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Research paper

Oral Bioavailability Enhancement of Efavirenz using Piperine coadministration in Experimental Rabbits

Mohammad Asif [1,], Hardik P Patel[2] and Rakesh K Patel[2]*

[1]Department of Pharmacognosy, Lachoo Memorial College of Science & Technology, Jodhpur, Rajasthan, 342001, India
[2]Department of Pharmacognosy, S.K. Patel College of Pharmaceutical Education & Research, Mahesana, Gujarat, 384012, India

*CORRESPONDING AUTHOR
Dr. Mohammad Asif, Department of Pharmacognosy, Lachoo Memorial College of Science & Technology Jodhpur, Rajasthan, 342001, India

ABSTRACT

Background: Efavirenz, a first line anti-retroviral drug has variable bioavailability owing to its limited aqueous solubility. Piperine, a bioavailability enhancer has been often used to enhance the bioavailability of many drugs.

Objective: The present study was aimed to investigate the possibility of improving the bioavailability of efavirenz using piperine.

Methods: Two doses of efavirenz 9.33 mg/kg and 28 mg/kg which corresponded to 200 and 600 mg/kg of human dose were selected. Single oral dose of efavirenz and piperine co administration was given to rabbit and at fixed time interval drug blood concentration was estimated by HPLC. Pharmacokinetic parameters of efavirenz and piperine co administration were determined.

Results: Efavirenz 9.33 mg/kg co administration with piperine 20.8 mg/kg increased area under the curve significantly at $p<0.01$ and C_{max} at $p<0.05$ compared to efavirenz control (9.33 mg/kg). The relative bioavailability of efavirenz and piperine co-administration was found to be 149.08%, i.e., higher than efavirenz control. $T_{1/2}$ of piperine co-administration was also increased significantly at $p<0.05$ compared to efavirenz control. T_{max} of piperine co-administration was found to be 0.5 h, followed by efavirenz control i.e., 1 h. Co-administration of efavirenz (28 mg/kg) with piperine (20.8 mg.kg) significantly increased AUC and C_{max} at $p<0.001$ compared to efavirenz control (28 mg/kg). The relative bioavailability of Piperine co-administration was found to be 158.92%, higher than efavirenz control. There was significant increase in $T_{1/2}$ of piperine co-administration at $p<0.01$ compared to efavirenz control. T_{max} of piperine co-administration and efavirenz were found to be same i.e., 1 h.

Conclusion: Based on the results it can be concluded that piperine co-administration significantly increases the oral exposure of efavirenz. Bioavailability of efavirenz with piperine was found to be higher than efavirenz control.

Keywords: *Efavirenz; Piperine; HPLC; Area under the curve; C_{max}; T_{max}*

Introduction

Bioavailability enhancers concept is derived from traditional system of Ayurveda. There action was first documented by Bose in 1929 where the anti-asthmatic properties of *Adhatoda vasika* leaves were enhanced by long pepper. These are known to enhance the activity of drug molecules through various ways such as

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potentiating the drug molecules by conformational interaction, making target cells more receptive to drugs, increasing the bioavailability of the drug across the membrane without exerting its own pharmacological activity.

Piperine is the world's first discovered and scientifically validated bioavailability enhancer by an Indian scientist in 1979 at Regional Research Laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine.). Piperine is an active principle present in *P. Longum*. Piperine has been reported to increase the bioavailability of several category of drug such as antitubercular, leprosy, antibiotics, NSAIDs. It has been reported to enhance the bioavailability of various drugs ranging from 30% to 200%. There are several reported mechanisms through which piperine is reported to enhance bioavailability of drugs *via* inhibition of drug metabolizing enzymes, stimulation of gut amino acid transporters, inhibition of cellular pump required for elimination of drug, inhibiting intestinal glucuronic acid production. It is reported to inhibit UDP-glucuronyltransferase and hepatic arylhydrocarbon hydroxylase activities, transferase activity, human p-glycoprotein and CYP3A4.Other studies suggests that Piperine also makes receptors more sensitive towards drug, itself act as a receptor, vasodilate the GIT vasculature to enhance absorption. [1]

Efavirenz is a first line antiretroviral drug belonging to category non-nucleoside reverse transcriptase inhibitor. It is one of the preferred constituents of high activity antiretroviral therapy (HAART) for human immunodeficiency virus (HIV) infections. [2]

