Clinical Pharmacokinetic Drug Interaction Potential of MenoAct851 in Adult, Female Healthy Volunteers

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ABSTRACT

Background: MenoAct851 (Varanasi BioResearch Pvt. Ltd., Varanasi, India) is a patented polyherbal formulation developed to manage menopause symptoms that can be taken along with other allopathic medicines.

Objective: The present study aims to evaluate the drug interaction potential of MenoAct851 to inhibit cytochrome (CY) P450 in vitro in rats, and to measure its effects on simvastatin pharmacokinetic parameters in healthy human volunteers.

Methods: CYP450-carbon monoxide assay of MenoAct851 was performed in rat liver microsomes to calculate the percentage inhibition. Fluorometric assays of CYP3A4 and CYP2D6 determined half maximal inhibitory concentration value. A double-blind, randomized, placebo-controlled drug interaction study of MenoAct851 was conducted in 24 healthy adult female volunteers aged 25 to 50 years. The selected volunteers were randomized to receive placebo or MenoAct851 500 mg BID PO for 14 days. On the 15th day, each group received 40 mg single-dose simvastatin. Blood samples were drawn at different intervals to measure simvastatin pharmacokinetic parameters.

Results: The mean (SD) CYP450 concentration of the diluted microsome sample was calculated and found to be 0.405 (0.12) nmol/mg. The inhibitory potential of MenoAct851 (41.16% [1.24%]) was found to be less than ketoconazole. Half maximal inhibitory concentration values of MenoAct851 on CYP3A4 and CYP2D6 were 11.96 (1.04) μg/mL and 15.24 (0.58) μg/mL, respectively, but they were higher than respective positive controls. There was no statistically significant difference between MenoAct851 and placebo groups concerning the pharmacokinetic parameters such as C_{max}, T_{max}, $t_{1/2}$, and mean residence time of simvastatin; however, AUC showed a significant difference ($P < 0.05$) between the groups.

Conclusions: MenoAct851 produced weaker interaction potential with CYP3A4 and CYP2D6 substrates based on in vitro assays, but the findings of clinical pharmacokinetic analysis indicate that MenoAct851 increased the AUC of simvastatin and simvastatin hydroxy acid. Therefore, coadministration of MenoAct851 might lead to drug-herb interaction, thereby affecting the therapeutic effect of CYP3A4 substrates. (*Curr Ther Res Clin Exp.* 2020; 81:XXX–XXX)

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Introduction

Transition to menopause is associated with a period of increased risk for problematic symptoms, including hot flashes, night sweats, sleep disturbances, sexual dysfunction, mood disorders, weight gain, and cognitive declines.[1] Hormone therapy is the first-line management for menopause symptoms. However, many women choose not to use it because of the adverse effects of hormone therapy.[2] Thus, some investigators/physicians attempt to substitute hormone therapy with polyherbal formulations (PHFs). PHFs have been used all around the world due to their medicinal and therapeutic applications.[3] Hence, PHFs (eg, MenoAct851, Varanasi BioResearch Pvt. Ltd., Varanasi, India), which contains freeze-dried hydroalcoholic extracts of *Dioscorea bulbifera* tubers, *Terminalia arjuna* bark, *Bambusa arundinacea* leaves, and *Withania somnifera* roots were developed. The rationale behind this product is that a combination of herbs may act on multiple targets simultaneously to provide relief superior to what is possible with maximal doses of a single herb. *Dioscorea* sp have an abundance of diosgenin—a steroidal saponin and a precursor in the synthesis of corticosteroids, estrogen, contraceptives, and spironolactone that reduces the incidence of cardiovascular complications in menopausal women.[4] *T arjuna* is reported to promote effective cardiac functioning, regulate blood pressure, and treat osteoporosis and other bone-related disorders because it can improve the synthesis and secretion of female hormones.[5] *B arundinacea* contains phytoestrogens that bind to estrogen receptors. Patients who consume such a supplement can compensate for their estrogen paucity because phytoestrogens are reported to alleviate postmenopause symptoms.[6,7] Stress is a significant cause of menopause symptoms, leading to mood disorders such as anxiety and depression.[8] In an 8-week, randomized, double-blind, placebo-controlled study, *W somnifera* was associated with greater reductions in anxiety, morning cortisol, C-reactive protein, pulse rate, and blood pressure in chronically stressed adults when compared with the placebo.[9]

PHFs use is more common in developing countries because of easy accessibility, availability, affordability, and societal acceptability [10]; however, when the PHFs are administered in combination with allopathic drugs, this increases the potential of herb-drug interactions.[11] Drug metabolism inhibition/induction is a common cause of clinically important pharmacokinetic herb-drug interactions.[12,13] However, enzyme induction is usually less important than inhibition of cytochrome (CY) P450s, because enzyme inhibition can cause rapidly elevated drug levels in the blood, leading to excessive toxicity.

The MenoAct851 formulation could increase the potential for an herb-drug interaction when it is coadministered with an allopathic medication if the herbal product affects the allopathic drug's metabolism. CYP450 enzymes play a significant role in the Phase I oxidative metabolism of a wide variety of exogenous chemicals, including drugs; carcinogens; toxins; and endogenous compounds such as steroids, fatty acids, and prostaglandins. Isoforms of CYP450 such as CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP1A2 are primarily responsible for metabolizing a majority of the drugs used to treat human beings.[14,15] Fifty percent of drugs are metabolized by CYP3A4, followed by 25% and 20% by CYP2D6 and CYP2C family enzymes, respectively. Existing knowledge also indicates that herbal formulations must not be taken at the same time as other drugs that are substrates of CYP450 isoforms. Therefore, we hypothesized that coadministration of MenoAct851, an inhibitor of the microsomal CYP3A4 enzyme system, could affect the elimination rate of simvastatin-sensitive CYP3A4 probe substrates. The present study was designed to assess the potential drug interaction of MenoAct851 by in vitro high throughput fluorometric assay, and by evaluating the changes of pharmacokinetic parameters of simvastatin in adult, healthy female volunteers when administered placebo versus MenoAct851.

