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Impact of N-acyl piperidine (Piperine) from *Piper nigrum* on the Pharmacokinetics of CYP3A Substrate Almotriptan in Rats

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ABSTRACT

Almotriptan belongs to second-generation triptans, which were discovered and developed by Almirall for the treatment of severe migraine headaches. Piperine (N-acyl piperidine) is a plant alkaloid and a natural bioenhancer, which was found to reinforce the bioavailability of structurally and therapeutically different drugs. The study developed a validated high-performance liquid chromatography (HPLC) method for assessment of the pharmacokinetic profile after oral administration of almotriptan (1.2 mg/kg) alone and in combination with Piperine (10 mg/kg) in rats. Pharmacokinetic profile was determined at 0 (predose), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, and 24 hours post-treatment using blood samples. The results indicated that the percentage change of Peak Concentration (C_{max}), Maximum Time for maximum concentration (T_{max}), Area Under Curve (AUC_{0-24}, $AUC_{0-\infty}$, AUC%) , Area Under Moment Curve ($AUMC_{0-24}$, $AUMC_{0-\infty}$) , Half-life ($T_{1/2}$), Mean Residence Time (MRT_{0-24}, $MRT_{0-\infty}$), and volume of distribution (V_D) were increased approximately 67.63%, 26.04%, 72.12%, 88.71%, 100.37%, 93.40%, 163.72%, 52.79%, 12.89%, 39.53%, and 25.80%, respectively. In contrast, clearance decreased by 50% when almotriptan was co-administered with Piperine. Piperine significantly improved the fraction of almotriptan that reached the rat's systemic circulation. Therefore, co-administration of piperine improved the bioavailability of almotriptan and could be attributed to the inhibition of CYP3A and P-gp in rats.

Keywords: Almotriptan, Piperine, Bioavailability, Pharmacokinetics, P-glycoprotein, Cytochrome P-450.

Introduction

Migraine headache is an outrageous, pounding, characteristically uneven cerebral pain.[1] It is a neurovascular problem that includes dilatation of cerebral arteries, regularly incorporates queasiness, retching, affectability to light and sound.[1] A few people have visual indications before the cerebral pain, such as irregular lights or wavy lines called an aura. Headache assaults keep going for a considerable length of time or barely even for over a day. They can return regularly. About 8% of men and 20% of ladies experience the sporadic episodes of migraine headaches. Migraine headache occurs across the board in 30 to 50 years old patients because of stress.[2,3]
In headaches, the action of almotriptan is perceived as an agonist impact at 5-HT 1B/1D receptors on the walls of blood vessels: extracerebral and intracranial arteries and veins.[4] In migraine assaults, these veins are enlarged. It is retained and has moderate bioavailability (approximately 70%) after oral administration because of the metabolism by CYP3A enzyme in the hepatocytes before going into the blood circulation.[5]
Piperine (PIP) from *Piper nigrum* Linn (Black pepper) and nourishing alkaloid, has been utilized as a flavor and supplement enhancer.[3] Piperine has reported pharmacological properties, such as hostility to oxidation and reducing the frequency of mutation, ulcer, inflammation, and tumor formation.[6,7] Piperine upgrades the bioavailability of different medications, for example, phenytoin,[8-10] carbamazepine,[11] midazolam,[12] propranolol, and theophylline.[13]
Piperine is an inhibitor of pathways interceded by a few cytochrome P450 proteins (CYP) and P-glycoprotein (P-gp) an efflux layer transporter, as well as of phase II metabolism.[14-17] Piperine represses CYP3A4, CYP2C9, and UDP glucuronyl transferase subordinate metabolism.[18] It has been reported to enhance the pharmacokinetics of domperidone[19] and fexofenadine[20] (P-gp and CYP3A substrates) in rodents improved by restraint of CYP3A metabolism and P-gp interceded medication efflux. The study developed a validated HPLC method for assessment of the pharmacokinetic profile after oral administration of almotriptan alone and in combination with piperine in rats. It also assessed the impact of piperine when co-administered with almotriptan.