It mainly acts on HIV-1 reverse transcriptase noncompetitively but lack significant inhibitory effect on HIV-2 reverse transcriptase. Various studies suggest that EFV based regimen with two NRTIs have shown better virologic response compared to triple-NRTI-based regimen or PI based regimen. As per BCS it belongs to Class II drug (high permeability and low solubility). EFV has reported to have T_{max} of approximately 3-5 hours. The AUC and C_{max} increases proportionally with dose till 1600 mg shedding light that the absorption of efavirenz decreases after this dose. It is more than 99% protein bound. EFV 400 mg has reported to be 14-34% excreted in the urine as metabolite and 16-61% was excreted in faeces as revealed by radiolabelled dose of EFV. EFV is mainly metabolised in liver mainly by CYP3A4 and CYP2B6 to hydroxylated metabolite (inactive) and is reported to have oral bioavailability of 40-45%. [3] Therefore,

citing to low bioavailability of efavirenz, the present study was undertaken to evaluate the effect of piperine on pharmacokinetics parameters of efavirenz in rabbit after oral administration of efavirenz at two different doses with or without piperine.

Materials and Methods

Materials

Efavirenz was received as gift sample from Al-Chem Pharmaceuticals, Diazepam was purchased from Santham Pharma Pvt. Ltd., Piperine was obtained from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was obtained from S.D Fine Chem Ltd., Water (HPLC grade), Methanol (AR) and potassium EDTA (AR), potassium dihydrogen phosphate (AR) and Tween 80 were obtained from Finar Chemicals Ltd., Ethyl acetate (AR) and hydrochloric acid (AR) were obtained from S.D Fine Chem Ltd., Ethanol (AR) was obtained from Baroda Chemical Industries Ltd.

Experimental animals

Rabbits (2000-3500 gm), were procured from Central Animal Facility, S.K. Patel College Education and Research, Kherva and housed under standard conditions of temperature (25±1°C), relative humidity (50 ± 10 %), 12 hr of alternate light and dark cycle. Rabbits were fed with carrot, cauliflower, fresh leaves of vegetables. The care and the use of these animals were in accordance with the guidelines of the CPCSEA (Reg.no. 197/PO/Re/S/2000/CPCSEA).

Calculation of dose

The dose to be given to experimental rabbits on the basis of surface area was extrapolated as per description of Paget et al. For determination of absolute dose of the experimental rabbit, the absolute dose was multiplied by the factor mentioned as per Paget et al. [4]

Dose to be given to rabbit = Dose of man (70 kg) x 0.07

Dose to be given to rabbit = Dose of rat (200 gm) x 3.9

Based on above calculation the dose of efavirenz to be administered to rabbit was calculated to be 9.33 mg/kg (Dose corresponding to 200 mg in humans) and 28 mg/kg of rabbit (Dose corresponding to 600 mg in humans); dose of Piperine to be administered was calculated to be 20.8 mg/kg of rabbit (Dose corresponding to 8 mg in rats); The treatment was

administered as suspension using Tween 80 as suspending agent.

Experimental design

The experimental rabbits were divided into six group containing six rabbits in each group. Group I rabbits were administered with efavirenz (9.33 mg/kg); Group II rabbits were administered with efavirenz + Piperine (9.33mg/kg + 20.8 mg/kg); Group III rabbits were efavirenz (28 mg/kg); Group IV rabbits were administered with efavirenz + Piperine (28 mg/kg + 20.8 mg/kg).All the group received single oral dose of above drug and blood was collected at 0, 0.5, 1, 2, 4, 6, 8, 10, 24 and 48 hours respectively from marginal ear vein of rabbit. Blood collected was subjected to plasma separation followed by series of extraction process and sample prepared was finally subjected to estimation of drug by using High Performance Liquid Chromatography.

HPLC assay

Preparation of calibration curve

Preparation of standard stock solution

A stock solution (50 mg/ml) was prepared by dissolving pure efavirenz in methanol. The working standards of efavirenz in concentrations ranging from 5 to 20000 ng were prepared in rabbit plasma.

Sample preparation of efavirenz

For each calibration standard, 250 µl plasma was taken in 2 ml micro centrifuge tube followed by spiking of 100 ng Diazepam (Internal Standard). Mixture was vortexed for 60 seconds. Standard stock solution of efavirenz, 5, 25, 250, 500, 1000, 2500, 5000, 10000, 15000 and 20000 ng were added separately. Above Plasma sample was extracted by 1000 µl ethyl acetate, vortexed well for 60 seconds followed by centrifugation at 12000 rpm for 10 minutes. Supernatant organic solution was taken in another 2 ml micro centrifuge tube and evaporated to dryness under gentle nitrogen stream at 37°C on water bath. The dried residue was reconstituted in 200 µl mobile phase, vortex for 60 seconds followed by centrifugation at 12000 rpm for 10 minutes. Clear solution was subjected to HPLC analysis [5,6]

