pump (series 200), a UV detector (series 200), and series 600 LINK chromatography interface connected to a PC (software Total Chrome version 6.2). The stationary phase, Bondclone C18 column (300 mm x 3.9 mm, 10 µm - Phenomenex, CA, USA), was kept at 25°C. The mobile phase was a mixture of methanol:
double distilled water : acetic acid (50:50:1)\textsuperscript{21}. The flow rate was 1.0 ml/min and the run time was 30 min. Effluent was monitored at 286 nm. The volume of each injection was 20 µl. Before injecting solutions, the column was equilibrated for 30 min with the mobile phase flowing through the system. Quantitation was accomplished using silibinin as an external standard. Five concentration levels of silibinin were used (5, 10, 20, 30, 40 µg/ml) to construct the standard calibration curve. Standard and sample were dissolved in and diluted with mobile phase to obtain the working solution in a concentration of 30 µg/ml. Each solution was injected in triplicate.

Construction of Langmuir adsorption isotherm
Ethanolic solutions containing different concentrations of SES (10, 20, 30, 40, 50, and 60 µg/ml) were added to 100 mg of FLR in 100 ml volumetric flasks. The flasks were shaken at 50 strokes/min in a water bath previously adjusted at 37°C for 6 hrs (equilibrium time was found to be 4 hrs). Samples were withdrawn and assayed spectrophotometrically at \( \lambda_{\text{max}} \) of 286 nm. Control solution containing SES only was treated similarly. Blank solution containing Florite only was prepared and subjected to the same treatment.

Preparation of co-adsorbates
Aqueous solutions of Tween 80 containing 1, 3 or 5% w/v Tween 80 were prepared. From each solution, a volume of 5 ml was added to the specified amount of activated FLR (1, 3 and 5 g) in a porcelain dish. The obtained slurry was, infrequently, stirred using a glass rod for 2 hrs. The slurry was then dried in an oven at 110°C until constant weight was obtained. The resulted dry material was pulverized and sieved using 100 mesh (150 µm) sieve. The final powdered materials were coined as 1:1, 1:3, 1:5, 3:1, 3:3, 3:5, 5:1, 5:3 and 5:5 Tween 80 : FLR powders. These sieved powders were used instead of dried FLR to prepare the co-adsorbates. The prepared co-adsorbates were coined as 1:1:1, 1:3:1, 1:5:1, 1:1:3, 1:3:3 and 1:5:3 drug : Tween 80 : FLR. By this way, drug : FLR ratio did not exceed 1:3.

Characterization of the prepared systems drug content
The actual drug content was determined by shaking 30 mg sample in 50 ml ethanol for 4 hrs. A volume
of 2 ml of the supernatant solution was appropriately diluted with water and assayed for drug content spectrophotometrically. Samples containing 100 ± 5% of the claimed drug content were subjected to further investigations.

**Differential scanning calorimetry (DSC) study**

The DSC patterns of various systems were obtained with a Shimadzu model DSC-50 (Japan), at a heating rate of 10°C/min from 30°C to 300°C under stream of nitrogen gas at a flow rate of 40 ml/min. Samples of about 4-8 mg were encapsulated into aluminum pans with crimped-on lids. The procedures involved heating of the sample contained in an aluminum pan and a similar empty reference pan at a predetermined heating rate and temperature range. The temperature difference between the sample and the reference is presented graphically in relation to differential heat flow.

**Infrared spectroscopy (IR) study**

Infrared-spectroscopy was used to investigate the type of interaction between SES and FLR. It was carried out using potassium bromide disc method. Samples of 1-2 mg were mixed with potassium bromide, compressed at a pressure of 6 ton/cm² into discs and scanned over the range of 4000-600 cm⁻¹ with an empty pellet holder as a reference, using Shimadzu IR-470 spectrophotometer (Shimadzu, Japan).

**X-ray diffraction study**

The x-ray diffractograms were obtained using Philips 1710 diffractometer (Endohoven, Netherlands). The target was CuKα radiation; operating at 40 KV and a current of 40 mA and a single crystal graphite monochromator were employed. The diffraction patterns were achieved using continuous scan mode with 2θ ranging from 8° - 40° at a rate of 2°/minute.

