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Pharmacokinetic Interaction Study for Simvastatin and Parsley and Its Plasma Quantification Using LC-MS: A Focus on Drug Metabolic Enzymes

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Abstract

Background: Parsley (*Petroselinum crispum* L) (PAR) is used widely as an antioxidant, antihyperlipidemic, diuretic, and medication to reduce hypertension. At the same time, conventional drugs such as Simvastatin (SV) are also used to manage hyperlipidemia in hypertensive patients. However, no studies have reported any interactions for the concomitant use of PAR and SV.

Objectives: The study aimed to evaluate the enzyme-inducing or inhibiting role of PAR for SV at the level of cytochrome P-450 metabolic enzyme.

Methods: This is an open-label (unblinded) study conducted with 24 healthy albino rats (4X6 = 24), at Imam Abdulrahman Bin Faisal University, Saudi Arabia, in the year 2018 with protocol approval No. IRB-2018-145-Pharm and approval date 18/04/2018. The animals were administered with a loading single oral dose of SV (80 mg), Clarithromycin (CLAR; 80 mg), Carbamazepine (CBZ; 80 mg), and PAR, i.e., dried herb powder (200 mg), in order to achieve a steady state concentration in the blood. Blood samples were collected at specified time points including pre-dose (0.0) and after-treatment, i.e., 0.5, 1, 1.5, 2, and 3 hours, and centrifuged. The plasma was extracted to analyze for SV by LC-MS using a previously developed method.

Results: The analysis of pharmacokinetic parameters revealed enhanced C_{max} (mean plasma maximum concentration) and $AUC_{0-\infty}$ (area under the concentration-time curve) as 2 and 2.2 folds, respectively, for SV+PAR ($P < 0.01$) as compared to SV alone. A decrease in CL/F (total-body clearance of drug from the plasma) for SV ($P < 0.01$) and an increased $t_{1/2}$ (mean half-life) from 3.20 to 6.12 hours for SV co-administered with PAR were observed.

Conclusions: Parsley combined with Simvastatin increased the C_{max} and AUCs for Simvastatin as 2 and 2.5 folds; thereby, the moderating enzyme-inhibiting role of Parsley for CYP 3A enzyme may be proposed. Hence, caution is required when administering Parsley with Simvastatin for any therapeutic purpose.

Keywords: Cytochrome P450, CYP3A4, Hyperlipidemia, Parsley, Pharmacokinetics, Simvastatin

1. Background

Human beings are dependent on plants not only as oxygen producers for breathing but also as sources of food, shelter, and medicine. That indigenous people decide to use which plant and how to use it is a cultural variation. People in remote areas depend mostly on plants as their basic element of food, as well as medicine for combating various diseases (1).

Petroselinum crispum L. (Umbelliferae) commonly known as Parsley (PAR) originates from Mediterranean regions (2). The plant has been reported to possess many phytochemicals; however, flavonoids are found to be more dominant in this plant (3). Similarly, carbohydrates such as D-glucose and apiose (4) and essential oils, i.e., apiol and myristicin are also present as the main active compounds (5), which are responsible for its antioxidant activity (6).

Parsley as a green herbaceous plant, apart from its usage as a vegetable and garnish, exhibits various potential pharmacological activities and it is used in traditional and conventional practice for many therapeutic purposes (7). The traditional uses include seeds for antimicrobial, an-

titussive and antispasmodic purposes (7-9), and leaf used for the treatment of skin disorders (10) whereas the whole plant is used to treat hypertension and diabetes (11, 12). In most of the cases, the patients use herbs along with conventional medications for a prompt and better outcome; however, there has been increasing evidence and clinical concern in terms of herb-drug interactions when used improperly (13). Few of such drug-herb interactions are related to warfarin; as reported in almost 50 drug-herb interaction studies (14), when it is co-administered with garlic (15), it results in bleeding in patients (16, 17) whereas its use along with *Panax ginseng* and ginger have been reported with a decrease in INR (18) and altered platelet aggregation (19), respectively. Similarly, serotonin syndrome has been reported in the case of St. John's wort (*Hypericum perforatum*) along with serotonin-reuptake inhibitors. Furthermore, the concomitant use of digoxin, cyclosporine, and theophylline with *Panax ginseng* in depressed patients using antidepressants will lead to mania. A high risk of hypertension is observed in patients using Yohimbine (*Pausinystalia yohimbe*) with tricyclic antidepressants (20).

