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Influence of hesperetin on the pharmacokinetics of diltiazem in rats

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ABSTRACT

Cytochrome P-450 3A4 (CYP3A4) and P-gp substrates exhibit poor bioavailability. Diltiazem, is a benzothiazepine derivative used in various cardiovascular conditions. It is a heart muscle and blood vessel relaxant that improves blood pumping to the heart and from the heart. It has low bioavailability because it is a substrate of CYP3A4 and P-gp. Hesperetin (a flavanone) inhibited CYP3A4 and P-gp in previous investigations. The major goal of this research was to see how hesperetin affected diltiazem pharmacokinetics (PK) in rats and employing everted intestinal sac. Male Wistar rats were given diltiazem (15 mg/kg) alone or in combination with hesperetin (12.5, 25, and 50 mg/kg) once daily for 15 days to hesperetin-pretreated animals. Blood samples were collected on the 1st day in single-dose PK study and on 15th day in multiple dosage PK studies. *In vitro*, diltiazem was incubated with rat everted intestinal sacs in the presence and absence of hesperetin and conventional P-gp blockers. Diltiazem C_{max}, area under the curve, and half-life ($T_{1/2}$) rose two-fold in rats pretreated with Hesperetin compared to the diltiazem control group, although T_{max} did not alter significantly. The value of Mean Residence Time increased by 37% to 47%. There has been a significant reduction in clearing and distribution rates. The results of an *in vitro* study showed that the transport of diltiazem was significantly increased in the presence of hesperetin and standard P-gp inhibitors. The current study found that hesperetin significantly increased diltiazem systemic absorption by inhibiting CYP3A4 and P-gp.

INTRODUCTION

Diltiazem is majorly prescribed to treat angina, supraventricular arrhythmias, and hypertension (Chaffman and Brogden, 1985; Pool, 1996; Weir, 1995). It undergoes an extensive presystemic metabolism because it is a substrate of cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein (P-gp) (Lefebvre *et al*., 1996; Buckley *et al*., 1990). The bioavailability of diltiazem is approximately 40% and was found to be metabolized mainly into N-demethyldiltiazem in humans and dogs. The most common metabolites in rabbits and rats were desacetyldiltiazem and O-diacetyl-N-monomethyl diltiazem, correspondingly (Yeung *et al*., 1998). CYP3A4 is the major human variant of diltiazem N-demethylation in liver microsomes, and it's also located in the gut (Pichard *et al*., 1990). The metabolism of diltiazem can be more in the proximal segment of the small intestine than in the distal section (Kolars *et al.,* 1992; Watkins *et al.,* 1987). The P-gp and CYP34A might decrease the oral absorption of diltiazem. The calcium channel blockers verapamil, nicardipine, and diltiazem compete to suppress P-gp multidrug resistance, according to Yusa and Tsuruo (1989).

Diltiazem is not only an Multidrug Resistance modulator but also a precursor for CYP 3A4 and the P-gp efflux transporter Wacher *et al*. (2001) Flavonoids are produced by a huge variety of plants in large quantities (Dixon and Steele, 1999). Hesperetin is a flavanone found naturally in citrus and grapefruits that have been shown to have anti-cancer, antioxidant, anti-inflammatory, anti-hypertensive, anti-atherogenic, hepatoprotective, and neuroprotective properties (Choi and Ahn, 2008) . Hesperetin can pass the blood-brain barrier. Furthermore, earlier research has shown that hesperetin and naringenin are CYP enzyme and P-gp

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inhibitors (Sridhar *et al.,* 2014; Surya *et al.,* 2014). The present study aimed to investigate the influence of hesperetin on the diltiazem pharmacokinetics (PK) in rats.

MATERIALS AND METHODS

Drugs and chemicals

Throughout the investigation, analytical grade compounds were employed. Hesperetin was furnished by Sigma Aldrich. Diltiazem and ritonavir were provided as complimentary samples by Sipra Labs in Hyderabad, India. Finisar Chemicals Ltd. of Ahmadabad, India, provided acetonitrile for high-performance liquid chromatography (HPLC).