Materials and Methods

Drugs, chemicals, and animals
The almotriptan was acquired from LUPIN Pharma, Hyderabad (India). Almotriptan malate *d*6 (internal standard) and Piperine were bought from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. All the HPLC solvents like Acetonitrile, Sodium Lauryl Sulfate, Ortho Phosphoric Acid, and water were obtained from MERCK. Wistar rats were acquired from the National Institute of Nutrition, Hyderabad, India.

Pharmacokinetic study in rats
Preparation of drugs
Almotriptan was dissolved in distilled water. The Piperine (10 mg) was triturated in a spotless dry mortar with tween 80 (30 µL). A 10 mL of Sodium CMC (0.9%) was added and triturated again to suspend the Piperine in it. After completion of preparation, the suspension was

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put into plastic vials. Piperine suspension was utilized within 10 minutes of preparation.

Experimental procedure
Wistar rats of either sex (150 - 250 grams) were used. Standard conditions of temperature (25 ± 2ºC), relative humidity (50 ± 15%), and 12 h light/dark cycle. The rats were randomly allotted into two groups of six animals per group. The animals have fasted for 18 h, and however, had access to water *ad libitum*. The Institutional Animal Ethics Committee of the Vignan organization of pharmaceutical innovation approved the protocols with approval number 2003/PO/Re/S/18/CPCSEA. The study was done according to the rules of the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), India. The animals were treated with a single oral dose of almotriptan only (1.2 mg/kg b.w, Group I), or with both almotriptan (1.2 mg/kg b.w) and piperine (10 mg/kg b.w, Group II).

Collection of blood samples from rats
The blood samples were taken from retro-orbital sinuses at 0 (Predose), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24 hours post-treatment into the Eppendorf tubes (2 mL) containing sodium citrate. The blood plasma was separated by centrifugation at 6000 rpm for 10 min and stored at -20°C until further analysis. The amount of almotriptan in the plasma at different time intervals was predictable by a validated HPLC method.

Detection of almotriptan in plasma by a sensitive HPLC method
The almotriptan in plasma samples was determined by the internal standard HPLC method.[21] A calibration curve was developed for the estimation of almotriptan in blood samples.[21]

Standard solutions
The primary stock solution of almotriptan (1 mg/mL) was prepared in methanol. Appropriate dilutions of almotriptan from stock solution were prepared using the mobile phase to produce working stock solutions of 0.5, 1, 2, 4, 6, 8, 10, 20, 40, and 60 µg/mL. These dilutions were used to spike plasma in the preparation of the calibration of the curve. Almotriptan spiked plasma samples were prepared by mixing 1 mL blank plasma with appropriate volumes of the standard almotriptan solution (100 µL) on the day of the analysis. A blank was also prepared to contain 1 mL of plasma.

Extraction procedure
Plasma was spiked with changing concentrations of almotriptan stock solution to give a progression of medication concentrations going from 0.5 to 60 µg/mL. 125 µL of spiked plasma was taken, and to this 25 µL of internal standard (Almotriptan malate-*d*6 stock solution 1 mg/mL in methanol) was included and afterward vortexed (Vortex blender, Genei, Mumbai) for 60 sec. At that point 500 µL of methanol was added to precipitate the proteins and vortexed for 5 min and centrifuged at 6000 rpm in a microcentrifuge (REMI Scientifics, India) for 15 min. Supernatant was taken and dried in a vacuum stove at 40^{o}C. The dried material was then redispersed in 100 µL methanol and vortexed. The supernatant was moved into a microcentrifuge tube, and from this, 20 µL was infused for HPLC investigation.

Chromatographic conditions
A quaternary grade HPLC (Shimadzu) with a Rheodyne manual injector (Rheodyne, Cotati, CA) joined with a 100 µL sample loop was utilized for stacking the sample. A variable frequency programmable SPD-20A Prominence UV-VISIBLE detector and reversed-phase column of C-18 (Enable Make C18G (250 X 4.6 mm; 5 µm) was utilized. The HPLC framework, which was furnished with the EMPOWER 2 programming, was utilized for data acquisition and processing. The mobile phase was a blend of Methanol: Water: Acetic acid (60:30:10) v/v/v. The separated mobile phase components were siphoned from the particular reservoirs at the flow rate of 1 mL/min. The column temperature was kept at room temperature (30^{o}C). An SPD detector identified the eluent at a frequency of 235 nm.

