Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Evaluation of Bioenhancing Effect of Piperine and its Analogs on the Pharmacokinetics of Verapamil

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ARTICLE INFO

Article history:

Received: 12 October, 2022

Revised: 16 April, 2023

Accepted: 25 April, 2023

Published: 30 May, 2023

Keywords:

Verapamil, Piperine, Bioenhancer, HPLC, Validation, Pharmacokinetic

DOI:

10.25004/IJPSDR.2023.150305

ABSTRACT

A present study was undertaken to understand the impact of piperine and some of its derivatives on the bioavailability of verapamil used in the treatment of cardiac disorders. On oral administration, it undergoes fast biotransformation resulting in its low bioavailability. Its bioavailability varies between 20 and 35%. Piperine (a well-known bioenhancer) has been reported to improve the bioavailability of a wide range of structurally and therapeutically varied medicines. Hence some derivatives of piperine were synthesized and evaluated for their bioenhancing effect on verapamil. The bioenhancing effect of piperine and synthesized derivatives was investigated and compared using a validated HPLC method per USFDA guidelines. The pharmacokinetic characteristics of verapamil alone and when combined with piperine and its synthetic analogs were also investigated. When administered to wistar rats, it was discovered that verapamil bioavailability was 1.54 times higher when given with piperine and 2.42 times higher when given with morpholine derivative. The study undertaken indicates that some synthetic derivatives of piperine can be successfully used to enhance the bioavailability of verapamil to a considerable extent.

Introduction

Verapamil (Calcium channel blocker) prevents the movement of calcium ions in the cells. It regulates the entry of ionic calcium through the cell membrane of arterial smooth muscle cells and conductive and contractile cardiac cells. Found application in the treatment of arrhythmias, angina, and hypertension. Upon oral administration, maximum plasma concentration reached between 1 to 2 hours. It undergoes fast biotransformation at the time of first transit through the portal circulation system, having low bioavailability which varies between 20 and 35%.[1-4] After oral dosing, nearly 70% of the administered drug is excreted in the urine as metabolites and about 3 to 4% of it is eliminated unaltered in the urine. About 16% or more of verapamil is eliminated as metabolites in the feces. Verapamil is bound to plasma proteins in about 90% of cases. Hepatic insufficiency causes the metabolism to be slowed and the elimination half-life to be extended up to 14 to 16 hours. People with liver dysfunction may be able to obtain therapeutic plasma concentrations with one-third of the daily oral dosage. It is due to an increased volume of distribution and decreased plasma clearance to roughly 30% of normal verapamil clearance values. Titration should be used to tailor the dose to such persons. In clinical investigations, the typical initial monotherapy dosage was 80 mg thrice a day (240 mg/day). Studies have used 360 and 480 mg doses daily; however, there is no indication that doses more than 360 mg had any further effect.[5-10] The first bioavailability enhancer discovered was 'piperine', a bioactive compound derived from black pepper (*Piper nigrum* and *Piper longum*). Most of the herbal remedies were identified as having trikatu which contains black pepper, long pepper, and ginger containing piperine. Trikatu was thought to boost the efficacy of

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

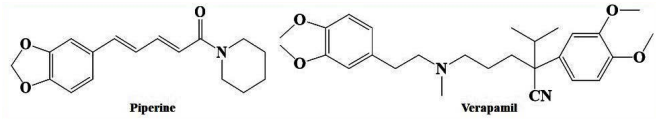

Fig. 1: Structure of piperine and verapamil

formulations and used to treat a variety of diseases.[11-23] Piperine is a powerful drug metabolism inhibitor. After adding long pepper, Vasaka leaves (*Adhatoda vasica*) boost their antihistaminic properties.[24-26] piperine is used as a bioenhancer in a variety of API formulations from various classes.[27] Piperine bioenhancing effectiveness in conjunction with non-steroidal anti-inflammatory drugs was described as clinically relevant at a dosage of 5 to 20 mg/kg in a study that then compared it to currently available marketed therapies.[28] A commercially available formulation Risorine® containing rifampicin 200 mg, isoniazid 200 mg, and piperine 10 mg is used for the treatment of tuberculosis. Because of the enhanced bioavailability, the formulation contains a 60% lower dose.[29] Piperine appears to be a pharmacologically important chemical of natural origin with extremely low toxicological consequences, and it has long been used in the form of black pepper to enhance food palatability around the world.[30]

Piperine as a scaffold for bio-enhancers is yet to be investigated. Continuous investigation of this structure could lead to significant advancements in drug discovery efforts.[31] Upon a thorough literature survey, the present study was performed. Certain piperine derivatives were synthesized, and a pharmacokinetic comparison of verapamil alone, with piperine, and with the synthesized derivatives was carried out.