Laboratory animals

Wister rats (Male) weighing 160–200 g were procured from CPCSEA registered breeder. Animals were quarantined, acclimatized for at least 1 week, housed six per cage. The approved animal experiments protocol was KVSRSCOPS/11-03-14-008.

Study design

The current investigation involves two experiments, acute (single dose) and sub-acute (once daily for 15 days) administration of diltiazem and hesperetin, as previously reported (Challa *et al*., 2013).

In vivo pharmacokinetic studies

Male Wistar rats were used throughout the study and randomly assigned to four groups of six animals each per study. After overnight fasting, the animals in group 1 was treated with 15 mg/kg (oral) diltiazem in 1% Sodium Carboxymethylcellulose (SCMC) while the other groups viz., groups 2, 3, & 4 were administered with 12.5, 25, and 50 mg/kg hesperetin in 2% SCMC p.o, respectively, followed by diltiazem, 15 mg/kg p.o in single-dose PK study (SDS). Similarly, in multiple dosing PK studies (MDS), the animals were given the same drugs once a day for 15 days.

Everted rat gut sac study

Preparation of gut sac

The rat-everted gut sac model, a simple and effective *in vitro* model for evaluating drug absorption and processes by assessing drug content in the colon and conveying it via intestinal tissue, is used to assess diltiazem transport across the intestine (Barthe *et al*., 1999). Babu *et al*. (2013) presented a modified method for preparing everted sacs of rat ileum. The ileum of a rat was removed and flushed with ice-cold saline multiple times (0.9%). Under phenobarbitone (40 mg/kg) anesthesia, the distal part of the ileum from the rat intestines (approximately 15 cm each) was removed from an over day fasted male Wistar rat weighing around 180–220 g, everted after removal of fat and mesenteric connectors, and fused with a silk incision to make into sacs (Capraro *et al*., 2011).

Effect of hesperetin on diltiazem transport across gut sac

The everted sacs were supplied with a mixture containing diltiazem 50 µg/ml in the presence or absence of ritonavir 50 µg/ml, conventional CYP3A4, and P-gp blocker (Kharasch *et al.,* 2008; Kumar *et al.,* 1996; Li *et al.,* 2012), and hesperetin 50, 100, and 200 µg/ml. Diltiazem travel from the serosal to the mucosal side of the everted sac was measured by collecting 1 ml of the outer medium [replaced by 1 ml Krebs–Ringer bicarbonate buffer (KRB) buffer] at 10, 20, 30, and 60 minutes from an Erlenmeyer flask comprising 30mL of oxygenated (O_2/CO_2; 95:5) KRB and incubating in a shaker bath at 37°C for 60 minutes. Each experiment was repeated three times.

Analytical methods

Diltiazem plasma concentrations were measured with changes using a technique published by Li *et al*., 2003). Briefly, a Shimadzu HPLC system with a pump (LC-20AT VP), a C_{18} column (Kromasil 150 × 4.6 mm) with a particle size of 5 µm and a dual-wavelength ultraviolet (UV) visible detector (SPD-10A VP) was used. liquid chromatography solution software was used to gather and process the data. The mobile phase consisted of 0.2% formic acid solution in acetonitrile and water (80:20 *v*/*v*) that was ultrasonically degassed and filtered through a 0.45 µm membrane filter. The effluent was monitored at 235 nm with a UV detector at a flow rate of 1 ml/minute. The total run time was 5.0 minutes and the diltiazem eluted at 4.8 minutes (Fig. 1). The analysis was performed at room temperature.

Extraction of diltiazem from plasma

Diltiazem was extracted from rat plasma using the liquid-liquid extraction method (Kallem *et al*., 2013). 1.5 ml tert-butyl methyl ether was added to a 50 µl plasma aliquot, vortexed, and centrifuged at 6,000 rpm for 5 minutes in each step. The residue (1.2 ml) was dried in a moderate nitrogen stream at 40°C. The dried residue was reconstituted and used for chromatography (Fig. 1).

