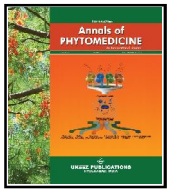
Annals of Phytomedicine 11(2): 654-660, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.2.80

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Effect of herbal bioenhancer (naringenin) on the pharmacokinetics of diltiazem in rats *via* CYP3A4 and P-glycoprotein inhibition

Ravindra Babu Pingili*, Sridhar Vemulapalli, Surya Sandeep Mullapudi***, Krishna Mohan Chinnala**** and Naveen Babu Kilaru*****◆

**Department of Pharmacology, School of Pharmacy and Technology Management, SVKM's NMIMS, Babulde, Shirpur-425405, Maharashtra, India*

***Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, Nebraska-68198, United States of America*

****Department of Pharmaceutics and Pharmaceutical Biotechnology, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada-520010, Andhra Pradesh, India*

*****Department of Pharmacology, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad-500088, Telangana State, India*

Article Info

Article history

Received 8 August 2022

Revised 25 September 2022

Accepted 26 September 2022

Published Online 30 December-2022

Keywords

CYP3A4

P-glycoprotein

Diltiazem

Naringenin

Everted sacs

Abstract

CYP3A4 and P-gp substrate is diltiazem and naringenin has been observed to affect both CYP3A4 and P-gp. The goal of this study was to observe how naringenin affected the pharmacokinetics (PK) of diltiazem in rats as well as absorption using everted gut sacs of rat *in vitro*. After 20 min, rats were administered orally with naringenin (12.5, 25 and 50 mg/kg) and then 15 mg/kg of diltiazem was also administered for 15 repeated days orally. In a single dosage PK study (SDS), blood was taken from the tail vein on the 1st day and in a multiple dosing PK study (MDS) on the 15th day. Thermokinetic was used to calculate the PK parameters. In a dose-dependent manner, naringenin pretreatment enhanced the Cmax and the AUC of diltiazem. At a dosage of naringenin 100 mg/kg, the Cmax of diltiazem increased from 39.276 ± 2.485 to 72.394 ± 5.152 and 44.982 ± 5.348 to 85.372 ± 5.263 ng/ml in SDS and MDS, respectively. In SDS and MDS, the AUC of diltiazem increased considerably from 818.206 ± 69.247 to 1448.781 ± 91.588 and 1730.362 ± 145.314 to 2677.052 ± 122.625 (ng/ml/h). *In vitro* test results revealed that diltiazem absorption was increased with naringenin and verapamil (a known standard P-gp and CYP3A4 inhibitor). The findings suggested that naringenin enhanced diltiazem absorption in the intestine due to P-gp and CYP3A4 inhibition.

1. Introduction

The bulk of medications' first-pass metabolism (FPM) and pharmacokinetics (PK) are influenced by cytochrome P-450 (CYP) and P-glycoprotein (P-gp). CYP3A4 is the important enzyme present in the human gut and liver (Paine *et al*., 2006; Rostami and Tucker, 2007; Shimada *et al*., 1994). A vast range of xenobiotic substances, including many medicinal medications, are known to be metabolized by CYP3A4 (Nebert and Russell, 2002). Drugs that undergo metabolism by CYP3A4 may combine with other medications that stimulate or inhibit CYP3A4, resulting in clinically relevant PK effects. Due to its broad-specificity and effect on drug PK characteristics, P-gp is an excellent example of a clinically useful drug transporter (Srivalli and Lakshmi, 2012). P-gp is an efflux transporter found in a variety of organs that have pharmacokinetic importance (Fardel *et al*., 2012). P-gp acts as a unidirectional efflux pump and play an important role in the PK of drugs (Aller *et al*., 2009). The Food and Drug Administration (FDA) further recommends that a screening to determine if prospective bioactive chemicals are P-gp substrates be carried out as early as feasible in the drug discovery pipeline (U.S. Food and Drug Administration, 2012).

Prescription and non-prescription medicines, as well as other xenobiotics present in some herbal remedies and food items, are CYP3A4 and P-gp modulators that can have clinically significant effects on some substrates (Rouveix, 2007). Diltiazem is a calcium channel blocker (CCB) that is commonly used to treat angina, supraventricular arrhythmias and hypertension (Millard *et al*., 1983, Buckley *et al*., 1990). Diltiazem has 40% absolute bioavailability because of first pass metabolism (FPM) mediated by CYP3A4 in the small intestine and liver (Pichard *et al*., 1990; Lefebvre *et al*., 1996). P-gp may be responsible for diltiazem's limited bioavailability in addition to extensive metabolism and diltiazem might be a substrate for both CYP3A4 and P-gp (Gottesman and Pastan, 1993; Gan *et al*., 1996; Wacher *et al*., 1998). Both P-gp and CYP3A4 are present in the small intestine, they may work together to increase FPM, resulting in drug absorption being reduced (Wacher *et al*., 2001; Ito *et al*., 1999). Naringenin is a citrus flavonoid that has an excellent antioxidant property. Several lines of research show that using naringenin supplements can help with obesity, diabetes, hypertension and metabolic syndrome (Paramita *et al*., 2021; Alam *et al*., 2014). In prior research, we found that naringenin inhibited CYP enzymes and P-gp (Surya *et al*., 2014). Previous studies suggested that some of

Corresponding author: Dr. Kilaru Naveen Babu

Professor, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada-520010, Andhra Pradesh, India

E-mail: naveenbabukvsr@gmail.com

Tel.: +91-9666212999

Copyright © 2022 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

the compounds acts as bioenhancers such as trikatu (Jatin *et al*., 2019), rutin (Falguni *et al*., 2019), piperine (Nitin *et al*., 2021; Megha *et al*., 2019), and catechin (Raseshkumar *et al*., 2021). However, there is no indication in the literature that naringenin has an effect on the PK of diltiazem. As a result, the goal of this study was to see how naringenin affected the PK of diltiazem in animal models.