An Indian scientist Atal invented the phrase "bioavailability enhancer" in 1985, piperine, a key ingredient of *P. nigrum* and *P. longum*, changes the pharmacokinetics of medicines *in-vitro* and/or *in-vivo.*[32-35] piperine increases the bioavailability of drugs by delaying their excretion and increasing their plasma half-life. It has been demonstrated to boost the bioavailability of a wide range of xenobiotic medications used to treat a wide range of human illnesses. Piperine's ability to act as a bioenhancer could be related to its ability to boost absorption while slowing the pace at which the drug is eliminated from the body.[36-37] The role of piperine as a scaffold for bio-enhancers is yet to be investigated. Continuous exploration of this structure could lead to significant advancements in drug discovery efforts.[31]

Materials and Methods

Chemicals and Reagents

Sami laboratories, Banglore, provided piperine as a gift sample. Emcure Pharmaceuticals, Ltd., Pune, provided verapamil as a free sample. All chemicals and reagents needed for the synthesis of derivatives were procured from Merck Lifesciences Pvt. Ltd., LOBA Chemie Pvt. Ltd., and Sigma Aldrich chemicals Pvt. Ltd. Anhydrous solvents and oven-dried borosilicate glassware were used in the reactions. Thin-layer chromatography (TLC) was used to monitor the reactions. Pre-coated silica gel plates-60 F254 were used to carry out TLC analysis. Plates were observed under UV light at 254 nm. Crude-synthesized products were purified on a silica gel column (mesh size #100-200).

Instruments

Piperine derivatives were synthesized on a Microwave synthesizer (Anton PaarMonowave 200) and a Combinatorial synthesizer (Redleys Discovery Technologies, RR98072). The melting points were determined for all synthesized compounds using the Veego VMP-PM digital melting point apparatus and are uncorrected. The absorbance maxima of the synthetic amide derivatives were measured in methanol using a JASCO V530 UV–vis double-beam spectrophotometer. IR spectra were recorded as potassium bromide pellets using the JASCO V-530 FTIR model. ^{1}H and ^{13}C-NMR i.e. nuclear magnetic resonance spectra were captured using the Bruker Avance III (500 MHz) spectrometer.the BrukerAvance III (500 MHz) spectrometer. Chloroform-d ($CDCl_3$) and DMSO-d^6 were used as solvents, whereas TMS i.e. tetramethylsilane, was used as the internal standard. Coupling constants values were recorded in Hz, whereas chemical shifts values were recorded as δ values. Mass spectra were recorded with Agilent technologies LC/MS (6460 Triple Quad, 1260 infinite QQQ G6460C model) in deconvoluted form using an ESI (Electro Spray Ionisation) source. JASCO HPLC system with a UV detector was used to determine the purity of the final compounds.

Animals

To carry out the pharmacokinetic study, approval was taken from IAEC i.e., Institutional Animal Ethics Committee of Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy (IAEC/PCP/PCH04/2021-2022). National Institute of Biosciences, Sinhagad Road, Pune-51, provided male wistar rats (weight 180-220 gm; n = 12). The animals were kept in ventilated cages for one week. The temperature of the room was maintained at 22 to 25°C. Relative humidity was between 55 to 60%. The cycle of 12 hour dark and light was also maintained. Animals were given food pallets. Tap water was given *ad libitum*.