Calculation of PK parameters

Thermo Kinetica was used to perform a non-compartmental PK analysis of each rat's plasma concentrations versus time data.

Statistical analysis

GraphPad Prism software was used for data analysis and p-value < 0.05 was considered significant.

RESULT

Influence of hesperetin on the PK of diltiazem in SDS

Diltiazem plasma concentrations versus time curves after oral administration of diltiazem alone and pre-treatment with hesperetin 12.5, 25, and 50 mg/kg in SDS are shown in Figure 2. Except for T_{max}, all PK parameters were logarithmically converted and compared using one-way ANOVA and Dunnett's multiple comparisons test. The mean plasma PK parameters are shown in Table 1. Hesperetin raised the C_{max}, area under the curve (AUC) 0-24, AUC0-, $t_{1/2}$, and Mean Residence Time (MRT) of diltiazem and lowered the clearance and volume of distribution of diltiazem considerably ($p < 0.001$). The C_{max} of diltiazem was changed from 39.273 ± 4.265 to 49.715 ± 6.855 and 39.273 ± 4.265 to 89.946 ± 7.222 ng/ml at a dose of hesperetin 25, 50 mg/kg correspondingly. The AUC_{0-24} of Diltiazem was significantly increased from 454.722

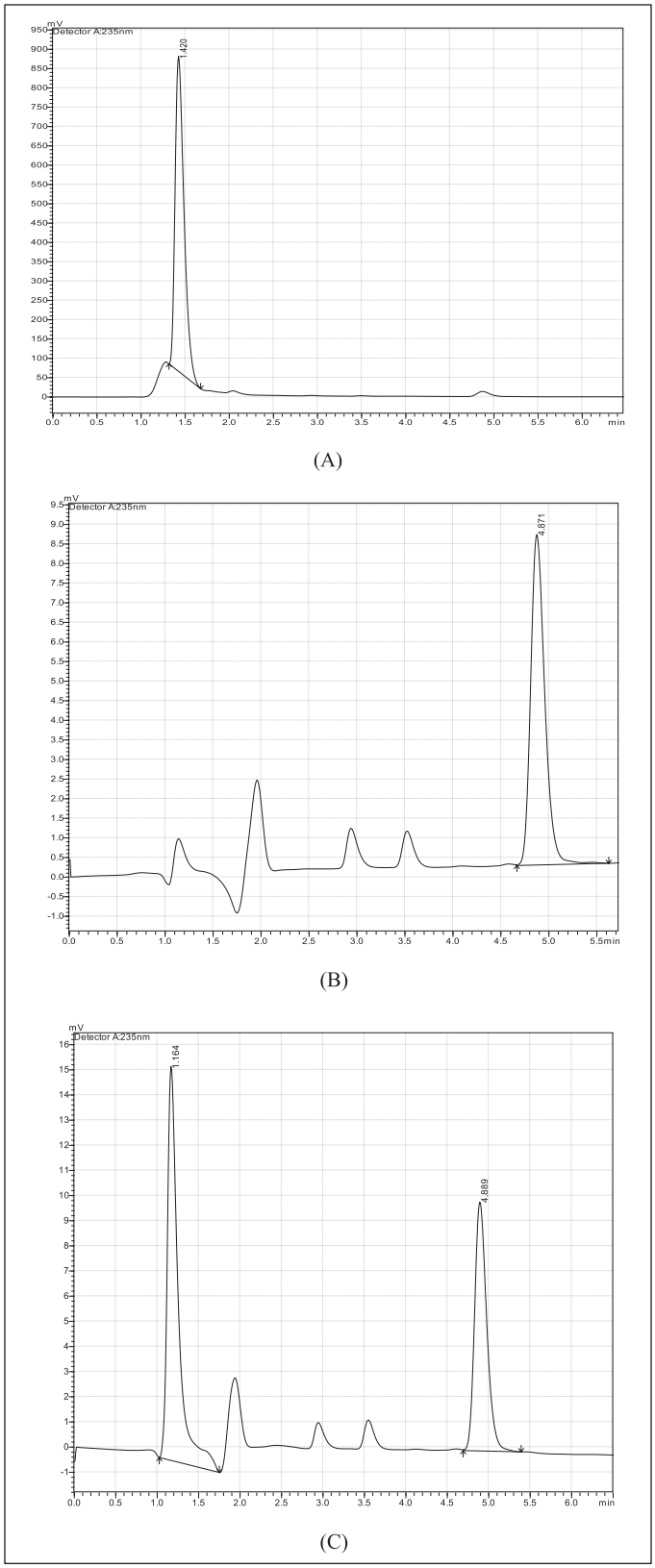

Figure 1. Chromatograms: (A) Blank plasma; (B) Diltiazem hydrochloride (2 µg/ml); (C) Plasma + Diltiazem hydrochloride 2 µg/ml.

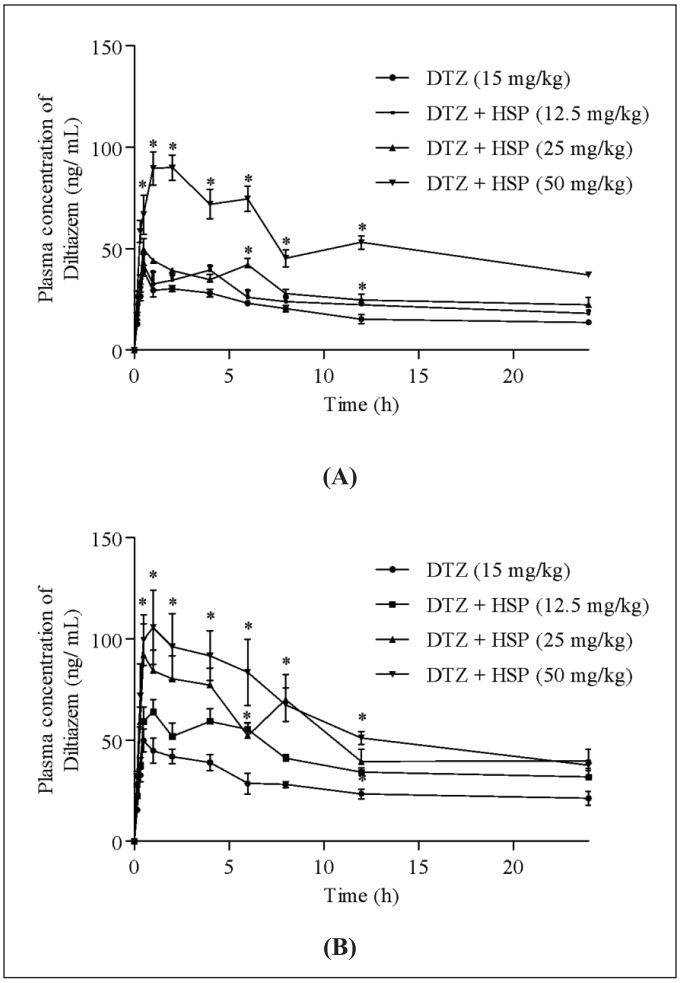

Figure 2. After oral administration of diltiazem (15 mg/kg), plasma concentration-time curves in rats treated with or without hesperetin. (A) on the first day; (B) on the fifteenth day. HSP, Hesperetin; DTZ, Diltiazem. $^{*}p < 0.05$ compared to diltiazem control group.

