Bioavailability Enhancement of Labetalol Using A Bioenhancer Piperine in Rat Plasma

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Abstract

Labetalol is a first antihypertensive drug with combined α and β adrenoceptor blocking properties. Although the drug is readily absorbed after oral administration, it undergoes considerable hepatic first pass metabolism. Approximately 85% of Labetalol in the blood is removed during a single passage through the liver, and therefore low bioavailability of approximately 25-30% is achieved. The enzyme cytochrome P450 is responsible for the metabolism of the Labetalol in liver. The bioenhancer, piperine, is known to inhibit this metabolizing enzyme cytochrome P450. Thus, makes the rationale for present study involving combination of Labetalol along with a bioavailability enhancer, piperine. RP-HPLC method was developed for both analytical as well as bioanalytical analysis by using Agilent column, Eclipse plus C18 (250 mm × 4.6 mm, i.d,5 µm). The mobile phase 0.1% OPA in methanol: 0.1% OPA in the ratio 80:20 was optimized for the analysis, pH 3.5 was adjusted by using 0.1% OPA, flow rate of 0.8 mL/min and wavelength of 302nm was optimized for the analysis. Pharmacokinetic study was performed by collecting the blood samples from retro-orbital plexus of Wistar rats at different time intervals and the plasma protein was precipitated by using 100% cold ACN for extraction of Labetalol from the plasma sample. The results from pharmacokinetic study indicated increase in AUC from 0.5145 µg-h/ml to 0.7925 µg-h/ml and increase in Cmax from 0.1246 µg/ml to 0.2799 µg/ml for Labetalol alone and when Labetalol combined with piperine respectively. So, the bioavailability of the drug can be predicted to be increased twice as that of the original bioavailability of the drug. Also, this combination will help to reduce the dose of the Labetalol, which is high as compare to other antihypertensive drugs, thus the side effect will be reduced. The patient compliance and cost reduction of drug will also be achieved.

Keywords - Labetalol, piperine, HPLC- method development, bioenhancer, bioavailability, pharmacokinetics.

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Indroduction :

Labetalol has been widely used for the past two decades and is generally regarded as a safe and effective drug, but it undergoes extensive pre-systemic and systemic metabolism leading to its low oral bioavailability. Also, compared to other nonselective antihypertensive drug, the dose of Labetalol is also high (100-300mg) as it has comparatively less potency. Patients have to take the dose of 100mg thrice a day while 200mg twice a day leading to non-patient compliance.[1,2] Hence, considering the pharmacokinetics and disadvantages pertaining to Labetalol, there is a need to focus on enhancing its bioavailability, because the increase in bioavailability will lead to reduction in its dose and thereby reducing its side effects. The current study is based on Reversed phase HPLC (RP-HPLC) which has a non-polar stationary phase and an aqueous, moderately polar mobile phase. The work encompasses HPLC method development for simultaneous estimation of Labetalol and piperine, and HPLC method development for estimation of Labetalol in plasma followed by the pharmacokinetic study for Labetalol alone and its combination with piperine.

1. Materials And Methods

Reagents: The reagents for HPLC analysis were of HPLC grade such as methanol, orthophosphoric acid (OPA) and water. Reference standard of piperine was purchased from Sigma-Aldrich with 97% purity.

Instruments: The HPLC system consisted of Agilent HPLC 1200 Series Quaternary Gradient pump equipped with 20 µL loop and G1314B Multi Wavelength Detector. A C18 column (Agilent column, Eclipse plus, C18 column having dimension of 250 mm × 4.6 mm, with particle size of 5µ was used for the analysis.

1.1. Bioanalytical Method Development and Validation

1.1.1. *Bioanalytical method development*

a) Preparation of mobile phase, diluents and standard solutions

i) Preparation of mobile phase: The mobile phase consisted of a mixture of 0.1% OPA in methanol and 0.1% OPA (pH= 3.5) in the ratio 80:20 v/v. It was filtered through a 0.45 µ membrane filter and degassed.

ii) Preparation of diluent: The diluent consisted of a mixture of methanol and 0.1% OPA in the ratio of 80:20 (same as the mobile phase).