**Dissolution of the prepared systems**

The prepared systems and the untreated SES dissolution profiles were obtained in different dissolution media, containing 0.02% w/v Tween 80 as a wetting agent. Dissolution studies were conducted in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) using USP II dissolution apparatus (Model DT-06, Hanson Research, CA., USA).

An accurately weighed sample of particle size range between 125-250 μm, equivalent to 70 mg silibinin, was sprinkled over the surface of 900 ml of the dissolution medium. The medium was kept at 37 ± 0.5°C and rotated at a rate of 100 ± 2 rpm. At specific time intervals, aliquots (5 ml each) were withdrawn using a volumetric pipette fitted with a soft cotton plug, and immediately replaced by an equal volume of pre-warmed fresh dissolution medium. The withdrawn samples were appropriately diluted and analyzed spectrophotometrically for the drug content. A cumulative correction factor was applied to compensate for...
the previously withdrawn samples. The final results were the average of at least three determinations.

**Hepatoprotective effect**

Male albino rats weighing 200 ± 20 g were obtained from University Animal House and fed standard pellet diet and supplied water *ad libitum*. The animals were classified into five groups, each containing 5 rats. Group I served as a control group and received 1 ml of blank starch mucilage. Group II received hepatotoxin (paracetamol), group III pre-treated with silibinin, group IV pre-treated with SES, and group V pre-treated with 1:3:5 co-adsorbate. Pre-treatments were given at a dose level equivalent to 10 mg of silibinin per kg body weight. Pretreatments were administered orally at 48, 24 and 0.5 hrs prior to the oral administration of paracetamol. The animals were fasted overnight (14 hrs) and then received paracetamol as 1 ml of starch mucilage. Paracetamol was given in a dose of 600 mg/kg body weight to the rats of groups II-V. Twenty four hrs following the hepatotoxin administration and under light anesthesia with ether; blood (3 ml) was collected from each rat by cardiac puncture and then animals were killed by decapitation. Serum was separated by centrifugation (3000 rpm for 10 min). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were estimated using commercial diagnostic kits. Results are expressed as means ± SD. Significance of the differences between values was assessed by a two way analysis of variance. Differences were considered significant when *p* < 0.05.

**The writhing test**

Adult albino rats weighing 200 ± 20 g were obtained from the University Animal House. The rats were classified randomly into three groups (5 rats each). Control group received 1.0 ml of saline. Paracetamol was given orally at a dose of 50 mg/kg as 1.0 ml solution in saline (standard group). The test group received the same dose of paracetamol as 1:1 w/w physical mixture with an amount of 1:3:5 SES-co-adsorbate equivalent to 2 mg of silibinin (10 mg/kg body weight) in 1.0 ml of saline. Acetic acid (3% v/v) was, then, injected intraperitoneally (10 ml/kg body weight) at a predetermined time points. The percentage response was determined at 15, 30 45, 60, 90, 120, and 180 min. Number of writhing displayed by each rat was counted for 10 min after the administration of acetic acid. Percentage response was calculated considering the peak response by paracetamol saline solution as the maximal possible effect. Data were presented as mean ± S.E.M. and analyzed statistically.

**RESULTS AND DISCUSSION**

**Preparation of SES**

The aqueous solubilities of the major silymarin components at 30°C and pH 3 were reported to be 20.4, 147.9 and 637 mg/l for silibinin (SB),
silydianin (SD) and silychristin (SC), respectively. These values represent the sum of the solubilities (total solubility, $S_T$) of the ionized and un-ionized species of each component. The $pK_a$ of silymarin components is 6.4. Therefore, at pH 3, the aforementioned solubilities can be considered as the solubilities of the un-ionized species ($S_o$). The total solubility ($S_T$) was calculated at pH 6.5 (the pH of the distilled water) using the following form of Henderson-Hasselbalch equation:

$$S_T = S_o + S_o \cdot 10^{pH-pK_a}$$

The calculated solubilities were 40.8, 295.8 and 1274 mg/L for SB, SD and SC, respectively. It is interesting to mention that the solubility of SB in distilled water was reported to be 39.56 mg/L.

It is clear that the solubility of SB is much less than that of SD or SC. Therefore, cold water was used to extract both SD and SC from silymarin. By this way, the content of silibinin in the left-over material of silymarin (after extraction) increases and silibinin enriched-silymarin (SES) was prepared. It should be mentioned that the higher the percentage of SD and SC in silymarin, the higher the degree of enrichment by SB.