± 52.362 to 593.578 ± 42.841 and 454.722 ± 52.362 to 665.135 ± 84.250 and 454.722 ± 52.362 to 1373.784 ± 142.545 ng/ml/hour at the dose of hesperetin 12.5, 25, 50 mg/kg respectively. The $AUC_{0-\infty}$ of diltiazem was increased from 818.206 ± 65.241 to 993.643 ± 74.325 and 818.206 ± 65.241 to 1472.611 ± 121.471 and 818.206 ± 65.241 to 2613.662 ± 187.266 ng/hour/ml at a dose of hesperetin 12.5, 25, 50 mg/kg, individually. The t_{max} does not change from 0.5 ± 0.1 hour. The $t_{1/2}$ of diltiazem was raised from 18.471 ± 1.584 to 30.463 ± 3.674 and 18.471 ± 1.584 to 24.886 ± 4.278 and 18.471 ± 1.584 to 23.090 ± 3.625 hour at a dose of hesperetin 12.5, 25, 50 mg/kg correspondingly. The MRT of Diltiazem was increased from 28.128 ± 2.639 to 28.450 ± 3.485 and 28.128 ± 2.639 to 37.702 ± 5.475 and 28.128 ± 2.639 to 32.588 ± 4.556 hour at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The clearance of diltiazem was decreased from 3.000 ± 0.341 to 0.009 ± 0.0001 and 3.000 ± 0.341 to 0.002 ± 0.0001 and 3.000 ± 0.341 to 0.001 ± 0.0001 l/hour/kg at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The volume of distribution of diltiazem was decreased from 0.111 ± 0.01 to 0.007 ± 0.0001 and 0.111 ± 0.01 to 0.083 ± 0.001 and 0.111 ± 0.01 to 0.040 ± 0.001 ml/kg at a dose of hesperetin 12.5, 25, 50 mg/kg respectively.

Effect of hesperetin on the PK of diltiazem in MDS

Diltiazem plasma concentrations versus time curves in MDS patients after an oral dose of Diltiazem alone and

Table 1. PK parameters of diltiazem on 1st day.

Parameter	DTZ (15 mg/kg)	DTZ + HSP (12.5 mg/kg)	DTZ + HSP (25 mg/kg)	DTZ + HSP (50 mg/kg)
C_{max} (ng/ml)	39.273 ± 4.265	26.126 ± 2.341	49.715 ± 6.855*	89.946 ± 7.222***
AUC_{0-24} (ng. hour/ml)	454.722 ± 52.362	593.578 ± 42.841*	665.135 ± 84.250***	1,373.784 ± 142.545***
$AUC_{0-\infty}$ (ng. hour/ml)	818.206 ± 65.241	993.643 ± 74.325*	1,472.611 ± 121.471**	2,613.662 ± 187.266***
t_{max} (hour)	0.5 ± 0.1	0.5 ± 0.1^{NS}	0.5 ± 0.1^{NS}	0.5 ± 0.1^{NS}
$t_{1/2}$ (hour)	18.471 ± 1.584	30.463 ± 3.674***	24.886 ± 4.278*	23.090 ± 3.625*
MRT (hour)	28.128 ± 2.639	28.450 ± 3.485	37.702 ± 5.475**	32.588 ± 4.556*
CL/F (l/h/kg)	3.000 ± 0.341	0.009 ± 0.0001***	0.002 ± 0.0001***	0.001 ± 0.0001***
V_z/F (ml/kg)	0.111 ± 0.01	0.007 ± 0.0001***	0.083 ± 0.001***	0.040 ± 0.001***

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{NS}p > 0.05$ compared to diltiazem control group.

Table 2. PK parameters of diltiazem on the 15th day.

Parameter	DTZ (15 mg/kg)	DTZ + HSP (12.5 mg/kg)	DTZ + HSP (25 mg/kg)	DTZ + HSP (50 mg/kg)
C_{max} (ng/ml)	49.822 ± 4.368	69.213 ± 5.477**	92.000 ± 7.254***	105.662 ± 9.571***
AUC_{0-24} (ng. hour/ml)	654.148 ± 45.265	987.381 ± 57.248***	1,509.941 ± 85.479***	1,736.974 ± 98.246***
$AUC_{0-\infty}$ (ng. hour/ml)	1,965.421 ± 94.258	2,882.222 ± 158.695**	3,670.254 ± 241.587***	4,038.111 ± 271.328***
t_{max} (hour)	0.5 ± 0.100	0.5 ± 0.100^{NS}	0.5 ± 0.100^{NS}	1.0 ± 0.200*
$t_{1/2}$ (hour)	22.651 ± 5.374	23.591 ± 2.565^{NS}	30.083 ± 2.478**	27.715 ± 2.851*
MRT (hour)	30.562 ± 2.555	30.311 ± 2.652^{NS}	44.191 ± 4.274**	41.202 ± 3.625***
CL/F (l/hour/kg)	1.000 ± 0.1	1.000 ± 0.1^{NS}	0.800 ± 0.2*	0.800 ± 0.2*
V_z/F (ml/kg)	0.1001 ± 0.1	0.052 ± 0.005*	0.038 ± 0.005**	0.033 ± 0.005**

