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EFFECT OF PIPERINE ON ORAL BIOAVAILABILITY OF DILTIAZEM HCL IN RABBITS.

Muneer babu. C and V.P Pandey

Department of Pharmacy, Annamalai University, Annamalainagar, Tamil Nadu, India
Corresponding author: Email**:**muneermpharm@yahoo.com Tel. No: 00966554009218

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ABSTRACT

Purpose: The bioavailability of oral diltiazem is mainly affected by CYP 3A4 and P-gp at the first-pass metabolism. The aim of this study was to investigate the effect of Piperine an alkaloid, on the pharmacokinetics of Diltiazem HCl in rabbits.
Methods: Rabbits were divided in four groups of six each: one control group, one co-administration group, one pretreatment group, and one of I.V group. A high performance liquid chromatography was employed to measure the plasma concentrations of diltiazem.
Results: The plasma concentrations of diltiazem were not significant when diltiazem was co-administered with piperine but it decreased significantly ($p < 0.04$) in the pretreated groups compared with the control. Conclusions: In the present investigation, piperine significantly decreased the in vivo bioavailability of diltiazem in the pretreatment group. These experimental findings explicitly convince that there is a possible interaction between diltiazem and piperine which has resulted in decreased serum diltiazem levels. However, further detailed research is needed to study the mechanism of decreased bioavailability of diltiazem via its combination with piperine as well as its effect on diltiazem metabolism.

Key words: Diltiazem; Piperine; Bioavailability; Pharmacokinetics; Rabbit.

[I] INTRODUCTION

Diltiazem is a calcium channel antagonist that is widely used in the treatment of angina, supraventricular arrhythmias and hypertension. Diltiazem undergoes an extensive presystemic metabolism and the absolute bioavailability is approximately 40%, with a large inter individual variation. It was reported that in humans and dogs, *N*-demethyldiltiazem was the most abundant metabolite in plasma. In contrast, desacetyldiltiazem and *O*-deacetyl-*N*-monodemethyl diltiazem were most predominant in the rabbits and rats, respectively. CYP3A4 is the main human isoform of the *N*-demethylation of diltiazem in liver microsomes. CYP 3A4 is mainly located in the liver, but it is also found in the intestine. Diltiazem could be metabolized in small intestine; the proximal segment is larger than the distal section [1],[2].

The reduced bioavailability of diltiazem after administering diltiazem orally might not only be due to the metabolizing enzyme CYP 3A4 but also to the P-glycoprotein (P-gp) efflux transporter in the small intestine [3]. Yusa and Tsuruo (1989)

reported that the calcium channel blockers verapamil, nicardipine and diltiazem competitively restrain the multidrug resistance of P-gp [4]. Saeki et al. (1993) reported that diltiazem is not only a MDR modulator but also a substrate for the efflux of P-gp [5]. Wacher et al. (2001) also reported that diltiazem is both a CYP 3A and P-gp substrate [6].

P-gp is found in the secretory epithelial tissues, including the brush border of the renal proximal tubules, the canalicular membranes in the liver and the apical membranes lining the gut. In addition, it is also found in the adrenal gland, placental trophoblast and endothelial blood barrier in the brain and testes. In the small intestine, P-gp is co-localized at the apical membrane of the cells with cytochrome P450 (CYP 3A4) P-gp and CYP3A4 might act synergistically to the presystemic drug metabolism to make the substrate of P-gp circulate between the lumen and epithelial cells, leading to prolonged exposure to CYP 3A4, resulting in a reduced absorption of the drug[7][8][9].

The bioavailability of oral diltiazem is mainly affected by CYP 3A4 and P-gp at the first-pass metabolism. When piperine administered with diltiazem orally, it might influence the bioavailability of diltiazem. However, there has been no report about if the piperine effluences the bioavailability of diltiazem in rabbits. The aim of this study was to examine the bioavailability of diltiazem when diltiazem was either co-administered or pretreated with piperine.