Validation of HPLC method
Detection of wavelength
The standard solutions of almotriptan and Piperine were scanned in the range of 200-400 nm against the mobile phase as a blank. Almotriptan showed maximum absorbance at 235 nm. So, the wavelength taken for the estimation of almotriptan was 235 nm.

The specificity of the method
It was shown that the method was specific, as the chromatogram of blank plasma without added almotriptan failed to show any relevant peaks (Figure 2) where the relevant peaks were expected (Figure 4) with almotriptan spiked plasma.

Limits of detection (LOD) and quantitation (LOQ)
The LOQ and LOD were determined according to ICH Q2 guidelines. The standard deviation of intercept (σ) and the slope of the calibration curves was used for the calculation of LOD (3.3.σ.s-1) and LOQ (10.σ.s-1). The LOD was defined as a signal/noise ratio of 3:1, and LOQ was with a signal/noise ratio of 10:1. For the HPLC-SPD detection method, the calibration curve of almotriptan was generated using the peak area at concentrations of 0.5-60 µg/mL.

*Pharmacokinetic **data analysis***
Pharmacokinetic data of almotriptan were determined using the Try-Kinetica software version 5.0 by NCA Assistant non-compartmental extravascular method.

Statistical Analysis
The data are mean ± S.D. The data were analyzed using two-way ANOVA followed by Bonferroni post hoc test. The correlations of pharmacokinetic parameters were interpreted using an unpaired t-test. *P < 0.05, **P < 0.01, ***P < 0.001 were considered as statistically significant. The Graph Pad Prism version 8.2.1 was used for statistical analysis.

Results and Discussion

HPLC method accuracy and precision
The HPLC assay method's accuracy and precision results are presented in Tables 2 and 3, respectively.

Calibration curve
The run time was set at 10 min, and almotriptan and internal standard (Almotriptan malate *d*6) appeared on the chromatogram at 3.154 min and 5.741 min, respectively, as shown in Figures 2 to 4. There was no interference of any other peak with the drug peak. When the same sample containing drug was injected six times, the drug's retention time was almost the same for all the six injection samples. A high correlation coefficient was observed (r = 0.999) in the range of 0.5-60 µg/mL. The regression of almotriptan concentration over its peak area was shown in Table 1 and Figure 1.

Effect of piperine on plasma concentration-time profiles of almotriptan
The plasma concentration vs. time profile of almotriptan in rats following oral treatment with almotriptan with and without piperine are shown in Table 4 and Figure 5. From the comparison of plasma concentration profiles of almotriptan alone and in combination with piperine, it is clear that there was a significant increase in the plasma concentration of almotriptan in the combination group at the following time points 0.5 h (^{ns}P > 0.05), 1 h (^{ns}P > 0.05), 1.5 h (***P < 0.001), 2 h (***P < 0.001), 2.5 h (***P < 0.001), 3 h (***P < 0.001), 3.5 h (***P < 0.001), 4 h (***P < 0.001), 6 h (***P < 0.001), 9 h (**P < 0.01), and 12 h (^{ns}P > 0.05).

Effect of piperine on pharmacokinetic parameters of almotriptan
Cmax
The Cmax of almotriptan significantly (***p < 0.001) increased in the rats treated with piperine and almotriptan (211.06 ± 3.92 ng/mL) compared to Cmax of almotriptan alone treated group (126.72 ± 4.91 ng/ml). The increase was almost 1.6 times. The percentage change of

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Cmax after treatment with piperine was found to be 67.63% (Histogram 1).

Tmax
The Tmax of almotriptan significantly (**p < 0.01) increased in the rats treated with piperine and almotriptan (2.42 ± 0.08 h) compared to the Tmax of almotriptan alone treated group (1.92 ± 0.08 h). The increase was almost 1.2 times. The percentage change of Tmax after treatment with piperine was found to be 26.04% (Histogram 1).

AUC0-24
The AUC0-24 of almotriptan significantly *** (p < 0.001) increased in the rats treated with piperine and almotriptan (1420.58 ± 59.59 ng/mL/h*h) compared to AUC0-24 of almotriptan alone treated group (825.11 ± 63.71 ng/ml/h*h). The increase was almost 1.7 times. The percentage change of AUC0-24 after the treatment with piperine was found to be 72.12% (Histogram 1).

