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Effect of Herbal Bio-enhancer (Piperine) on the Bioavailability and Pharmacokinetic of Simvastatin in Wistar rats.

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KEYWORDS

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ABSTRACT:

Simvastatin is a well-known lipid lowering drug. Because of its extensive presystemic metabolism, CYP3A4 exhibits low oral bioavailability. Piperine, an alkaloid isolated from black and long pepper, is known to inhibit CYP3A4 and therefore it is expected to improve the bioavailability of simvastatin. Based on this assumption, a pharmacokinetic study of simvastatin (10 mg/kg) alone and in combination with piperine (20 mg/kg) was performed in fasted Wistar rats on days 1 and 7 of repeat oral dose administration. Blood sampling was done at different time points up to 24 hours, and the plasma samples collected were analyzed on LC-MS/MS for the estimation of simvastatin. When given together with piperine, the plasma Cmax and AUC of simvastatin was 0.9 ng/mL and 11.4 ng·h/mL on day 1 and 21.6 ng/mL and 57.8 ng·h/mL on day 7, respectively. This Cmax and AUC were 1.4- and 2.9-fold higher on day 1 and 6.4- and 4.7-fold higher on day 7, respectively, compared to simvastatin alone. In the current study, piperine was found to significantly enhance the circulating levels of simvastatin. Therapeutically this interaction could be beneficial in terms of improving the efficacy and can have implication on toxicity. A clinical study can further help to investigate these aspects

Introduction

Oral medications that are efficacious need to have good bioavailability. Bioavailability is the term used to describe the portion of medication that enters the bloodstream and is accessible to produce its desired medical outcomes [1]. In simple terms, it measures how much of the drug is absorbed into the bloodstream and can be utilized by the body. Factors like poor aqueous solubility, low intestinal penetrability, efflux of drugs by intestinal transporters, first-pass effects, and interactions with food or concomitant medications can greatly influence the bioavailability of drugs taken orally [2]. It is therefore necessary to have knowledge of the drug's aqueous solubility, lipophilicity, or interaction with various transporters and metabolizing enzymes. In the intestine, several efflux transporters play an important role in the process of oral absorption and may greatly limit the permeability of drugs [1]. In some cases, the drugs may have good oral absorption but display low bioavailability due to extensive first-pass metabolism by the intestinal or hepatic enzymes. A common drug administered to people with cardiovascular disease to decrease cholesterol levels is simvastatin. This medication belongs to the statin class of drugs and functions by inhibiting 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, a liver-specific enzyme that causes the production of cholesterol [3]. However, despite its proven efficacy, simvastatin's bioavailability and pharmacokinetics can vary among individuals. In

Journal of Chemical Health Risks

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humans, simvastatin has shown poor oral bioavailability as a result of low aqueous solubility and extensive intestinal and hepatic first-pass metabolism [4]. It is also a P-gp substrate and an inhibitor. Several herbal products have been reported to increase the bioavailability of drugs that are either substrates of intestinal efflux transporters or CYP isoenzymes [5]. Piperine, an active ingredient present in black and long pepper, has also gained recognition as a bio-enhancer due to its ability to improve oral absorption. Piperine is reported to inhibit P-gp and CYP isoenzymes, which include CYP3A4.[6] It also helps to increase the blood flow towards the gastrointestinal tract, which aids in drug absorption through the intestine. Clinically, piperine has also been proven to be beneficial in a combination product of rifampicin and isoniazid, which is currently available in India. In this combination, piperine helped to increase the rifampicin absorption by 60%, thereby facilitating the lowering of the amount of rifampicin from 450 to 200 mg [7]. A similar approach to improving the bioavailability can be applied for drugs like simvastatin, which is a substrate of both P-gp and CYP3A4. [8] Therefore, in the present report, the pharmacokinetic interaction between simvastatin and piperine was investigated to explore the possibility of combining piperine with simvastatin so as to enhance the bioavailability of simvastatin.

Material and method

Chemicals

Piperine powder was purchased from Green Heaven India Pvt. Ltd., Nagpur. Simvastatin and atorvastatin were procured from the Wockhardt Research Centre, India. HPLC-grade methanol and acetonitrile for bioanalysis were bought from MERCK, India, and carboxymethyl cellulose was purchased from Sigma, US. Ammonium formate was received from Thermo Fisher Scientific, India. Simvastatin and piperine suspensions were prepared separately in carboxymethylcellulose (1% w/v) using a pestle and mortar for oral administration to rats.