2. Materials and Methods

2.1 Drugs and chemicals

Sigma Chemical Co. provided the naringenin (St. Louis, MO). Diltiazem and verapamil are provided by the Orchid Health Care, Chennai, India as gift samples and analytical grade solvents used for this study.

2.2 Laboratory animals

Animal studies were carried out in accordance with CPCSEA guidelines at Vijayawada. Mahaveer Enterprises in Hyderabad, India, provided male Wistar rats weighing 150-180 g. For at least one week before to the commencement of the tests, the animals were housed in typical laboratory settings. KVSRSCOPS/11-03-14-008 was the protocol approval number.

2.3 Study protocol

This investigation consisted of acute (single) and chonic (once daily for 15 days) administration of diltiazem and naringenin as previously described (Challa *et al*., 2013).

2.4 Single-dose PK study

Animals were divided into four groups (n = 6). For oral administration, diltiazem and naringenin were suspended in 0.5% SCMC. The rats were given the following treatment: Pretreated with naringenin (12.5 mg/kg), followed by diltiazem (15 mg/kg); Group III: Pretreated with naringenin (25 mg/kg), followed by diltiazem (15 mg/kg); Group IV: Pretreated with naringenin (50 mg/kg), following injection, 100 µl blood was withdrawn from the tail vein at various periods (0.16, 0.33, 0.5, 1, 2, 4, 6, 8, 12 and 24 h). The plasma was separated and stored at –20°C till analysis (R- 4C Compact, India).

2.5 Multiple dose PK study in rats

The rats in the multiple dose PK study (MDS) were given the same medications once a day for 15 days. The remaining procedure is same like SDS.

2.6 Drug absorption study using everted rat gut sacs *in vitro*

2.6.1 Gut sac preparation

The method that was described previously by Babu *et al*. (2013) for the preparation of everted gut sacs of rat ileum, followed for this study. Pentobarbital sodium 40 mg/kg was used to anaesthetize the male Wistar rats and the small intestine was torn out (Capraro *et al*., 2011). The intestinal digesta was removed and the distal ileums (about 15 cm each) were extracted and everted using glass rod.

2.6.2 Influence of naringenin on the intestinal absorption

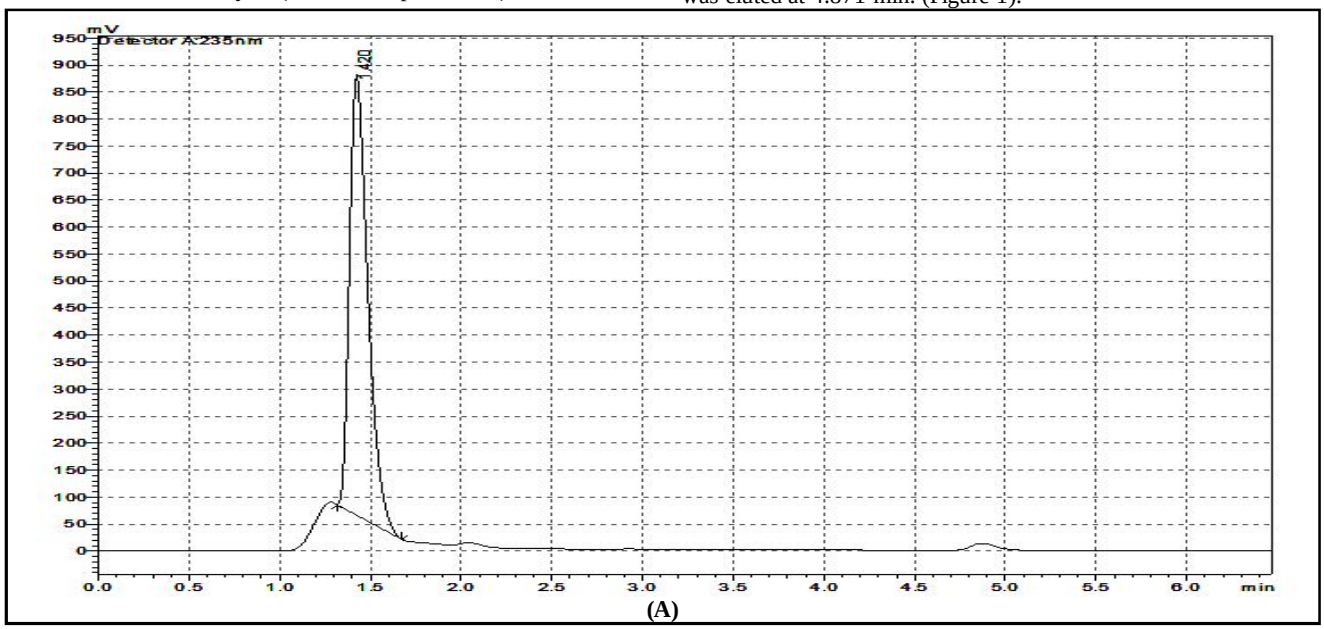
Krebs-Ringer bicarbonate buffer containing diltiazem (50 µg/ml) was filled in everted sacs and incubated in a shaker bath at 37°C for 60 min. At 10, 20, 30, 40, 50, and 60 min, 1 ml collected from the outer medium and the 1 ml buffer was replaced. The movement of diltiazem was determined using RP-HPLC. Each experiment was carried out three times. The same study was repeated with and without of verapamil (50 µg/ml) and naringenin 25, 50, and 100 µg/ml.