AUC0-∞
The AUC0-∞ of almotriptan significantly (***p < 0.001) increased in the rats treated with piperine and almotriptan (1689.88 ± 57.6 ng/mL/h*h) compared to AUC0-∞ of almotriptan alone treated group (895.28 ± 64.64 ng/ml/h*h). The increase was almost 1.8 times. The percentage change of AUC0-∞ after treatment with piperine was found to be 88.71% (Histogram 1).

AUC%
The AUC% of almotriptan significantly (**p < 0.01) increased in the rats treated with piperine and almotriptan (15.99 ± 1.67%) compared to AUC% of almotriptan alone treated group (7.98 ± 1.33%). The increase was almost 2.1 times. The percentage change of AUC% after the treatment with piperine was found to be 100.37% (Histogram 1).

AUMC0-24
The AUMC0-24 of almotriptan significantly (***p < 0.001) increased in the rats treated with piperine and almotriptan (11152.99 ± 580.24 ng/mL/h*h) compared to AUMC0-24 of almotriptan alone treated group (5766.19 ± 471.73 ng/ml/h*h). The increase was almost 1.9 times. The percentage change of AUMC0-24 after the treatment with piperine was found to be 93.40% (Histogram 1).

AUMC0-∞
The AUMC0-∞ of almotriptan significantly (***p < 0.001) increased in the rats treated with piperine and almotriptan (21280.28 ± 1414.99 ng/mL/h*h) compared to AUMC0-∞ of almotriptan alone treated group (8069.99 ± 646.39 ng/ml/h*h). The increase was almost 2.6 times. The percentage change of AUMC0-∞ after treatment with piperine was found to be 163.72% (Histogram 1).

$T_{1/2}$
The t1/2 of almotriptan was significantly (**p < 0.01) increased in the rats treated with piperine and almotriptan (9.03 ± 0.87 h) compared to t1/2 of almotriptan alone treated group (5.91 ± 0.34 h). The increase was almost 1.8 times. The percentage change of $t_{½}$ after treatment with piperine was found to be 52.79% (Histogram 1).

MRT0-24
The MRT0-24 of almotriptan significantly (***p < 0.001) increased in the rats treated with Piperine and almotriptan (7.88 ± 0.16 h) compared to MRT0-24 of almotriptan alone treated group (6.98 ± 0.10 h). The increase was almost 1.16 times. The percentage change of MRT0-24 after the treatment of Piperine was found to be 12.89 % (Histogram.1).

MRT0-∞
The MRT0-∞ of Almotriptan significantly (**p<0.01) increased in the rats treated with piperine and almotriptan (12.60 ± 0.72 h) compared to MRT0-∞ of almotriptan alone treated group (9.03 ± 0.42 h). The increase was almost 1.3 times. The percentage change of MRT0-∞ after treatment with piperine was found to be 39.53% (Histogram 1).

Clearance
The clearance of almotriptan significantly (***p < 0.001) decreased in the rats treated with piperine and almotriptan (0.0007 ± 0.00001 L/h) compared to the clearance of almotriptan alone treated group (0.0014 ± 0.0001 L/h). The decrease was almost 0.5 times. The percentage decrease in clearance after the treatment with piperine was found to be 50% (Histogram 1).

Volume of distribution
The volume of distribution of almotriptan did not significantly (P > 0.05) increased in the rats treated with piperine and almotriptan (0.0117 ± 0.0010 L) compared to the volume of distribution of almotriptan alone treated group (0.0093 ± 0.0010 L). The increase was almost 1.2 times. The percentage change of volume of distribution after treatment with piperine was found to be25.80% (Histogram 1).

