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Effect of hesperetin on the pharmacokinetics of metoprolol succinate in rats

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Abstract

Background Metoprolol is a substrate of CYP3A4, 2B6, CYP2D6, CYP2C9, and P-glycoprotein (P-gp). Hesperetin was reported as an inhibitor of cytochrome P-450 (CYP) enzymes and P-gp. The objective of this study was to investigate the effect of hesperetin on the pharmacokinetics of metoprolol in rats and in vitro models. In in vivo studies, male Wistar rats were treated with metoprolol (30 mg/kg) once a day for 15 consecutive days alone and in combination with hesperetin (25, 50, and 100 mg/kg). Blood samples were withdrawn from the tail vein on the 1st day in the single-dose pharmacokinetic study and on the 15th day in the repeated-dose pharmacokinetic study. In in vitro studies, metoprolol was incubated in the presence or absence of hesperetin and traditional P-gp inhibitors using rat-everted gut sacs. Reverse phase-high-performance liquid chromatography (RP-HPLC) was used to determine the amounts of metoprolol in the plasma and incubated samples (RP-HPLC).

Results The C_{max}, AUC, and half-life ($t_{1/2}$) of metoprolol significantly increased by twofold compared to the metoprolol group in rats pre-treated with hesperetin. The clearance and volume of distribution both decreased significantly. Metoprolol transport was dramatically increased in the presence of hesperetin and quinidine (standard P-gp inhibitor) in in vitro study.

Conclusion The present study results revealed that hesperetin significantly increased the absorption of metoprolol in rats and everted gut sacs in vitro might be due to the inhibition of CYP and P-gp.

Keywords Metoprolol, Hesperetin, CYP3A4, CYP2C9, P-glycoprotein

Background

Metoprolol succinate, a β_1-selective (cardio-selective) adrenoceptor antagonist, is widely used to treat mild to severe hypertension, heart failure, and angina pectoris. Heart rate, myocardial contractility, and cardiac output are all reduced when the β_1 receptor is blocked. It lowers renin activity in the blood [1] and is rapidly and nearly completely absorbed in the intestine. It has a considerable beta-blocking action within 60 min of dosing. However, due to substantial first-pass metabolism, it has a bioavailability of only about 50%. CYP2D6 is the enzyme that metabolizes metoprolol the most. These enzymes can be inhibited by several drugs or compounds. Concomitant use of CYP3A4, CYP2B6, CYP2C9, and CYP2D6 inhibitors (quinidine, fluoxetine, paroxetine, and propafenone) will increase blood levels of metoprolol several-folds [2].

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Flavonoids have a variety of biological actions, and they may have a role in the prevention of chronic diseases through diet [3, 4]. Flavonoids are abundantly synthesized by a variety of plants. Hesperetin and naringenin are the most common dietary flavanones, found virtually exclusively in citrus fruits. Hesperetin has hypolipidemic [5], anticancer [6], anti-aromatase [7], neuroprotective [8], anti-inflammatory [9], antioxidant [10], anti-atherosclerotic [11], anti-thrombotic [12], and anti-cyclooxygenase or lipoxygenase properties [13]. Hesperetin also increases nitric oxide production [14], and high-density lipoprotein in plasma [15]. In addition to pharmacodynamic effects, hesperetin also has inhibitory effects on CYP3A4 [16], CYP2C9 [17], and CYP2B6 [18]. Therefore, the present study was conducted to investigate the effect of hesperetin on the pharmacokinetics of metoprolol in rats and in vitro models.

Methods

Drugs and chemicals

Metoprolol and quinidine are gifted by Orchid Health Care, Chennai, India. Hesperetin was purchased from Sigma Chemical Co. (St. Louis, MO). The required analytical grade solvents for this study were purchased from Finar chemical Ltd, India.

