CHAPTER 8

A RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED DRUG INTERACTION STUDY OF MENOACT450 IN HEALTHY, ADULT, FEMALE, HUMAN SUBJECTS

8.1 INTRODUCTION

Although *in vitro* models permit rapid screening tools for interactions, and can be used to guide expensive *in vivo* studies; the use of *in vitro* tests has some limitations, and therefore, may not always be reliable. First, it is not always possible to know the concentration of an interacting compound and its primary metabolites in specific tissues. Second, the demonstration of an *in vitro* effect does not tell whether the effect is likely to occur in clinical practice, that is, the clinical significance of an *in vitro* interaction is unknown. Third, there could be a contribution by some physiological factors like plasma protein binding, hepatic blood flow and presystemic extraction to the development of a drug interaction that is unaccounted for by *in vitro* studies alone [207].

Probe-substrates/inhibitors can be used to explore the effects of herbs on the activity of specific CYP enzymes *in vivo*. Serum and/or urinary metabolite/parent ratios obtained at specific time points after oral administration provide good clearance estimates for various CYP substrates, including midazolam or simvastatin (CYP3A4), caffeine (CYP1A2), chlorzoxazone (CYP2E1) and dextromethorphan (CYP2D6). The probe-drug clearance can also be assessed simultaneously by a ‘cocktail’ approach, which has become a widely accepted methodology for evaluating drug-drug interactions. Recently, a rapid and reliable *in vivo* screening method that utilizes single-time point phenotypic metabolic ratios was described for identification of botanical supplements capable of modulating CYP activity [208]. However, the value of the cocktail approach
may be limited by marked intra-individual variability and the possibility of interaction between the co-administered probes. For example, it has been reported that chlorzoxazone significantly altered the pharmacokinetics of oral CYP3A4 substrates [209].

Predicting the clinical significance of pharmacokinetic drug interactions is sometimes difficult, especially for drugs for which there are no robust methods to quantify effects or adverse effects. In the US, efforts have recently been made by the FDA and the Pharmaceutical Research and Manufacturers of America (PhRMA) to establish some general guidelines to help drug companies, prescribers and patients interpret the clinical significance of drug interactions [165, 210].

These are based on clinical experience of some well-known drug interactions, such as the inhibition of CYP3A4. For this interaction, it could be shown that the benzodiazepine midazolam or HMG-COA inhibitors simvastatin is a reproducible probe that allows quantitative determination of the interaction potential of an enzyme inhibitor. The degree of interaction can be measured in the form of an increase in the AUC of the midazolam or simvastatin serum concentrations. AUC changes that range from 2- to 5-fold, which occur after simvastatin co-administration with erythromycin, diltiazem, fluconazole, verapamil, large portions of grapefruit juice, and cimetidine, are classified as ‘moderate’. Changes that exceed a 5-fold increase in the simvastatin AUC are labelled as ‘strong’. Examples of strong midazolam interactions include ketoconazole, itraconazole, mibefradil, clarithromycin and nefazodone. This FDA proposed classification system for the clinical significance of drug interactions determined from in vivo studies is identical to that presented by industry [165].

In vivo studies are important for several reasons. First, they take into account all the biological and physiological factors that are not usually considered by the in vitro models and may influence the outcome of such studies. Second, in vivo studies can test for induction as well as inhibition whereas most of the in vitro studies assess only inhibition. A drug that both induces and inhibits a specific CYP enzyme may have offsetting effects in vivo which would not be apparent in many in vitro studies. For example, although the direct effects of ritonavir (a potent CYP3A4 inhibitor) on alprazolam (a CYP3A4 substrate) [211] metabolism were not studied in vitro, it was anticipated that ritonavir would increase alprazolam exposure in vivo [212]. However, in a drug
interaction study, a multiple dose regiment of ritonavir (500 mg every 12 hours) decreased the AUC of alprazolam by only 12 %, and produced no significant changes in its psychomotor or sedation tests. Since ritonavir auto induces CYP3A and may also induce CYP1A2, 2C9, 2C19, and glucuronyltransferase, it is possible that induction of drug metabolizing enzymes by the multiple dose regimen of ritonavir in the clinical study offset any CYP3A4 inhibition of alprazolam [213]. Third, *in vivo* studies can incorporate pharmacodynamic measures to assess the potential clinical significance of a change in the level of the affected drug. For example, *in vivo* studies testing the effect of a drug on the clearance of warfarin could also include measuring the clotting time [165, 214].