Materials and Methods

PHF

MenoAct851is a novel PHF developed at the National Facility for Tribal and Herbal Medicine, Banaras Hindu University, Varanasi, India, in collaboration with Interdisciplinary Institute of Indian System of Medicine and SRM Institute of Science and Technology, Chennai, India, for managing menopause symptoms. The formulation was put into capsule doses from freeze-dried, hydro-alcoholic *D bulbifera* tuber extracts, *T arjuna* bark, *B arundinacea* leaves, and *W somnifera* roots through a memorandum of understanding with a traditional health practitioner using high ethical standards.[16] The phytochemistry lab of the Interdisciplinary Institute of Indian System of Medicine, SRM Institute of Science and Technology, carried out the quality control analyses. The acute, short-term toxicity, pharmacological profile in animals, and clinical studies were conducted to establish the safety and efficacy of MenoAct851 (patent No. 851/DEL/2007 and dossier No. T.12016/26/2010-DCC AYUSH).[17,18]

Chemicals

All of the chemicals and solvents for the preparation of rat liver microsomes and CYP450-carbon monoxide complex assay were of analytical grade (potassium chloride [catalog No. P9333], and sodium hydrosulfite [catalog No. 71699]), and purchased from Sigma-Aldrich, Mumbai, India. Vivid CYP450 Screening Kit and Vivid Substrates were purchased from Invitrogen Drug Discovery Solutions (Waltham, Massachusetts). Vivid CYP3A4 Red (catalog No. P2856) and Vivid CYP2D6 Blue (catalog No. P2972) screening kits included baculosome respective isozymes and nicotinamide adenine dinucleotide phosphate-P450 reductase; regeneration system glucose-6-phosphate; glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide phosphate were used for the study. Ninety-six well black, flat-bottom, polystyrene, untreated microplate were obtained from Corning (Corning, New York). Ketoconazole was obtained as a gift sample from M/s Micro Labs Pvt Ltd, Hosur, Tamil Nadu, India, and quinidine was obtained as a gift sample from M/s Trigenesis Life Sciences Pvt Ltd, Bangalore, Karnataka, India. LC-MS grade methanol (catalog No. 34860-2.5L-R) and ammonium acetate (catalog No. V800034) were used for the pharmacokinetic parameters study. The probe drug, simvastatin, and the internal standard, lovastatin, were obtained from Mankind Pharmaceuticals Pvt Ltd, Bangalore, India.

CYP450-carbon monoxide complex assay

Liver microsomes were isolated from male Wistar rats weighing 200 to 250 g, based on the method described by Pandit et al [14] by the Institutional Animal Ethical Committee (approval No. IAEC151/2015), SRM College of Pharmacy, SRM Institute of Science and Technology approved the experimental protocol. Based on the method described by Vijayakumar et al,[19] screening the inhibitory activity of MenoAct851was performed with pooled rat liver microsomes in 96-well microplates. The following is the formula used to calculate the concentration of CYP450:

$$CYP450\ mM) = \frac{\Delta APC - \Delta AP}{91}$$

ΔA_{PC} is the absorbance difference of the sample kept in the incubator (PC sample) and ΔA_P is the absorbance difference of the

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sample kept at room temperature (P sample). The percentage inhibition is calculated using the following formula:

$$\% \ inhibition = \frac{Blank - Test}{Blank} \times 100$$

High throughput fluorometric assay of CYP3A4 and CYP2D6

High throughput screening assays were performed in black 96-well microplates. Fluorescence readings were obtained on BioTek-FLx 800 fluorescence microplate reader (BioTek, Winooski, Vermont) using appropriate excitation/emission wavelength (λ). The assay was performed according to a protocol provided by Invitrogen Drug Discovery Solutions. All measurements were performed in triplicate. Product formation from the fluorogenic probes was determined from the fluorescence data at 7 different concentrations of the inhibitors and tests. The following formula determines the half-maximal inhibitory concentration (IC_{50}):

$$\% \ inhibition = \left(1 - \frac{RFU \ in \ presence \ of \ test \ compound}{RFU \ in \ absence \ of \ test \ compound}\right) \times 100$$

where RFU = relative fluorescence units.

Clinical study protocol

SRM Medical College Hospital and Research Centre and SRM Institute of Science and Technology, Kattankulathur ethics committees approved the protocol (approval No. 476/IEC/2013) and the study was conducted in accordance with the Declaration of Helsinki in its revised edition, the Guidelines of Good Clinical Practice (CPMP/ICH/135/95), and directives 2001/20/EC and 2005/28/EC and with international and local regulatory requirements. Following a full explanation of the study, volunteers provided written consent to participate. Volunteers were compensated per Institutional Ethics Committee (IEC) guidelines.[20] This study is registered in the clinical trial registry of India (CTRI/2014/02/004406).