2.7 Extraction of diltiazem from plasma

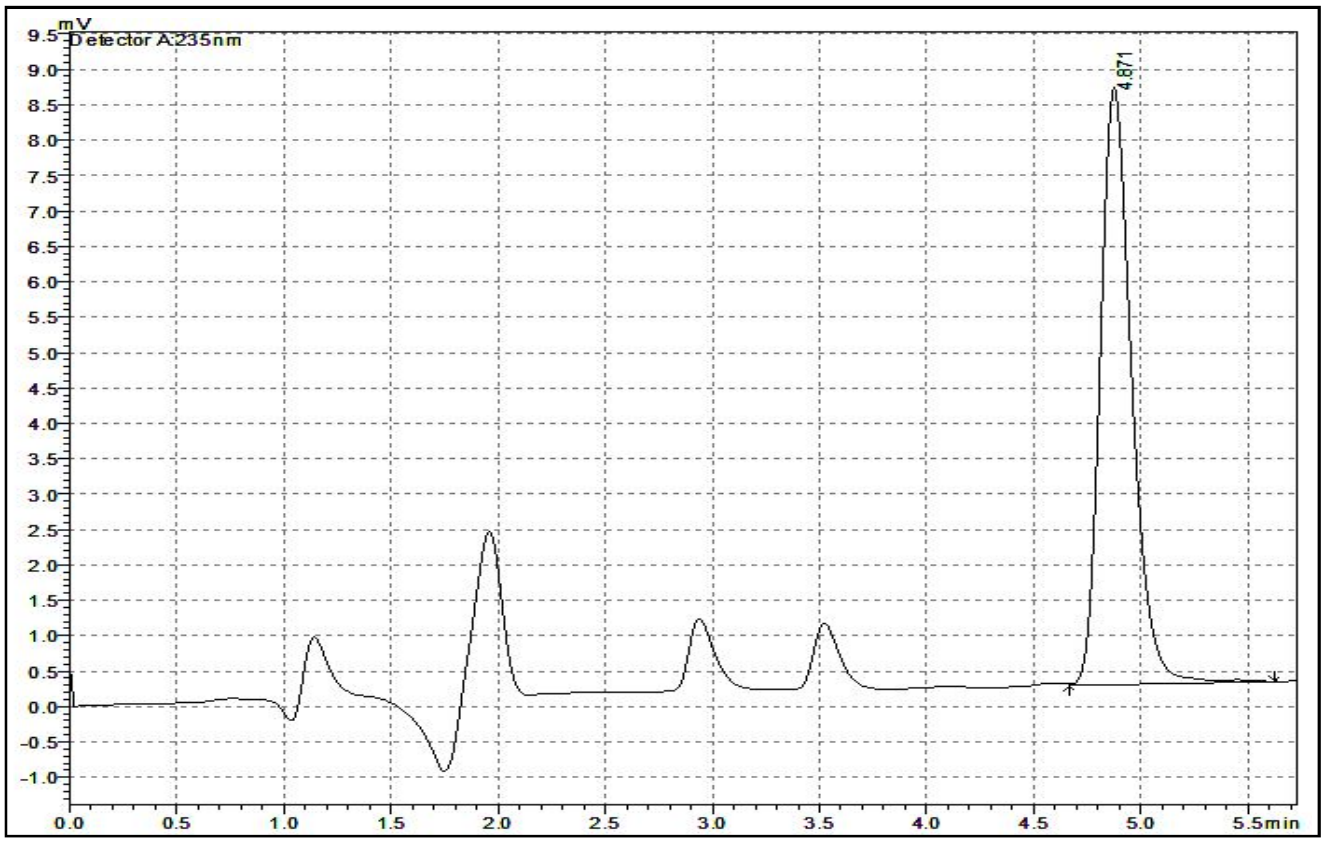
Diltiazem was extracted from rat plasma using a liquid–liquid extraction technique. To a 50 µl plasma, 1.5 ml ter-butyl methyl ether was added and vortex mixed for 5 min. The supernatant (1.4 ml) was dried and the residue was reconstituted in 50 µl of mobile phase.

2.8 Analytical methods

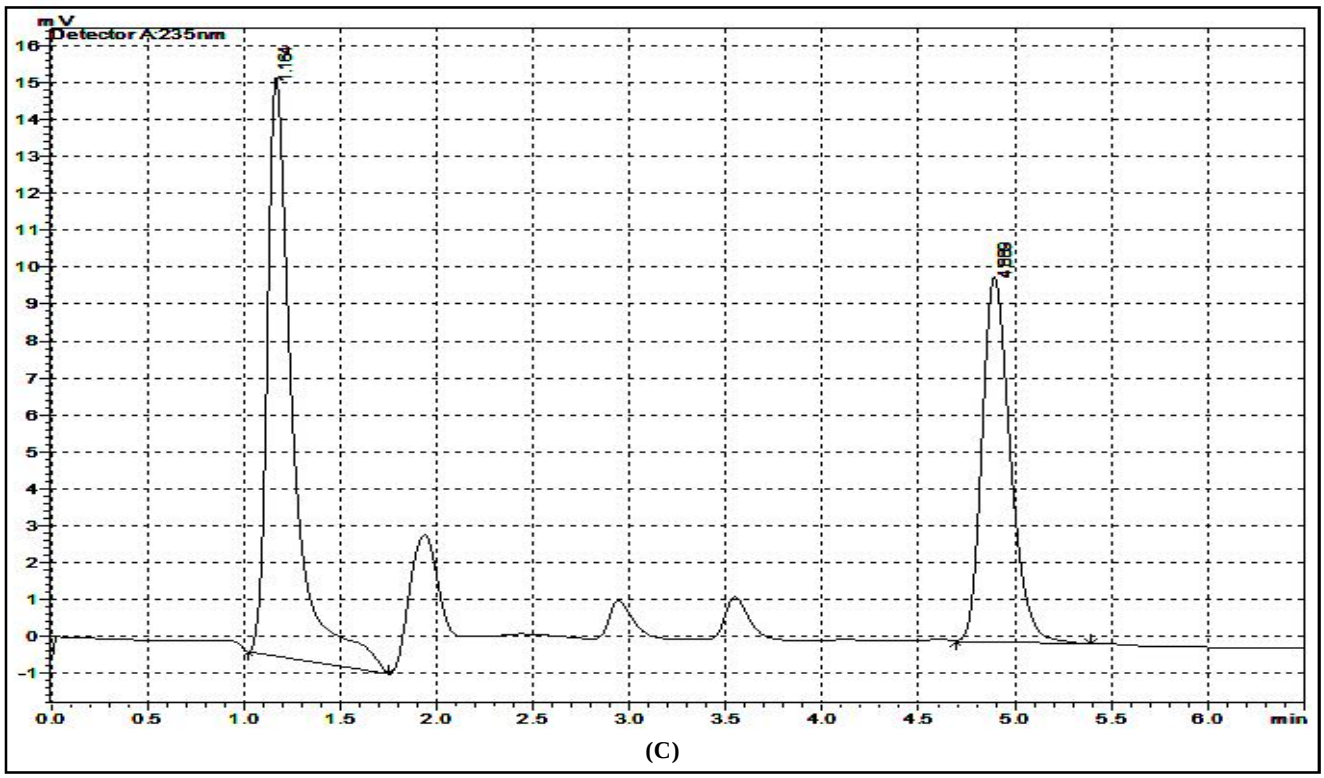
The concentrations of diltiazem in plasma samples were determined using a modified version of a previously reported technique (Babu *et al*., 2013; Kallem *et al*., 2013). The data was collected and processed using LC solution software (Tokyo, Japan). 0.2 % formic acid in acetonitrile and water (80:20 v/v) made up the mobile phase and the effluent was monitored at 235 nm with a UV detector and diltiazem was eluted at 4.871 min. (Figure 1).