While a host of clinical studies have evaluated the interaction potential of botanicals on CYP3A4 substrates [4, 215-224], fewer have evaluated the effect of botanical supplementation on human CYP2D6 activity *in vivo* [103, 225], and most of these have focused only on St. John’s wort. Interestingly, CYP2D6 does not appear to be readily inducible; [226, 227] therefore, herb-mediated effects on this enzyme are likely to be limited to either inhibition or no effect at all.

We therefore hypothesized that co-administration of MenoAct450, as an inhibitor of the microsomal CYP3A4 enzyme system, could affect the elimination rate of Simvastatin (sensitive CYP3A4 probe substrate). The present clinical study was designed to assess the CYP3A4 mediated potential pharmacokinetic interaction by evaluating the simvastatin plasma concentration vs time profiles after co-administration with MenoAct450.
8.2 MATERIALS AND METHODS

8.2.1 Study protocol

The protocol was approved by the ethics committee of the SRM Medical College Hospital and Research Centre, SRM University, Kattankulathur (476/IEC/2013) and conducted in accordance with the declaration of Helsinki in its revised edition, the Guidelines of Good Clinical Practice (CPMP/ICH/135/95) and the Directives 2001/20/EC and 2005/28/EC and with International and local regulatory requirements. Subjects consented to participating in the study in writing after a full explanation of the study had been given. This study was registered in the clinical trial registry of India (CTRI/2014/02/004406).

8.2.2 Study Participants

12 female subjects were included if they were healthy nonsmokers aged 25 to 50, with a body mass index of 18 to 29, inclusive, with negative tests for alcohol and drugs of abuse. Subjects were excluded if they had any clinically significant history or presence of a cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, or neurologic disease/disorder. Subjects also were excluded if they had a psychological, psychiatric, or metabolic disorder (including eating disorders) or if they had experienced any acute illness within 4 weeks. Female subjects were excluded if they were pregnant (positive test for serum human chorionic gonadotropin at screening or check-in), breast feeding, or planning to conceive a child within 30 days of cessation of treatment. The subjects were forbidden to use any medications or herbal products for 15 days before and during the study. Drugs known to cause enzyme induction or inhibition and grapefruit juice were not allowed for 30 days before the study. Consumption of coffee, tea, alcohol, and cola drinks were not allowed during the study days.

8.2.3 Inclusion Criteria

- Healthy female volunteers of 18 to 45 years (both years inclusive) and body weight > 50 kg.
- Willing to give informed written consent and complies with the study requirements.
- Subject should be able to communicate effectively.
- Non-smokers or individuals who smoke less than 5 cigarettes per day
- Teetotalers or individuals who drink less than 60 ml of high-alcohol-content liquor or 120 ml of low-alcohol-content liquor
- Body Mass Index (BMI) between 18.50 and 24.99 Kg/m2
- Healthy individuals as evaluated by personal history, medical history and general clinical examination.
- Vital parameters - BP should be within the range of 100 – 139 mmHg systolic and 60 – 89 mmHg diastolic. Pulse rate should be within the range of 60 – 100 / min. Oral temperature between 97.8° F and 99.0 ° F. Respiratory rate should be within the range of 14-18/min.
- Normal biochemical, hematological and urinary parameters.
- Normal chest X-ray PA view and ECG in 12 leads.
- Negative test for HIV 1 and 2, Hepatitis B, Hepatitis C and Syphilis tests.
- Negative urine test for drugs of abuse for Morphine, Barbiturates, Benzodiazepines, Amphetamine, THC & Cocaine (to be performed on the day of check in).
- Negative alcohol breath analysis (to be performed on the day of check in).