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{NS}p > 0.05$ compared to diltiazem control group.

pretreatment with hesperetin 12.5, 25, and 50 mg/kg are also shown in Figure 2. Except for T_{max}, all PK parameters were logarithmically converted and compared using one-way ANOVA and Dunnett's multiple comparisons test. The mean plasma PK parameters are shown in Table 2. Hesperetin raised the C_{max}, AUC0-24, AUC0-, $t_{1/2}$, and MRT of Diltiazem and lowered the clearance and volume of distribution of Diltiazem in studies ($p < 0.001$). The C_{max} of Diltiazem was increased from 49.822 ± 4.368 to 69.213 ± 5.477 and 49.822 ± 4.368 to 92.000 ± 7.254 and 49.822 ± 4.368 to 105.662 ± 9.571 ng/ml at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The AUC_{0-24} of Diltiazem was increased from 654.148 ± 45.265 to 987.381 ± 57.248 and 654.148 ± 45.265 to 1509.941 ± 85.479 and 654.148 ± 45.265 to 1736.974 ± 98.246 ng. hour/ml at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The $AUC_{0-\infty}$ of Diltiazem was increased from 1,965.421 ± 94.258 to 2,882.222 ± 158.695 and 1,965.421 ± 94.258 to 3,670.254 ± 241.587 and 1,965.421 ± 94.258 to 4,038.111 ± 271.328 ng. hour/ml at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The t_{max} does not change from 0.5 ± 0.100 hour. The $t_{1/2}$ of Diltiazem was increased from 22.651 ± 5.374 to 23.591 ± 2.565 and 22.651 ± 5.374 to 30.083 ± 2.478 and 22.651 ± 5.374 to 27.715 ± 2.851 hour at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The MRT of Diltiazem was increased from 30.562 ± 2.555 to 30.311 ± 2.652 and 30.562 ± 2.555 to 44.191 ± 4.274 and 30.562 ± 2.555 to 41.202 ± 3.625 hour at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The clearance of diltiazem was decreased from 1.000 ± 0.1 to 1.000 ± 0.1 and 1.000 ± 0.1 to 0.800 ± 0.2 and 1.000 ± 0.1 to 0.800 ± 0.2 l/hour/kg at a dose of hesperetin 12.5, 25, 50 mg/kg respectively. The volume of distribution of diltiazem was decreased from 0.1001 ± 0.1 to 0.052 ± 0.005 and 0.1001 ± 0.1 to 0.038 ± 0.005 and 0.1001 ± 0.1 to 0.033 ± 0.005 ml/kg at a dose of hesperetin 12.5, 25, 50 mg/kg respectively.

Hesperetin's effect on P-gp-mediated diltiazem transport

P-gp operates as a barrier in the gut, lowering net absorption of xenobiotics and medicines into the intraluminal space, which can have a substantial influence on P-gp substrate bioavailability and therapeutic uses. Diltiazem bowel absorption was assessed using everted gut sacs from the mucosal to the serosal compartments. Hesperetin increased diltiazem absorption in a concentration-dependent manner (Table 3). When

Table 3. Transport of diltiazem (n = 3).