Piperine is the alkaloid responsible for the pungency of black pepper along with chavicine (an isomer of piperine). Piperine has also been found to inhibit human CYP3A4 and P-glycoprotein, enzymes important for the metabolism and transport of xenobiotics and metabolites. In animal studies, piperine also inhibited other enzymes important in drug metabolism. By inhibiting drug metabolism, piperine may increase the bioavailability of various compounds [10][11][12]. Notably, piperine may enhance bioavailability of curcumin by 2000% in humans. Several studies have reported enhancement of blood levels of drugs like vasicine, sparteine, phenytoin, propranolol, theophylline, rifampicin, sulphadiazine and tetracycline when co administered with piperine. The enhanced bioavailability of drugs is a result of inhibition of the drug metabolizing enzymes (Cytochrome P450) by piperine [13][14][15][16].

Therapeutic efficacy of diltiazem depends more upon, the peak plasma concentration of diltiazem attained, rather than, on the period for which the minimal inhibitory concentration are maintained. Thus any pharmacological interaction that may enhance or reduce the peak plasma concentration of diltiazem would have therapeutic implications. This study investigates the influence of piperine on the bioavailability of diltiazem in rabbits. If the results of the study are positive, then a reduction in the dosage and therefore the toxicity and cost of diltiazem can be envisaged.

[II] MATERIALS AND METHODS

2.1. Materials:

Diltiazem hydrochloride and imipramine hydrochloride was purchased from CEEAL LABS, Chennai. Piperine or 1 - peperoyl piperidine was purchased from the SAMI LABS ,Banglore. All other chemicals - Acetonitrile (HPLC grade), methanol (HPLC grade), Tert-butylmethylether (HPLC grade), HCl (AR) - were of reagent grade and were used without further purification.

2.2. Apparatus:

The apparatus used in this study were a high performance liquid chromatograph (LC-10AD liquid chromatograph pump, SIL-10A autoinjector, SPD-10A UV–vis detector, CBM-10A communications bus module), a mechanical stirrer, a centrifuge, a microcentrifuge, a sonicator and a rotamix.

2.3. Animal experiments and drug administration:

The male New Zealand white rabbits were given access to a normal standard chow diet and tap water ad libitum. Throughout the experiment, the animals were housed, two per cage, in laminar flow cages maintained at 22 ± 2 °C, 50–60%

relative humidity, under a 12 h light:12 h dark cycle. The animals were kept in these facilities for at least 1 week prior to the experiment. The research adhered to the Principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985).

Rabbits were divided in four groups of six each: one control group (diltiazem 15 mg/kg, oral), one co-administration groups (15 mg/kg diltiazem co-administered orally with piperine10 mg/kg), one pretreatment groups (15 mg/kg diltiazem pretreated orally with 10mg/kg piperine 0.5 h before), and one of i.v. group (intravenous administration of 5 mg/kg diltiazem).

Diltiazem dose (15 mg/kg) was chosen to keep plasma concentrations above the limit of detection at the time variation from 0 to 24 h in rabbits' plasma. The rabbits were fasted for at least 24 h prior to experiments and given free access to water. Diltiazem solutions were prepared by adding diltiazem (15 mg/kg) to distilled water (10 ml) and stirring for 1 h, and then administered orally through a catheter for the control. The mixtures for co-administered group were prepared by adding diltiazem (15 mg/kg) and piperine (10mg/kg) in distilled water (10 ml) and stirred for 1 h before administration. The piperine suspensions for pretreated groups were prepared by adding piperine (10mg/kg) to distilled water (5 ml) and stirring for 1 h, and piperine suspensions were administered orally 30 min prior to administration of diltiazem solutions. In order to estimate the absolute bioavailability (AB %), diltiazem (5 mg/kg) was injected through the ear vein by dissolving diltiazem in the saline solution [17].