**Chromatographic study**

Figure (1) shows the chromatogram of silibinin characterized by four peaks at retention times of 17.54, 19.4, 23.3 and 25.2 min. The peaks were labeled as silibinin A (SB$_A$) silibinin B (SB$_B$), isosilibinin A (ISB$_A$) and isosilibinin B (ISB$_B$), respectively.

The total area under the aforementioned peaks was used to construct the calibration curve (Fig. 2), and the obtained data were shown in Table (1).

**Fig. 1:** Chromatograms of Silymarin, SES, and Silibinin.

**Fig. 2:** Calibration curve for silibinin.
Table 1: Chromatographic data of silibinin used to construct the calibration curve

<table>
<thead>
<tr>
<th>Concentration of silibinin (µg/ml)</th>
<th>Area under the peaks (µV.s x 10⁻³) ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1108 ±66</td>
</tr>
<tr>
<td>10</td>
<td>2310 ±127</td>
</tr>
<tr>
<td>20</td>
<td>4622 ± 231</td>
</tr>
<tr>
<td>30</td>
<td>6934 ± 277</td>
</tr>
<tr>
<td>40</td>
<td>9249 ± 451</td>
</tr>
</tbody>
</table>

Table 2: Chromatographic data obtained by injecting 20 µl of 30 µg/ml solutions of silibinin, silymarin and SES sample.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Assigned component</th>
<th>Area under the peaks (µV.s x 10⁻³) of retention times ≥17.0 min for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Silibinin</td>
</tr>
<tr>
<td>5.3</td>
<td>Taxifolin (TAX)</td>
<td>1429418</td>
</tr>
<tr>
<td>6.3</td>
<td>Silychristin (SC)</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>Isosilychristin (ISC)</td>
<td></td>
</tr>
<tr>
<td>8.46</td>
<td>Silydianin (SD)</td>
<td></td>
</tr>
<tr>
<td>17.54</td>
<td>Silibinin A (SBₐ)</td>
<td></td>
</tr>
<tr>
<td>19.4</td>
<td>Silibinin B (SBₐ)</td>
<td></td>
</tr>
<tr>
<td>23.3</td>
<td>Isosilibinin A (ISBₐ)</td>
<td></td>
</tr>
<tr>
<td>25.2</td>
<td>Isosilibinin B (ISBₐ)</td>
<td></td>
</tr>
</tbody>
</table>

Figure (1) shows the chromatogram of the SES sample. Compared to that of silymarin, it can be observed that the peaks of silymarin at retention time of 5.3 min disappeared indicating that taxifolin was washed out during the process of SES preparation. In addition, the peaks of ISC, SC and SD were found but with smaller areas. Interestingly, the area under the four peaks characteristic for silibinin increased. Calculation showed that silibinin content of the SES sample is 78% w/w.

Preparation of adsorbates

Florite® (FLR) is a porous calcium silicate. It has an extensive surface area of 140 m²/g and good flowability. It is pure synthetic inorganic un-absorbable material so it is considered safe for oral administration. Molecular dispersion of drugs on the extremely large surface area of porous silica has been utilized to enhance dissolution and
absorption of poorly soluble drugs. Furthermore, Florite was used to prepare adsorbates of paracetamol. The dissolution of paracetamol from ground mixtures of the drug and FLR was studied.

In adsorption from solution, physical adsorption usually occurs rather than the chemical one and results in monolayer coverage on the surface. Multilayer adsorption can occur, however, in some cases, and can be distinguished from monolayer coverage by the shape of the adsorption isotherm. The Langmuir equation is frequently used to analyze adsorption data from solution.

The equilibrium time of adsorption was found to be 4 hrs. However, samples were shaken for 6 hrs to ensure equilibrium. Control test showed no decrease in drug concentration. This indicates no drug loss was observed due to adsorption to the glass container or due to degradation of the drug.