Study participants

Twenty-four female volunteers who were healthy, nonsmokers, had undergone the tubal ligation method of contraception, tested negative for alcohol and drug abuse, aged between 25 and 50 years, and had a body mass index of 18 to 29, were included in this study. Volunteers 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. A volunteer also was excluded if she had a psychological; psychiatric; or metabolic disorder, including eating disorders; or if she had experienced any acute illness within the previous 4 weeks. Female volunteers were excluded if they were pregnant (positive test for serum human chorionic gonadotropin at screening or check-in), breastfeeding, or planning to conceive a child within 30 days of treatment cessation. The volunteers were forbidden from using any medications or herbal products for 15 days before and during the study. Drugs that are known to cause enzyme induction or inhibition and grapefruit juice were not allowed for the 30 days leading up to the study. Consuming coffee, tea, alcohol, and cola drinks were not allowed during the study. Nicotine use was assessed by the Fagerstrom test for nicotine dependence.[21]

Study design and procedure

The study was a double-blind, randomized, placebo-controlled, single-dose simvastatin and multiple-dose MenoAct851 drug-herb interaction study. The selected volunteers were randomized to placebo (inert substitute for a treatment or intervention) or MenoAct851 formulation (500 mg BID for 14 days in double-blind fashion) groups. Double-blinded treatments were allocated using sequentially numbered drug containers. The randomization code was concealed to avoid selection bias, which secured the trial's blinding codes. On day 15 of the trial, each group was received a 40 mg single-dose simvastatin. Per the schedule, the selected volunteers fasted overnight on those days. As soon as the volunteers assembled, Venflon Paul Medical Systems, Chennai, India was inserted by vein puncture into each volunteer's forearm vein before the drug administration, and 5 mL blood was drawn at T_0. Next, the simvastatin tablet was administered to the volunteers, with 200 mL water. They were seated and instructed to remain seated for 3 hours after receiving the drug. A warm meal was served after the 3 hours and a light meal was served 7 hours after taking simvastatin. All volunteers 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 volunteers were recorded throughout the study.

Sample collection

Following the administered dose of simvastatin, blood samples were taken at the following intervals: 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after the administration of the drug, including predose (total of 13 samples, each 3 mL). All of the samples were collected by trained personnel using an intravenous cannula placed in a forearm/arm vein using prelabeled vacutainers containing K_3EDTA (Becton, Dickinson, and Company, Franklin Lakes, New Jersey) at the scheduled time points. Blood samples collected during the study were centrifuged at 4000 rpm for 10 minutes at 4°C ($\pm$2°C) 5430R Centrifuge, (Eppendorf, Germany). Plasma was separated into single aliquots and stored at approximately –80°C.

Bioanalytical methods

Simvastatin and simvastatin hydroxy acid with lovastatin as an internal standard were determined in plasma by validated LC-MS method.[22,23] The LC-MS 2020 system (Shimadzu, Kyoto, Japan) was equipped with LC10ADVP binary pump, photodiode array detector, a Prominence-7725i Spinco Biotech Pvt Ltd, Chennai, India, injection valve with a sample loop of 20 uL, and Phenomenex-Luna (Torrance, California) RP C18 column i.d. (250 × 4.6 mm, 5 μm particle size) was used as a stationary phase for separation. The MS compartment consisted of a single quadrapole mass spectrometer with electrospray ionization ESI source and nitrogen gas to assist nebulization with a flow rate of 1.5 L/min. The temperature was set for curved desolvation line and heat block at 2800°C and 3200°C. All data were collected and processed by Lab Solution Software version 7.1 (Shimadzu). The chromatographic separation was achieved by mobile phase, consisting of methanol:water:ammonium acetate 5 mM or 90:10:0.1 v/v by isocratic elution with a flow rate of 0.8 mL/min and the detector was set at 238 nm with the temperature column oven of 30°C.

Determination of simvastatin and simvastatin hydroxy acid

An amount of 50 μL internal standard lovastatin was added to tubes containing 200 μL plasma. After a thorough vortex mixing for 30 seconds, mixtures were extracted with 2 mL acetonitrile, vortex-mixed for 3 minutes, and centrifuged at 4000 rpm for 10 minutes. An amount of 1.5 mL of the 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. The dried residue obtained was dissolved in 100 μL methanol, vortex-mixed for 2 minutes,

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centrifuged at 4000 rpm for 10 minutes, and 20 μL of the supernatant liquid was injected into the LC-MS system. The created strategy was linear in the fixation scope of 0.100 to 74.626 ng/mL for simvastatin and 0.100 to 48.971 ng/mL for simvastatin hydroxy acid, with connection coefficient more prominent than 0.99 for both analytes. The approach demonstrated considerable reproducibility for all analytes, with intra- and interday precision with <5.6%, and intra- and interday precision of ±10.1% of nominal values. Quantitation was performed by multiple reaction monitoring mode of precursor–production transitions at m/z 435.2 → 281.2 for simvastatin, m/z 412.3 → 286.3 for simvastatin hydroxy acid at 150 ms per transition. Limit of quantitation was 0.24 ng/mL and 0.22 ng/mL for simvastatin and simvastatin hydroxy acid, respectively.

Pharmacokinetic parameter analysis

Simvastatin and simvastatin hydroxy acid concentrations were calculated for each volunteer when simvastatin was dosed with the placebo, and in combination with MenoAct851 formulation, a noncompartmental method was applied using WinNonlin professional version 5.0.1 (Pharsight Corp, Mountain View, California). The key pharmacokinetic parameters calculated for simvastatin and simvastatin hydroxy acid included C_{max}, the maximum observed concentration; T_{max}, the time at which C_{max} occurs; $t_{1/2}$, the elimination half-life; $AUC_{0-\infty}$, the AUC curve extrapolated to infinity; and mean residence time (MRT).

Statistical analysis

The results are presented as the mean (standard deviation). IC_{50} values concentration required to cause a 50% inhibition in enzyme activity were obtained using mean enzyme activity versus inhibitor concentration curves created in GraphPad prism version 5.01 (GraphPad Prism Software Inc, La Jolla, California). The difference in the C_{max}, T_{max}, $T t_{1/2}$, $AUC_{0-\infty}$, and MRT were analyzed by paired Student t test. $P < .05$ was considered statistically significant.