(A)



(B)



(C)

Figure 1: Chromatograms: (A) Blank plasma, (B) Diltiazem hydrochloride (2 µg/ml) and (C) Plasma + Diltiazem hydrochloride 2 µg/ml.

2.9 Calculation of PK parameters

Thermokinetic (Version 5.1) was used to carry out a non-compartmental PK analysis.

2.10 Data analysis

Graph Pad Prism 5.0 was used to calculate all statistics (San Diego, CA). For multiple comparisons, one-way (ANOVA) and two-way ANOVA were used to compare the PK parameter values and plasma concentrations, respectively. $^{***}p<0.001$, $^{**}p<0.01$ and $^{*}p<0.05$ are considered significant.

3. Results

3.1 PK of diltiazem in single dose study

The plasma concentrations of diltiazem vs time profiles were shown in Figure 2. Naringenin raised diltiazem's Cmax, AUC_{0-24}, $AUC_{0-\infty}$, T_{max}, $t_{1/2}$ and MRT in a dose-dependently ($p<0.001$). The Cmax of diltiazem was significantly increased from 39.276 ± 2.485 to 42.482 ± 3.588, 58.425 ± 6.251 and 72.394 ± 5.152 ng/ml at 12.5, 25 and 50 mg/kg of naringenin, respectively. The AUC_{0-24} of diltiazem enhanced from 454.722 ± 58.365 to 689 ± 48.635 ng/ml/h (with 12.5 mg/kg), 671.603 ± 63.524 ng/ml/h (with 25 mg/kg), 920.574 ± 57.250 ng/ml/h (with 50 mg/kg) and $AUC_{0-\infty}$ of diltiazem raised from 818.206 ± 69.247 to 886.699 ± 74.258, 1107.681 ± 85.362, 1448.781 ± 91.588 ng/ml/h when naringenin pretreated at 12.5, 25 and 50 mg/kg, respectively. The T_{max} of diltiazem increased from 0.5 ± 0.1 to 1 ± 0.2 h at naringenin 50 mg/kg. The $t_{1/2}$ of diltiazem increased from 18.471 ± 2.657 to 20.493 ± 2.635 h (with 25 mg/kg) and 22.733 ± 2.165 h (with 50 mg/kg). The MRT of diltiazem also significantly increased from 28.128 ± 3.145 to 34.754 ± 4.366 and 38.810 ± 2.855 h with naringenin 25 and 50 mg/kg, respectively. The CL/F of diltiazem was significantly decreased from 3.954 ± 0.256 to 2.934 ± 0.522 ml/h/kg (at 25 mg/kg) and 2.243 ± 0.363 ml/h/kg (at 50 mg/kg) and the V_z/F was decreased significantly from 0.117 ± 0.01 to 0.102 ± 0.01, 0.072 ± 0.01 and 0.053 ± 0.01 ml/kg at 12.5, 25 and 50 mg/kg, respectively.

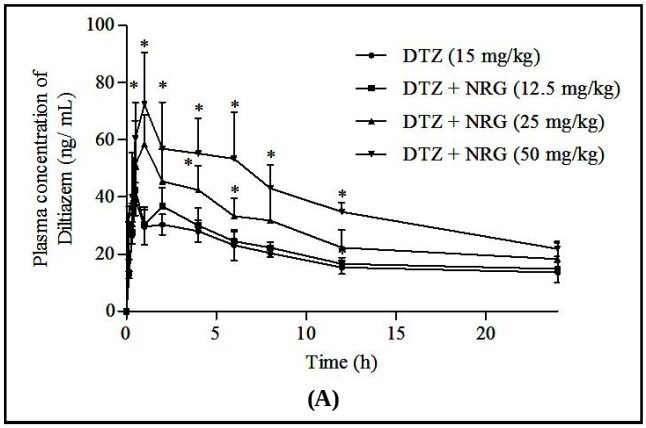
Table 1: PK parameters of diltiazem in single day study

PK parameter	DTH (15 mg/kg)	DTH + NRG (12.5 mg/kg)	DTH + NRG (25 mg/kg)	DTH + NRG (50 mg/kg)
C_{max} (ng/ml)	39.276 ± 2.485	42.482 ± 3.588^{NS}	58.425 ± 6.251**	72.394 ± 5.152***
AUC_{0-24} (ng/ml/h)	454.72 ± 58.37	495.69 ± 48.64^{NS}	671.60 ± 63.52***	920.57 ± 57.25***
$AUC_{0-\infty}$ (ng/ml/h)	818.21 ± 69.25	886.7 ± 74.26*	1107.68 ± 85.36***	1448.78 ± 91.58***
T_{max} (h)	0.5 ± 0.1	0.5 ± 0.1	1 ± 0.2*	1 ± 0.2*
$t_{1/2}$ (h)	18.471 ± 2.657	19.263 ± 2.415^{NS}	20.493 ± 2.635*	22.733 ± 2.165*
MRT (h)	28.128 ± 3.145	29.841 ± 3.657^{NS}	34.754 ± 4.366*	38.810 ± 2.855**
CL/F (ml/h/kg)	3.954 ± 0.256	3.665 ± 0.354^{NS}	2.934 ± 0.522*	2.243 ± 0.363**
V_z/F (ml/kg)	0.117 ± 0.01	0.102 ± 0.01^{NS}	0.072 ± 0.01**	0.053 ± 0.01***

DTH, Diltiazem, NRG, Naringenin.