A General Method of Synthesis

Synthesis of Piperic Acid (PA) from Piperine

To piperine (1-g, 3.5 mmol), 25% ethanolic NaOH (30 mL) was added and kept in a microwave at 78°C for 3 hours to complete the reaction. Once the reaction was complete, the mixture was cooled and the progress of the reaction was checked by TLC using *n*-hexane: acetone (3:2, v/v) as the mobile phase. Ethanol was evaporated under reduced pressure, leaving a yellow residue. This residue was

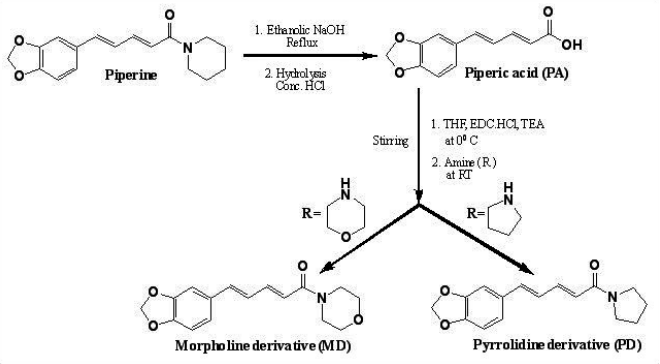

Fig. 2: Scheme for the synthesis of piperine derivatives

diluted with water, filtered, and the filtrate was acidified with concentrated HCl to produce a yellowish piperic acid precipitate. This was filtered under a mild vacuum. This crude piperic acid obtained yellow needles of pure material by recrystallization from methanol. Microwave-assisted synthesis reduced time, solvents, and reagents and improved yield from 70 to 85% as compared to the conventional method.

Synthesis of Derivatives from Piperic Acid

Dry THF i.e. tetrahydrofuran (20 mL) is used to dissolve Piperic acid (750 mg, 3.44 mmol). To this 693 mg (4.45 mmol) EDC. HCl [1-ethyl-3-(3-dimethyl (aminopropyl) carbodiimide hydrochloride] and (1.215 mL, 7.57 mmol) of TEA (triethylamine) were mixed at a temperature of 0 to 4°C and the mixture was stirred well for an hour. After completion of 1-hour, amines (4.13 mmol) were added with stirring at RT and further stirred for 6 hours. TLC was used to monitor the reaction [mobile phase *n*-hexane: ethyl acetate (3:2, v/v)]. THF was evaporated. Ethyl acetate was used to dissolve the residue before brine was used to extract it. Over anhydrous sodium sulphate, the ethyl acetate layer was recovered and dried. The crude derivatives were obtained by evaporating the solvent. Pure amides were obtained by column chromatography using silica gel (100–200 mesh) and *n*-hexane: ethyl acetate as the mobile phase.[38-40]

Analytical Method Development

Chromatographic Conditions

Analysis was performed on the JASCOHPLC system with UV/VIS (JASCO UV 1575) detector. Borwin (version 15) software was used to analyze the data. ThermoHypersil GOLD C18 column was used (250 × 4.6 mm, i.d., 5µm) along with Phenomenex Security Guard column (C18, 4 x 3.0 mm ID). Acetonitrile (ACN): Ammonium acetate buffer (pH 4.5), 60:40 (v/v), was used as the mobile phase in the analysis. The mobile phase was passed through a 0.22 µ filter and ultrasonically degassed for 30 minutes. The flow rate was set to a 1-mL/min. The run time was set to 10 minutes. The detection wavelength was 280 nm. The retention time of verapamil and piperine was 5.9 and 8.2 minutes, respectively.

Calibration Standards and Quality Control Sample Preparations

The standard stock solution was made by carefully dissolving 10 mg of verapamil in acetonitrile (10 mL). Further dilutions were made from the stock solution, which was properly stored away from light. Diluted solutions were spiked with 100 µL of blank plasma to produce a calibration curve with concentrations ranging from 2 to 32 µg/mL. Quality control samples were prepared by the same process. All samples were prepared in concentrations of 4, 16, and 24 µg/mL (low, medium, and high).

Plasma Sample Pretreatment

Rat plasma samples were extracted after protein precipitation with acetonitrile prior to HPLC analysis. After being vortexed for 5 minutes, the plasma and acetonitrile combination was centrifuged at 15000 rpm for 15 minutes at 40°C. The supernatant was collected after centrifugation and put through 0.22 syringe filters. The filtered supernatant was injected onto the HPLC column for analysis.