Time (Minute)	DTH (50 µg/ml)	DTH + RTV (50 µg/ml)	DTH + HSP (25 µg/ml)	DTH + HSP (50 µg/ml)	DTH + HSP (100 µg/ml)
10	3.896 ± 0.884	6.110 ± 1.232**	5.022 ± 0.457NS	5.756 ± 1.064*	6.305 ± 1.176**
20	5.452 ± 1.635	8.544 ± 1.844**	6.266 ± 0.925NS	8.265 ± 1.265**	9.580 ± 1.452**
30	6.588 ± 1.748	10.600 ± 2.362**	7.462 ± 1.143NS	8.821 ± 1.755**	11.326 ± 1.826**
40	7.895 ± 2.141	13.525 ± 2.455***	9.126 ± 1.647NS	10.552 ± 2.615**	14.866 ± 2.647***
50	9.662 ± 1.685	16.220 ± 2.526***	10.587 ± 2.634*	13.732 ± 2.365**	16.783 ± 2.362***
60	11.026 ± 1.811	18.362 ± 3.652***	12.235 ± 1.854*	16.541 ± 2.203**	17.425 ± 2.820***

DTH, diltiazem; RTV, ritonavir, and HSP, hesperetin. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS$p > 0.05$ compared to diltiazem control group.

administered alone, the transport of Diltiazem was found to be 11.026 ± 1.811 at a concentration of 50 µg/ml over a time interval of 60 minutes. The transport of Diltiazem increased from 11.026 ± 1.811 to 12.235 ± 1.854 and 11.026 ± 1.811 to 16.541 ± 2.203 and 11.026 ± 1.811 to 17.425 ± 2.820 when pretreated with hesperetin at concentrations of 25, 50, 100 µg/ml at a time interval of 60 minutes. To validate the role of P-gp in diltiazem transport, the studies were performed in the presence of 50 µg/ml ritonavir, a P-up blocker. When administered in the presence of ritonavir, the amount of Diltiazem was increased from 11.026 ± 1.811 to 18.362 ± 3.652 at a concentration of 50 µg/ml at a time interval of 60 minutes. The findings show that ritonavir increased absorption at the incubation time.

DISCUSSION

In the current investigation, Hesperetin dramatically changed the PK of Diltiazem in rats owing to an inhibition of CYP3A4 and P-gp. These results are consistent with previous study reports. Morin, a flavonoid, significantly increased diltiazem oral exposure. The C_{max} and AUC have significantly increased from 173 ± 41.5 to 374 ± 55.2 ng/ml and 358 ± 56.9 to 642 ± 76.6 ng. hour/ml respectively at a dose of 7.5 mg/kg. the $T_{1/2}$ of Diltiazem was decreased from 13 ± 2.9 to 11 ± 3.2 hour and clearance was decreased from 710 ± 93.4 to 393 ± 38.5 ml/minute. Kg at a dose of morin 7.5 mg/kg. The increased oral absorption of Diltiazem is due to the inhibition of the CYP3A4-mediated metabolism of Diltiazem (Choi *et al*., 2005a). The bioavailability of diltiazem increased considerably in rabbits pretreated with quercetin compared to the control, but not in rabbits co-administered with quercetin. The *C*max and AUC significantly increased from 94.2 ± 23.5 to 99.3 ± 24.8 ng/ml and 232 ± 58 to 287 ± 71 ng/ml hour respectively at a dose of 2 mg/kg. The $t_{1/2}$ of the Diltiazem was increased from 11.3 ± 2.8 to 12.3 ± 2.9 hour at a dose of 2 mg/kg. The increased bioavailability of Diltiazem in rabbits treated with quercetin could be attributed to quercetin's inhibition of the efflux pump P-gp and the first-pass metabolizing enzyme CYP 3A4 (Choi *et al*., 2005b).

The concomitant use of hesperetin significantly enhanced the oral exposure of Diltiazem in rats. The C_{max} and AUC of Diltiazem were raised from 173 ± 41.5 to 375 ± 61.1 ng/ml and 358 ± 56.9 to 682 ± 54.8 ng/ml hour respectively at a dose of naringenin 15 mg/kg. There is no significant change in $T_{1/2}$ and T_{max} (Choi *et al*., 2005c). The concurrent use of fluvastatin significantly enhanced the oral exposure of Diltiazem in rats. At a dosage of fluvastatin 2 mg/kg, the C_{max} and AUC of Diltiazem rose from 174 ± 35.8 to 310 ± 62.1 ng/ml and 363 ± 63.9 to 628 ± 130 ng. hour/l, correspondingly, when fluvastatin was used concurrently. Diltiazem bioavailability increased considerably in rabbits pretreated with quercetin compared to the control, but not in rabbits co-administered with quercetin. Fluvastatin may inhibit presystemic metabolism during intestinal absorption, according to these studies. According to Lee *et al*. (1991) Diltiazem extraction ratios in the small intestine and liver were about 85% and 63%, significantly, following oral therapy of rats, suggesting that Diltiazem is widely extracted in both the small intestine and the liver . In conclusion, simultaneous fluvastatin medication may contribute, at least in part, to the increased oral exposure to Diltiazem by reducing both intestinal and hepatic extraction (Choi *et al.,* 2006; Lee *et al.,* 1991).