3.2 PK of diltiazem in multiple dose study

Figure 2 represents plasma concentrations of diltiazem vs time profiles in MDS. Naringenin raised diltiazem's Cmax, AUC_{0-24}, Tmax, $t_{1/2}$ and MRT in a dose-dependently ($p<0.001$). The Cmax of diltiazem was significantly increased from 44.982 ± 5.348 to 51.075 ± 3.622, 61.773 ± 5.685 and 85.372 ± 5.263 ng/ml at 12.5, 25 and 50 mg/kg of naringenin, respectively. Diltiazem's AUC_{0-24} rise from 535.184 ± 34.241 to 595.066 ± 56.360 ng/ml/h (with 12.5 mg/kg), 905.392 ± 63.252 ng/ml/h (with 25 mg/kg), 1219.665 ± 68.325 ng/ml/h (with 50 mg/kg) and the $AUC_{0-\infty}$ of diltiazem increased from 1730.362 ± 145.314 to 1396.677 ± 75.245, 1942.561 ± 68.458, 2677.052 ± 122.625 ng/ml/h when naringenin pretreated at the dose of 12.5, 25 and 50 mg/kg, respectively. At a dosage of naringenin 50 mg/kg, the Tmax of diltiazem rose from 0.5 ± 0.1 to 1 ± 0.2 h. Diltiazem's $t_{1/2}$ rose from 20.659 ± 3.847 to 28.093 ± 3.584 h (for 25 mg/kg) and 28.536 ± 3.584 h (for 50 mg/kg). With naringenin 25 and 50 mg/kg, the MRT of diltiazem rose dramatically from 38.290 ± 5.652 to 44.620 ± 4.635 and 47.943 ± 3.120 h, respectively. The CL/F of diltiazem was significantly reduced from 2.087 ± 0.1 to 1.373 ± 0.254 ml/h/kg (at 25 mg/kg) and 1.214 ± 0.325 ml/h/kg (at 50 mg/kg) and the V_z/F was reduced significantly from 0.828 ± 0.01 to 0.096 ± 0.01, 0.595 ± 0.04 and 0.460 ± 0.03 ml/kg at 12.5, 25 and 50 mg/kg, respectively.



(A)

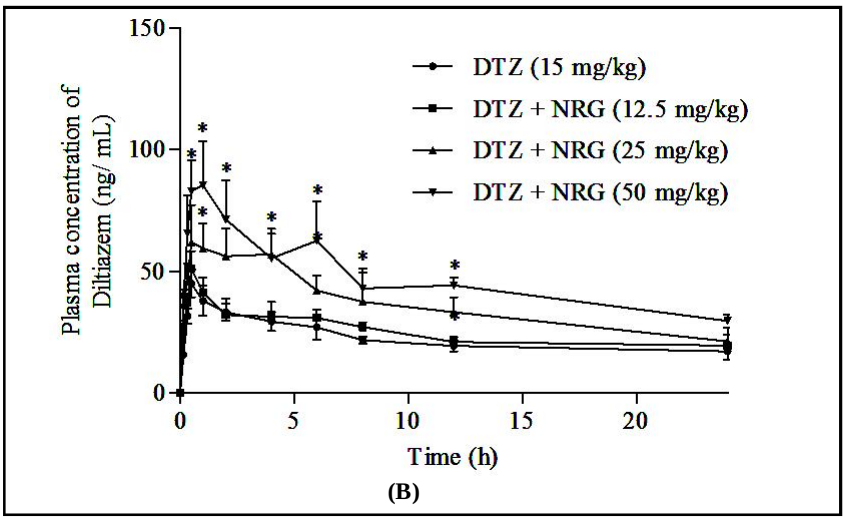


Figure 2: Diltiazem plasma concentration and time plots (A) 1st day and (B) 15th day.

Table 2: PK parameters of diltiazem on 15th day

PK parameter	DTH (15 mg/kg)	DTH + NRG (12.5 mg/kg)	DTH + NRG (25 mg/kg)	DTH + NRG (50 mg/kg)
C_{max} (ng/ml)	44.982 ± 5.348	51.075 ± 3.622^{NS}	61.773 ± 5.685**	85.372 ± 5.263***
AUC_{0-24} (ng/ml/h)	535.18 ± 34.24	595.06 ± 56.36^{NS}	905.39 ± 63.25**	1219.66 ± 68.33***
$AUC_{0-\infty}$ (ng/ml/h)	1730.36 ± 145.31	1396.68 ± 75.25	1942.56 ± 68.46*	2677.05 ± 122.63***
T_{max} (h)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	1.0 ± 0.2*
$t_{1/2}$ (h)	20.659 ± 3.847	27.603 ± 3.685*	28.093 ± 3.584*	28.536 ± 3.584*
MRT (h)	38.290 ± 5.652	40.669 ± 3.241^{NS}	44.620 ± 4.635*	47.943 ± 3.120*
CL/F (ml/h/kg)	2.087 ± 0.1	1.472 ± 0.362*	1.373 ± 0.254*	1.214 ± 0.325*
V_z/F (ml/kg)	0.828 ± 0.01	0.096 ± 0.01*	0.595 ± 0.04*	0.460 ± 0.03*

DTH, Diltiazem, NRG, Naringenin.