Method Validation

The bioanalytical method was developed and validated in compliance with the US Food and Drug Administration's (USFDA) guidelines.[41-43]

Selectivity

Selectivity of the HPLC method was assessed to examine endogenous substances in the sample if any, is interacting with the drug. Six blank rat plasma samples and plasma samples spiked at LLOQ (lower limit of quantitation) were utilized to evaluate selectivity.

Carryover Effect

It was assessed to examine any possible interference on the retention time of the analyte due to carryover. To check this, the samples were injected in the following order: blank plasma, LLoQ, ULoQ (upper limit of quantitation), and blank plasma.

Linearity

Linearity was plotted as the peak area of verapamil versus its concentration in the 2 to 32 µg/mL range. The correlation coefficient was used to check the linearity of plasma samples.

Accuracy and Precision

Interday and intraday precision and accuracy of three different concentrations of QC samples were measured by applying six replicates.

Extraction Recovery

The results of extracted analytical QC samples and blank matrix samples were compared with the results of post-

extracted analytes. To determine verapamil recovery, six replicates of QC samples at three concentration levels were used.

Stability

One of the most important aspects in the development of a bioanalytical method and its validation is solution stability. Many solutes degrade rapidly in plasma prior to analysis. Analyte stability investigations are complicated by many variables (e.g., sample storage conditions such as duration of storage and temperature used, method of extraction). The analyte's stability studies were done for:

- *Benchtop stability*

Plasma samples were stored for 8 hours at room temperature and then processed for study.

- *Long-term stability*

Plasma samples were stored for 15 days at –20°C.

- *Freeze-thaw stability*

The freeze-thaw stability of prepared plasma samples was tested for three freeze-thaw cycles. Samples were kept at room temperature to thaw and then stored again at –20°C.

In-vivo Pharmacokinetic Study

Single Oral Administration

Before the experiment, the animals were fasted for 24 hours and separated into five groups (Vehicle Control, Verapamil, Verapamil + Piperine, Verapamil + MD, Verapamil + PD). A single dose of verapamil (9 mg/kg; orally) was given to one group of rats. Other groups got a mixture of verapamil and piperine and verapamil and synthetic piperine derivatives. The retro-orbital venous plexus technique was used to collect rat blood samples (0.5 mL per animal) at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours. After 4 to 5 hours, the animals were given food. The blood samples were collected in marked tubes containing Ca-EDTA in order to prevent blood coagulation. The tubes were centrifuged at 4°C and 10,000 rpm, for 2 to 5 minutes to collect plasma. The extracting solvent (acetonitrile, 200 µL) was introduced to the 200 μL plasma and vortexed (5 minutes). Samples were centrifuged for 15 minutes at a speed of 15,000 rpm at 4°C. Precipitated proteins settled down and the supernatant was collected further it was filtered through 0.22 µ syringe filters. 20 µL *of* the solution from the filtrate was used for HPLC analysis.

Statistical Analysis of Data

A WinNonlin Noncompartmental Analysis (NCA) tool was utilized to estimate various pharmacokinetic parameters. The trapezoidal rule was used to calculate the pharmacokinetic parameters. The area under the plasma-drug concentration versus time curve i..e. AUC_{0-t}, C_{max} (maximum concentration of drug in plasma), T_{max} (time required to reach maximum concentration of drug in plasma) were calculated.

Results

Synthesized Compounds Characterization

Piperic Acid (PA): (2E, 4E)-5-(benzo[d] [1, 3] dioxol-5-yl) penta-2,4-dienoic acid