The HPLC method developed was validated for Intra-day and inter-day variation. The results indicated that the HPLC method was highly reproducible. The developed LOD and LOQ HPLC method is simple, sensitive, precise, and highly accurate and requires only a small quantity of plasma samples. The method applies to the pharmacokinetic evaluation of almotriptan in rat plasma.
The results demonstrated that piperine pretreatment increased bioavailability and decreased the clearance of almotriptan when compared to the control. The findings suggest a connection between almotriptan concentration and CYP3A action.[22]

Table 1: Calibration of the HPLC method for the estimation of almotriptan in plasma using almotriptan malate *d*6 (Internal standard)

Plasma concentration of Almotriptan (µg/mL)	Mean peak area of Almotriptan	Mean peak area of Internal standard	Mean peak area ratio
0.5	69701.45	395212.25	0.18
1	139412.90	404381.06	0.34
2	278815.80	410293.08	0.68
4	557621.60	399571.37	1.40
6	836407.40	393094.02	2.13
8	1087335.62	405212.38	2.68
10	1413545.41	419062.72	3.37
20	2827060.81	402849.16	7.02
40	5654151.62	415802.35	13.60
60	8481280.44	399624.04	21.22

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Table 2: Recovery of Almotriptan at selected concentrations

Amount of drug added (µg)	Amount of Almotriptan	
	Amount of drug (µg) recovered	% of drug recovered
2	1.91 ± 0.15	99.04 ± 0.31
6	5.82 ± 0.11	99.08 ± 1.59
10	8.97 ± 0.046	99.72 ± 0.15
40	38.79 ± 0.21	99.85 ± 0.13

Data represent Mean ± SD, n = 6.

Table 3: Precision of the proposed HPCL method for Almotriptan

Drug concentration (µg/mL)	Amount of Almotriptan (µg/mL) found on	
	Intra-day (%CV)	Inter-day (%CV)
2	1.95 (0.81)	1.94 (1.26)
6	5.94 (0.28)	5.92 (0.48)
10	9.65 (3.49)	9.79 (1.49)
40	38.98 (1.28)	38.56 (0.82)

n = 6

Table 4: Summary of mean plasma concentrations of almotriptan treated group and almotriptan with piperine treated group: a single-dose study

Time Points (h)	Almotriptan (1.2 mg/kg)	Almotriptan (1.2 mg/kg) + Piperine (10 mg/kg)
0	0	0
0.5	41.53 ± 4.40	51.43 ± 4.67$^{n.s}$
1	72.20 ± 6.48	84.30 ± 3.07$^{n.s}$
1.5	105.71 ± 6.60	151.97 ± 3.87***
2	125.47 ± 5.00	189.47 ± 5.13***
2.5	111.70 ± 5.09	206.06 ± 6.81***
3	100.84 ± 4.91	176.39 ± 7.73***
3.5	85.12 ± 4.88	148.50 ± 9.69***
4	72.58 ± 6.17	110.88 ± 8.67***
6	56.09 ± 6.64	87.58 ± 5.32***
9	37.96 ± 4.83	66.56 ± 5.54**
12	19.79 ± 2.64	37.33 ± 3.21$^{n.s}$

CYP3A has a significant role in microsomal medication metabolism. CYP3A4 is dynamic in the catabolism of lipophilic substrates, such as fentanyl, alfentanil, oxycodone, and methadone.[23-25] Almotriptan is one of the substrates of CYP3A and P-gp. The metabolism of almotriptan can determine the pace of hepatic CYP3A activity in the liver and P-gp activity in the digestive tract. Any adjustments in the CYP3A and P-gp enzymatic pathway may influence almotriptan metabolism. [26]

It has been reported that piperine interrupts some CYP450 mediated pathways.[27] Piperine is a non-specific inhibitor of CYP3A; however, it has lower action on the other microsomal enzymes. The results showed that piperine significantly increased the bioavailability of almotriptan and prolong the half-life of almotriptan. Piperine hinders P-glycoprotein and CYP3A actions in GIT and liver,[28] thus playing a vital role in the degradation of drugs before entering the blood circulation.