Laboratory animals

Animal experiments were carried out following CPCSEA guidelines at KVSR Siddhartha College of Pharmaceutical Sciences ((993/PO/Re/S/06/CPCSEA). Mahaveer Enterprises in Hyderabad, India, provided male Wistar rats weighing 180–220 g and housed in an animal house. For at least one week before the commencement of the studies, the animals were housed in conventional laboratory settings (12/12 h light/darkness, 22 °C, and 50–60% humidity).

Study protocol

The present investigation was divided into a single-dose pharmacokinetic (SDPK) study and a repeated-dose pharmacokinetic (RDPK) study as previously mentioned in rats [19].

Single-dose pharmacokinetic study

Wistar rats were randomly divided into four groups of six animals each in SDPK. Metoprolol and hesperetin were suspended in 1% SCMC for oral administration.

Group I treated with metoprolol (30 mg/kg).

Group II hesperetin (25 mg/kg) and metoprolol (30 mg/kg).

Group III hesperetin (50 mg/kg) and metoprolol (30 mg/kg).

Group IV hesperetin (100 mg/kg) and metoprolol (30 mg/kg). About 100 µL of blood was collected from the tail vein at various times (0.16, 0.33, 0.5, 1, 2, 4, 6, 8, 12, and 24 h) after administration. The plasma was separated and stored at − 20 °C until analysis.

Repeated-dose pharmacokinetic study

In the RDPK study, rats were treated with the above drugs once a day for 15 days. On the 15th day, 100 µL of blood was drawn from the tail vein at various intervals (0.16, 0.33, 0.5, 1, 2, 4, 6, 8, 12, and 24 h). The plasma was separated and stored at − 20 °C until analysis.

Drug absorption study in vitro

Gut sac preparation

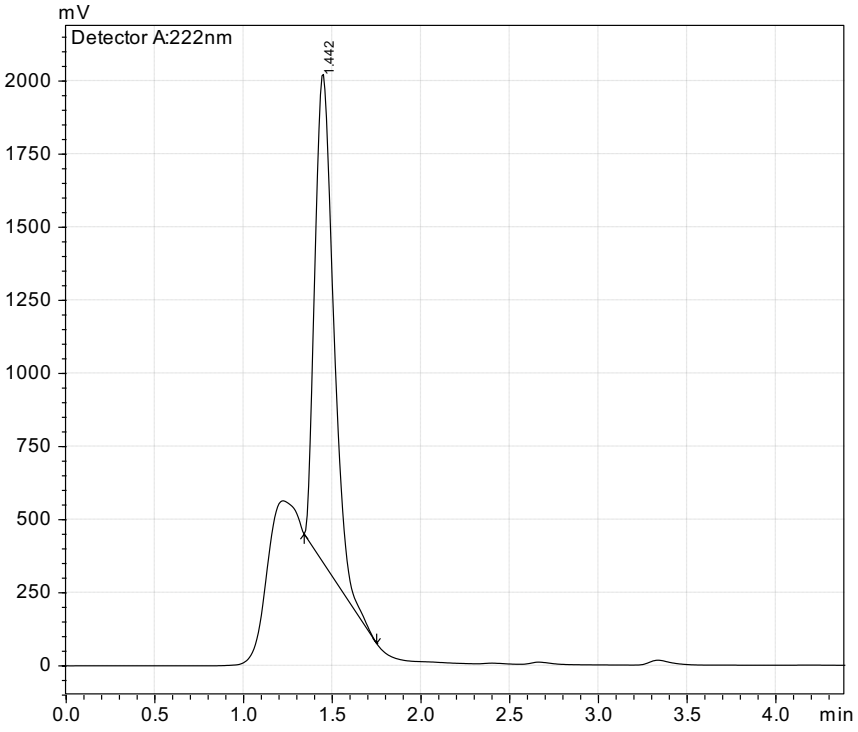
The method that was described previously by [20] for the preparation of everted gut sacs of rat ileum was followed for this study also. Pentobarbital sodium 40 mg/kg was used to anesthetize the rats, and the small intestine was removed [21]. The intestinal digesta was removed and cleaned with ice-cold saline, and the distal ileum (about 15 cm each) was extracted and everted using a glass rod.