To piperine (1-g, 3.50 mmol), 25% ethanolic NaOH (30 mL) was added and kept in a microwave at 78°C for 3 hours. Ethanol was removed from the reaction mixture once the reaction was over under the reduced pressure. The resulting yellow-colored residue was dissolved in water and then filtered. Further, the filtrate was processed for acidification using concentrated HCl to get crude piperic acid in the form of a yellow precipitate. It was filtered under a mild vacuum. and recrystallization was done using methanol to get yellow crystals of pure piperic acid. Yield (850 mg, 85%); R_f value: 0.65 (mobile phase- *n*-hexane: acetone 3:2, v/v); percentage purity (HPLC): 99%; melting point: 210.8-211.4°C; UV (acetonitrile) λ_{max}: 338 nm; IR (anhydrous KBr) cm^{-1}: 3526.2 (-COOH), 3020.94 (=C-H stretching), 1680.66 (C=O stretching), 1611.23 (C=C stretching), 1267 (CH_2-O stretching); ^{1}H-NMR (500MHz, DMSO-d^6) δ: 12.22 (s, 1H), 7.324 (dd, J=7Hz & 15Hz, 1H), 7.244 (d, J=1.5Hz, 1H), 7.018 (dd, J=1.5Hz & 8Hz 1H), 6.986-6.971 (m, 2H), 6.938 (d, J=8Hz, 1H), 6.057 (s, 2H), 5.940 (d, J=15Hz, 1H); ^{13}C-NMR (500 MHz, DMSO-d^6) δ: 168.109, 148.577, 148.452, 145.114, 140.282, 130.980, 125.313, 123.599, 121.576, 108.988, 106.143, 101.840; MS ESI (-ve) *m/z*218.31($C_{12}H_{10}O_4$,218.21).

Morpholinederivative(MD):(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-1-morpholinopenta-2,4-dien-1-one

Piperic acid (750 mg, 3.44 mmol) was dissolved in 20 mL dry THF. To it EDC.HCl (693 mg, 4.45 mmol) and TEA (1.215 mL, 7.57 mmol) were added at 0-4°C and stirred for an hour. After completion of 1-hour, morpholine (360 µL, 4.13 mmol) was added with stirring at RT and further stirred for 6 hours. THF was evaporated and the residue was dissolved in ethyl acetate. It was extracted with brine. Ethyl acetate was collected and dried over anhydrous sodium sulfate. The solvent was evaporated to obtain a crude derivative. The derivative was purified by column chromatography with silica gel (100–200 mesh) and mobile phase *n*-hexane: ethyl acetate (60:40, v/v), to obtain pure crystals of MD.

Yield (555 mg, 74%); $R_f$value 0.582 (mobile phase *n*-hexane: ethyl acetate 3:2, v/v); percentage purity (HPLC): 97%; melting point: 145.5-146.5°C; UV (acetonitrile) λ_{max}: 340.6 nm; IR (anhydrous KBr) cm^{-1}: 3025.15 (=C-H stretching), 2987.2 (C-H stretching), 1641.10 (C=O stretching), 1593.88 (C=C stretching), 1496.49 (C-N stretching), 1275 (CH_2-Ostretching), 1117.56 (C-O-C stretching);^{1}H-NMR: (500MHz, $CDCl_3$) δ: 7.443 (dd, J=9.5Hz & 14.5 Hz, 1H), 6.992 (d, J=1.5Hz, 1H), 6.909 (dd, J=1.5Hz & 8Hz, 1H), 6.799-6.713 (m, 3H), 6.434 (d, J=14.5Hz, 1H), 5.981(s, 2H), 3.116 (s, 4H), 3.042 (s, 4H); ^{13}C-NMR (500 MHz, $CDCl_3$)

δ: 165.697, 148.305, 148.220, 143.503, 139.134, 130.787, 124.957, 122.735, 118.696, 108.525, 105.674, 101.328, 66.855, 46.138, 42.377, 29.691; MS ESI (+ve) *m/z* 287.19 ($C_{16}H_{17}NO_4$, 287.32).

Pyrrolidonederivative (PD): (2E, 4E)-5-(benzo[d][1, 3] dioxol-5-yl)-1-(pyrrolidin-1-yl)penta-2,4-dien-1-one

Piperic acid (750 mg, 3.7 mmol) was dissolved to this EDC in 20 mL dry THF. HCl (693 mg, 4.45 mmol) and TEA (1.215 mL, 7.57 mmol) were added at 0-4°C and stirred for an hour. After completion of 1-hour, pyrrolidine (345 µL, 4.13 mmol) was added with stirring at RT and further stirred for 6 hours. Tetrahydrofuran was evaporated and the residue was subjected to dissolution in ethyl acetate. Brine was used to extract it. The ethyl acetate layer was collected and dried over anhydrous sodium sulfate. The excess solvent was evaporated to obtain a crude amide derivative. By using column chromatography with silica gel (100–200 mesh) technique, purification of derivative was done using mobile phase *n*-hexane: ethyl acetate (60:40, v/v),to obtain pure crystals of PD.