3.3 Effect of naringenin on the transport of diltiazem

Table 3 illustrates the transport/absorption of diltiazem in rat gut sacs *in vitro*. Naringenin boosted diltiazem transport considerably (p<0.001). In the presence of naringenin at 25, 50 and 100 µg/ml, the transport of diltiazem rose dramatically from 3.896 ± 0.884 to 4.226 ± 0.578, 5.677 ± 1.220, and 6.522 ± 1.032 µg/ml, respectively, following a 10 min incubation period. After 60 min of incubation, diltiazem transport increased considerably from 11.026 ± 1.811 to 13.695 ± 2.633 µg/ml (with 25 µg/ml of naringenin), 16.114 ± 2.556 µg/ml (with 50 µg/ml of naringenin), and 18.956 ± 3.651 µg/ml (with 100 g/ml of naringenin). Verapamil, a common P-gp and CYP3A4 inhibitor, boosted diltiazem transport from 3.896 ± 0.884 to 6.110 ± 1.232 g/ml after a 10 min incubation period and from 11.026 ± 1.811 to 18.362 ± 3.652 g/ml after a 60 min incubation period.

Table 3: Effect of naringenin on the absorption of diltiazem

Time (Min)	DTH (50 µg/ml)	DTH + VER (50 µg/ml)	DTH + NRG (25 µg/ml)	DTH + NRG (50 µg/ml)	DTH + NRG (100 µg/ml)
10	3.89 ± 0.88	6.11 ± 1.23**	4.22 ± 0.57^{NS}	5.67 ± 1.22*	6.52 ± 1.03**
20	5.45 ± 1.63	8.54 ± 1.84**	5.58 ± 1.12^{NS}	7.45 ± 1.65**	8.85 ± 1.45**
30	6.58 ± 1.74	10.60 ± 2.36**	7.36 ± 1.63^{NS}	9.58 ± 2.25**	10.78 ± 1.48**
40	7.89 ± 2.14	13.52 ± 2.45***	8.66 ± 2.84^{NS}	10.88 ± 1.47**	14.52 ± 1.36***
50	9.66 ± 1.68	16.22 ± 2.52***	11.32 ± 2.42*	13.58 ± 2.62**	16.77 ± 2.58***
60	11.02 ± 1.81	18.36 ± 3.65***	13.69 ± 2.63*	16.11 ± 2.55**	18.95 ± 3.65***

DTH, diltiazem, VER, verapamil and NRG, naringenin.

4. Discussion

The pharmacokinetics of diltiazem in rats was considerably influenced by naringenin in this investigation. These findings are in line with previous research. Because of inhibition of both CYP3A4-mediated metabolism and P-gp-mediated transport in the intestine and/or liver, resveratrol significantly increased the Cmax (from 165 ± 37.8 to 259 ± 60.6 ng/ml), AUC (342 ± 80.2 to 547 ± 131 ng/ml) and bioavailability of diltiazem at the dose 10 mg/kg in rats (Soon *et al*., 2008). In rats, lovastatin dramatically increased the systemic availability of diltiazem. At a dosage of lovastatin (1 mg/kg), the $AUC_{0-\infty}$ of diltiazem was raised from 355 ± 69 to 508 ± 107 ng h/ml, and the Cmax was increased from 165 ± 35 to 234 ± 53 ng/ml. At a dosage of lovastatin (1 mg/kg), the volume of distribution of diltiazem was reduced from 52.2 ± 14.9 to 42.4 ± 12.2 ml/kg and the clearance was reduced from 45.2 ± 13.8 to 38.0 ± 9.9 ml/min per kg. The enhanced bioavailability of diltiazem in the presence of lovastatin might be due to suppression of CYP3A4 and P-gp-mediated efflux pump in the gut and/or liver's (Soon *et al*., 2011).

In another investigation, simvastatin also increased the diltiazem bioavailability in rats and Cmax and AUC were raised from 182 ± 33 to 246 ± 44 ng/ml and 270 ± 51 to 392 ± 74 ng. h/ml, respectively. The increase in diltiazem oral bioavailability might be due to increased absorption in the small intestine due to P-gp inhibition, as well as reduced FPM of diltiazem in the small intestine and/or liver due to CYP3A subfamily suppression in the small intestine and/or liver (Dong *et al*., 2011). Similarly, the influence of atorvastatin on diltiazem PK was studied to see whether there were any possible pharmacological interactions between atorvastatin and diltiazem. Atorvastatin dramatically increased diltiazem oral exposure in rats (Soon *et al*., 2007). Cimetidine has a considerable impact on diltiazem rabbits' pharmacokinetic characteristics (Choi and Jin, 2004). In comparison to control rabbits, the Cmax of diltiazem in rabbits pretreated with cimetidine rose and lasted substantially longer ($p< 0.05$). In the presence of hesperidin (5 or 15 mg/kg), the $AUC_{0-\infty}$ of diltiazem was considerably (5 mg/kg, $p< 0.05$; 15 mg/kg, $p< 0.01$) raised by 48.9 to 65.3 %, and the Imax was significantly ($p< 0.05$) increased by 46.7 to 62.4 %. As a result, due to inhibition of P-gp and CYP3A4, the absolute bioavailability (F) of diltiazem was considerably (5 mg/kg, $p<0.05$; 15 mg/kg, $p< 0.01$) greater than in the control group and the relative bioavailability (RB) of diltiazem was enhanced by 1.49-1.65 times by hesperidin (Young *et al*., 2009). Naringenin raised the Imax, AUC, $t_{1/2}$, and lowered the clearance and volume of distribution of diltiazem in rats in the current research.