Influence of hesperetin on the intestinal absorption

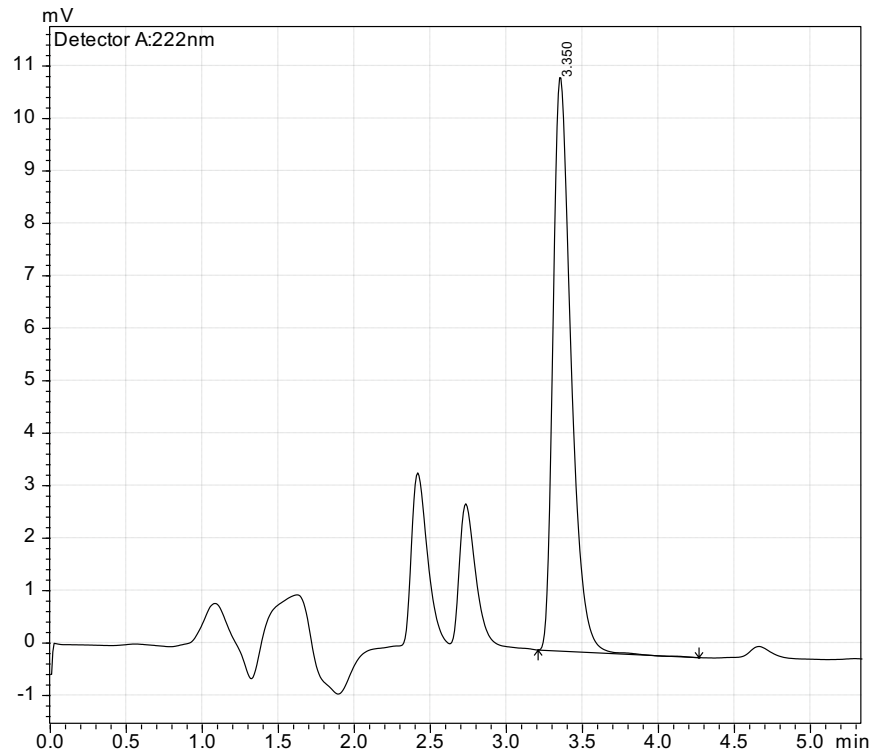
Krebs–Ringer bicarbonate (KRB) buffer containing metoprolol 50 µg/mL was filled in everted sacs. Each sac was placed in a 50-mL Erlenmeyer flask containing 30 mL oxygenated (O_2/CO_2; 95:5) KRB and incubated in a shaker bath at 37 °C for 60 min. At 10, 20, 30, and 60 min, 1 ml of sample was collected from the outer medium and the 1 ml KRB buffer was replaced. The movement of metoprolol from the serosal to the mucosal side was determined using RP-HPLC after centrifugation at 6000 rpm for 10 min. Each experiment was carried out three times. The same study was repeated with and without of quinidine 50 µg/ml and hesperetin 25, 50, and 100 µg/ml.

Analytical methods

The plasma concentrations of metoprolol were determined using a previously described method [22]. The data were collected and processed using LC solution software (Tokyo, Japan). 0.2% in acetonitrile and water (80:20 v/v) WAS the mobile phase. Before use, the prepared mobile phase was ultrasonically degassed and filtered through a 0.45 µm membrane filter. The injection volume was 20 µL, and the effluent was monitored at a flow rate of 1 ml/min at 222 nm with a UV detector. The chromatographic run duration was 5.0 min, while metoprolol was eluted at 3.35 min (Fig. 1). The experiment was conducted out at room temperature.