Yield (645 mg, 86%); R_f value: 0.573 (mobile phase *n*-hexane: ethyl acetate 3:2, v/v); percentage purity (HPLC) : 92%; melting point: 128.3-129.9°C; UV (acetonitrile) λ_{max} : 338.4 nm; IR (anhydrous KBr) cm^{-1}: 3010.75 (=C-H stretching), 2971.77 (C-H stretching), 1633.41 (C=O stretching), 1593.88 (C=C stretching), 1495.53 (C-N stretching), 1249.65 (CH_2-O stretching), 1038.48 (C-O-C stretching); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.459 (dd, J=10Hz and 15Hz, 1H), 6.991 (d, J=1.5Hz, 1H), 6.907 (dd, J=1Hz and 8Hz, 1H), 6.803-6.708 (m, 3H), 6.274 (d, J=14.5Hz, 1H), 5.977 (s, 2H), 3.578 (m, 4H), 2.012-1.855 (m, 4H); ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 164.568, 148.161, 147.756, 141.813, 138.721, 130.979, 125.348, 122.595, 121.373, 108.367, 105.678, 101.224, 46.499, 45.938, 26.263, 24.919; MS ESI (+ve) *m/z* 271.09 ($C_{16}H_{17}NO_3$, 271.32).

HPLC Method Validation

The HPLC method of bioanalysis to estimate the concentration of verapamil in rat plasma was validated as per the USFDA guidelines.

Selectivity

During method development, the selectivity of the method was tested to ensure that analytes did not interfere with the verapamil peak. The chromatograms of six blank plasma samples with the spiked plasma samples were compared to determine selectivity. At retention times of 5.9 minutes (verapamil) and 8.2 minutes (piperine), there was no interference observed.

Carryover Effect

The carryover effect was evaluated in terms of interference at the retention time of verapamil. For this, the samples were injected in the following order: blank plasma sample, LLoQ sample, ULoQ sample, and the same blank plasma sample. The absence of interference showed that the carryover effect was absent.

Linearity

The linear range for verapamil in rat plasma was 2 to 32 µg/mL. The correlation coefficient (R^2) was calculated using calibration curve data (R^2 = 0.999). The regression equation for verapamil was found to be y = 18612x + 5101.1. The LLoQ was 1.10 µg/mL and the LoD value was found to be 3.29 µg/mL.

Accuracy and Precision

Rat plasma at 4, 16, and 24 µg/mL doses was used in the interday and intraday precision investigations. The percentage of relative standard deviation (% RSD) was found to be below the range at all examined levels. The %RSD for intra-day and inter-day were found to be between 2.35 to 2.80% and 2.43 to 5.05%, respectively. The %accuracy for intra-day and inter-day were found to be within 15% of the acceptance limit i.e. between 97.19 to 97.64% and 94.35 to 97.57%, respectively. This demonstrated that the procedure for verapamil in the biological matrix was repeatable and precise.

Stability

The stability of verapamil in rat plasma was studied using 4, 16, and 24 µg/mL QC samples, and the results showed no degradation. The %RSD for benchtop stability samples was between 1.67 to 4.83%. These results indicate that verapamil was stable under benchtop conditions at room temperature for 8 hours. The %RSD for freeze-thaw stability samples was 1.79 to 5.54% after three freeze-thaw cycles. The results also proved that verapamil remained stable for 15 days in plasma when stored at -20°C as %RSD for long-term stability samples was found to be between 2.77 to 3.45%.