Blood samples (1.2 ml) were withdrawn from the marginal ear vein at 0, 0.5, 1, 2, 3, 4, 8, and 12 h after the oral administration of the diltiazem to each of all rabbits. The blood samples were centrifuged at 13,000 rpm for 5 min. The plasma samples (0.5 ml) were stored at −40 °C until analyzed by the HPLC.

2.4. HPLC assay:

The plasma concentrations of diltiazem were determined by a HPLC assay and a modification of the method reported by Goebel and Kolle (1985). Briefly, 50 µl of imipramine (2 µg/ml), as the internal standard, and 5 ml of *tert*-butylmethylether were added to 0.5 ml of the plasma sample. It was then mixed for 20 min using a rotamix and centrifuged at 5000 rpm for 10 min. 4.5 ml of the organic layer were transferred to another capped tube, 0.3 ml of 0.01 N hydrochloride was added and the mixture was vortexed for 2 min. fifty microlitres of the water layer were injected into the HPLC system.

The chromatographic system was composed of LC-10AD liquid chromatograph pump, SIL-10A autoinjector, SPD-10A UV–vis detector, CBM-10A communications bus module (Shimadzu, Kyoto, Japan). The detector wavelength was set to 237 nm; and the column, a µ-bondapack C_{18} (3.9 × 300 mm, 10 µm, Waters Co., Ireland) was used at room temperature. Mixtures of methanol: acetonitrile:0.04 M ammonium bromide:triethylamine (24:31:45:0.1, v/v/v, pH 7.4, adjusted with acetic acid) were used as the mobile phase at a flow rate of 1.5 ml/min. The retention times are as follows: internal standard, 10.5 min; diltiazem, 8.0 min. The calibration curve of diltiazem was linear within range 5–400 ng/ml (r = 0.9999). Detection limit was defined below 5 ng/ml. The within-day (n = 5) and day-to-day (n = 5) coefficients of variation were less than 5% for diltiazem and 2% for imipramine. Recovery (%) was assessed from replicate analysis (n = 5) for 5 days by adding 20 and 200 ng/ml of diltiazem to rabbit's plasma shown 106 ± 5.7 and 101 ± 4.9, respectively[18].

2.5. Pharmacokinetic analysis:

Non-compartmental pharmacokinetic analysis was performed and trapezoidal rule was used to calculate the AUC of the plasma concentration (C_p) as a function of time (t). 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 elimination rate constant (K_{el}) was calculated from the slope of the line by regression analysis and the half-life ($t_{1/2}$) of the drug was obtained by $0.693/K_{el}$. The absolute bioavailability of diltiazem after being administered orally compared to the diltiazem that is injected intravenously was calculated as follows:

Absolute bioavailability (A.B %)

$$= \frac{AUC_{oral}}{AUC_{IV}} \times \frac{IV\ dose}{Oral\ dose} \times 100$$

The relative bioavailability of diltiazem administered orally was calculated as follows:

Relative bioavailability (R.B %)

$$= \frac{AUC_{pre\ treated}}{AUC_{control}} \times 100$$

2.6. Statistical analysis:

All the means are presented with their standard deviation (mean ± S.D.). An unpaired Student's *t*-test was used to determine the significant difference between the controls and the rabbits either co-administered or pretreated with piperine. A p value < 0.05 was considered significant.

[III] RESULTS

3.1. Group: I - Control Group:

[Table: I]

S.No	Time (Hrs)	Peak Area	Concentration (ng/ml)
1	0	0	0
2	0.5	92.67	75
3	1	83.82	66
4	2	72.078	55
5	4	53.907	40
6	6	24.090	20
7	8	23.226	15
8	10	21.797	10
9	12	-	0