Parsley also has some documented interactions. Since PAR has antidiabetic effects on pancreatic beta cells, it is used among Turkish diabetic people. Medicinal amounts of PAR should be used with moderate caution if it is combined with antidiabetic medications (like Glimepiride, Glyburide, Pioglitazone, Rosiglitazone, insulin, and so on) due to its additive effect on lowering blood glucose (21, 22). PAR extracts might prolong the action of some medications like paracetamol, aminopyrine, and pentobarbital (Nembutal), which may increase the effect and side effects of these agents (23). In addition, PAR acts as a "water pill" and too much amount of water may be lost if it used in combination with diuretic medications without caution (24).

Despite the numerous research studies in the literature, still, most of the plants lack information regarding proper use along with conventional medications. PAR is one of these plants, which has an unexplored mechanism of interaction when used along with SV.

2. Objectives

The current study aimed to investigate the nature of PAR as inducer or inhibitor for SV at the hepatic level. The study deals with the in vivo drug-herb interaction in an animal model to determine the AUC (area under the curve) and C_{max} (maximum plasma concentration) in animal plasma using LC/MS. Till date, no studies have reported such interactions, and it will be a first-time study to report the PAR and SV interaction in an animal model.

3. Methods

3.1. Chemical and Solvent Used

Simvastatin (CYP3A substrate) as a standard drug (Sigma-Aldrich Merck), CLAR (potent inhibitor for CYP3A enzyme), and CBZ (inducer for CYP3A enzyme) were prepared. PAR was used as a dried herb powder. HPLC-grade acetonitrile, ammonium acetate, ammonium formate, and methanol were purchased from Sigma-Aldrich (St Louis, MO). A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for the purification of deionized water.

3.2. Instruments and Equipment Used

We used an HPLC apparatus (LCMS-2020 Shimadzu, Japan) fitted with a degasser unit (DGU-20A3R), binary pumps (LC-20AD), autosampler (SIL-20AC HT), and a PDA detector. Moreover, column (Ultra C-18, Particle diameter 3μm, and dimensions 150 X 4.6 mm), sonicator (Bransonic® Ultrasonic cleaner 5510E-DTH, USA), centrifuge machine (Kubota Japan 400X; Model 4000; Frequency 50/60 Hz), Vortex mixer (Corning® LSE™ with standard tube head, Sigma-Aldrich Germany), balance (A & D weighing GR-200 lab balance, USA), and blender (Blender HGB660, 60 Hz, USA) were employed.

3.3. PAR Powder Preparation

The herb was purchased from the local market and identified by Dr. Rizwan Ahmad and Dr. Amir (Pharmacognosy department, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, Saudi Arabia). A voucher specimen (Voucher ID: IAU-PHG-P005) has been submitted to the natural products lab at Imam Abdulrahman Bin Faisal University, Saudi Arabia. The herb was made to spread over a large sheet of filter paper and kept for 15 days, to dry in shade, under controlled room temperature conditions. The dried herb was powdered with the help of a stainless steel blender and the powder, thus, obtained was properly stored in a refrigerator (2°C - 8°C) with controlled humidity.

3.4. Mass Spectrophotometer (MS) Conditions

MS with SIM (selected-ion monitoring) and Quadrupole operating conditions were as follows: high-resolution mass with positive (+ve) mode, scan time (1.0 minute), inter-scan delay (0.02 second).