Sample preparation for Group-I-IV

Healthy rabbits were fasted overnight with free access and were administered single oral dose of respective treatment. Blood samples (1.5 ml) were collected at 0, 0.5, 1, 2, 3, 4, 5, 6, 10, 24 and 48 hours after drug administration in anticoagulant treated micro centrifuge tube and plasma was separated by centrifugation at 12000 rpm for 10 minutes. Above Plasma sample was extracted by 1000 µl ethyl acetate, vortexed well for 60 seconds followed by centrifugation at 12000 rpm for 10 minutes. Supernatant organic solution was taken in another 2 ml micro centrifuge tube and evaporated to dryness under gentle nitrogen stream at 37°C on water bath. The dried residue was reconstituted in 200 µl mobile phase, vortex for 60 seconds followed by centrifugation at 12000 rpm for 10 minutes. Clear solution was subjected to HPLC analysis. [5,6]

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of two pumps (LC 20 AD), diode array detector (SPD M20A) and degasser (DGU-20A5). A rheodyne manual injector (7725i) (Rheodyne, Cotati, CA, USA) attached with 20 µl sample loop was used for loading the sample by 25 µl syringe (Hamilton, Bonaduz, Switzerland). Class VP LC workstation was used for data collection and acquisition. The analytical column was a Kromasil C18, 250 x 4.6 mm ID, 5µ, 100 Å particle size (Phenomenex, CA, USA) protected by a compatible security guard cartridge system, C18, 4 x 3.0 mm (Phenomenex, CA, USA). The mobile phase consisted of 10 mM phosphate buffer, pH 2.4 (adjusted with I N HCl) and acetonitrile (55:45, v/v). Wavelength was set at 247 nm. The chromatogram was run for 25 minutes with flow rate of 2.4 ml/min at 25°C. Unknown concentration was derived from linear regression analysis of the calibration curve.

Pharmacokinetic analysis

Pharmacokinetic parameters in terms one compartment open model were calculated with the area under the plasma concentration–time curves (AUC) were calculated by trapezoidal rule. The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were determined by a visual inspection of the experimental data. The terminal phase rate constant (K_{el}) was calculated as the negative of the slope of the log linear terminal portion of the plasma concentration versus time curve using least square regression analysis. The terminal phase half-life ($T_{1/2}$) was calculated as $0.693/K_{el}$. The absorption rate constant (K_a) was calculated by the residual method. The relative bioavailability of efavirenz after oral administration was calculated as follows: [7,8]

$$Relative\ bioavailability = \left(\frac{AUCcoadmin}{AUCcontrol}\right) \times 100$$

Statistical analysis

All the means are presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett's correction.
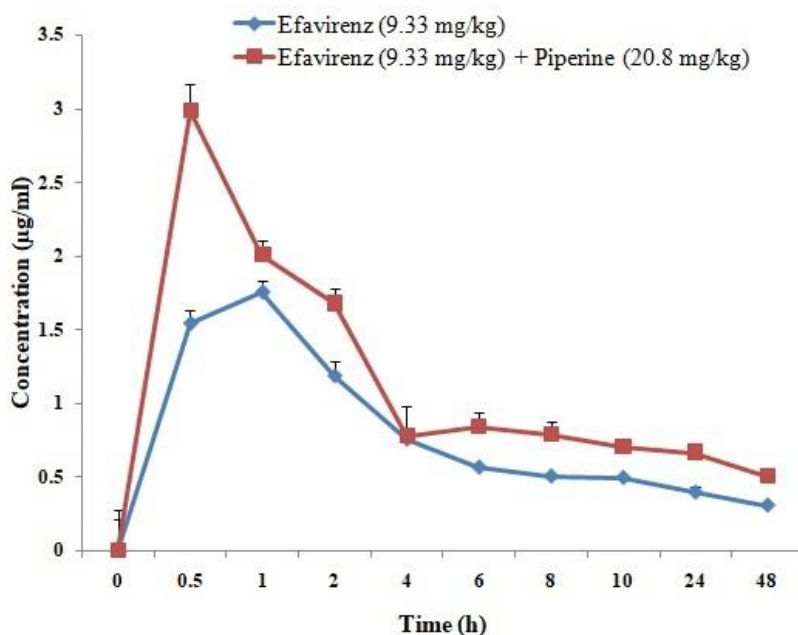