In the current *in vitro* investigation, diltiazem transport was also greatly boosted in the presence of naringenin. Surya *et al*. (2014) used rat everted gut sacs *in vitro* to explore the influence of naringenin on the transit of felodipine. Because P-gp and CYP3A4 were inhibited in the presence of naringenin, felodipine transport was enhanced (Surya *et al*., 2014). Due to inhibition of P-gp and CYP enzymes, naringenin increased the transport of rasagiline, a substrate of P-gp and CYP enzymes, in another *in vitro* investigation employing rat everted gut sacs (Pingili *et al*., 2016). Naringenin enhanced diltiazem transport/absorption in the current research,

5. Conclusion

Both CYP3A4 and P-gp inhibitors greatly influence the systemic availability of drugs. The current investigation found that naringenin raised the Cmax, AUC, $t_{1/2}$, MRT and lowered the clearance, $V_{z/F}$, of diltiazem in rats, which might be attributed to inhibition of CYP3A4 and P-gp. *In vitro* investigations utilizing rat everted gut sacs showed that diltiazem transport was greatly boosted in the presence of naringenin and verapamil, both of which are P-gp and CYP3A4 inhibitors.

Acknowledgements

We are very grateful to the management of SVKM's NMIMS and Siddhartha College for facilities provided to carry out this work.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

Abbreviations

CYP3A4, Cytochrome P-450 3A4; P-gp, P-glycoprotein; PK, Pharmacokinetics; SDS, Single dosage PK study; MDS, Multiple dosing PK study; FPM, first-pass metabolism; FDA, Food and Drug Administration; CCB, Calcium channel blocker; DTZ, Diltiazem; NRG, Naringenin and VER, Verapamil.

References

Alam, M.A.; Nusrat, S.; Rahman, M.M.; Shaikh, J.U.; Hasan, M.R. and Satyajit, D. S. (2014). Effect of citrus flavonoids, naringin and naringenin on metabolic myndrome and their mechanisms of action. Adv. Nutr., **5** (4):404-417.

Aller, S.G.; Yu. J.; Ward, A.; Weng, Y.; Chittaboina, S. and Zhuo, R. (2009). Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science, **323**:1718-1722.

Babu, P.R.; Babu, K.N. and Peter, P.L. (2013). Influence of quercetin on the pharmacokinetics of ranolazine in rats and *in vitro* models. Drug Dev. Ind. Pharm., **39**:873-879.

Buckley, M.M.T.; Grant, S.M.; Goa, K.L.; McTavish, D. and Sorkin, E.M. (1990). Diltiazem: A reappraisal of its pharmacological properties and therapeutic use. Drugs, **39**:757-806.

Capraro, J.; Clemente, A. and Rubio, L.A. (2011). Assessment of the lupin seed glucose-lowering protein intestinal absorption by using *in vitro* and *ex vivo* models. Food Chemistry, **125**:1279-1283.

Challa, V.R.; Babu, P.R. and Challa, S.R. (2013). Pharmacokinetic interaction study between quercetin and valsartan in rats and *in vitro* models. Drug Dev. Ind. Pharm., **39**:865-872.

Choi, J.C. and Jin, P.B. (2004). The influence of cimetidine on the pharmacokinetics of diltiazem and its main metabolite in rabbits. Arch. Pharm. Res., **27**:254-258.

Dong, H.C.; Jin, S.C.; Cheng, L. and Jun, S.C. (2011). Effects of simvastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, after oral and intravenous administration in rats: possible role of P-glycoprotein and CYP3A4 inhibition by simvastatin. Pharmacological Reports, **63**:1574-1582.

Falguni, D.M.; Shailesh, K.B.; Jatin, H.P.; Rasesh. D.V.; Lalit, C.M.; Megha, M. and Nitin, K. (2019). Pharmacokinetic profile of rutin after intramuscular administration in rats favours its *in vivo* anti-inflammatory activity in carrageenan-induced rodent model of inflammation. Ann. Phytomed., **8**(1):185-192.

Fardel, O.; Kolasa, E. and Le, V.M. (2012). Environmental chemicals as substrates, inhibitors or inducers of drug transporters: implication for toxicokinetics, toxicity and pharmacokinetics. Expert Opin Drug Metab. Toxicol., **8**:29-46.

Gan, L.S.; Moseley, M.A.; Khosla, B.; Augustijns, P.F.; Bradshaw, T.P.; Hendren, R.W. and Thakker, D.R. (1996). CYP3A-like cytochome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport. Drug Metab. Dispos., **24**:344-349.

Gottesman, M.M. and Pastan, I. (1993). Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev. Biochem., **62**: 385-427.

Ito, K.; Kusuhara, H. and Sugiyama, Y. (1999). Effects of intestinal CYP3A4 and P-glycoprotein on oral drug absorption theoretical approach. Pharm Res., **16**:225-231.

Jatin, H.P.; Priti, D.V.; Kamlesh, A.S.; Urvesh, D.P.; Rasesh, D.V.; Shailesh, K.B.; Ashwin, M.T. and Jitendra, K.R. (2019). Pretreatment with trikatu augments pharmacokinetic profile and bioavailability of orally administered levofloxacin in goat. Ann. Phytomed., **8**(1):172-177.

Kallem, R.R.; Ramesh, M. and Seshagirirao, J.V.L.N. (2013). Validated LC-ESI-MS/ MS method for simultaneous quantitation of felodipine and metoprolol in rat plasma: Application to a pharmacokinetic study in rats. Biomed Chomatogr., **27**:784-791.

Kharasch, E.D.; Bedynek, P.S. and Walker, A. (2008). Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics. II. ritonavir effects on CYP3A and P-glycoprotein activities. Clin. Pharmacol. Ther., **84**:506-12.

Kumar, G.N.; Rodrigues, A.D.; Buko, A.M. and Denissen, J.F. (1996). Cytochome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. J. Pharmacol. Exp. Ther., **277**:423-431.

Lefebvre, M.; Homsy, W.; Caillé, G. and Souich, P. (1996). First-pass metabolism of diltiazem in anesthetized rabbits: role of extrahepatic organs. Pharm. Res., **13**:124-128.

Li, F.; Lu. J. and Ma, X. (2012). CPY3A4-mediated lopinavir bioactivation and its inhibition by ritonavir. Drug Metab. Dispos., **40**:18-24.