iii) Preparation of standard solutions: 100mg of Labetalol and piperine each was accurately weighed and transferred to 100 mL volumetric flask each and the volume was made up to the mark with diluent to give standard stock solution of 1000 µg/ml of Labetalol and piperine. From the standard stock solution, mixed standard solution was prepared by taking 1mL aliquot from standard solution of Labetalol and piperine each and transferring to 10 mL volumetric flask and making up to the mark to contain 100 µg/ml of Labetalol and piperine.

iv) Preparation of sample solutions of piperine and Labetalol: Stock solutions (1000 µg/ml) of Labetalol and piperine were prepared in diluent. Working solutions of Labetalol and piperine were prepared by appropriate dilutions of the stock solution with the diluent. All the solutions were prepared freshly prior to the analysis.

b) Selection of stationary phase: A reverse phase C18 (octadecylsilane) column was selected for analysis due to its flexibility in solvent selection with different range of polarity and pH. The column dimensions were 250 × 4.6 mm with a particle size of 5 µ.

c) Preparation of plasma sample for the determination of Labetalol: 300 µL of rat plasma was transferred into a 1.5 mL eppendorf tube. 300 µL of precipitating agent was added (precipitating agent was 100% cold ACN) and vortexed for 5 min and the mixture was centrifuged at 2000 rpm for 20 min. The supernatant layer was transferred into another tube and reconstituted with 300 µL of diluent and vortexed for 1 min. This was filtered through a 0.2 µ syringe filter. A 20 µL of the filtered solution was injected into the HPLC column.

1.1.2. *Optimization of Method:*

1. Selection of column: Agilent column, Eclipse plus C18, 250 mm × 4.6 mm, i.d,5 µm was used. C18 column was used for the analysis as it is rugged, retentive, and widely used for analysis when an extended pH range is required. Also, it is a most commonly used type of column due to efficiency in terms of selectivity, lifetime and operating pressure.
2. Selection of mobile phase: From the various trials performed, the mobile phase consisting of 0.1% OPA in methanol and 0.1% OPA (80:20) resulted into a sharp peak with better resolution for simultaneous estimation of Labetalol and piperine. Hence, 0.1% OPA in methanol: 0.1% OPA in the ratio 80:20 was optimized as the mobile phase for the analysis.
3. Selection of pH: In the trails that was performed during the development of mobile phase, pH 3.5 adjusted by using 0.1% OPA gave the good retention time. For further bioanalytical method, higher retention time for Labetalol was an important criterion to avoid interference with plasma components. Hence, the pH of 3.5 was optimized.
4. Selection of flow rate: The analysis was carried out at 0.8, 0.9 and 1 mL/min, considering the system suitability parameters like number of theoretical plates and peak symmetry, were better when run at 0.8 mL/min; hence 0.8 mL/min was optimized as flow rate.
5. Selection of detector wavelength: The λ_{max} of Labetalol and piperine are 302 nm and 342 nm respectively. Labetalol was less detected with wavelength other than 302 nm, while piperine was detected even at 302 nm. Hence, 302 nm was selected as an optimized wavelength.
6. Selection of column temperature: The analysis was carried out at ambient temperature.

The retention time of Labetalol and piperine was at 5.82 and 6.91 respectively. The plasma peak retained at 3.92 min. Thus, there was no interference of the plasma peak with that of the drug peak. **(figure 1)**

The HPLC method was validated as per US FDA guidelines for various parameters such as selectivity, accuracy, precision, recovery, calibration curve and stability of the analyte in rat plasma sample

1.1.3. *Bioanalytical Method Validation (As per FDA guidelines)* **[10]**

The developed bioanalytical method using HPLC was validated for various parameters such as selectivity, accuracy, precision, recovery, calibration curve and stability of the analyte in rat plasma sample.