Figure 1: Plasma concentration of efavirenz (9.33 mg/kg) and efavirenz co-administration with piperine.
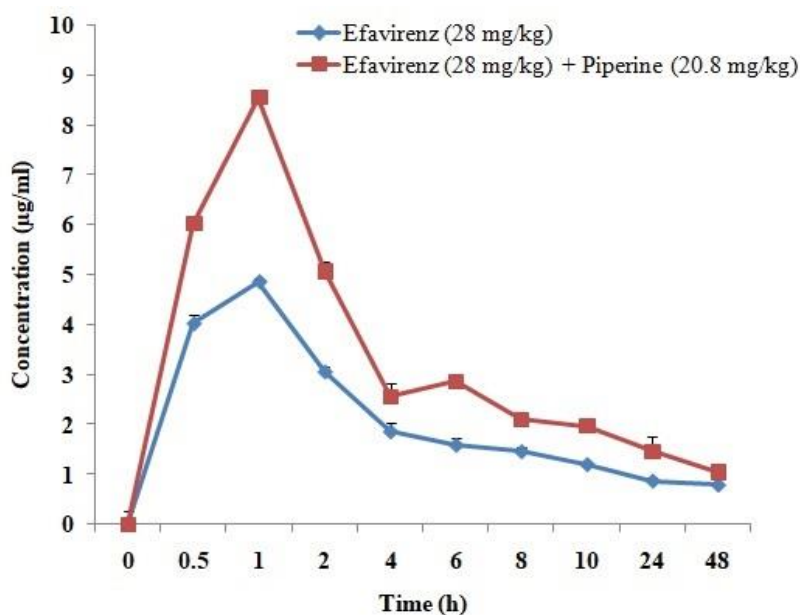

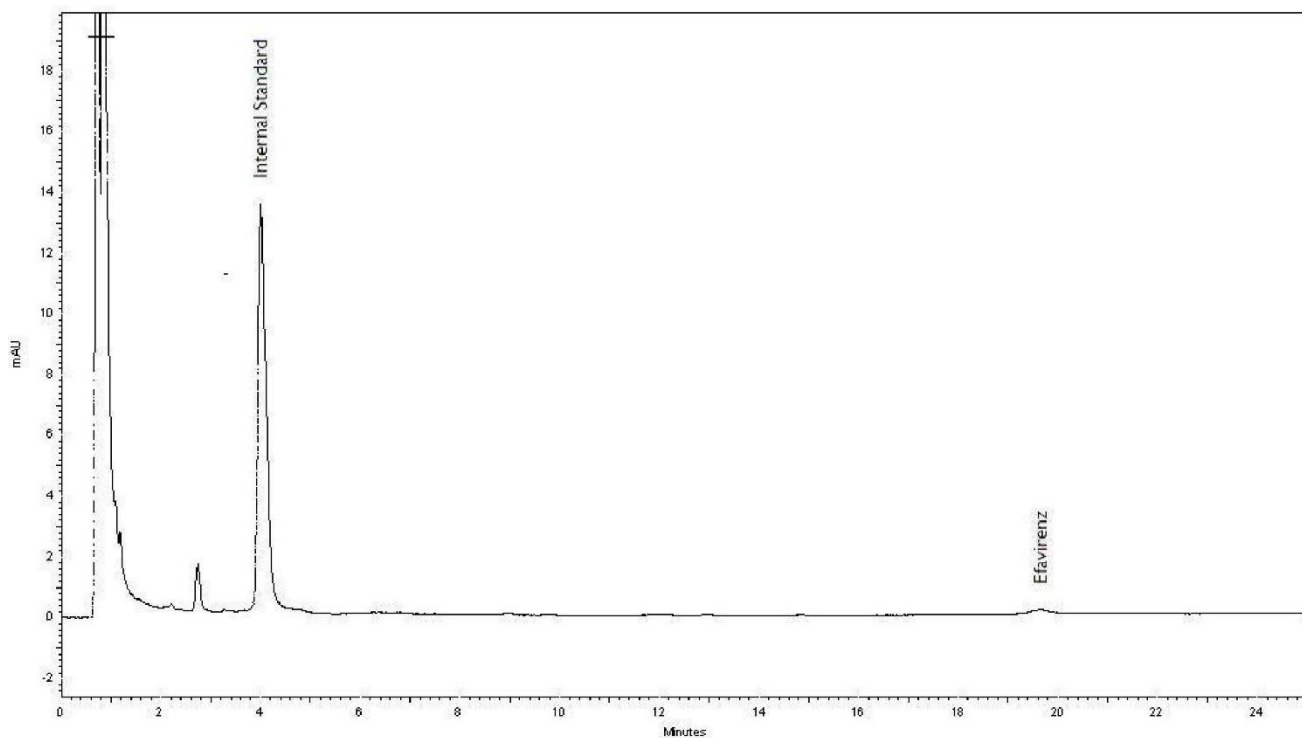**Figure 2:** Plasma concentration of efavirenz (28 mg/kg) and efavirenz co-administration with piperine.