8.2.4 Exclusion Criteria
- Subjects incapable of understanding the informed consent.
- History of any major surgical procedure in the past 3 months.
- History of diabetes mellitus, tuberculosis and systemic hypertension.
- Pregnancy and lactating women.
- History suggestive of cardiac, gastrointestinal, respiratory, hepatic, renal, endocrine, neurological, metabolic, psychiatric or hematological systems, judged to be clinically significant.
- History of dysphagia.
- History of any medical disorder that is of significance in the investigator’s opinion.
- Present or past history of drug abuse.
- History of hypersensitivity to study formulation.
- History of allergy to vegetables and / or food substances and / or any other manifestations suggestive of hypersensitivity reactions.
- Present or past history of intake of drugs which potentially modify kinetics / dynamics of study medication or any other medication judged to be clinically significant by the investigator.
- Consumption of grapefruit / its products within 48 hours prior to the start of study.
- Intake of any prescription drug within 14 days or over-the counter (OTC) drugs within 7 days prior to study and / or intake of any drug in the past, which could affect the kinetics or dynamics of Simvastatin, in view of investigator.
- Subject with clinically significant abnormal values of laboratory parameters.
- Subject who had participated in any other clinical trial during the last 3 months.
- Subject who had bled in the past 3 months from the date of start of study either for blood donation or for any other reason.
- History of habituation to coffee, tea or other xanthine containing products and inability to withhold the intake during the - in house - stay.
- Drugs that can potentially affect the hepatic metabolism of other drugs are as listed below (not limited to the list, though)
  - Hepatic microsomal enzyme inducers (which can reduces the systemic bioavailability):- Barbiturates, Carbamazepine, Ethanol (chronic), Inhalational anaesthetics, Griseofulvin, Phenytoin, Primidone, Rifampicin
  - Hepatic microsomal enzyme inhibitors (which can increases the systemic bioavailability):- Amiodarone, Cimetidine, Albendazole, Dextropropoxyphene, Ethanol (acute), Etomidate, Erythromycin, Fluconazole, Ketoconazole, Metronidazole.

### 8.2.5 Study Design

The study was a double-blind, randomized, placebo controlled, single dose (Simvastatin) and multiple-dose (MenoAct450) drug-herb interaction study. The selected subjects were randomized into placebo or MenoAct450 formulation (500 mg b.i.d) for 7 days in double blind fashion (Figure 8.1). On 8th day each group was received 40 mg single dose Simvastatin (ZOCOR® 40 mg, Mankind Pharmaceuticals Pvt. Ltd, India).
As per the schedule, the selected volunteers were requested for overnight fasting on those date. As soon as the volunteers assembled, Venflon was inserted by vein puncture into the forearm vein of each volunteer before the drug administration and 5ml of blood was withdrawn ($T_0$). Then, the Simvastatin tablet was administered with 200 ml of water to the volunteers in sitting position and the volunteers were asked to be seated for 3 hours after administration of the drug. A standardized warm meal was served 3 hours and a standardized light meal was served 7 hours after Simvastatin intake. All subjects were monitored for safety during the study. Adverse events with special attention to signs of drowsiness either observed by the investigator or reported spontaneously by the subjects were recorded throughout the study.

Figure 8.1 Flow chart of the clinical pharmacokinetic drug interaction study
8.2.6 Blood Samplings

Blood samples were taken at the following time points after Simvastatin dose administration at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24th hour after the administration of the drug (Including pre-dose, total 13 samples, each 3 ml). All the samples were collected by trained personnel using intravenous cannula placed in a forearm / arm vein using pre-labeled vacutainers containing K3EDTA (Becton, Dickinson and company, USA) at the scheduled time points. Blood samples collected during the study was centrifuged at 4000 rpm for 10 minutes at 4 ± 2°C (5430R, Eppendorf, Germany). Plasma was separated into single aliquot and stored at about -80°C deep freezer (Thermo Fisher Scientific, USA).