1. Selectivity

Selectivity is the ability of an analytical method

to differentiate and quantify the analyte in the presence of other components in the sample. Six plasma samples were chromatographed to check for endogenous components which might interfere with Labetalol and piperine. Spiked plasma samples representing a low (0.5 µg/ml), medium (1.5 µg/ml) and high (5 µg/ml) concentration were analyzed to verify the selectivity of the method of analysis. The HPLC chromatograms of blank plasma and for plasma sample spiked with Labetalol and piperine are shown in **Figure 2** and **Figure 1** respectively**.**

2. Linearity

The linearity was determined from the constructed standard calibration curve. Samples were prepared and injected on the same day. Six concentrations (0.1, 0.5, 1, 1.5, 2.5 and 5µg/ml) which included the lower limit of quantification (LLOQ i.e. 0.1 µg/ml) were used to plot the calibration curve (n= 3). From the calibration curve plotted, the regression equation for Labetalol was found to be y = 39.792x +49.701 and the residual sum of squares of correlation coefficient (R^2) was found to be 0.99 and similarly for piperine it was found to be y = 215.95x + 125.16 and R^2 was found to be 0.99. Hence, the method was found to be linear in the concentration range 0.1-5.0 µg/ml.

3. Lower limit of quantification (LLOQ) and lower limit of detection (LLOD)

The lowest limit of quantification (LLOQ) is the analyte concentration that produced a signal to noise ratio greater than 5 and the lowest limit of detection (LLOD) is the analyte concentration at which the signal to noise ratio was more than 3.The LLOQ and LLOD as per these criteria were found to be 0.1 µg/mL and 0.06 µg/mL, respectively when 20 µL of sample was injected.

4. Extraction efficiency

The extraction efficiency was evaluated by comparing the % recovery of Labetalol after extraction from plasma sample corresponding to three concentration levels (low, medium, and high) with unextracted standards that represent 100% recovery. The % extraction recovery for the three concentration levels was found tobe 83.902%- 99.453%. **(Table 1)**

5. Accuracy

Accuracy was measured using five determinations per concentration. The three concentrations selected were 0.5, 1.5 and 4.5 µg/ ml. The % recovery of Labetalol from the plasma sample was found to be 99.5-100.6% at the three QC levels and it was found to be 98.56% at LLOQ. The mean value for % recovery was found to be within 15% of the actual value at three QC levels and was within 20 % of actual value at LLOQ (0.1µg/ ml). Hence, the method was found to be accurate for Labetalol

The % recovery of piperine from the plasma sample was found to be 100.48-102.62 at the three QC levels and it was found to be 97.10 % at LLOQ. The mean value for % recovery was found to be within 15% of the actual value at three QC levels and was within 20 % of actual value at LLOQ (0.1µg/ ml). Hence, the method was found to be accurate for piperine**. (Table 2)**

6. Precision

The concentration levels for precision studies included LLOQ (0.1µg/ml) and three QCsamples 0.5, 1.5 and 5µg/ml. The mean value for intra-day and inter-day precision studies of Labetalol did not exceed 20% of RSD at LLOQ and it did not exceed 15%of RSD at all the three QC levels. Hence, the method was found to be precise. Also, for piperine the mean value did not exceed 20%

of RSD at LLOQ and it did not exceed 15% of RSD at all the three QC levels. Hence, the method was found to be precise. **(Table 3 and 4 respectively)**

7. Stability

Freeze thaw stability of the spiked quality control samples was determined during three freeze thaw cycles stored at temperature below -20 ± 5°C. Stability was assessed by comparing against the freshly spiked quality control samples. The results for the freeze thaw stability of Labetalol in plasma are shown in **(Table 5)** The concentration changes in comparison to the nominal concentration were found to be less than 15%, indicating no significant loss of the drug during the study. Hence, the drug was found to be stable in all the three freeze thaw cycles for low QC as well as the high QC samples.

2. PHARMACOKINETIC STUDIES

1. Procurement of animals: Rats were procured and were transported using cool cab in polycarbonate cages as per the CPCSEA guidelines. The study protocols were approved by Institutional Animal Ethics Committee.

2. Animals Housing and preparation for study: Albino rats (Wistar strain) were procured and housed in animal house of C. U. Shah College of Pharmacy. Animals were acclimatized to the experimental room for one week and conditioned at room temperature and natural photoperiods. Animals were randomly selected, marked to permit individual identification, and caged in polypropylene cages containing paddy husk as bedding with maximum of three animals in each cage and provided free access to standard food pellets as basal diet and water.

3. Preparation of doses and its administration: Labetalol was dissolved in distilled water for oral administration. Doses were prepared freshly prior to administration. The drug was administered as a single dose by oral gavage using a suitable oral feeding needle.