3.5. High-Performance Liquid Chromatography (HPLC) Conditions

For bioanalysis, a previously developed and reported method by Jemal et al. (25) was reproduced. HPLC with binary solvent system, tunable mass detector, and column was used as mentioned earlier whereas chromatographic conditions for separation were as follows: degassed and sonicated solvents with isocratic mobile phase, i.e., acetonitrile (95%): 5 mM ammonium acetate (5% v/v), flow rate of 0.600 mL/min, injection volume of 10 μL/run, and a retention time of 5.022 min with a total chromatographic run time of 8.00 minutes (Figure 1A).

3.6. Venue and Year of Study

This study was performed at the Natural Products and Alternative Medicine laboratory at Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia, in the year 2018.

3.7. Study Design

The study was designed to compare in-vivo drug-herb interaction with CLAR, CBZ, and PAR and without the presence of interacting substances, i.e., SV alone. In addition, the study was open-label (unblinded), i.e., without pharmacodynamics endpoints, with a loading dose of the drug, i.e. maximum single dose for a steady-state concentration of the drug, and parallel design, i.e. SV alone, SV+PAR, SV+CLAR, and SV+CBZ administered to animals (26, 27).

3.8. Ethical Approval

A proper ethical approval was obtained from the Ethics Committee of Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia, which ensured that the ethical guidelines confirmed the National Guidelines on the Care and Use of Laboratory Animals, with protocol approval No. IRB-2018-145-Pharm and approval date 18/04/2018.

3.9. Study Population

One week before experiments, Wistar rats (weight: 200 - 250 g and age: 8 - 10 weeks) were maintained in an environment with controlled room temperature (25°C $\pm$ 2°C) and humidity (60% $\pm$ 5%) for a 12-hour dark-light cycle. In addition, standard pelleted diet and water were used to feed animals. Before experiments, the rats were kept on fasting overnight (26, 28).

3.10. Sample Size Calculation and Power of the Study

The power of a study intends to find an effect. Though it is kept mostly between 80 and 99%, the most acceptable power in many studies, as used here, is 80% (29). Similarly, a series of software is available to calculate the number of animals for a study such as G Power, Open Epi, Biomath, and StatPages; however, the calculation with the mentioned software needs various factors. These factors include the power of the study, the magnitude of response, expected attrition or death, type-I error, etc. As this study was conducted for the first time and no previous data (factors mentioned above) are available to be used for calculation through such a method, "resource equation" was applied in our study. This method is applicable in studies that are novel and have no previous reports regarding the factors needed for sample size calculation (29, 30). This method involves ANOVA and E value (degree of freedom) which must be between 10 and 20 for animals; that is it may not be less the 10 and can go above 20. The formula used is as follows:

$$E = Total\ number\ of\ animals - Total\ number\ of\ groups$$

Based on the formula, the number of animals required for this study was 20, which was the required sample size.

3.11. Choice of the Substrate and Interacting Drugs

The study focused on drugs associated with the P450 system of enzymes as either an inhibitor or an inducer. In order to assess the impact of the interacting drug, it is recommended to select very sensitive substrates for CYP P450 systems. Herein, the substances/drugs selected were entirely related to the CYP system. SV is considered as the best substrate for CYP3A4/CYP3A5 whereas the drugs with the potential to either inhibit or induce the metabolism for SV at the CYP level are CLAR and CBZ, respectively, as reported elsewhere (26).

3.12. Route of Administration

The route of administration is very important for any herb-drug interaction and mainly depends on the product-labeled method. In addition, the herbal products mostly available in the market are in the oral dosage form. Based on the availability of the route of administration for the test substance and substrate, the oral route was chosen (26).