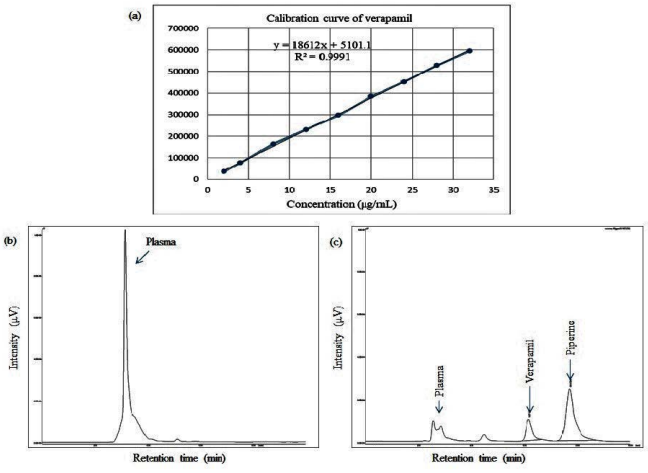

Fig. 3: Calibration curve of verapamil (a), HPLC Chromatogram of Blank Plasma (b) and ULOQ (c)

Table 1: Accuracy, precision, and stability study of verapamil in rat plasma

Concentration (µg/ml)	*Mean area ± SD*	*%RSD*	*%Accuracy*
Inter-day			
4	77043 ± 3887	5.05	94.95
16	308723 ± 8666	2.81	97.19
24	450497 ± 10960	2.43	97.57
Intra-day			
4	76935 ± 2155	2.80	97.19
16	319611 ± 7518	2.35	97.65
24	441942 ± 11038	2.49	97.50
Benchtop Stability			
4	77745 ± 3754	4.83	95.17
16	324567 ± 9517	2.93	97.07
24	455330 ± 7637	1.68	98.32
Freeze-thaw Stability			
4	78242 ± 4337	5.54	94.46
16	312894 ± 15103	4.83	95.17
24	454830 ± 8120	1.79	98.21
Long-term Stability			
4	80458 ± 2389	2.97	97.03
16	330246 ± 11388	3.45	96.55
24	455275 ± 12613	2.77	97.23

Extraction Recovery

The extraction efficiency of an analyte process is referred to as recovery. Higher recovery indicates effective extraction and sensitivity. At the LQC, MQC, and HQC levels, recovery is a comparison of the instrument response associated with extracted samples to that of matrix samples spiked with a known amount of analyte post-extraction. The extraction recoveries of verapamil from LQC, MQC, and HQC samples ranged from 98.00 to 101.82%.

In-vivo Pharmacokinetic Study of Verapamil

The validated HPLC method of analysis was used to carry out *in-vivo* pharmacokinetics of verapamil in male wistar rats. The plasma drug concentration and time profiles were plotted using WinNolin software. Pharmacokinetic parameters were studied for verapamil alone and with piperine and other synthetic analogs of piperine. According to these findings, when piperine and synthetic analogs of piperine are given with verapamil, the pharmacokinetic parameters of verapamil change considerably.

A non-compartment model using NCA in the WinNolin program was used to predict pharmacokinetic parameters. The trapezoidal rule was used to compute the pharmacokinetic parameters. Maximum plasma concentrations i.e. C_{max} and time to reach the maximum concentration i.e. t_{max} were assessed in wistar rats.

Table 2: Extraction recovery of verapamil in rat plasma

Concentration (µg/mL)	*%Drug*	*Total Amount (µg/mL)*	*Amount Recovered (µg/mL)*	*%Recovery*
	80.00	3.2	3.22	100.63
4	100.00	4.0	3.92	98.00
	120.00	4.8	4.71	98.13
	80.00	12.8	12.55	98.05
16	100.00	16.0	15.92	99.50
	120.00	19.2	19.55	101.82
	80.00	19.2	18.95	98.70
24	100.00	24.0	23.55	98.13
	120.00	28.8	28.49	98.92

C_{max} of verapamil was found to be 14.24 µg/mL at t_{max} of 4 hour with AUC_{0-t} 53.44 µg*hr/mL when administered alone orally. C_{max} increased to 16.824 µg/mL, AUC_{0-t} to 82.35 µg*hr/mL when piperine was combined with verapamil. C_{max} increased to 17.94 µg/mL in the presence of the synthetic derivative PD, while AUC_{0-t} increased to 111.13 µg*hr/mL. In the presence of the synthetic derivative MD, C_{max} increased to 18.47 µg/mL and AUC_{0-t} to 129.39 µg*hr/mL. Finally, verapamil bioavailability was shown to be 2.42 times greater with morpholine derivative, 2.08 times higher with pyrrolidine derivative, and 1.54 timeshigher with piperine.