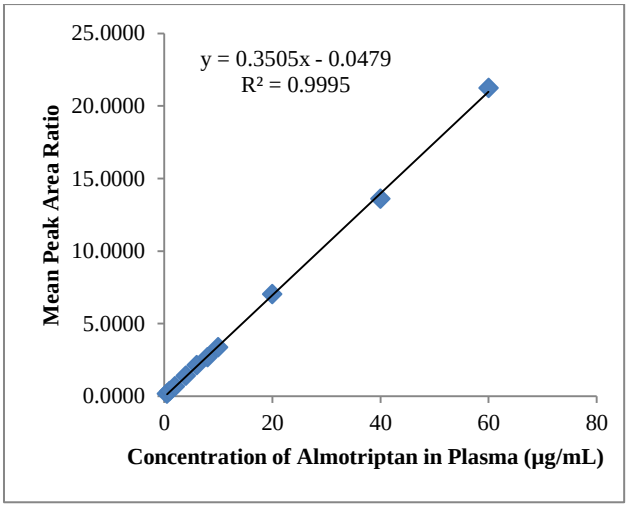

Figure 1: Calibration curve for the estimation of Almotriptan in plasma

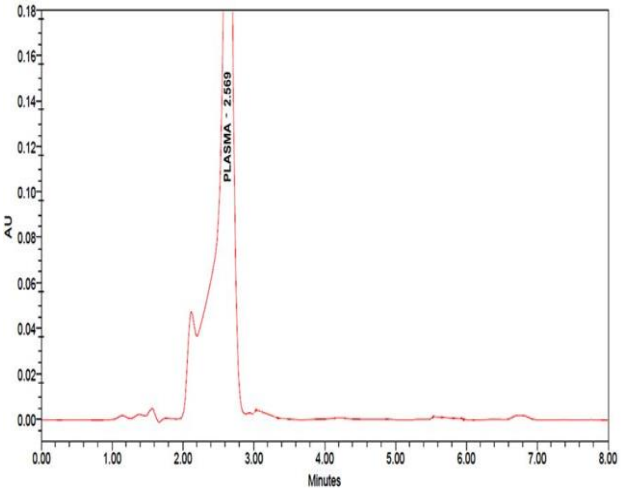

Figure 2: HPLC chromatogram of blank plasma

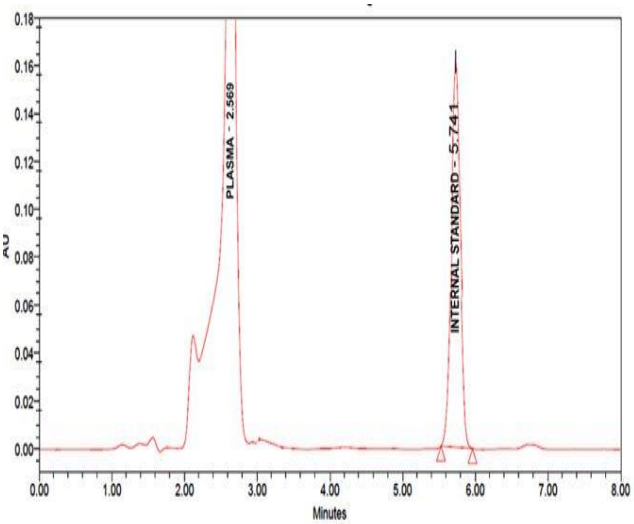

Figure 3: HPLC chromatogram of blank plasma with internal standard (Almotriptan malate *d*6)

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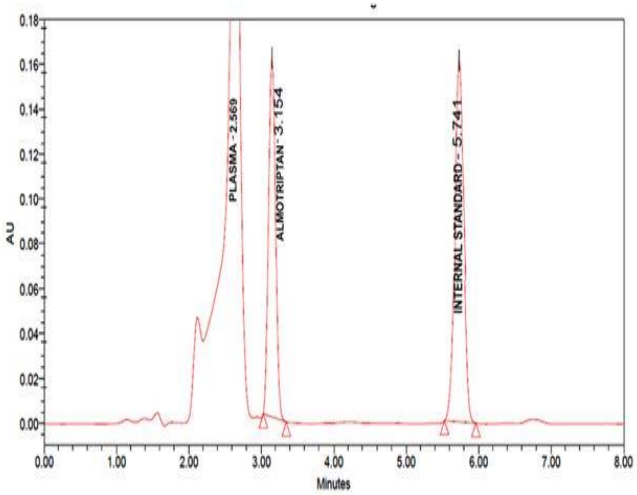