3.13. Dose Selection and Sampling Time Point

Generally, it is preferred to have a maximum planned dose for finding an interaction. The animals were administered with an oral loading dose of PAR powder (200 mg),

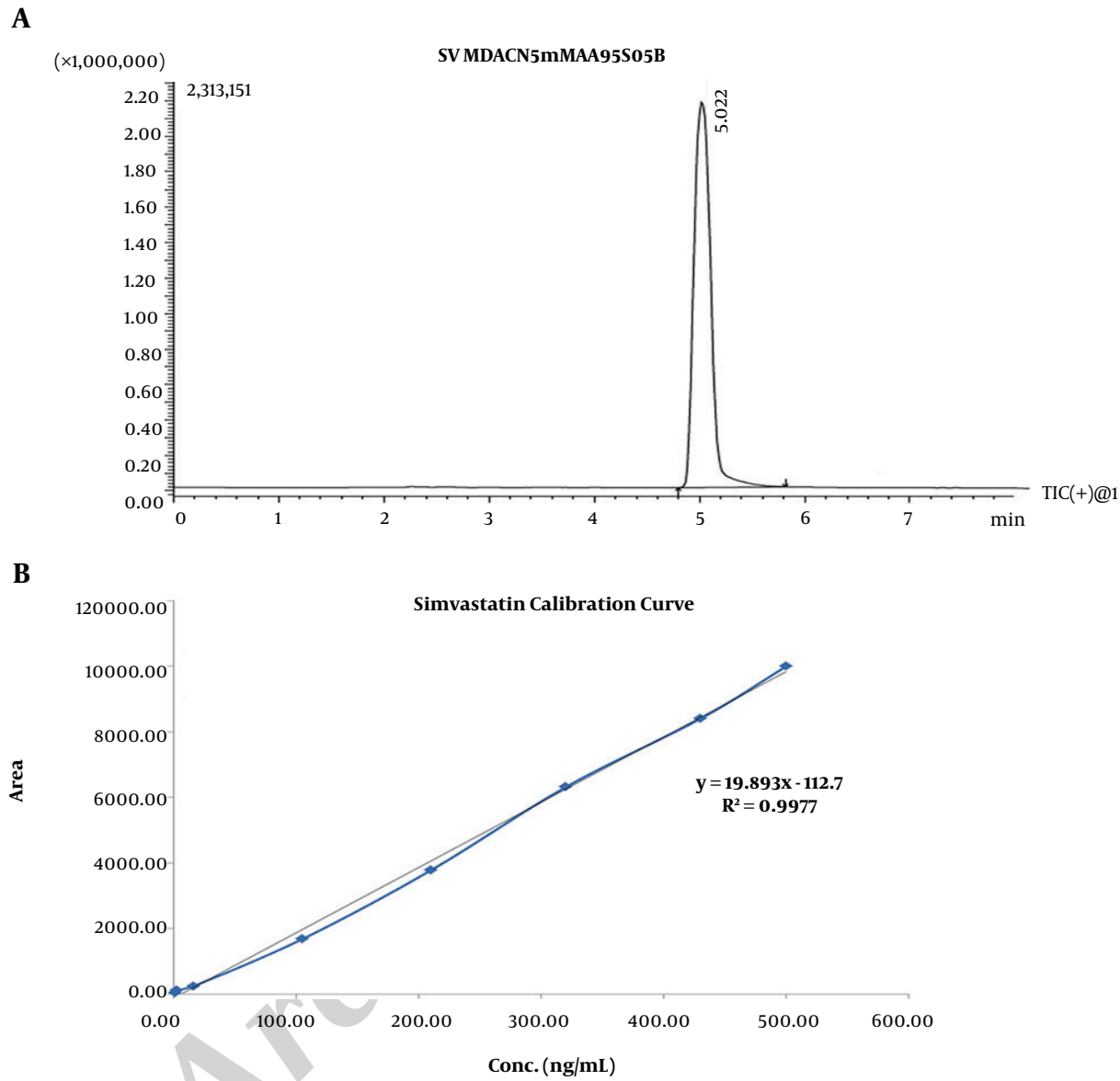

Figure 1. (A) Plasma Extracted SV, (B) calibration graph (1.0 - 500.0 ng/mL)

SV (80 mg), CLAR (80 mg), and CBZ (80 mg) in order to evaluate the inhibitive or inductive nature for PAR in described groups. The required drugs and PAR were dissolved in water and filtered. 2 mL of each drug (equal to 80 mg) and PAR (equal to 200 mg) were administered to respective groups. The blood samples (1 - 2 mL) were collected in proper vacutainer using a cardiac puncture subsequently for each specified time point, i.e. pre-dose (0.0) and after-treatment including 0.5, 1, 1.5, 2, and 3 hours, washed twice using normal saline solution and made free from adhering (31), followed by centrifugation (3500 rpm, 10 minutes), separation of plasma, and storage at -40°C in a deep freezer until further analysis (26, 28).