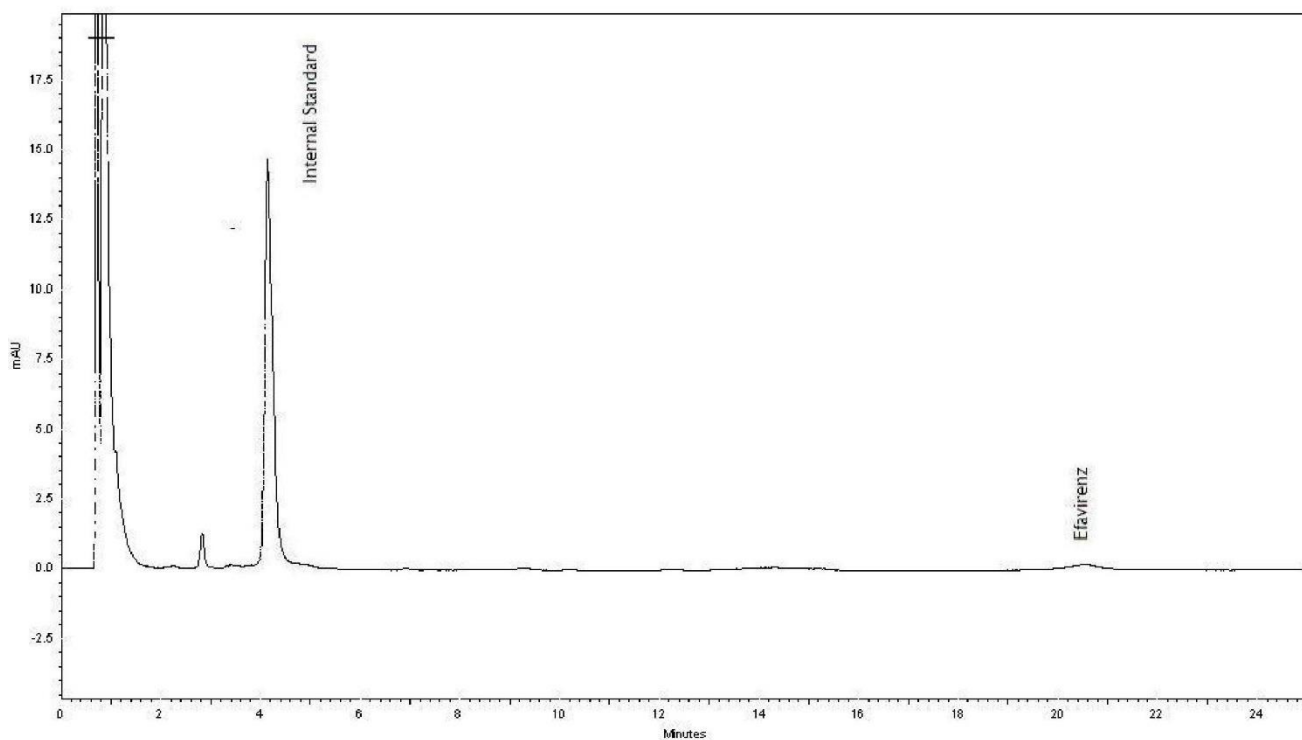**Figure 3:** Chromatogram showing efavirenz 9.33 mg/kg at 1 hour.