8.2.7 Bioanalytical methods

Simvastatin and simvastatin hydroxy acid with Lovastatin as internal standard were determined in plasma by validated Liquid Chromatography–Mass Spectrometry (LC-MS) method. The LC-MS 2020 system (shimadzu, Japan) was equipped with LC10ADVP binary pump, photo diode array detector (PDA), a Prominence – 7725i injection valve (USA) with a sample loop of 20uL and Phenomenex-Luna (Torrance, CA, USA), RP C18 column (i.d. 250 X 4.6 mm, 5µm particle size) was used as stationary phase for separation. The MS compartment consist of single quadrapole mass spectrometer with electrospray ionization (ESI) source and nitrogen gas was used to assist nebulization with a flow rate of 1.5 L/min. The temperature was set for curved desolvation line (CDL) and heat block at 2800 C and 3200 C. All the data were collected and processed by Lab Solution Software (Version 7.1, Shimadzu). The chromatographic separation was achieved by mobile phase consist of [Methanol: Water: Ammonium acetate (5mM), 90:10:0.1 v/v] by isocratic elution with a flow rate of 0.8 mL/min and detector was set at 238 nm with the temperature (Column Oven) of 30°C.

8.2.8 Determination of Simvastatin and Simvastatin hydroxy acid

50 µl internal standard (Lovastatin) was added to tubes containing 200 µl of plasma. After a thorough vortex mixing for 30 s, mixtures were extracted with 2 ml of acetonitrile, vortex-mixed for 3 min, and centrifuged at 4000 rpm for 10 min. 1.5 ml of organic layer was removed to another centrifuge tube and evaporated under a stream of nitrogen gas in the thermostatically controlled water-bath maintained at 40°C until
completely dry (Figure 8.2). The dried residue obtained was dissolved in 100 µl of methanol, vortex-mixed for 2 min, centrifuged at 4000 rpm for 10 min, and 20 µl of the supernatant liquid was injected into the LC-MS system.

8.2.9 Pharmacokinetic Analysis

Simvastatin and Simvastatin hydroxy acid were calculated for each subject when Simvastatin was dosed with placebo and in combination with MenoAct450 formulation by applying a non-compartmental method using WinNonlin professional version 5.0.1 (Pharsight Corp., Mountain View, CA). The key Pharmacokinetic parameters calculated for Simvastatin and Simvastatin hydroxy acid included $C_{\text{max}}$ (the maximum observed concentration), $T_{\text{max}}$ (the time at which $C_{\text{max}}$ occurs), $T_{1/2}$ (the elimination half-life),
AUC \((0-\infty)\) (the area under concentration-time curve extrapolated to infinity) and MRT (Mean Residence Time).

### 8.2.10 Statistical Analysis

The results were presented as the mean ± standard deviation (Mean ± SD). All the data were analyzed by one-way ANOVA followed by paired t-test. A difference was considered significant at \(p<0.05\). GraphPad prism Version 5.01 (GraphPad Prism Software Inc., USA) software was used for statistics.

### 8.3 RESULTS AND DISCUSSION

#### 8.3.1 Drug safety and tolerability

MenoAct450 was well tolerated throughout the study when co-administered with Simvastatin. No serious adverse events occurred. The only adverse events reported by more than one subject during the treatment period was nausea and abdominal discomfort. These events were reported and documented more commonly by subjects during the periods when MenoAct450 was administered with placebo or in combination with Simvastatin. All other mean laboratory parameters for serum chemistry, hematology and urinalysis remained within reference range. There were no remarkable findings in the vital signs, ECGs, physical examinations, visual acuity tests, and slit-lamp examinations in this study. None of the adverse events resulted in discontinuation from the study.