The results of adsorption were fitted to Langmuir equation:

\[ Y = \frac{X}{m} = \frac{n k C_{eq}}{1 + n C_{eq}} \]

Where \( Y \) is the amount of drug (X in mg) adsorbed per m gram of the adsorbent; \( C_{eq} \) is the equilibrium concentration of the drug (mmole/L); \( k \) is the association constant and \( n \) is the maximum amount of drug adsorbed to form monolayer under experimental condition (limiting adsorption capacity). Figure 3 shows the plot of \( C_{eq} \) vs. \( Y \) gave the typical type I Langmuir isotherm as classified by Brunauer. Therefore, fitting the data using Langmuir equation was appropriate. This approach was confirmed by the linear transformation of the experimental data.

The linear form of Langmuir equation can be written as:

\[ \frac{C_{eq}}{Y} = \frac{C_{eq}}{k} + \frac{1}{nk} \]

The linear plot of \( \frac{C_{eq}}{Y} \) vs. \( C_{eq} \) is shown in Figure (4).

**Fig. 3:** Isotherm for adsorption of SES onto Florite in water at 37°C.

**Fig. 4:** Linear transformation of Langmuir plot for the adsorption of SES onto Florite at 37°C.
Differential scanning calorimetry (DSC)

Figure (5) shows the DSC thermograms of SES, Florite, their physical mixture, adsorbates and co-adsorbates. There were no peaks in the thermogram of Florite\textsuperscript{29}. The thermogram of SES-Florite systems showed complete disappearance of the drug peaks in both physical and loaded mixtures. This finding indicates that the drug was transformed to the amorphous state. Similar results were obtained with co-adsorbates (Fig. 5). In both cases there were no new peaks appeared indicating the compatibility of SES with Florite.

![Fig. 5: Thermograms of SES (A), FLR (B), SES-Florite 1:1 physical mixture (C), 1:5 physical mixtures (D), 1:1 adsorbates (E), 1:5 adsorbates (F), 1:1:1 co-adsorbates (G), and 1:1:5 co-adsorbates (H).](image)

Infra-red spectroscopy (IR)

The IR spectra of SES, Florite, their physical and loaded mixtures (adsorbates) are shown in Figure 6. The spectrum of Florite is characterized by a complete absence of IR absorption bands (trace B) due to the inorganic nature of Florite. The physical mixtures of SES and Florite showed great reduction in the intensity of the drug absorption bands (Traces C, D and E). The loaded adsorbates showed more reduction in the intensity of the drug bands (Trace F).

![Fig. 6: IR spectra of SES, Florite, SES-Florite physical mixtures, and adsorbates. SES (A), Florite (B), SES-Florite 1:1 physical mixture (C), SES-Florite 1:5 physical mixture, SES-Florite 1:1 adsorbate (E), SES-Florite 1:5 adsorbate (F).](image)

The IR spectra of SES co-adsorbates are shown in Figure 7. It was observed that the intensity of the drug bands was greatly reduced. In addition, the drug band at 1634 cm\textsuperscript{-1} appeared as a forked band at 1634 and 1614 cm\textsuperscript{-1}. 

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Fig. 7: IR spectra of SES, Florite, SES-Florite-Tween 80 co-adsorbates. SES (A), Florite (B), SES-Florite-Tween 80 1:1:1 co-adsorbate (C), 1:1:5 co-adsorbate (D), 1:3:5 co-adsorbate (E).

X-ray diffraction

The x-ray diffraction patterns of SES-Florite systems are shown in Figure 8. It is clear that Florite has a characteristic sharp diffraction peak at 28.8 2θ value (Trace b). The diffraction patterns of 1:1 drug : Florite physical or loaded systems (adsorbates) revealed the disappearance of the diffraction peaks of the drug with the appearance of a new peak at 21.6 2θ value. The interesting feature in the Figure is the disappearance of the Florite characteristic peaks. The disappearance of the drug characteristic diffraction peaks indicates that the drug transferred from the crystalline state to the amorphous state. However, the appearance of the new diffraction peak could not be explained.

Fig. 8: X-Ray diffractograms of SES, Florite, and SES-Florite physical mixture and adsorbates. SES (A), Florite (B), SES-Florite 1:1 physical mixture (C), 1:1 adsorbate (D), 1:5 adsorbate (E).