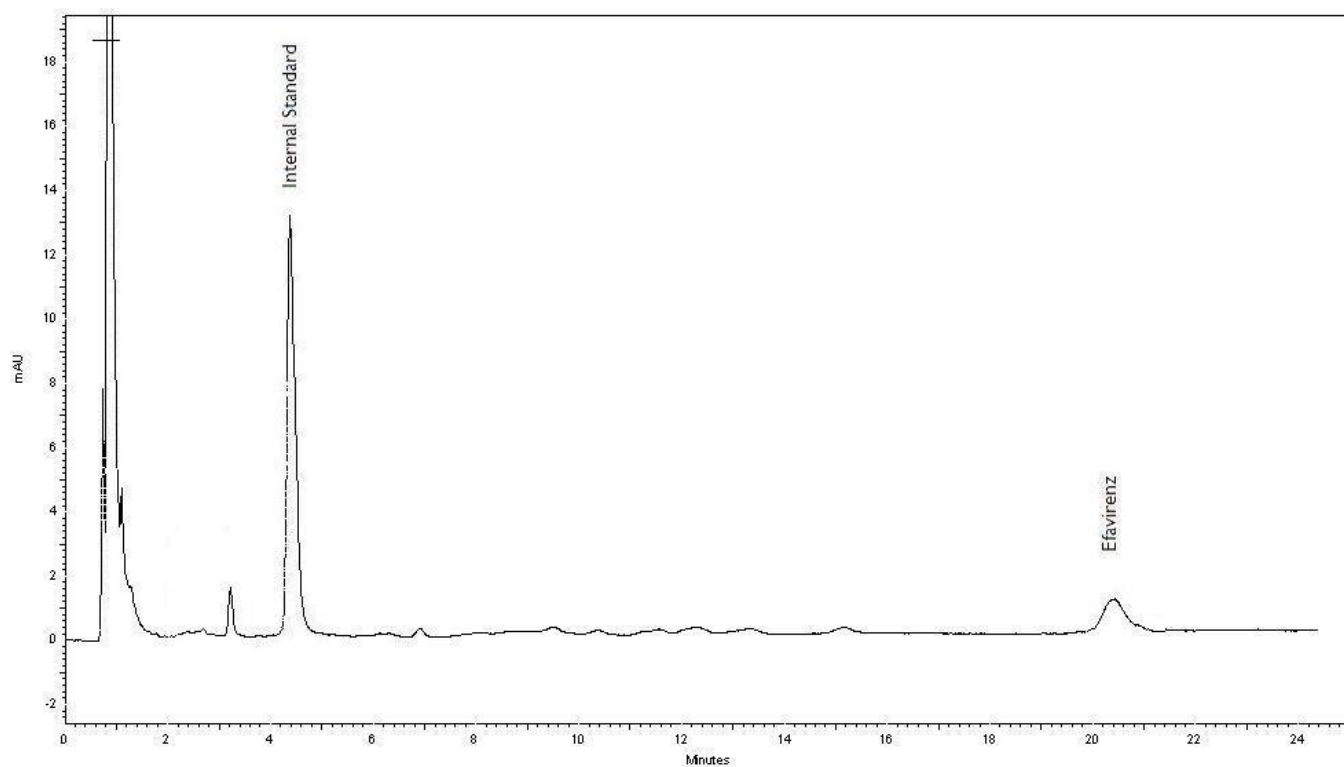**Figure 4:** Chromatogram showing efavirenz 9.33 mg/kg + piperine 20.8 mg/kg at 1 hour.

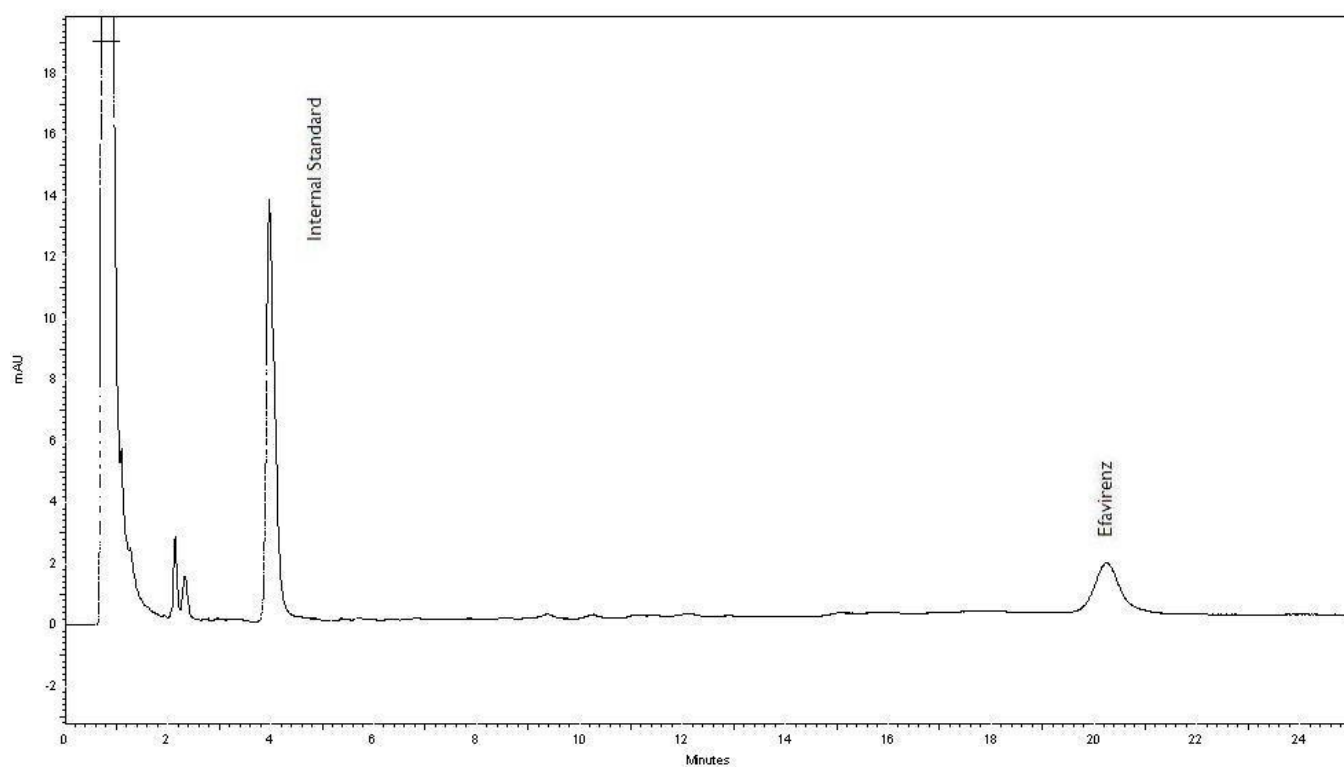**Figure 5:** Chromatogram showing efavirenz 28 mg/kg at 1 hour.