Results

Inhibition of CYP450 by MenoAct851

The mean (SD) concentration of protein in isolated rat liver microsome was 6.2 (0.81) mg/mL (n = 3). CYP450-carbon monoxide complex assay was used to assess the inhibitory potential of MenoAct851. The mean (SD) CYP450 concentration of the diluted microsome sample was calculated and was found to be 0.405 (0.12) nmol/mg protein (n = 3). Two different solvents ethanol and dimethyl sulfoxide were used for CYP450 inhibition assay to confirm the solvent effect.[24] The percentage inhibition of MenoAct851 on CYP450 showed a significant difference when compared to ketoconazole in ethanol (95% CI, 19.20–35.28; $P = 0.015$) and dimethyl sulfoxide (95% CI, 0.5609–11.87; $P = 0.042$. However, no significant difference was noted with quinidine in both solvents (Figure 1).

Determination of MenoAct851 inhibitory potency on CYP3A4 and CYP2D6

MenoAct851 and positive controls were assayed between concentrations ranging from 1.5 to 25 μg/mL. All samples were assayed in triplicate, the end point mode was selected, and IC_{50} values were calculated (Table 1). Concentration-dependent percentage inhibitions of the test compound on both isozymes were observed in Figure 2. Percentage inhibition of the samples on CYP3A4 and CYP2D6 increases with the rise of concentration. Mean (SD) IC_{50} values of MenoAct851 dissolved in dimethyl sulfoxide on CYP3A4
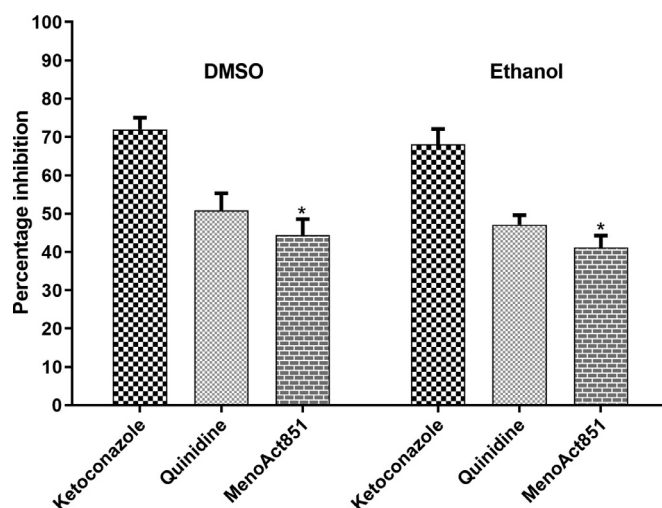

Figure 1. Percentage inhibition of MenoAct851 versus positive controls (n = 3). Values are expressed in mean (SD). ANOVA followed by Dunnett's multiple comparison test. DMSO = dimethyl sulfoxide. *Level of significance at $P < 0.05$.

Table 1
Half maximal inhibitory concentration (IC_{50}) value of positive controls and MenoAct851 (Varanasi BioResearch Pvt. Ltd., Varanasi, India).

Test sample	IC_{50} (μg/mL)*	
	Cytochrome P3A4	Cytochrome P2D6
MenoAct851	11.96 (1.04)	15.24 (0.58)
Ketoconazole	0.74 (0.66)	–
Quinidine	–	3.32 (1.42)

* Values are expressed as mean (SD).

and CYP2D6 were 11.96 (1.04) μg/mL and 15.24 (0.58) μg/mL, respectively. IC_{50} values of the positive controls were in good agreement with the literature.[24] In the high throughput fluorometric assay, MenoAct851 showed a weaker interaction potential on CYP3A4 and CYP2D6 when compared with ketoconazole and quinidine, respectively.