3.14. Experimental Protocol

Prior to pharmacokinetic studies, rats fasted for 12 hours. It is a kind of parallel study including groups of albino rats (six in each group with total number of animals up to 4 x 6 = 24) including G-I (SV only as the control group), G-II (SV + enzyme inducer), G-III (SV + enzyme inhibitor),

and G-IV (SV + PAR). The animals were held from the back in slanted position during oral administration/dosing.

3.15. Quality Control (QC) Sample and Standard Sample Preparation

For stock solution preparation, the required amount of SV was dissolved in acetonitrile to prepare a solution of 1 mg/mL and sonicated (20 minutes; 44 kHz/ 250W). For working solutions, water and acetonitrile (1:9) were used as a mixture in order to dilute the stock solution. For CC (calibration curve), eight-non-zero (A-H) concentrations were prepared from the standard solution as a 2% aqueous analyte (20 μL) was spiked with blank homogenate, i.e. rat plasma (980 μL), thus yielding different concentrations ranging from 1 to 500 ng/mL for SV (1, 2, 16, 105, 210, 320, 430, and 500 ng/mL). Four QC level samples were prepared as high-quality control (HQC; 400 ng/mL), middle-quality control (MQC; 200 ng/mL), low-quality control (LQC; 2.9 ng/mL), and LLOQC (1.01 ng/mL) (28). The storage at 2°C - 8°C was ensured for all solutions until use.

3.16. Sample Preparation Protocol for Plasma

For sample preparation, a 600 μL sample in a glass tube with 200 μL of 5% formic acid (for breaking plasma protein binding) was vortexed (300 rpm for 5 minutes), followed by addition of 5 mL of an extraction solution (ethyl acetate) in order to extract out the analyte. The glass tubes were placed in a centrifuge (400 rpm; 4°C) for 10 minutes, and the supernatant organic layer (4 mL) was poured off into a clean glass tube. The obtained supernatant was dried with the help of nitrogen stream (psi $\leq$ 20; 50°C $\pm$ 2.0°C), reconstituted in 600 μL of the respective mobile phase, and finally transferred (10 μL) to HPLC vials for injections (28).

3.17. Endpoint and Data Analysis

All the plasma samples were included in the pharmacokinetic data analysis. For plasma concentrations of SV, the following PK-parameters were determined: AUC (0 - t) (area under the concentration-time curve from time zero to t), $AUC_{(0-\infty)}$ (area under the concentration-time curve from time zero to infinity), C_{max} (maximum plasma drug concentration), T_{max} (time to reach maximum concentration following drug administration), $t_{1/2}$ (half-life), and elimination rate constant, in order to assess the effects of PAR herb drug powder, CLAR, and CBZ on the PK of SV. The results of drug interaction studies were reported with a 90% confidence interval (26, 28).

3.18. Statistical Analysis

The data were entered into IBM SPSS Statistical for Windows, Version 22.0 (IBM Corp., Armork, N.Y., USA). S.D (standard deviation), along with 90% CI and the P value of 0.01, was used to report the results of herb-drug interaction whereas, for inter-rat variations, a CV (coefficient of Variation, %) was applied. The results were analyzed and expressed as means $\pm$ standard error of the mean (SEM). The student's *t* test was applied for differences between unpaired observations using ANOVA (P value $<$ 0.05).

4. Results

4.1. Pharmacokinetics Endpoints Analysis

4.1.1. HPLC Analysis

The reproduced method showed a retention time (RT) of 5.02 minutes whereas the calibration curve for SV reproduced method showed a linearity in a concentration range of 1 - 500 ng/mL with least-squares regression $r^2 \geq 0.997$; also, accuracy and precision for SV calibration curve standards were within acceptable limits as per the 2001 FDA guidelines (28) as shown in Figure 1B.