Animals

Male Wistar rats (body weight: 220–250 g and age: 8–10 weeks) were obtained from the animal house facility of the Wockhardt Research Center, India. The Institute Animal Ethics Committee of the Wockhardt Research Center had approved the experimental protocol prior to the conduct of the study. Rats were kept in polypropylene cages that had a stainless steel top grill placed over the rice husk bedding. The environmental conditions were well controlled. The temperature in the room was maintained at 22 ± 2°C, while the percent of humidity varied between 40 -70 %. The recycled, filtered air was replaced about 20 times per hour. A 12-hour artificial day/night cycle (7 a.m. to 7 p.m. and vice versa) was maintained for at least 7 days. All the rats had free access to rodent feed except 12 hours before and during the experimental period, while drinking water was given *ad libitum*.

Experimental procedure

Twenty-four male Wistar rats were equally divided into two groups (n=12/group). Each group of 12 rats was divided into two sets of six rats each. Group 1 received simvastatin suspension orally at a dose of 10 mg/kg, and group 2 received both simvastatin and piperine suspension separately at a dose of 10 and 20 mg/kg, respectively, for the duration of 7 days. The dosing volume kept for both formulations was 2.5 mL/kg. The blood sampling for the pharmacokinetic analysis was performed on days 1 and 7 after the drug administration. From each group, blood samples were obtained via retro-orbital plexus in heparin tubes at 0.5, 2, 6, and 12 hours time point from 6 rats (set 1) and at 1, 4, 8, and 24 hours from the other 6 rats (set 2). The blood samples collected from six rats at each timepoint were centrifuged at 10,000 rpm for five minutes. The plasma samples thus obtained were equally pooled from two rats so as to get three samples per timepoint. The plasma samples were stored at -70°C until the samples were processed for bioanalysis on LC-MS/MS.

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Bioanalysis

A standard solution of 1 mg/ml of simvastatin was made in methanol and stored at 20 °C. With the help of this stock solution, linearity samples were prepared by serial dilutions in blank plasma to achieve concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.312, and 0.156 ng/mL. These

Journal of Chemical Health Risks

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JCHR (2024) 14(1), 581-588 | ISSN:2251-6727

plasma samples were processed using the acetonitrile precipitation technique. In brief, 50 µL of each of the linearity samples and blank plasma was transferred to labeled 1.5 mL eppendorf tubes. In these tubes, 400 µL of acetonitrile containing 25 ng/ml of internal standard (Atorvastatin) was added. In another separate eppendorf tube, 50 µL of blank plasma was processed with 400 µL of acetonitrile without an internal standard. Plasma samples collected during the pharmacokinetic study were also processed in a similar way. After the addition of acetonitrile, the linearity and study samples were mixed for 30 seconds and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was subsequently analyzed on LC-MS/MS for the estimation of simvastatin.

LC-MS/MS method

The LC-MS/MS system (AB SCIEX QTRAP 4500-LCMS), consisting of an HPLC unit (UFLC, Shimadzu) and a quadruple MS detector, was used to estimate simvastatin from the processed plasma samples. The mobile phase consisted of 10 mM ammonium formate and acetonitrile (10:90 v/v %). The mobile phase flow rate was adjusted to 1 mL/min, and the injection volume was kept at 1 µL. Chromatographic separation was carried out using a Zorbax SB C18 column measuring 75 x 4.6mm with a particle size of 3.5µm. Additionally, a Fortis C18 guard column with a diameter of 4mm was connected prior to the column. To maintain optimal conditions, the column oven temperature was maintained at 30°C. The run time of the method was 2.8 minutes. The analytical data were processed using Analyst 1.6.1 software (AB SCIEX).

Statistical analysis

Using the plasma concentration-time data, pharmacokinetic parameters such as area under the concentration-time curve up to the last measurable concentration (AUC_{last}), time to achieve maximum concentration ($_{Tmax}$), and maximum concentration (C_{max}) were estimated by a non-compartmental method using the WinNonlin software (Professional version 2.1, Pharsight Inc., U.S.). The statistical significance between simvastatin alone and the combination group was determined by an unpaired Student's t-test using GraphPad Prism 5 software. All the results are expressed as mean ± S.D., and $p < 0.05$ was considered statistically significant.