4. Number of animals and dose levels: The rats were divided into three groups (6 rats per group) as detailed below:

Group 1: Control group (treated with 1mL of dextrose-normal saline solution)

Group 2: Treatment group I (treated with Labetalol)

Group 3: Treatment group II (treated with Labetalol and piperine combination)

The standard doses of Labetalol and piperine were selected by calculating it from the human dose. [43, 9] It was calculated based on the total body surface area of the rat using the conversion factor 0.018. [44] The maximum human dose selected for Labetalol and piperine were 300 mg and 2.2 g respectively for a 70 kg human. Dose for Labetalol was calculated as 0.0177mmol/kg and the dose for piperine was calculated as 0.1401mmol/kg.

2.1. Methodology followed for the pharmacokinetic study: Albino rats (wistar strain) weighing 180-200 g were randomly selected, marked to permit individual identification and quarantined for one week. The animals were divided into three groups and each group contained six animals. The animals were fasted 12 hours prior to dosing with free access to water. The selected doses of Labetalol (mmol/kg) and combination of Labetalol (0.0177mmol/kg) and piperine (0.1401mmol/kg) were administered orally usingoral gavage.

The plasma concentration-time profiles of Labetalol in Group II and Group III are shown in **Figure 3** and **Figure 4** respectively. The mean

plasma concentration at each sampling time was plotted. The concentration of Labetalol was monitored in plasma from the first post dose sampling time point (0 h), and followed up to 8 h after oral administration for both the groups.

Maximum plasma concentration (Cmax) of 0.1246 µg/ml was obtained at (tmax) 1 h for Group II which received Labetalol (0.0177 mmol/kg) alone. On the other hand, maximum plasma concentration (Cmax) of 0.2799µg/ml was obtained at (tmax) 1 h for Group III which received a combination of Labetalol (0.0177mmol/kg) and piperine (0.1401mmol/kg). Area under the curve (AUC 0-48) was calculated by trapezoidal rule. The plasma concentrations (µg/ml) of Labetalol for six different rats in the Group II at different time intervals are given in **Table 6.** The mean plasma concentrations (µg/ml) of Labetalol at different time intervals in Group II are tabulated in **Table 7.**

Plasma concentration - time curve for Group II was plotted as mean plasma concentrations (µg/ml) of Labetalol at different time intervals versus the time intervals (h). The plasma concentration - time curve, thus obtained is shown in **Figure 7.**

Discussion: As indicated in **Figure 7,** for Group II, maximum plasma concentration (C_{max}) of 0.1246 µg/mL was obtained at (t_{max}) 1 h and the area under the curve for Labetalol was found to be 0.5145 µg-h/mL. **Table 8** indicates that the plasma concentrations of Labetalol decreased gradually after 1h. There was decrease in absorption of Labetalol after 1h and Labetalol was not detected in the plasma samples collected at 24^{th} hour, indicating the complete elimination of the drug. The plasma concentrations (µg/ml) of Labetalol for six different rats in the Group III at different time intervals are tabulated in **Table 9.**

The mean plasma concentrations (µg/ml) of Labetalol at different time intervals in Group IIIare tabulated in **Table 10.**

The plasma concentration - time curve for group III was plotted as mean plasma concentrations (µg/ml) of Labetalol at different time intervals versus the time (h). The plasmaconcentration- time curve for group III, thus obtained is shown in **Figure 8.**

Discussion: For group III rats, the maximum plasma concentration (C_{max}) of 0.2799 µg/ml was obtained at 1 h (T_{max}). Group III rats received a combination of Labetalol (0.0177 mmol/kg) and piperine (0.1401 mmol/kg). (**Figure 8)** The area under the curve for Labetalol was found to be 0.7925 µg-h/ml

The plasma concentrations of Labetalol decreased gradually after 1h for group III. This indicated decrease in absorption of Labetalol after 1h and Labetalol was not detected in the plasma sample collected at 24^{th} hour, indicating the complete elimination of the drug. While,

for group II, maximum plasma concentration (C_{max}) of 0.1246 µg/ml was obtained at (T_{max}) 1h and the area under the curve for Labetalol was found to be 0.5145 µg-h/ml.

The results from the pharmacokinetic study show the evidence for increase in the AUC and C_{max}, for Labetalol in the group III (piperine administered group). On statistical analysis the p value or calculated probability was found to be 0.04 which is less than 0.05 ($p<0.05$) and thusthe increase was found to be statistically significant.