Pharmacokinetic parameters of simvastatin and simvastatin hydroxy acid

LC-MS chromatograms of standard simvastatin, plasma-spiked simvastatin, and the volunteers' plasma samples are shown in Figure 3. The main pharmacokinetic parameters of simvastatin and its hydroxy acid metabolite for the 24 female volunteers were determined by noncompartmental model analyses listed in Tables 2 and 3. The mean (SD) for each parameter was given for the 2 groups in which simvastatin was administered. The mean (SD) plasma concentrations of simvastatin, its metabolite simvastatin hydroxy acid, and internal standard lovastatin are shown in Figure 4. Maximal simvastatin levels were observed in the placebo group after 1.32 (0.44) hours and 1.92 (0.26) hours in MenoAct851 group (Figure 5). Maximal simvastatin hydroxy acid levels were observed in placebo group after 1.28 (0.66) hours and 1.51 (0.24) hours in MenoAct851 group (Figure 6). Simvastatin and simvastatin hydroxy acid's peak plasma concentration in the 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 MenoAct851 group was slightly higher than the placebo group.
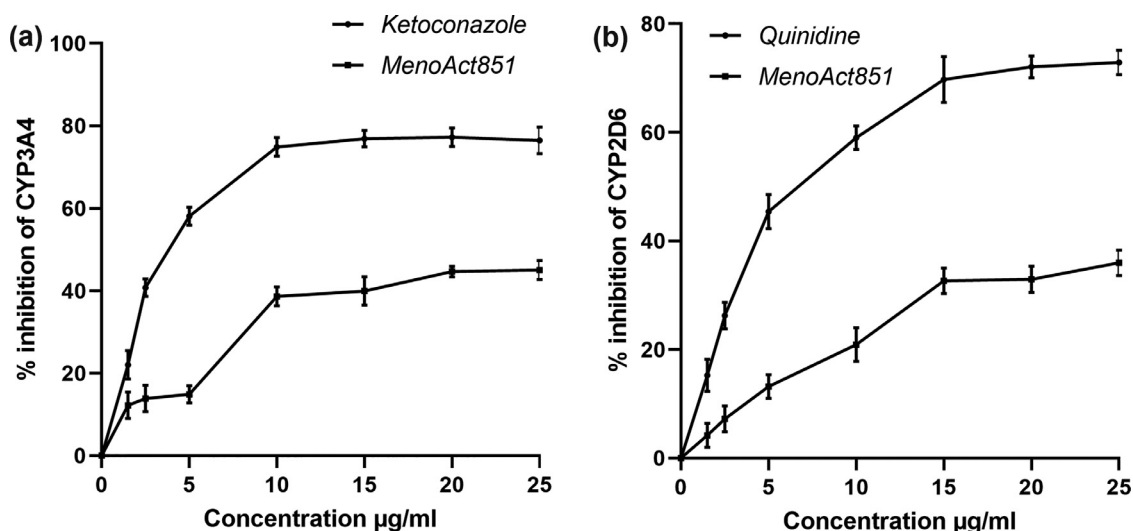
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Figure 2. Concentration dependent inhibitory effect of MenoAct851 and positive controls dimethyl sulfoxided (DMSO). (A) cytochrome (CY) P3A4. (B) CYP2D6. Values are expressed as mean (SD) (n = 3).
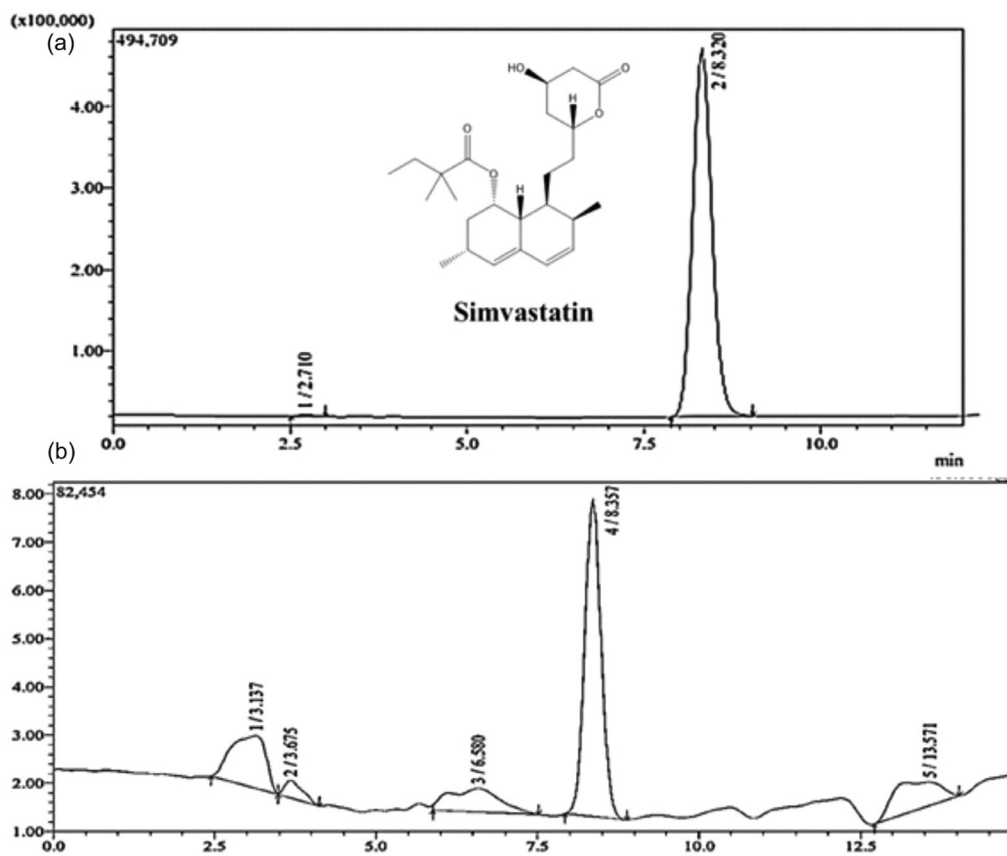

Figure 3. Chromatogram. (A) Standard simvastatin. (B) Blank plasma spiked with simvastatin. x-axis = runtime; y-axis = mV.

Table 2
Pharmacokinetic parameters of simvastatin with placebo and in combination with MenoAct851 (Varanasi BioResearch Pvt. Ltd., Varanasi, India) formulation.

| Pharmacokinetic parameter	Simvastatin + placebo*	Simvastatin + MenoAct851*	P value
T_{max}, h	1.32 (0.44)	1.92 (0.26)	0.250
C_{max}, ng/mL	17.51 (2.65)	18.44 (1.56)	0.174
$AUC_{0-\infty}$	56.16 (1.42)	65.44 (0.88)	0.0417[†]
$t_{1/2}$, h	5.74 (0.79)	5.77 (0.88)	0.342
Mean residence time	8.68 (1.32)	8.16 (2.66)	0.508

* Values are expressed as mean (SD).
[†] A difference was considered significant at $P < 0.05$.

Table 3

Pharmacokinetic parameters of simvastatin hydroxy acid with placebo and in combination with MenoAct851 (Varanasi BioResearch Pvt. Ltd., Varanasi, India) formulation.