Result

The day 1 mean plasma concentration-time profiles of simvastatin after an oral dose of simvastatin (10 mg/kg, p.o.) alone and simvastatin (10 mg/kg, p.o.) in combination with piperine (20 mg/kg, p.o.) in Wistar rats are presented in Table 2 and Figure 1. Similarly, the day 7 mean plasma concentration-time profiles of simvastatin following a repeat oral dose of simvastatin (10 mg/kg, p.o.) alone and simvastatin (10 mg/kg, p.o.) in combination with piperine (20 mg/kg, p.o.) are provided in Table 4 and Figure 2. The day 1 and day 7 plasma pharmacokinetic parameters of simvastatin derived from the concentration-time profiles of alone simvastatin group and simvastatin in combination with piperine groups are summarized in Tables 2 and 4, respectively.

On both days 1 and 7, the oral absorption of simvastatin from the gastrointestinal tract (GIT) appears to be fairly rapid, with a mean T_{max} of 1.0 ± 0.0 h for simvastatin alone and simvastatin together with piperine. There was no difference in the time to achieve C_{max} in both groups. The rats treated with piperine significantly increased the mean AUClast of simvastatin from 3.92 ng·h/mL (simvastatin alone) to 11.41ng·h/mL (simvastatin together with 20 mg/kg piperine) on day 1 and from 12.17 ng·h/mL (simvastatin alone) to 57.8 ng·h/mL (simvastatin together with piperine, 20 mg/kg) on day 7. In combination with piperine, the mean AUC of simvastatin increased by 2.91-fold on day 1 and by 4.74-fold on day 7 compared to the standalone group. Similarly, the maximum plasma concentration (C_{max}) of simvastatin was significantly elevated in the presence of piperine. The mean plasma C_{max} of simvastatin in the simvastatin alone group on day 1 was 0.64 ng/mL, and on day 7 it was 3.38 ng/mL. In the combination group, the simvastatin mean C_{max} was 0.92 ng/mL on day 1 and 21.59 ng/mL on day 7. Compared to the standalone simvastatin group, the increase in mean C_{max} of simvastatin in the combination group was 1.43-fold on day 1 and 6.4-fold on day 7. Significant accumulation of simvastatin was seen on day 7 in both the standalone and combination groups. However, the accumulation was

Journal of Chemical Health Risks

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JCHR (2024) 14(1), 581-588 | ISSN:2251-6727

much higher in the combination group. The ratio of day 7 to day 1 mean AUC in the standalone group was 3.1 and in the combination group was 5.1.

Table 1: Day 1 plasma concentration time profile of simvastatin following oral administration of simvastatin alone and simvastatin in combination with piperine.

Time (Hr)	Simvastatin	Simvastatin + Piperine
0	0	0
0.5	0.14 ± 0.02	0.46 ±.07
1	0.64 ± 0.1	0.92 ±.06
2	0.60 ± 0.2	0.63 ± 0.2
4	0.55 ± 0.14	0.58 ±0.19
6	0.38 ± 0.05	0.54 ± 0.1
8	0.27 ± 0.05	0.35 ± 0.15
12	0.02 ±.0.05	0.05 ± 0.05
24	0.0	0.01 ± 0.01

Each value is represented as Mean ± S. D. (n = 3.