Mason, R.P. (2005). A rationale for combined therapy with a calcium channel blocker and a statin: Evaluation of basic and clinical evidence. Curr. Drug Targets Cardiovasc Haematol Disord., **5**(6): 489-501.

Megha, A.M.; Nitin, V.K.; Jatin, H.P.; Rasesh, D.V.; Falguni, D.M. and Priti, D.V. (2019). Pharmacokinetics of ferulic acid following oral administration ethyl ferulate alone and in combination with piperine in rats. Ann. Phytomed., **8**(2):194-198.

Millard, R.W.; Grupp, G.T.L.; Disalvo, J.; DePover, A. and Schwartz, A. (1983). Chonotropic, inotropic and vasodilator actions of diltizem, nifedipine and verapamil: A comparative study of physiological responses and membrane receptor activity. Circul. Res., **52**:29-39.

Nebert, D.W. and Russell, D.W. (2002). Clinical importance of the cytochomes P450. Lancet, **360**:1155-1162.

Nitin, V.K.; Megha, A.M.; Jatin, H.P.; Rasesh, D.V.; Falguni, D.M. and Priti, D.V. (2021). Pharmacokinetics of protocatechuic acid following oral administration of protocatechuic ethyl ester alone and in combination with piperine in rats. Ann. Phytomed., **10**(2):456-461.

Paine, M.F.; Hart, H.L.; Ludington, S.S.; Haining, R.L.; Rettie, A.E. and Zeldin, D.C. (2006). The human intestinal cytochome P450 'pie'. Drug Metab. Dispos., **34**:880-886.

Paramita, D.; Preethi, K.; Angelin, A.K.; Nikhil, K. and Anjali, N. (2021). Flavonoids: An alternative pathway for the treatment of Alzheimer's disease. Ann. Phytomed., **10**(2):240-251.

Pichard, L.; Gillet, G.; Fabre, I.; Dalet B.I.; Bonfils, C.; Thenot, J.P. and Maurel, P. (1990). Identification of the rabbit and human cytochomes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. Drug Metab. Dispos., **18**:711-719.

Pingili, R.B.; Sridhar, V.; Surya, S.M.; Siddhartha, N.; Sivaprasad, P. and Naveen, B.K. (2016). Pharmacokinetic interaction study between flavanones (hesperetin, naringenin) and rasagiline mesylate in wistar rats. Drug Development and Industrial Pharmacy, **42**:1110-1117.

Raseshkumar, D.V.; Jatin, H.P.; Falguni, D.M.; Priti, D.V.; and Shailesh, K.B. (2021). *In vitro* and *in vivo* antibacterial and anti-inflammatory effect of catechin including pharmacokinetic profile in rat. Ann. Phytomed., **10**(2):472-478.

Rostami, H.A. and Tucker, G.T. (2007). Simulation and prediction of *in vivo* drug metabolism in human populations from *in vitro* data. Nat. Rev. Drug Discov., **6**:140-148.

Rouveix, B. (2007). Clinical implications of multiple drug resistance efflux pumps of pathogenic bacteria. J. Antimicrob Chemother., **59**:1208-1209.

Shimada, T.; Yamazaki, H.; Mimura, M.; Inui, Y. and Guengerich, F.P. (1994). Interindividual variations in human liver cytochome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. J. Pharmacol. Exp. Ther., **270**:414-423.

Soon, P.H.; Dong, H.C. and Jun, S.C. (2008). Effects of resveratrol on the pharmacokinetics of diltiazem and its major metabolite, desacetyldiltiazem in rats. Cardiovasc. Ther., **26**:269-275.

Soon, P.H.; Joon, S.Y.; Jung, Y.H.; Sung, I.H.; Joong, W.C.; Young, Y.K.; Kyong, S.C. and Dong, H.C. (2011). Effects of lovastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats: Possible role of cytochome P450 3A4 and P-glycoprotein inhibition by lovastatin. Journal of Pharmacy and Pharmacology, **63**:129-135.

Soon, P.H.; Kyoung, S.C.; Dong, H.C. and Jun, S.C. (2007). Effect of atorvastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats. Arch. Pharm. Res., **30**:90-95.

Srivalli, K.M.R. and Lakshmi, P.K. (2012). Overview of P-glycoprotein inhibitors: A rational outlook. Braz. J. Pharm. Sci., **48**:353-367.

Surya, S.M.; Sridhar, V. and Puneeth, Y. (2014). Enhanced oral bioavailability of felodipine by naringenin in Wistar rats and inhibition of P-glycoprotein in everted rat gut sacs *in vitro*. Drug Dev. Ind. Pharm., **40**:1371-1377.

U.S. Food and Drug Administration. (2012). Guidance for industry: Drug interaction studies - study design, data analysis, implications for dosing, and labeling recommendations. Silver Spring, MD: FDA.

Wacher, V.H.; Silverman, J.A.; Zhang, Y. and Benet, L.Z. (1998). Role of P-glycoprotein and cytochome P450 3A in limiting oral absorption of peptides and peptidomimetics. J. Pharm. Sci., **87**:1322-1330.

Wacher, V.J.; Salphati, L. and Benet, L.Z. (2001). Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv. Drug Deliv. Rev., **46**:89-102.

Wolozin, B.; Kellman, W.; Ruosseau, P.; Celesia, G.G. and Siegel, G. (2000). Decreased prevalence of alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. Arch. Neurol., **10**:1439-1443.

Young, A.C.; Dong, H.C. and Jun, S.C. (2009). Effect of hesperidin on the oral pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem in rats. J. Pharm. Pharmacol., **61**:825-829.

Citation **Ravindra Babu Pingili, Sridhar Vemulapalli, Surya Sandeep Mullapudi, Krishna Mohan Chinnala and Naveen Babu Kilaru (2022). Effect of herbal bioenhancer (naringenin) on the pharmacokinetics of diltiazem in rats via CYP3A4 and P-glycoprotein inhibition . Ann. Phytomed., 11(2):654-660. http://dx.doi.org/10.54085/ap.2022.11.2.80.**