Figure 4: HPLC chromatogram of spiked plasma with almotriptan

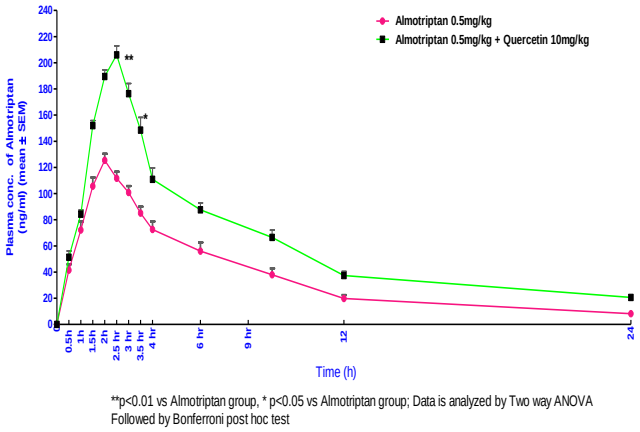
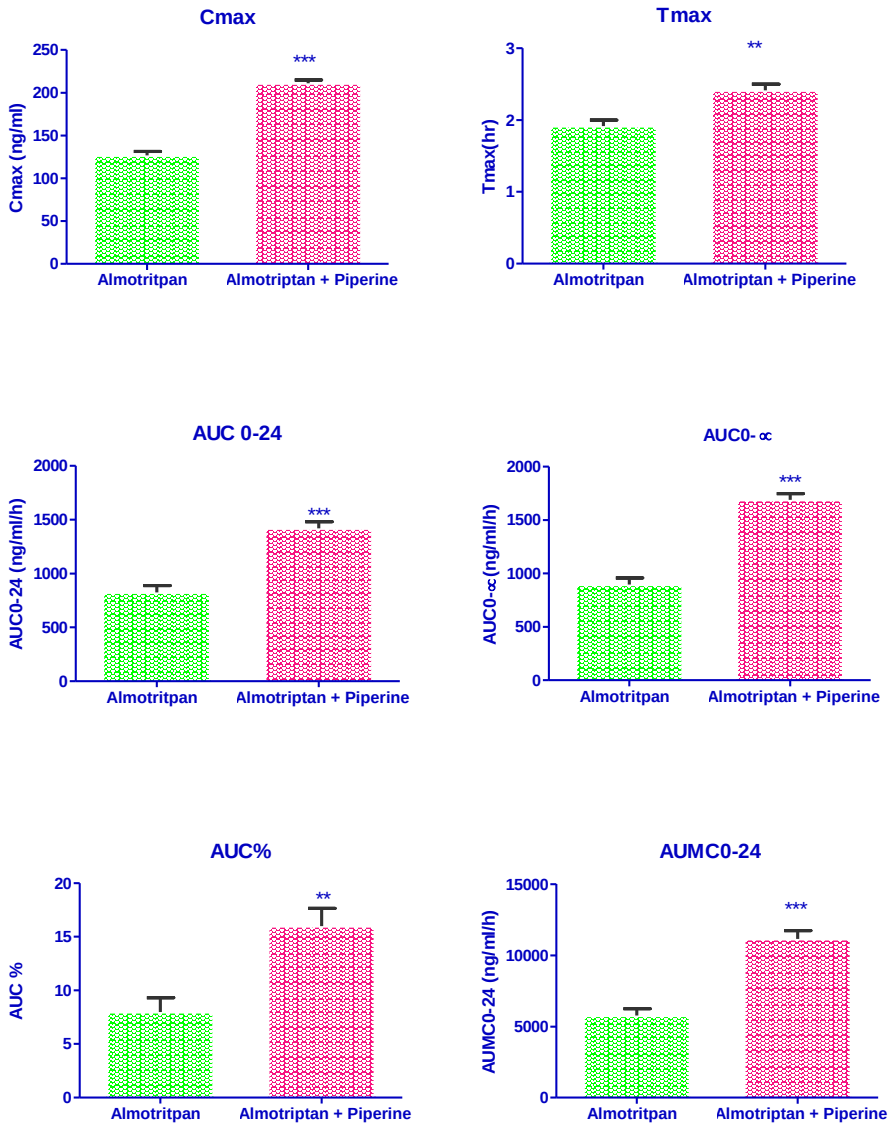