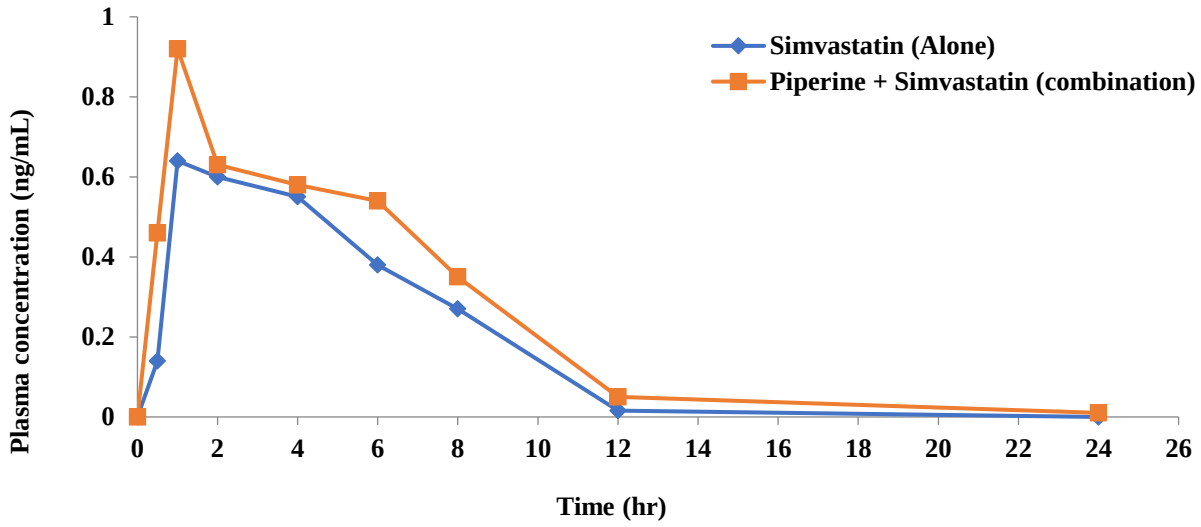

Figure 1. The day 1 mean plasma concentration-time curve of simvastatin when administered orally to rats at the dose of 10 mg/kg as alone and in combination with piperine (20 mg/kg, p.o.).

Table 2: The day 1 plasma pharmacokinetic parameters of simvastatin after oral administration of simvastatin alone and in combination with piperine.

Parameter	Simvastatin	Simvastatin + Piperine
AUC_{last} (ng·h/mL)	3.92±0.18**	11.41±0.85**
C_{max} (ng/mL)	0.64±0.1	0.92±0.006
Time (hr)	1	1

Each value is depicted as Mean ± S. D. (n = 3), * P <0.05, ***P <0.01 is regarded to be statistically significant compared to standalone simvastatin group.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 581-588 | ISSN:2251-6727

Table 3: Day 7 plasma concentration time profile of simvastatin following oral administration of Simvastatin alone and simvastatin in combination with piperine.

Time (Hr.)	Simvastatin	Simvastatin + Piperine
0	0	0
0.5	1.6 ± 0.29	15.81 ± 5.37
1	3.38 ± 1.6	21.59 ± 8.7
2	1.55 ± 0.165	9.33 ± 2.815
4	0.79 ± 0.065	3.58 ± 0.79
6	0.65 ± 0.06	2.56 ± 0.88
8	0.32 ± 0.07	1.44 ± 0.5
12	0.24 ± 0.03	0.45 ± 0.115
24	0.17 ± 0.07	0.31 ± 0.05

Each value is represented as Mean ± S. D. (n = 3).

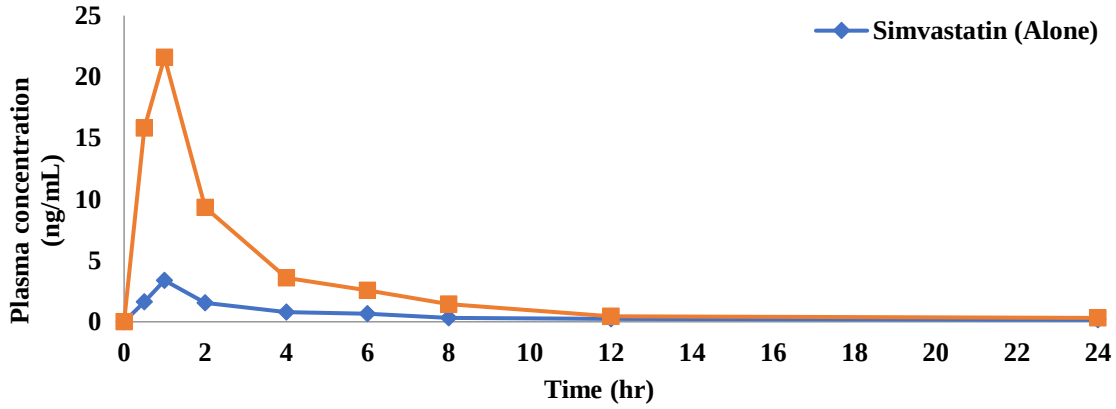

Figure 2. The day 7 mean plasma concentration-time curve of simvastatin when administered orally to rats at the dose of 10 mg/kg as standalone and in combination with piperine (20 mg/kg, p.o.).

Table 4: The day 7 plasma pharmacokinetic parameters of simvastatin after oral administration of simvastatin alone and in combination with piperine

Parameter	Simvastatin	Simvastatin + Piperine
AUC_{last} (ng·h/mL)	12.17±0.80**	57.8±0.8515**
C_{max} (ng/mL)	3.38±0.2275*	21.59±1120*
Time (hr)	1	1

Each value is depicted as Mean ± S. D. (n = 3). * P <0.05, ***P <0.01 is regarded statistically Significant compared to control.