#### 8.3.2 Pharmacokinetics of Simvastatin and Simvastatin hydroxy acid

LC-MS chromatograms of standard Simvastatin, plasma spiked Simvastatin and from the subject plasma samples were shown in Figure 8.3 & 8.4. The main pharmacokinetic parameters of Simvastatin and its hydroxy acid metabolite for the 12 female subjects were determined by non-compartmental model analyses, are listed in Tables 8.1 and 8.2. The mean and standard deviation for each parameter are given for the two groups in which Simvastatin was administered. Figures 8.5 and 8.6 showed the mean plasma concentrations of Simvastatin and its metabolite (Simvastatin hydroxy acid), respectively. Maximal Simvastatin levels were observed in placebo group after 1.32 ± 0.44 hr and 1.92 ± 0.26 hr in MenoAct450 group.
Maximal Simvastatin hydroxy acid levels were observed in placebo group after 1.28 ± 0.66 hr and 1.51 ± 0.24 hr in MenoAct450 group. The peak plasma concentration of Simvastatin and Simvastatin hydroxy acid in placebo group was 17.51 ± 2.65 ng/ml and 12.58 ± 0.88 ng/ml respectively. The peak plasma concentration of Simvastatin (18.44 ± 1.56 ng/ml) and Simvastatin hydroxy acid (14.11 ± 2.12 ng/ml) in MenoAct450 group was slightly higher than placebo group.

The purpose of this study was to assess the effect of multiple doses of MenoAct450 formulation on the pharmacokinetics of Simvastatin, a substrate of CYP3A4. Simvastatin was chosen as an *in vivo* probe drug for this study because its pharmacokinetics are very sensitive to inhibition of CYP3A4 [228].
Elevated $C_{\text{max}}$, $T_{\text{max}}$ and AUC showed that Simvastatin with co-administration of MenoAct450 herbal formulation was expected to increase the pharmacokinetic parameters of CYP3A4 substrate. Co-administration of Simvastatin with multiple doses of polyherbal formulation has increased Simvastatin and Simvastatin hydroxy acid AUC without altering the elimination $T_{\text{max}}$, $T_{1/2}$ of Simvastatin but the difference was statistically insignificant (Figure 8.7 and 8.8). Previous studies confirmed that prolonged garlic oil supplements (500 mg, three times daily for 28 days) inhibited human CYP2E1 activity in both young and elderly adults by 40% and 25%, respectively. No significant effects were observed in the pharmacokinetic of Simvastatin or Pravastatin. Hence, no modulatory effects were noted for CYP1A2, CYP2D6, or CYP3A4 [229, 230].
Table 8.1 Pharmacokinetics parameters of Simvastatin with placebo and in combination with MenoAct450 formulation

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Simvastatin + Placebo</th>
<th>Simvastatin + MenoAct450</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hrs)</td>
<td>1.32±0.44</td>
<td>1.92±0.26</td>
<td>0.250</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>17.51±2.65</td>
<td>18.44±1.56</td>
<td>0.174</td>
</tr>
<tr>
<td>AUC (0-∞)</td>
<td>59.16±1.42</td>
<td>61.44±0.88</td>
<td>0.867</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (hrs)</td>
<td>5.74±0.79</td>
<td>5.77±0.88</td>
<td>0.342</td>
</tr>
<tr>
<td>MRT</td>
<td>8.68±1.32</td>
<td>8.16±2.66</td>
<td>0.508</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SD. A difference was considered significant at p<0.05