3. RESULT AND CONCLUSION

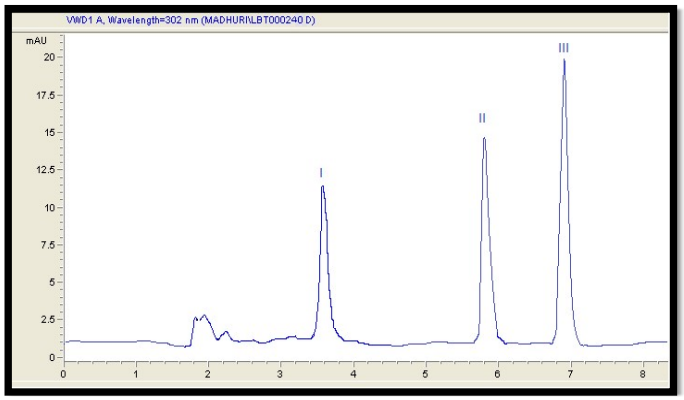

Figure 1: HPLC chromatogram of plasma sample spiked with Labetaloland piperine using mobile phase by 0.1% OPA in Methanol: 0.1% OPA pH=3.5 (80:20)

Retention time for plasma peak (I): 3.92 min. Retention time of Labetalol (II): 5.82 min.

Retention time of piperine (III): 6.91 min

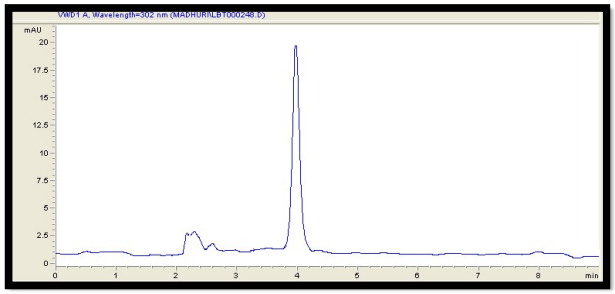

Figure 2: HPLC chromatogram of blank plasma

Table 1: Results for extraction efficiency of the extraction method

Concentration (µg/ml)	%Recovery	
	Mean	SD
0.5	93.83	1.79
1.5	83.90	0.99
5	99.45	0.15

Theoretical concentration (Cth, µg/ml)	Concentration after spiking	Area	Calculated concentration (Cexp µg/ml)	Mean Cexp (µg/ml)	SD	% RSD	% recovery
LLOQ 0.1	0.6	109.3	1.497	1.468	0.113	7.697	98.56
		112.2	1.570				
		110.2	1.520				
		108.6	1.480				
		100.4	1.274				
QC 1 0.5	1	124.5	1.87	1.552	0.066	4.252	99.59
		128.2	1.96				
		129.6	2.00				
		130.6	2.03				
		126.6	1.93				
QC 2 1.5	2	283.4	5.87	5.854	0.026	4.441	100.6
		284.2	5.89				
		282	5.83				
		281.9	5.83				
		282.7	5.85				
QC 3 4.5	5	354.4	7.65	7.652	0.079	1.032	99.80
		350.1	7.54				
		358.2	7.75				
		353.2	7.62				
		356.4	7.70				

Theoretical Concentration (Cth, µg/ml)	Concentration after spiking	Area	Calculated concentration (Cexp µg/ml)	Mean Cexp (µg/ml)	SD	% RSD	% recovery
LLOQ 0.1	0.6	194.2	0.466	0.485	0.024	5.147	97.1
		197.6	0.480				
		195	0.469				
		198.2	0.483				
		194.9	0.528				
QC 1 0.5	1	326.6	1.01	1.004	0.008	0.890	100.58
		322.4	0.99				
		325.8	1.01				
		324.1	1				
		325.9	1.01				
QC 2 1.5	2	577.1	2.04	2.048	0.008	0.408	102.62
		580.8	2.06				
		579.2	2.05				
		578.0	2.05				
		576.9	2.04				
QC 3 4.5	5	1296.5	5.02	5.022	0.021	0.431	100.48
		1304.5	5.05				
		1290	4.99				
		1298.1	5.02				
		1300.0	5.03				