Figure 6: Chromatogram showing efavirenz 28 mg/kg + piperine 20.8 mg/kg at 1 hour.

Table 1: AUC of various groups at different concentration and time.

S. No	Time (h)	efavirenz (9.33 mg/kg)		efavirenz (9.33 mg/kg) + Piperine (20.8 mg/kg)		efavirenz (28 mg/kg)		efavirenz (28) + Piperine (20.8 mg/kg)	
		Conc (µg/ml)	AUC ±SD	Conc (µg/ml)	AUC ±SD	Conc (µg/ml)	AUC ±SD	Conc (µg/ml)	AUC ±SD
1	0	0	0	0	0	0	0	0	0
2	0.5	1.545	0.38625 ±0.6761	2.986799	0.74669975±0.5106	4.03	1.0075±0.3872	6.036	1.509±0.6689
3	1	1.758387	0.82584675±0.2121	2.008764	1.24889075±0.4331	4.87	2.225±0.3972	8.56	3.649±0.3427
4	2	1.186863	1.472625±0.1934	1.67878	1.843772±0.2472	3.06	3.965±0.1786	5.07	6.815±0.1087
5	4	0.7546078	1.9414708±0.2489	0.778069	2.456849±0.2366	1.86	4.92±0.2381	2.565	7.635±0.4941
6	6	0.5653658	1.3199736±0.1226	0.8384286	1.6164976±0.4854	1.59	3.45±0.4231	2.86	5.425±0.6812
7	8	0.5045248	1.0698906±0.0034	0.7896346	1.6280632±0.2578	1.46	3.05±0.3235	2.1	4.96±0.0403
8	10	0.4942054	0.9987302±0.0351	0.7039215	1.4935561±0.2196	1.2	2.66±0.1936	1.97	4.07±0.1550
9	24	0.3949535	6.2241123±0.0136	0.665415	9.5853555±0.1100	0.87	14.49±0.1100	1.46	24.01±0.1361
10	48	0.3066233	8.4189216±0.1077	0.506565	14.06376±0.1629	0.79	19.92±0.1652	1.05	30.12±0.7210

Results

The mean plasma concentration – time profile of efavirenz at dose 9.33 and 28 mg/kg with and without piperine were characterized in rabbit and illustrated in Figures 1 and 2. Area under the curve (AUC) at different concentration and time has been detailed in table 1. HPLC analysis from each group at 1 hour is depicted in Figures 3-6. The pharmacokinetic parameters of efavirenz (9.33 mg/kg and 28 mg/kg), piperine co-administration have been detailed in Table 2.