4.1.2. MS Analysis

Mass spectrometry used for quantification showed a transition, i.e. RT at m/z 419.5, as shown in Figure 2.

4.2. Pharmacokinetics of SV

The main pharmacokinetic parameters for SV are listed in Figure 3 and Table 1. Figure 3 shows the mean plasma concentrations and $AUC_{0-\infty}$ for SV following the oral administration of SV alone and combined with PAR, an enzyme inducer (EI), and an enzyme inhibitor (EIn).

For SV, the co-administration with PAR resulted in increased C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ with a two-fold increase ($P < 0.01$) whereas the co-administration with CBZ resulted in a significant ($P < 0.01$) decrease in C_{max}. Similarly, the co-administration of SV and CLR showed an increased (> two folds) C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ for SV as shown in Table 1 and Figure 3 ($P < 0.01$).

The CV (coefficient of variation) showed an inter-rat variation for C_{max} and AUCs; however, the exact mechanism for this variability was not completely understood. It may be due to the variation of CYP3A4 activity among rats.

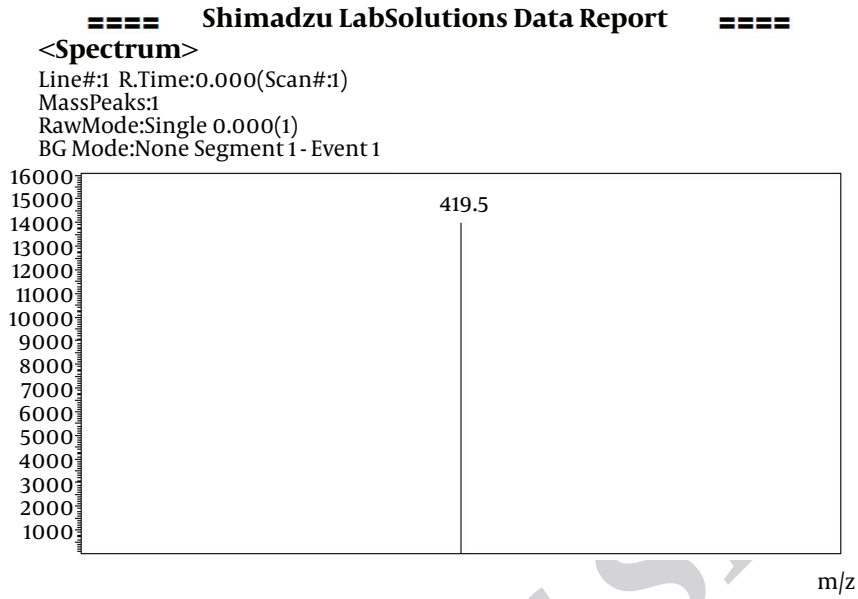

Figure 2. Mass spectra for SV at m/z 419.5 $[M+H]^+$

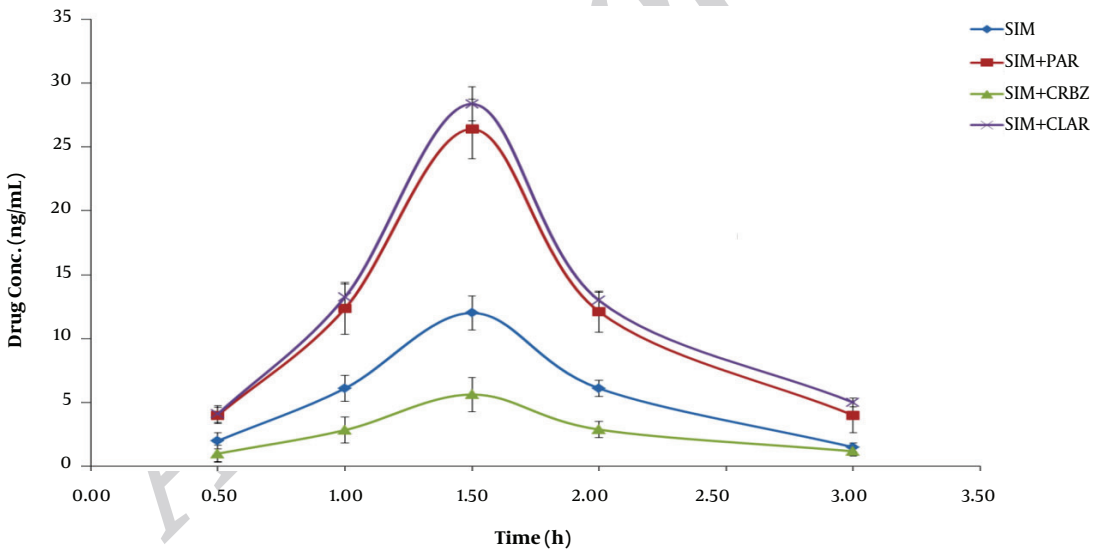

Figure 3. The PK profiles for plasma SV at different time intervals after oral administration in G-I (SV), G-II (SV + EI), G-III (SV + EIn), and G-IV (SV+ PAR)

5. Discussion

This study was performed to determine whether PAR could alter the pharmacokinetics of SV when given concomitantly in healthy albino rats. The study has an important implication in terms of outcome due to the potential interaction of PAR with the widespread use of SV in hypertensive patients