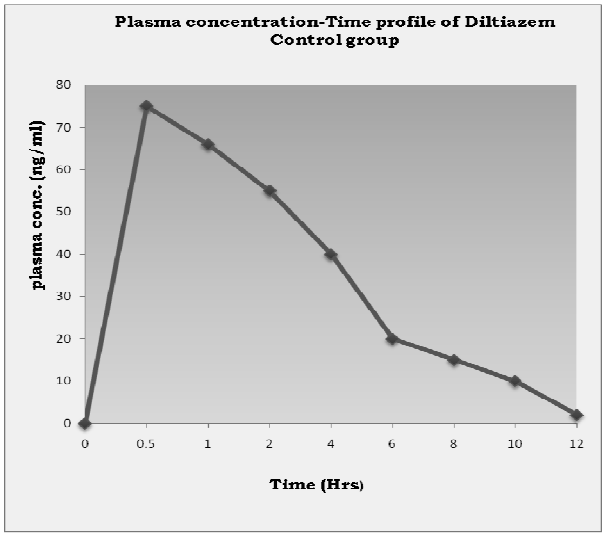

Figure -1

3.2. Group: II - Co Administration Group:

[Table: II]

S.No	Time (Hrs)	Peak Area	Concentration (ng/ml)
1	0	0	0
2	0.5	79.236	67
3	1	69.104	55
4	2	53	39
5	4	23.792	23
6	6	22.081	14
7	8	21	9
8	10	-	0
9	12	-	0

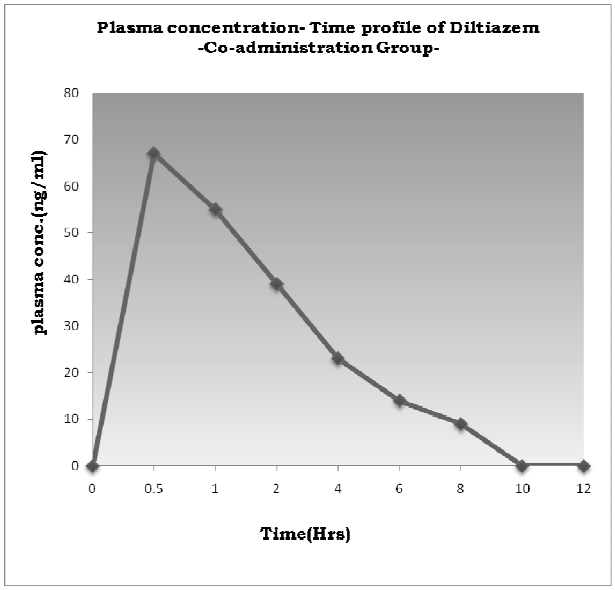

Figure -2

3.3. Group: III - Pretreatment Group:
[Table: III]

S.No	Time (Hrs)	Peak Area	Concentration (ng/ml)
1	0	0	0
2	0.5	72	62
3	1	60.117	51
4	2	53	39
5	4	23.492	19
6	6	22.690	13
7	8	8.875	8
8	10	-	0
9	12	-	0

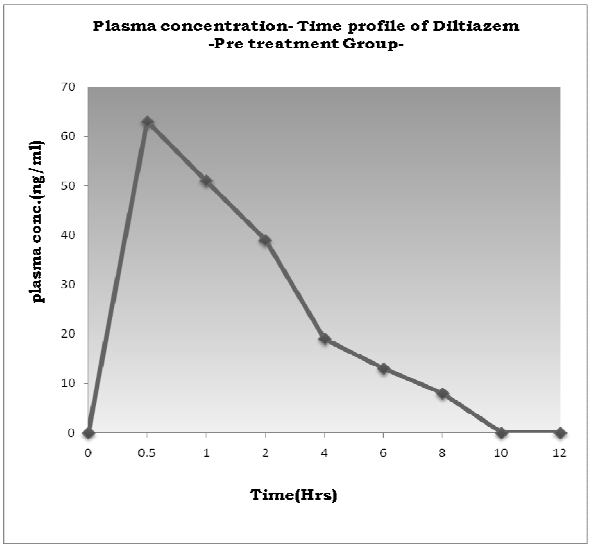

Figure-3

3.4. Group: IV – I.V Group:
[Table: IV]

S.No	Time (Hrs)	Peak Area	Concentration (ng/ml)
1	0	578-080	580
2	0.5	513.711	440
3	1	471.656	390
4	2	403.612	270
5	4	346.870	130
6	6	22.690	13
7	8	8.875	8
8	10	-	0
9	12	-	0