Table 2: Results for accuracy studies for piperine

Table 3: Results for intra-day and inter-day precision studies of Labetalol

% Level	Area		Mean ± S.D.		% RSD	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
LLOQ 0.1 ppm	75.4 78.2 81.6 79.2 80.4	79.4 82.7 80.2 81.4 76.2	78.96 ± 2.36	79.97 ± 2.45	2.993	3.068
QC1 0.5 ppm	100.2 101.9 95.4 98.2 97.6	101.6 100.8 97.2 96.4 98.9	98.66 ± 2.49	98.98 ± 2.23	2.521	2.259
QC 2 1.5 ppm	192.6 190.5 186.6 192.3 191.4	194.0 192.3 189.4 193.6 192.1	190.68 ± 2.42	192.28 ± 1.80	1.714	0.938
QC 3 5 ppm	694.4 690.0 710.5 700.6 702.8	697.6 699.2 711.8 710.6 707.1	699.66 ± 7.89	702.26 ± 6.52	1.128	0.924

Table 4: Results for intra-day and inter-day precision studies of piperine

% Level	Area		Mean ± S.D.		% RSD	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-Day	Inter-day
LLOQ 0.1 ppm	138.1 135.8 140.5 139.2 138.6	137.6 136.8 139.4 141.2 139.8	138.44 ± 1.727	138.96 ± 1.762	1.247	1.268
QC1 0.5 ppm	184.2 177.7 171.3 174.1 178.3	185.6 179.4 172.8 173.2 179.4	177.12 ± 4.869	178.80 ± 5.285	2.749	2.967
QC 2 1.5 ppm	389.8 377.5 369.6 370.4 374.8	390 376.4 369.2 372.8 371.4	376.42 ± 8.148	375.96 ± 8.273	2.164	2.200
QC 3 5 ppm	1304.5 1290.0 1300.8 1289.6 1269.4	1308.8 1300.6 1326.1 1284.1 1271.6	1290.86 ± 13.672	1294.24 ± 15.878	1.059	1.226

Table 5: Results for freeze- thaw stability of Labetalol in plasma Samples (n=3)

Concentration (µg/ml)	% Recovery		
	1st Cycle	2nd Cycle	3rd Cycle
0.5	94.61	90.9	85.2
5.0	100	98.42	97.2

Table 6: HPLC analysis of Labetalol for group II rats at different time intervals

Sr. no	Time intervals (h)	Plasma concentration of Labetalol for 6 different rats in group II (µg/ml)					
1	0	ND	ND	ND	ND	ND	ND
2	1	0.1278	0.1145	0.998	0.1668	0.1363	0.1028
3	2	0.0989	0.0814	0.0758	0.0989	0.0898	0.0978
4	4	0.0875	0.0741	0.0621	0.0852	0.0789	0.0868
5	6	0.0512	0.0389	0.0214	0.0563	0.0472	0.0356
6	8	0.0112	0.0126	0.0138	0.0184	0.0102	0.0128
7	24	ND	ND	ND	ND	ND	ND
8	48	ND	ND	ND	ND	ND	ND

Table 7: Mean plasma concentration (µg/ml) of Labetalol at differenttime intervals in group II rats

Sr. no.	Time interval (h)	Mean plasma concentration (µg/ml) ± S.D.
1	0	0
2	1	0.1246 ± 0.0246
3	2	0.09043 ± 0.0099
4	4	0.0591 ± 0.0098
5	6	0.04176 ± 0.0125
6	8	0.01316 ± 0.0028
7	24	ND
8	48	ND