Discussion

In the rapidly changing pharmaceutical world, attempts should be made to provide safe, effective medicine in a short period of time along with the consideration of treatment economics.

In this study, the derivatization of piperine was done with certain synthetic modifications. The conventional method for the synthesis of piperic acid takes 48 hours to complete the reaction, whereas the reaction using a microwave synthesizer takes only 3 hours. Microwave-assisted synthesis reduced time, solvents and improved yield from 75 to 85%. Synthesis of derivatives from piperic acid using thionyl chloride requires three steps. Acid chlorides are not stable in the environment for a long time and need to go for the next step of amidation immediately. Hence a coupling reagent EDC. HCl was used

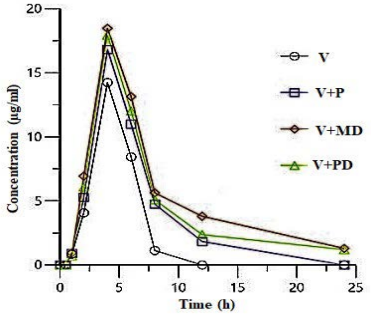

Fig. 4: Plasma concentration Vs Time Profile

Table 3: Pharmacokinetic Parameters of verapamil in presence of piperine and its synthetic derivatives

Name	*AUC_{0-t} (µg*hr/mL)*	*C_{max} (µg/mL)*	*T_{max} (hr)*	*K_{el} (hr^{-1})*	*$t_{1/2}$ (hr)*	*MRT (µg*hr^2/mL)*	*Fold increase in AUC with respect to verapamil alone*
V + P	82.35	16.824	4	0.29	2.39	5.36	1.54
V + MD	129.39	18.47	4	0.09	7.58	7.83	2.42
V + PD	111.13	17.94	4	0.083	8.37	7.45	2.08

for derivative synthesis. The use of EDC. HCl reduced the synthesis of derivatives to two steps, which also reduced time and solvents.

FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopy, and mass spectroscopy were used to characterize synthesized derivatives. Spectral data indicated that the intended product was obtained in stages. The piperidine ring of piperine was substituted by heterocyclic rings like morpholine and pyrrolidine to get MD and PD, respectively. Pharmacokinetic analysis of male wistar rats revealed that simultaneous administration of piperine as a bioenhancer and some of its synthetic analogs enhanced the bioavailability of verapamil. Current research showed an increase in C_{max} and AUC_{0-t} value of verapamil in presence of piperine. When given in combination with MD and PD, the C_{max} and AUC_{0-t} value of verapamil further increased as compared to a combination of verapamil and piperine. An increase in the AUC0-t of verapamil increases its bioavailability by 1.54-fold with piperine, 2.08-fold with PD, and 2.42-fold with MD.

Conclusion

The current study indicates that piperine increased the concentration of verapamil in plasma, slowed its clearance, and increased the C_{max}, AUC_{0-t}, $t_{1/2}$, and MRT, according to pharmacokinetic studies. In this study, the MD and PD were far more superior to piperine. The present study also opened the windows for the researchers to work on various piperine derivatives along with other medicines to understand the boosting of bioavailability.

Acknowledgment

The authors would like to express gratitude to the Indian Council of Medical Research/Department of Health Research i.e. DHR/Human Resource Development (HRD), Govt. of India, New Delhi for giving the financial support. We also express gratitude to the Principal, Poona College of Pharmacy, Pune for his encouragement and support.

Conflict of Interest

Nil

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HOW TO CITE THIS ARTICLE: Pharne A, Gabhe S, Gabhe SY. Evaluation of Bioenhancing Effect of Piperine and its Analogs on the Pharmacokinetics of Verapamil. Int. J. Pharm. Sci. Drug Res. 2023;15(3):260-267. **DOI:** 10.25004/IJPSDR.2023.150305