Dissolution study

Figures 9 and 10 show the dissolution profiles of silibinin adsorbates in 0.1 N HCl and phosphate buffer pH 7.4. It is clear that there is a remarkable improvement of drug dissolution as the concentration of Florite increases. This finding may be attributed to the increase in the surface area of the adsorbent available to interact (adsorb) with the drug. In addition, using the same ratio of Florite, loaded systems showed higher dissolution than the physical mixtures. These results indicate that adsorbates formation is more efficient to increase drug dissolution than physical
mixtures. The dissolution of the drug was found to increase as the pH of the medium increase as the drug is a weak acid.

The preparation of co-adsorbates has been used to enhance drug dissolution as it gathers the solubilizing effect of the surfactant to the exposed surface area of the adsorbent\(^{20,30}\). The technique depends on the adsorption of a surfactant on the surface of the adsorbent. By this way, it was assumed that surfactant may form different types of aggregates such as admicelles or bi-layers depending on the nature of both surfactant and the adsorbent. For this reason this process was called ad-solubilization\(^{31}\). Surfactant aggregates may incorporate drug molecules which otherwise would not adsorb spontaneously onto the solid/water interface. Various drugs were treated using this technique\(^{30,32,33}\).

Figures (11&12) illustrate the dissolution profiles of SES co-adsorbates with Tween 80 in distilled water, 0.1 N HCl and phosphate buffer pH (7.4). From the figures it is clear that co-adsorbates exhibited more pronounced improvement of the drug dissolution rate compared to adsorbates. This may be due to the dual effect of these systems, the high solubilizing effect of the surfactant plus the extremely large surface area provided by the adsorbent.

The dissolution rate increased as the concentration of surfactant increased, this can be attributed to the incorporation of the hydrophobic drug molecules in the cores of the surfactant’s admicelles\(^{30}\).
Hepatoprotective effect

Serum AST (aspartate aminotransferase) and ALT (alanine aminotransferase) levels drastically increased after acute intoxication with paracetamol. Although AST and ALT levels remain higher after 48 hrs of treatment hrs in the intoxicated animals (Table 3), the levels of enzymatic activity obtained after silibinin treatment are near the control.

The writhing test

The percentage response (relative to paracetamol solution) after the administration of the test formulations (paracetamol alone and paracetamol physical mixture with co-adsorbed SES) is listed in Table 4. The co-adsorbate preparation gave a comparable analgesic response to paracetamol mucilage. There was no significance difference in the response pattern of the two treatments, p > 0.05. These results indicate that co-administration of paracetamol with the fast soluble SES (as a co-adsorbate) does not affect the analgesic effect of paracetamol. This combination may have therapeutic value for hepatic patients who seek analgesic effect.
Table 3: Effect of various Silibinin pre-treatments on the serum activity of: (A) alanine amino-transferase (ALT), and (B) aspartate amino-transferase (AST). Determinations were performed after 48 hrs of paracetamol intoxication. Each determination represents the mean ± SD.

<table>
<thead>
<tr>
<th>Types of pre-treatment (Group number)</th>
<th>Serum level (IU/L) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT</td>
</tr>
<tr>
<td>None (Group I)</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>None (Group II)</td>
<td>311 ± 42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Silibinin (Group III)</td>
<td>136 ± 19&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SES (Group IV)</td>
<td>151 ± 28&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>SES 1:3:5 co-adsorbate (Group V)</td>
<td>109 ± 22&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: significantly different from group I (P<0.001)
<sup>b</sup>: significantly different from group II (P<0.001)
<sup>c</sup>: significantly different from group III (P<0.05)
<sup>d</sup>: significantly different from group IV (P<0.05)

Table 4: Percentage response of paracetamol aqueous solution and paracetamol: SES co-adsorbate physical mixture (1:1 w/w ratio) at different time points following their oral administration to rats.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Response ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paracetamol Solution (Standard)</td>
</tr>
<tr>
<td>15</td>
<td>67 ±5.7</td>
</tr>
<tr>
<td>30</td>
<td>81 ±6.4</td>
</tr>
<tr>
<td>45</td>
<td>89 ±7.3</td>
</tr>
<tr>
<td>60</td>
<td>87 ±7.2</td>
</tr>
<tr>
<td>75</td>
<td>84 ±5.8</td>
</tr>
<tr>
<td>90</td>
<td>82 ±6.5</td>
</tr>
<tr>
<td>120</td>
<td>78 ±5.9</td>
</tr>
<tr>
<td>180</td>
<td>62±5.6</td>
</tr>
</tbody>
</table>
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