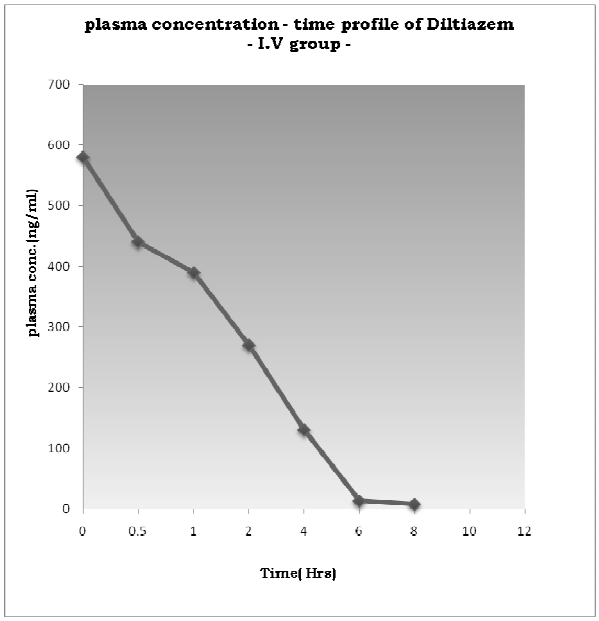

Figure- 4

3.5. Comparison of plasma concentration –time profile of different groups

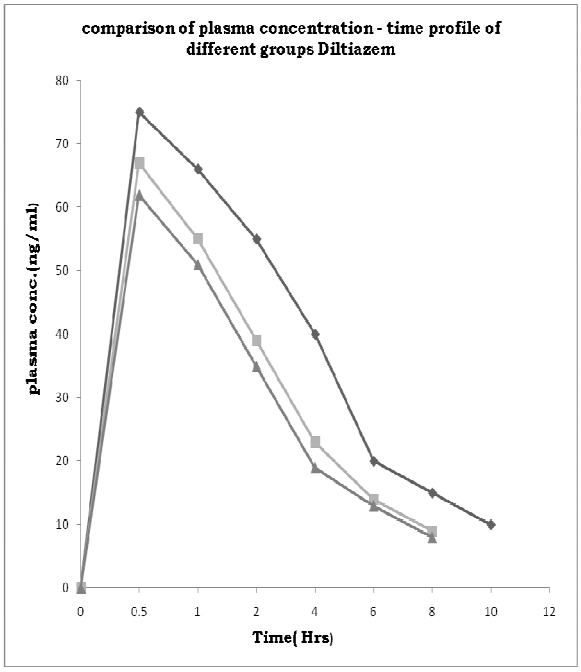

Figure- 5

Mean plasma concentration-time profiles of diltiazem after the oral co-administration and pretreatment with piperine (10mg/kg) to the rabbits.

(♦) - Control group
(■) - Co-administration group
(Δ) - Pretreatment group

3.6. Pharmacokinetic parameters of diltiazem after the oral co-administration of diltiazem (15 mg/kg) with piperine to the rabbits.
[Table: V]

Parameters	Diltiazem control	Piperine co-administration (10 mg/kg)	i.v. (5 mg/kg)
AUC (ng/ml h)	**321.5 ± 58**	**225.25 ± 71**	**1322 ± 467**
C_{max} (ng/ml)	**75 ± 23.5**	**67± 24.8**	**-**
T_{max} (h)	**0.5**	**0.5**	**-**
AB%	**8.1 ± 0.52**	**5.73 ± 0.88**	**100**
RB%	**100**	**70**	**-**

Mean ± S.D. (n = 8); AUC, area under the plasma concentration-time curve from 0 to 24 h. C_{max}, peak concentration; T_{max}, time to reach peak concentration; RB%, AUC rate compared to $AUC_{control}$; AB%, absolute bioavailability.