Pharmacokinetic parameter	Simvastatin hydroxy acid + placebo*	Simvastatic hydroxyl acid + MenoAct861*	P value
T_{max}, h	1.28 (0.66)	1.51 (0.24)	0.327
C_{max}, h	12.58 (0.88)	14.11 (2.12)	0.142
$AUC_{0-\infty}$	45.75 (2.21)	54.44 (2.05)	0.0422[†]
$t_{1/2}$, h	4.21 (1.76)	4.98 (2.67)	0.176
Mean residence time	6.05 (1.06)	7.77 (2.08)	0.759

* Values are expressed as mean (SD).
[†] A difference was considered significant at $P < 0.05$.
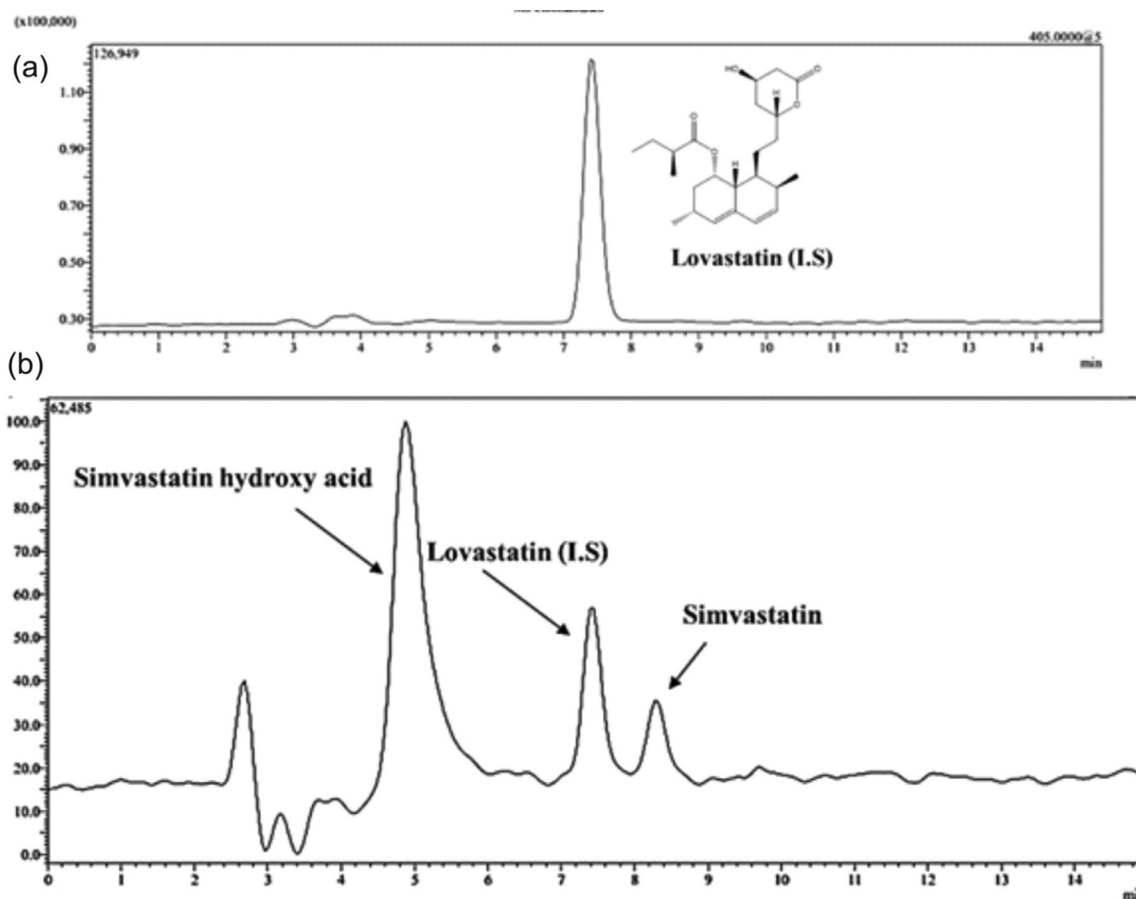
Figure 4. Chromatogram. (A) Internal standard lovastatin. (B) Plasma obtained from the volunteer received single oral dose of 40 mg simvastatin. *x*-axis = runtime; *y*-axis = mV.

Drug tolerability

MenoAct851 was well tolerated throughout the study and also when coadministered with simvastatin. No serious adverse events occurred. The only adverse events reported by more than 1 volunteer during MenoAct851 or placebo treatment period were nausea and abdominal discomfort. All mean laboratory parameters for serum chemistry, hematology, and urinalysis remained within reference range during their discharge. There were no remarkable findings in the vital signs, echocardiograms, physical examinations, visual acuity tests, and slit-lamp examinations in this study. None of the adverse events resulted in discontinuation from the study.