Co-administration of efavirenz (9.33 mg/kg) with piperine significantly increased area under the curve at $p < 0.01$, compared to efavirenz control. Similarly, C_{max} with piperine co-administration increased significantly at $p < 0.05$ compared to efavirenz control (9.33 mg/kg). The relative bioavailability of Piperine co-administration was found to be 149.08%, higher than efavirenz control. $T_{1/2}$ of piperine co-administration was also increased significantly at $p < 0.05$ compared to efavirenz control. T_{max} of piperine co-administration was found to be 0.5 h, followed by efavirenz control i.e., 1 h.

Table 2: Pharmacokinetic parameter of various groups.

Parameters	efavirenz control (9.33mg/kg)	Piperine co-admin	efavirenz control (28 mg/kg)	Piperine co-admin
K_a	0.3132 ± 0.073	$0.0437 \pm 0.009^{**}$	0.2625 ± 0.048	$0.1335 \pm 0.042^{**}$
K_{el}	0.3316 ± 0.083	$0.2924 \pm 0.067^{**}$	0.5135 ± 0.099	$0.3638 \pm 0.079^{**}$
AUC_{0-48} (µg/ml)	22.65 ± 4.65	$34.68 \pm 5.29^{**}$	55.68 ± 7.69	$88.19 \pm 10.38^{***}$
C_{max} (µg/ml)	1.75 ± 0.45	$2.98 \pm 0.53^{*}$	4.86 ± 0.81	$8.56 \pm 1.39^{***}$
T_{max} (h)	1	0.5	1	1
$T_{1/2}$ (h)	2.089 ± 0.55	$2.369 \pm 0.69^{*}$	1.349 ± 0.38	$1.904 \pm 0.42^{**}$
R.B%	100	149.058	100	158.95

Mean±S.D. (n = 6), K_a: Absorption rate constant, K_{el}: Elimination rate constant, AUC_{0-48} Area under the plasma concentration–time curve from 0 to 48 hours, C_{max}: Peak concentration, T_{max}: Time to reach peak concentration, $t_{1/2}$: Terminal half-life, RB: Relative bioavailability AUC rate compared to AUC control. * $p < 0.05$ compared to control, **$p < 0.01$ compared to control, ***$p < 0.001$ compared to control #=Not significant compared to control.

Co-administration of efavirenz (28 mg/kg) with piperine significantly increased AUC at $p<0.001$. C_{max} with co-administration of piperine increased significantly at $p<0.001$ compared to efavirenz control (28 mg/kg). The relative bioavailability of Piperine co-administration was found to be 158.92%, higher than efavirenz control. There was significant increase in $T_{1/2}$ of piperine co-administration at $p<0.01$ compared to efavirenz control. T_{max} of piperine co-administration and efavirenz were found to be same i.e., 1 h.

Discussion

The present study evaluated the effect of piperine on an anti-retroviral drug efavirenz which is prominently used in its class. Most of the currently used anti-retroviral drugs have a narrow therapeutic index. [9] Hence it is prudent to monitor the plasma drug levels to ensure that it is well within the therapeutic range. [10] Apart from studying pharmacokinetics it will also help to ensure that a minimum therapeutic concentration is achieved at the initiation of therapy and the maximum recommended therapeutic concentration is not exceeded during the dose escalation phase. [11] The enhancement of systematic availability of efavirenz by piperine assumes the additional significance to enhance the bioavailability of co-administered drugs, as reported in this and previous studies, can be considered from a different perspective. The results of this study demonstrated that efavirenz when co-administered with

piperine significantly increased the blood drug concentration. The enhanced absorption could be due to retardation of CYP3A4 by which efavirenz is metabolized and an alteration of membrane dynamics which aid in efficient permeability through membranes. The maximum concentrations reached in both the dose groups were significantly increased when piperine co-administered along with efavirenz, suggesting that the extent of absorption was also increased. It been postulated that piperine causes inhibition of the hepatic Cytochrome P450 enzyme system. This study may provide a therapeutic benefit whereby it enhances oral exposure and lowers the dose administered. Since the present study raised the awareness about the potential drug interactions by concomitant use of piperine, principal constituent of piper nigrum and piper longum [12], with efavirenz, the clinical significance of this finding needs to be further evaluated in the clinical studies.

Conclusion

Efavirenz (9.33mg/kg and 28 mg/kg) when administered along with piperine (20.8 mg/kg) significantly enhanced the oral exposure of drug in rabbits. Concomitant use of piperine or piperine-containing dietary supplements with efavirenz may require close monitoring for potential drug interaction. Further, oral efavirenz preparations along with bio-enhancer Piperine could be developed to ensure the less

adverse complications and lessening cost of medication with improved patient compliance.

Conflict of Interest

The author declares no conflict of interest, financial or otherwise.

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