Figure 5: Comparison of mean plasma concentrations of almotriptan treated group and almotriptan with Piperine treated group: a single-dose study



Histogram 1: Summary of pharmacokinetic parameters of Almotriptan alone group and Almotriptan with Piperine group: a single-dose study

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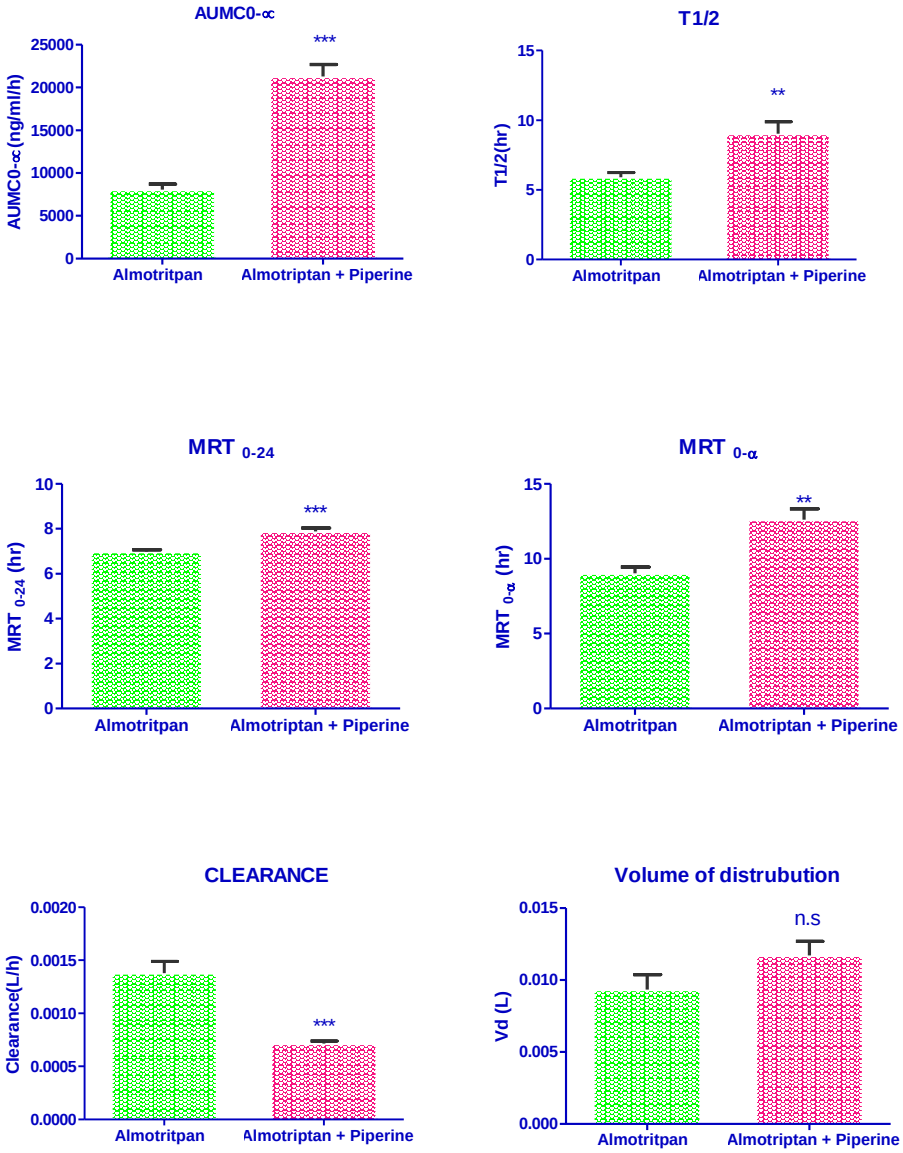

Histogram 1Cont'd: Summary of pharmacokinetic parameters of Almotriptan alone group and Almotriptan with Piperine group: a single-dose study

Conclusion

Piperine increased the oral pharmacokinetics of almotriptan, suggesting that the utilization of piperine and almotriptan could necessitate the use of lower doses of almotriptan thus reducing its adverse effects in migraine headache patients.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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