Discussion

Herb-drug interactions incidence was predicted to increase due to herbal medicines' worldwide popularity with the development of several herbal formulations with improved bioavailability. Numerous case studies have described harmful herb-drug interactions that could lead to morbidity or mortality.[25,26] Deciding whether herb-drug interactions occur based on data from CYP450-CO inhibition assays and in vitro studies is inadequate. Results from the above studies need to be evaluated further, using well-conducted clinical trials to validate their clinical significance. Hence, this study is designed to assess the effects of multiple doses of MenoAct851 on the pharmacokinetic parameters of simvastatin, a substrate of CYP3A4. Simvastatin was chosen as an in vivo probe drug for this study because its pharmacokinetic parameters is very sensitive to inhibition of CYP3A4.[27] Databases such as PubMed, Scopus, Medline, Cochrane Library, and ScienceDirect were scanned for literature containing articles published between 2008 and 2019. Keywords such as CYP450 mediated interaction, pharmacokinetic parameters of MenoAct851, drug-herb interactions etcetera, and found that this is the first report to evaluate the effect of MenoAct851 on simvastatin in female healthy adult volunteers.
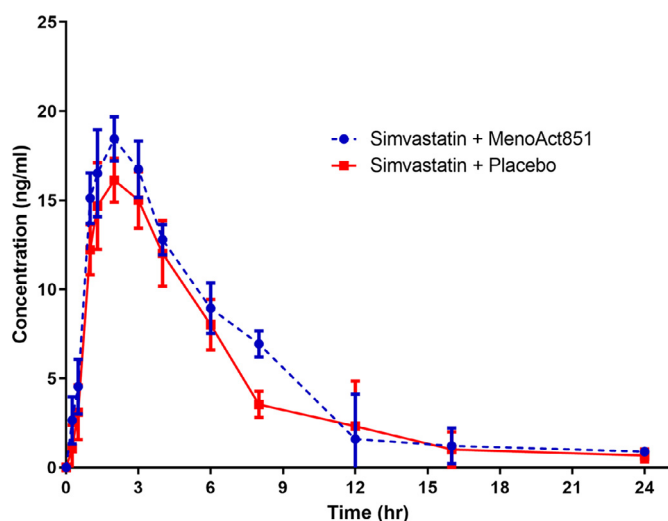
Figure 5. Mean plasma concentration time-profiles of simvastatin after a single oral dose of 40 mg simvastatin with placebo and after pretreatment with 500 mg MenoAct851 BID PO for 14 days.
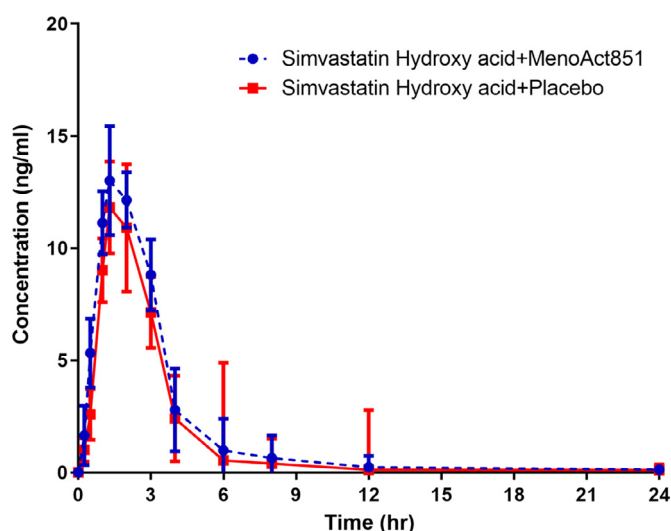

Figure 6. Mean plasma concentration time-profiles of simvastatin hydroxy acid after a single oral dose of 40 mg simvastatin with placebo and after pretreatment with 500 mg MenoAct851 BID PO for 14 days.

In the present study, an approach is made to evaluate the interaction potential of MenoAct851 on CYP450, through rat liver microsomes and high throughput fluorescence screening assays. Orally administered medicinal products were mostly metabolized by gut flora before being absorbed into the systematic circulation.[28] CYP450 had very significant role as a metabolizing enzyme that was involved in the biotransformation of substances taken by oral route, whereas the presystemic metabolism of these drugs took place before entering the liver cell.[13,29] Concentration dependent CYP450 inhibition was found in fluorescence screening assay by the MenoAct851 and standard positive controls.

There was no statistically significant difference between the MenoAct851 and the placebo groups with respect to the mean pharmacokinetic parameters like C_{max}, T_{max}, $t_{1/2}$, and MRT of simvastatin but AUC showed significant difference ($P < 0.05$) between the groups. Gurley et al [30] 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 [30] further demonstrate that 14 days of

H canadensis supplementation with ~209 mg isoquinoline alkaloids daily significantly increases midazolam AUC, C_{max}, and half-life elimination.

Study limitations

Lack of nicotine estimation in the volunteers was the limitation of the clinical pharmacokinetic parameters study. This study reports only the effects of MenoAct851 on CYP3A4 and CYP2D6 isoforms, the effects on other isoforms remains unknown. Further tests on other CY isoforms and transporters should be conducted to determine whether MenoAct851 has any additional effects on allopathic medicines' metabolism and pharmacokinetic parameters.

Conclusions

Coadministered, repeat 500-mg doses of MenoAct851 with a single 40-mg dose of simvastatin resulted in significant change in the plasma simvastatin and simvastatin hydroxy acid AUC, indicating an inhibitory effect of MenoAct851 on CYP3A4 activity. Thus, MenoAct851 is likely to affect the exposure of CYP3A4 substrates following coadministration. Accordingly, caution should be taken when MenoAct851 formulations are used in combination with allopathic drugs metabolized by CYP3A4.

Declaration of Competing Interest

Dr Govind Prasad Dubey holds the patent for MenoAct851. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

Author's contribution

All the authors contributed equally.