Figure 3: Mean concentration v/s time curve for Labetalol in group II rats

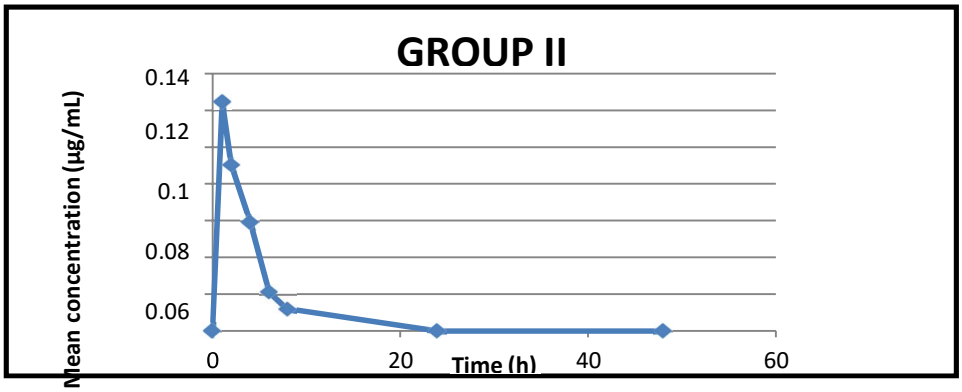

Table 8: HPLC analysis of Labetalol for group III rats at different time intervals

Sr. no	Time intervals (h)	Plasma concentration of Labetalol for 6 different rats in group III (µg/ml)					
1	0	ND	ND	ND	ND	ND	ND
2	1	0.2677	0.3251	0.2326	0.3774	0.2421	0.2345
3	2	0.1250	0.0928	0.1856	0.1294	0.1363	0.1023
4	4	0.0956	0.0812	0.0741	0.0912	0.0854	0.0912
5	6	0.0602	0.0621	0.0518	0.0424	0.0621	0.0542
6	8	0.0954	0.0245	0.0256	0.0352	0.0120	0.0236
7	24	ND	ND	ND	ND	ND	ND
8	48	ND	ND	ND	ND	ND	ND

Table 9: Mean plasma concentration for Labetalol at different time intervals ingroup III rats

Sr.no.	Time interval (h)	Mean plasma concentration (µg/ml) ± S.D.
1	0	0
2	1	0.2799 ± 0.05903
3	2	0.12856 ± 0.0325
4	4	0.08645 ± 0.0078
5	6	0.05546 ± 0.0076
6	8	0.03605 ± 0.0299
7	24	ND
8	48	ND

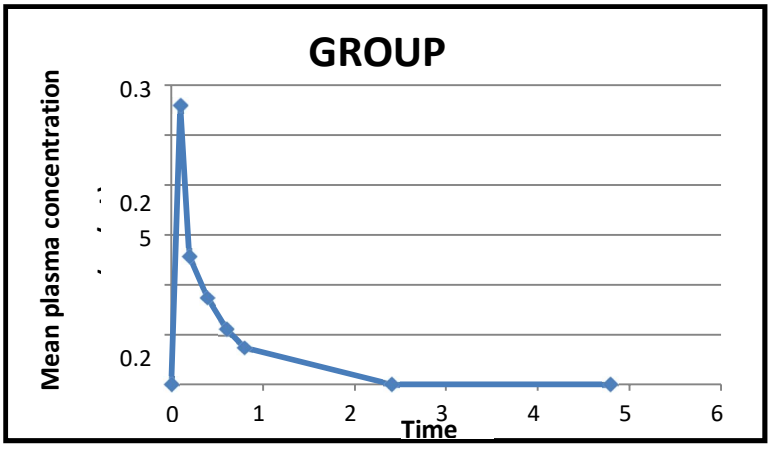

Figure 4: Mean concentration v/s time curve for Labetalol for group III rats

Table 10: Pharmacokinetic data of Labetalol

S.no	Parameter	Data	
		Group II	Group III
1	AUC	0.5145µg-h/ml	0.7925 µg-h/ml
2	C_{max}	0.1246 µg/ml	0.2799 µg/ml
3	t_{max}	1 h	1 h

Conclusion

It is concluded from the Pharmacokinetic study that the Cmax and AUC of Labetalol were found to be increased in the piperine administered group (Group III). On statistical analysis (Student t-test) of the results obtained, the p value was found to be 0.04. Since the p value was less than 0.05, the increase in Cmax and AUC in group III was found to be statistically significant. Also according to literature, piperine is known to have antihypertensive action, so it can be predicted that piperine can even show the synergistic effect with the Labetalol. Hence, we can conclude from the AUC and Cmax of Labetalol and Labetalol in combination with piperine that bioavailability of Labetalol has been increased. The increase in bioavailability of the Labetalol with piperine will lead to reduction in the dose of the drug thus reducing its side effects and thereby making it cost effect with better patient compliance. Thus, aim of the study has been successfully achieved

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