Figure 8.5 Mean plasma concentration time-profiles of Simvastatin after a single oral dose of 40 mg Simvastatin with placebo and after pretreatment with 500 mg MenoAct450 b.i.d. orally for 7 days.
Several prospective human trials have investigated the effects of *Panax ginseng* and *Panax quinquefolius* supplements on human drug disposition. Warfarin was examined the most. Results of the clinical trials were divided in two categories: no effect or mild induction of CYP2C9 substrate [231]. Gurley et al., 2005 [232] first observed that *Hydrastis canadensis* supplements significantly inhibited CYP2D6 and CYP3A4 in healthy volunteers. In subsequent investigations with the CYP3A4 substrate Midazolam, Gurley et al. further demonstrated that 14 days of *Hydrastis canadensis* supplementation (~ 209 mg isoquinoline alkaloids daily) significantly increased midazolam AUC, C<sub>max</sub>, and elimination half-life. The effects were similar to those noted for clarithromycin (1000 mg daily), a well-recognized inhibitor. Using the CYP3A probe midazolam, Gorski et al., 2004 [233] concluded that 8 days of *Echinacea purpurea* supplementation selectively modulated CYP3A activity in the intestine (inhibition) and liver (induction) of healthy volunteers. Mild inhibitory effects were observed for CYP1A2 and CYP2C9, while CYP2D6 was unaffected. Treatment at healthy adults with supplements with *Echinacea purpurea* for either 14 (800 mg extract daily) or 30 days (1500 mg extract daily) produced no clinically significant changes in CYP phenotypes [234].

**Table 8.2** Pharmacokinetics parameters of Simvastatin hydroxy acid with placebo and in combination with MenoAct450 formulation

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Simvastatin hydroxy acid + Placebo</th>
<th>Simvastatin Hydroxy acid + MenoAct450</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hrs)</td>
<td>1.28±0.66</td>
<td>1.51±0.24&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.327</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>12.58±0.88</td>
<td>14.11±2.12&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.142</td>
</tr>
<tr>
<td>AUC (0-∞)</td>
<td>48.75±2.21</td>
<td>50.44±2.05&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.688</td>
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<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (hrs)</td>
<td>4.21±1.76</td>
<td>4.98±2.67&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.176</td>
</tr>
<tr>
<td>MRT</td>
<td>6.05±1.06</td>
<td>7.77±2.08&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.759</td>
</tr>
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Values are expressed in Mean ± SD. A difference was considered significant at p<0.05.
Figure 8.6 Mean plasma concentration time-profiles of Simvastatin hydroxy acid after a single oral dose of 40 mg Simvastatin with placebo and after pretreatment with 500 mg MenoAct450 b.i.d. orally for 7 days.

Figure 8.7 Comparison of $AUC_{(0-\infty)}$ of Simvastatin with placebo group (White column) and Simvastatin with MenoAct450 group (Black column)
However, by comparing to standard regimens of Clarithromycin (500 mg daily for 7 days) or Rifampicin (600 mg daily for 7 days), *Cimicifuga racemosa* supplement (40–80 mg extract daily for 14 days) produced no demonstrable effects on digoxin and Midazolam pharmacokinetics. These findings suggest that, *Cimicifuga racemosa* is not a potent modulator of human CYP3A4 activity *in vivo* [235].

![Figure 8.8](image)

**Figure 8.8** Comparison of AUC \((0-\infty)\) of Simvastatin hydroxy acid with placebo group (White column) and Simvastatin hydroxy acid with MenoAct450 group (Black column).

Elevated C\(_{\text{max}}\), T\(_{\text{max}}\) and AUC showed that Simvastatin with co-administration of MenoAct450 herbal formulation was expected to increase the pharmacokinetic parameters of CYP3A4 substrate. Co-administration of Simvastatin with multiple doses of polyherbal formulation has increased Simvastatin and Simvastatin hydroxy acid AUC without altering the elimination T\(_{\text{max}}\), T\(_{1/2}\) of Simvastatin but the difference was statistically insignificant. We have demonstrated that pretreatment with 2 capsule of 500 mg MenoAct450 b.i.d or placebo 500mg b.i.d for 7 days in 12 normal volunteers does not significantly change the mean pharmacokinetic parameters (C\(_{\text{max}}\), AUC\(_{0-\infty}\), T\(_{\text{max}}\), T\(_{1/2}\) and MRT) of 40 mg of oral Simvastatin. This could be explained that, no clinical effect of MenoAct450 on pharmacokinetics of Simvastatin in our study.