Discussion

The findings of this study have significant implications for healthcare and medicine. Simvastatin is a widely prescribed drug to lower cholesterol levels in patients with hyperlipidemia. It also helps to prevent cardiovascular diseases that are associated with dyslipidemia. In humans, the absolute oral bioavailability of simvastatin is 5%, which is substantially low, and hence several formulation-based

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 581-588 | ISSN:2251-6727

approaches are being explored to improve its oral bioavailability. [9,10] Another possible approach to improving its systemic exposure is to combine simvastatin with herbal bioenhancers.

Herbal bioenhancers are active phytochemicals derived from herbal drugs that are used to enhance not only the oral bioavailability but also the therapeutic efficacy of drugs. The use of phytochemicals as bioenhancers in complementary and alternative medicine has gained popularity due to their safety profile and minimal side effects. It should be known that phytochemicals in dietary supplements and food can have an effect on the activity and expression of drug-metabolizing enzymes. CYP450 enzymes, particularly CYP3A4, are prone to interactions with various active components found in both food and dietary supplements. These interactions can result in notable effects when orally consumed along with drugs that are metabolized by this particular enzyme. The herb-drug interaction is also reported to occur due to the suppression of different efflux transporters present in the intestine. Piperine is also designated as an herbal bioenhancer, as it is reported to improve the plasma levels of many drugs in animals as well as humans. Piperine was found to increase the plasma exposures of drugs such as midazolam, diclofenac, rifampicin, pyrazinamide, propranolol, fexofenadine, theophylline, nimesulide, chlorzoxazone, carbamazepine, and phenytoin in humans. [11,12] This pharmacokinetic interaction of piperine can be attributed to its inhibitory effect on P-gp located in the intestine or to its CYP inhibitory potential. [13]. evaluated the effect of piperine on the P-gp transporter in a CaCO2 assay using digoxin and cyclosporin substrates. In this assay, piperine markedly inhibited the efflux of both digoxin and cyclosporine. Further, he had also determined the CYP3A4 inhibitory effect of piperine using human liver microsomes and verapamil as substrates. In this study, piperine was also found to significantly inhibit the CYP3A4 enzyme. [13] Besides the CYP3A4 inhibitory effect, piperine is also known to inhibit CYP2C9, CYP1A2, and uridine-diphospho glucuronosyl transferases (UGT). [14, 15, 16] In a study piperine was shown to inhibit the content of UDP-glucuronic acid in an in vitro study utilizing Guinea pig small intestines. [17]

Simvastatin undergoes extensive intestinal and hepatic first-pass metabolism predominantly through CYP3A4 and is also a substrate of P-gp at low doses. Simvastatin disposition is also known to be facilitated by the glucuronidation process through UGTs. [18]

The present study was planned to evaluate the pharmacokinetic interaction of piperine with simvastatin, as no information is available for both of these agents in combination. In this study, piperine was found to significantly enhance the plasma exposure of simvastatin in rats. It not only increased the plasma C_{max} but also markedly improved the AUC of simvastatin. The repeat dose of piperine with simvastatin was found to have a more pronounced effect on the circulating levels of simvastatin. As discussed above, the main reason for this positive interaction could be the inhibition of CYP3A4 and UGT enzymes or the P-gp transporter by piperine.

Piperine is also reported to have several therapeutic benefits, which also include lowering lipid levels. [19] In conjunction with piperine, the dose of simvastatin can be substantially lowered due to improved plasma levels of simvastatin and the possible synergistic antihyperlipedemic activity of both drugs together. However, further evaluation through clinical studies is necessary to fully understand the clinical implications of these findings.

Conclusion:

Piperine can improve Simvastatin absorption by suppressing P-gp and Cyp3A4 in the liver and gut. This improvement in oral pharmacokinetics suggests that using piperine alongside Simvastatin could potentially decrease the required dosage. Furthermore, piperine can potentially aid in the uptake of other drugs that have limited absorption when taken orally and are influenced by CYP3A4 and P-gp enzymes. In a study involving W. rat rats, administering Simvastatin (10 mg/kg) in combination with piperine (20 mg/kg). Led to a slight enhancement in the drug's oral exposure. By inhibiting the actions of P-GP and CYP3A4, piperine facilitated the more efficient passage of Simvastatin through the gastrointestinal mucosa. This is particularly significant since Simvastatin faces challenges in absorption due to its physicochemical properties and poor water solubility. Thus, when taking Simvastatin with piperine or dietary supplements containing piperine, care should be taken to

avoid any possible medication interactions. Furthermore, developing oral Simvastatin products with the addition of the bio-enhancer piperine could potentially reduce side effects and lower medication costs while improving patient satisfaction.

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