Acknowledgments

References

1. Cohen LS, Soares CN, Vitonis AF, Otto MW, Harlow BL. Risk for New Onset of Depression During the Menopausal Transition: The Harvard Study of Moods and Cycles. *Archives of General Psychiatry*. 2006;63:385–390.
2. Johnson A, Roberts L, Elkins G. *Complementary and Alternative Medicine for Menopause*. 2019;24 2515690X19829380.
3. Hu Z, Yang X, Ho PC, et al. Herb-drug interactions: a literature review. *Drugs*. 2005;65:1239–1282.
4. Vasanthi HR, Mukherjee S, Ray D, Jayachandran K, Lekli I, Das D. Protective role of air potato (Dioscorea bulbifera) of yam family in myocardial ischemic reperfusion injury. *Food & function*. 2010;1:278–283.
5. Nadkarni KM, Nadkarni AK. *Indian materia medica*. Popular Book Depot; 1955.
6. Jawaid T, Awasthi A, Kamal M. Estrogenic activity of a hydro-alcoholic extract of Bambusa arundinaceae leaves on female wistar rats. *Journal of advanced pharmaceutical technology & research*. 2015;6:19–24.
7. Patisaul HB, Jefferson W. The pros and cons of phytoestrogens. *Frontiers in neuroendocrinology*. 2010;31:400–419.
8. Dalal PK, Agarwal M. Postmenopausal syndrome. *Indian J Psychiatry*. 2015;57:S222–S232.
9. Auddy B, Hazra J, Mitra A, Abedon B, Ghosal S. A Standardized Withania Somnifera Extract Significantly Reduces Stress-Related Parameters in Chronically Stressed Humans: A Double-Blind, Randomized, Placebo-Controlled Study. *Journal of American Nutraceutical Association*. 2008;11:50–56.

10. Agrawal K, Goel D. *Herbal Use amongst Patients in a Tertiary Care Hospital*, 6, Pattern and Perceptions; 2016:129–131.

11. Fan J, Chen L, Lu X, Li M, Zhu L. The Pharmacokinetic Prediction of Cyclosporin A after Coadministration with Wuzhi Capsule. *AAPS PharmSciTech*. 2019;20:247.

12. Ilango K, Vijayakumar TM, Agrawal A, Dubey GP. Pharmacogenetic Comprised Prospect on Cytochrome P450 Facilitated Drug Interactions. *Journal of Bionanoscience*. 2013;7:127–139.

13. Mukherjee PK, Ponnusankar S, Pandit S, Hazam PK, Ahmmed M, Mukherjee K. Botanicals as medicinal food and their effects on drug metabolizing enzymes. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*. 2011;49:3142–3153.

14. Pandit S, Ponnusankar S, Bandyopadhyay A, Ota S, Mukherjee PK. Exploring the possible metabolism mediated interaction of Glycyrrhiza glabra extract with CYP3A4 and CYP2D6. *Phytotherapy research: PTR*. 2011;25:1429–1434.

15. Zhou SF, Xue CC, Yu XQ, Li C, Wang G. Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. *Therapeutic drug monitoring*. 2007;29:687–710.

16. *SCIENCES CCFRIA*. New Delhi: CCRAS; 2018:1–100.

17. Sandhiya S. *Prevention and Management of Neuropsychiatric and Cardiovascular Complications among Menopausal Women by a Polyherbal Formulation*. Kattankulathur: Department of Biotechnology, SRM Institute of Science and Technology; 2017 [Research Thesis].

18. Shukla A. Comparison of effect of raloxifene and herbal drug on BMD in postmenopausal CKD patients. *11th International Conference on Nephrology & Urology*; 2017:48.

19. Vijayakumar TM, Kumar RM, Agrawal A, Dubey GP, Ilango K. Comparative inhibitory potential of selected dietary bioactive polyphenols, phytosterols on CYP3A4 and CYP2D6 with fluorometric high-throughput screening. *Journal of food science and technology*. 2015;52:4537–4543.

20. . *Research DoH*. Participants NEGFBaHRIH. New Delhi: Ministry of Health; 2017.

21. Rustin TA. Assessing nicotine dependence. *American family physician*. 2000;62:579–584 591-572.

22. Yang H, Feng Y, Luan Y. Determination of Simvastatin in human plasma by liquid chromatography–mass spectrometry. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences.*. 2003;785:369–375.

23. Alakhali K.M. Method Validation for Analysis of Simvastatin in Human Plasma Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS). 2013;7:2739-2743.

24. Vijayakumar TM, Ilango K, Kumar RM, Agrawal A, Dubey GP. Effect of Dioscorea bulbifera and its Major Bioactive Compound, Diosgenin on CYP450 Mediated Drug Metabolism. *Journal of Biologically Active Products from Nature*. 2015;5:313–321.

25. Choi EJ, Park JB, Yoon KD, Bae SK. Evaluation of the in vitro/in vivo potential of five berries (bilberry, blueberry, cranberry, elderberry, and raspberry ketones) commonly used as herbal supplements to inhibit uridine diphospho-glucuronosyltransferase. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*. 2014;72:13–19.

26. Iwata H, Tezuka Y, Kadota S, Hiratsuka A, Watabe T. Mechanism-based inactivation of human liver microsomal CYP3A4 by rutaecarpine and limonin from Evodia fruit extract. *Drug metabolism and pharmacokinetics*. 2005;20:34–45.

27. Moron B, Verma AK, Das P, et al. CYP3A4-catalyzed simvastatin metabolism as a non-invasive marker of small intestinal health in celiac disease. *The American journal of gastroenterology*. 2013;108:1344–1351.

28. Ahmmed SM, Mukherjee PK, Bahadur S, et al. Interaction potential of Trigonella foenum graceum through cytochrome P450 mediated inhibition. *Indian journal of pharmacology*. 2015;47:530–534.

29. Harwansh RK, Mukherjee K, Bhadra S, et al. Cytochrome P450 inhibitory potential and RP-HPLC standardization of trikatu–a Rasayana from Indian Ayurveda. *Journal of ethnopharmacology*. 2014;153:674–681.

30. Gurley BJ, Gardner SF, Hubbard MA, et al. In vivo effects of goldenseal, kava kava, black cohosh, and valerian on human cytochrome P450 1A2, 2D6, 2E1, and 3A4/5 phenotypes. *Clinical pharmacology and therapeutics*. 2005;77:415–426.