who use SV for reducing lipid levels. Herein, for the first time, we report a practical interaction for PAR and SV at the level of the microsomal hepatic enzyme. This will help elucidate the theory for possible induction or inhibition between PAR and SV when used together. The study was conducted in strict compliance with USFDA (the United States food and drug administration) guidelines (28). The half-life of SV is low (two hours), and

Table 1. SV PK Parameters Following Oral Administration of SV Alone and in Combination with PAR, CBZ, and CLAR

PK- Parameters	SIM	SIM+PAR	SIM+ CRBZ	SIM+ CLAR
C_{max}, ng/mL	12.01 ± 1.33	26.39 ± 2.56	5.63 ± 1.03	28.36 ± 2.98
T_{max}, h	1.50	1.50	1.50	1.50
$t_{1/2}$	3.20 ± 0.69	6.12 ± 1.66	17.80 ± 3.69	16.23 ± 2.99
K_e, h^{-1}	0.2166	0.1132	0.0389	0.0427
AUC_{0-t}, ng h/mL	14.90 ± 2.59	31.46 ± 5.63	7.26 ± 1.89	34.08 ± 6.48
$AUC_{0-\infty}$, ng h/mL	21.87 ± 3.01	66.87 ± 9.56	37.81 ± 5.87	151.42 ± 15.64

thus, a loading single dose was administered in order to achieve a steady state concentration (26). Similarly, SV is considered the best substrate for CYP3A4 and A5; hence, the study focused on inducers and inhibitors for SV, which was a substrate for CYP3A4/A5. CLAR and CBZ are reported as the best inhibitor and inducer, respectively, for SV at CYP3A4/A5 enzyme level. The investigated drug here was PAR powder. PAR is widely used as a diuretic as mentioned in many studies in the literature (32, 33). However, no studies have reported herb-drug interaction for PAR in hypertensive patients using SV for lowering lipid levels. This is a first-time study of the interaction of PAR with SV at the level of CYP enzyme in the presence of inducers or inhibitors.