Simvastatin enhanced Diltiazem's oral absorption. At a dosage of 1 mg/kg, Diltiazem's C_{max} and AUC were raised from 182 ± 33 to 246 ± 44 ng/ml and 270 ± 51 to 392 ± 74 ng. hour/ml, accordingly. T_{max} and $t_{1/2}$ were similarly elevated, although the differences were not important. Increased absorption in the small intestine due to P-gp inhibition and reduced first-pass metabolism of Diltiazem due to CYP3A subfamily inhibition in the small intestine and/or liver, rather than renal elimination of Diltiazem by simvastatin, could explain the rise in Diltiazem oral bioavailability (Choi *et al*., 2011). The resveratrol had increased the bioavailability of Diltiazem. At a dosage of resveratrol 10 mg/kg, the C_{max} and AUC of Diltiazem were considerably enhanced from 165 ± 37.8 to 259 ± 60.6 ng/ml and 342 ± 80.2 to 547 ± 131 ng/ml, correspondingly. Resveratrol did not alter T_{max}. The resveratrol significantly increased the bioavailability of Diltiazem due to the inhibition of both the CYP3A4-mediated metabolism and the P-gp in the intestine and or liver (Hong *et al*., 2008).

The addition of lovastatin increased Diltiazem's systemic bioavailability. The $AUC_{0-\infty}$ of Diltiazem was increased from 342 ± 69 to 508 ± 107 ng hour/ml at a 1 mg/kg dose of lovastatin. The C_{max} of Diltiazem was increased from 165 ± 35 to 234 ± 53 ng/ml at a dosage of lovastatin 1 mg/kg. The T_{max} of Diltiazem was decreased from 0.33 ± 0.13 to 0.29 ± 0.10 hour 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 at a dose of lovastatin 1 mg/kg. The clearance of Diltiazem was decreased from 45.2 ± 13.8 to 38.0 ± 9.9 ml/minute per kg at a dose of lovastatin 1 mg/kg. The increased bioavailability of Diltiazem in the presence of lovastatin may be due to lovastatin's suppression of the P-gp mediated efflux pump in the bowel and/or suppression of CYP3A4-mediated metabolic in the gut and/or liver (Hong

et al., 2011). Rasagiline's concentration in the brain rose when it was coupled with hesperetin and significant naringenin. In the presence of hesperetin or naringenin, rasagiline transport from the mucosal to the serosal side did not change significantly *ex vivo* (rat-everted gut sacs used). Hesperetin and naringenin enhanced rasagiline systemic exposure via CYP1A2 inhibition but not P-gp suppression, according to our findings Ravindra *et al*., 2016). *In vitro* studies demonstrated that hesperetin enhanced felodipine intestinal absorption. Concurrent usage of hesperetin changed the PK of felodipine, resulting in increased systemic exposure (Sridhar *et al*., 2014).

CONCLUSION

Due to P-gp and CYP3A4 inhibition, hesperetin significantly increased the plasma concentration, AUC, $t_{1/2}$, MRT, and greatly lowered the clearance, Vz/F, of diltiazem in rats. According to *in vitro* study results, diltiazem transport was significantly increased in the presence of hesperetin and ritonavir owing to P-gp and CYP3A4 suppression.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The study was approved by the Institutional Animal Care. The approved protocol number was KVSRSCOPS/11-03-14-008.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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