3.7. Pharmacokinetic parameters of diltiazem after the oral administration of diltiazem (15 mg/kg) pretreated with piperine to the rabbits.
[Table: VI]

Parameters	Diltiazem control	piperine pre-treatment (10mg/kg)	i.v. (5 mg/kg)
AUC (ng/ml h)	**321.5 ± 58**	**211.25 ± 107***	**1322 ± 467**
C_{max} (ng/ml)	**75 ± 23.5**	**62 ± 43.4***	**-**
T_{max} (h)	**0.5**	**0.5**	**-**
AB%	**8.1 ± 0.64**	**5.32 ± 1.21***	**100**
RB%	**100**	**65**	**-**

Mean ± S.D. (n = 8), $^{*}p < 0.05$, $^{**}p < 0.01$, significant difference compared to control; AUC: area under the plasma concentration-time curve from 0 to 24 h; C_{max}, peak concentration; T_{max}, time to reach peak concentration; RB%, AUC rate compared to $AUC_{control}$; AB%, absolute bioavailability.

[IV]. DISCUSSION.

The plasma concentrations of diltiazem after the oral administration of diltiazem (15 mg/kg), either co-administered or pretreated with piperine (10 mg/kg) to rabbits are shown in Table-II and Table III. The bioavailability and the pharmacokinetic parameters of diltiazem after administering diltiazem, either co-administered or pretreated with piperine are shown in Table V and Table VI.

When diltiazem (15 mg/kg) was co-administered with piperine (10 mg/kg), the plasma concentrations of diltiazem were not significant. However, it decreased significantly ($p < 0.04$) in the pretreated groups compared with the control. In the pretreated group, the AUC and C_{max} of diltiazem decreased significantly compared to the control.

The absolute bioavailability (AB %) of the diltiazem control was 8.1%, which was decreased significantly ($p < 0.04$) from 8.1% to 5.32% by pretreatment of piperine.

By the co-administration of piperine, the AUC and C_{max} of diltiazem were not significantly affected compared to the control.

[V]. CONCLUSION

In the present investigation, it was observed that the administration of diltiazem with piperine to rabbits significantly decreased diltiazem serum levels as compared to diltiazem alone treated rabbits (Table V& VI and Fig. 5).

These experimental findings explicitly convince that there is a possible interaction between diltiazem and piperine which has resulted in decreased serum diltiazem levels. Our experimental findings are in agreement with Dahanukar et al. (1982)[14] , Karan et al. (1998)[15]and L.G.Lala &P. M. D'Mello et al.(2003) who have demonstrated that Piperine decreases the bioavailability of rifampicin, isoniazid and diclofenac respectively. It is likely that each individual component may elicit a differential effect on absorption, distribution, metabolism, excretion or transport of diltiazem. To site an example, piperine has shown to affect the metabolism of oxyphenylbutazone, pentobarbital and others, largely by inhibiting various forms of monooxygenase (hepatic microsomal enzyme) involved in the oxidation of aliphatic hydroxylation (Atal et al., 1989)[12]. From the experimental findings it has been observed that in the initial half an hour, the absorption of the drug is declined significantly by piperine. Alternatively,

one is tempted to predict that piperine might affect or interfere with the transport or other processes, like absorption, distribution or excretion of the drug, as demonstrated in the case of phenylbutazone (Mujumdar et al., 1999)[19].

In conclusion, it can be stated that a thorough understanding of herbal drug interactions with the synthetic drugs is of paramount importance, as these interactions may lead to alteration of therapeutic responses of these drug materials.

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[VI]. REFERENCES

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