The literature reports a well-established route of degradation for SV, i.e., CYP450 (CYP3A4-mediated) (34, 35). However, in some instances, the drug may be reversibly converted to SV-hydroxy-acid through esterase enzyme. The current study revealed an increase in the mean values of C_{max} and AUC_{0-t} (> two folds), as well as an increase in the mean $t_{1/2}$ (from 3.20 to 6.12 hours), for SV when co-administered with PAR; hence, it indicates the inhibition of the primary mechanism of CYP3A4 responsible for biotransformation of SV to its respective metabolites. It may be concluded that in the presence of PAR, plasma levels of standard doses of drugs (mainly degraded by the CYP3A4 system) may increase (Table 1); hence, it may be considered as a CYP 3A4 inhibitor for SV. The labeling implications as per USFDA and drug interaction studies requirements (26) state any investigational drug when administered at the highest dose if increases the AUC for oral CYP3A4 substrate between two and five folds (> two and < five folds) is labeled to be "moderate inhibitor." Based on the assumption, PAR inhibited CYP3A4 and enhanced the AUC for SV by two folds; thus, PAR may be identified as a moderate inhibitor of CYP3A4.

It is quite true to understand the complex nature of herbs in such studies; however, numerous plant food constituents such as flavonoids (36), isothiocyanates (37), and allyl sulfides (38) have exhibited a potent role of CYP monooxygenases modulator, both in vitro and in animal models. Sometimes, the effects of such phytochemicals are complex as they may inhibit certain CYP enzymes at high concentrations and activate moderately the same enzyme at lower concentrations (39). Up to now, numerous compounds have been reported as competitive CYP inhibitors even at low concentrations that their actions may become pronounced at the CYP level when combined with other compounds/drugs (40). A similar CYP1A2 inhibitory activity for PAR has already been reported by Peterson et al. using apiaceous vegetables. Cytochrome P-450 1A2 is known as a biotransformation enzyme responsible for the activation of several pro-carcinogens, such as aflatoxin-B1 (41). Furanocoumarin derivatives present in PAR plant have been reported to inhibit the CYP3A4 system (42). The mentioned studies confirm and support the current observations for PAR, as an inhibitor at the CYP level for drugs including SV.

The design of the study allows only for the limited interpretation of results, i.e. the effects of PAR on the plasma level of SV, and no correlation or evidence was established to report the efficacy of SV in the presence of PAR in reducing the lipid levels. The main aim was to mark the inhibitory or inducing role of PAR for SV at the already established and reported CYP system. For more PK and PD outcomes as associated with the concomitant use of PAR and SV, more in-depth studies using cell culture systems and different enzyme models may be employed. Furthermore, the study is a preliminary study conducted in animal models and may not have the same inhibitory or inducing effects in humans as reported here. Thus, further clinical trials are needed to establish the interaction of PAR and SV in the view of inducing or inhibitory effect for each other. This will further confirm the role of PAR in patients using SV and dose adjustment, as well as the effect on the therapeutic value of SV when used together with PAR.

5.1. Conclusions

The co-administration of PAR herb powder at a steady state with SV significantly ($P < 0.01$) increases the exposure

(C_{max} and AUCs) to SV by more than two folds confirming the inhibitory role of PAR for CYP3A4-mediated biotransformation. PAR as a potential inhibitor reported in animal models may have a therapeutic application in case of avoiding drug interaction for patients using SV and PAR together. Therefore, caution and consultation with a physician are required when administering PAR in any kind of use for patients who take SV.

Footnotes

Authors' Contribution: Rizwan Ahmad and Niyaz Ahmad designed the study, searched the literature, and performed experimental work. Dhafer Mahdi Alshayban, Ali Hassan Mohammed Alkhalifah, Muntathir Ali Alamer, and Hamzah Almomatten were involved in experimental work and animal and analytical instruments handling. Rizwan Ahmad, Niyaz Ahmad, and Dhafer Mahdi Alshayban analyzed the data. Rizwan Ahmad and Niyaz Ahmad wrote the manuscript whereas Dhafer Mahdi Alshayban reviewed the manuscript for any grammatical or linguistic errors.

Conflict of Interests: The authors declare no conflict of interests.

Ethical Considerations: A proper ethical approval was obtained from the Ethics Committee of Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia, which ensured the confirmation of the ethical guidelines with the National Guidelines on the Care and Use of Laboratory Animals, with a protocol approval No., IRB-2018-145-Pharm and approval date 18/04/2018.

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