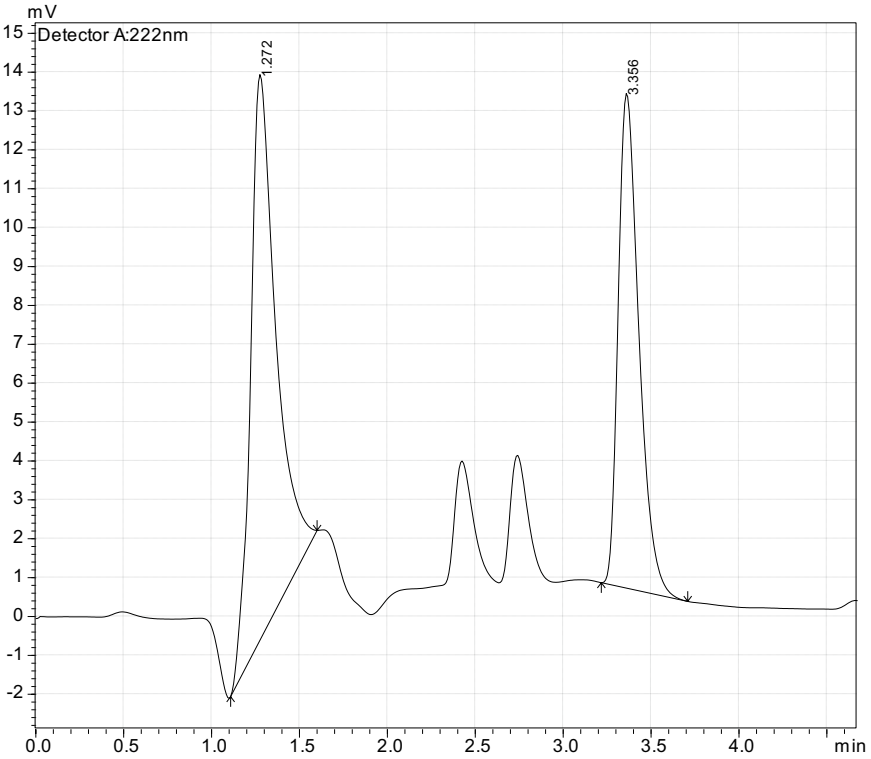


(A)



(B)

Fig. 1 Representative peaks of **A** Blank plasma; **B** Metoprolol Succinate (4 µg/mL); **C** Plasma + Metoprolol Succinate (4 µg/mL)



(C)

Fig. 1 continued

Extraction of metoprolol from plasma

The metoprolol was extracted from rat plasma using a liquid–liquid extraction technique [22]. 1 mL tert-butyl methyl ether was added to a 50 µL plasma sample, vortex mixed for 5 min, and then centrifuged at 6000 rpm for 5 min. The supernatant (500 µL) was dried at 40 °C under a moderate nitrogen stream. The dried residue was reconstituted in 50 µL of mobile phase (80:20, v/v), and 20 µL of this was used to run the HPLC.

Calculation of PK parameters

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

Data analysis

Graph Pad Prism 5.0 was used to calculate all statistics (San Diego, CA). For multiple comparisons, one-way analysis of variance (ANOVA) and two-way ANOVA were applied to compare the PK parameter values and plasma concentrations, respectively. Significant was defined as a $p < 0.05$.

Results

Influence of Hesperetin on the PK of Metoprolol in SDPK

Figure 2 shows the plasma concentrations of metoprolol vs time after oral administration of metoprolol alone and pre-treatment with hesperetin 25, 50, and 100 mg/kg in SDPK. The mean PK parameters are summarized in Table 1. Hesperetin enhanced the C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, $t_{1/2}$, and MRT of metoprolol and lowered the clearance and volume of distribution of metoprolol in the present study ($p < 0.001$). The C_{max} of metoprolol was increased from 3.871 ± 0.856 to 6.696 ± 2.544 and 3.871 ± 0.856 to 14.086 ± 4.362 and 3.871 ± 0.856 to 18.962 ± 4.115 µg/mL at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The AUC_{0-24} of metoprolol was significantly increased from 27.876 ± 3.685 to 70.797 ± 6.955 and 27.876 ± 3.685 to 114.615 ± 9.690 and 27.876 ± 3.685 to 138.743 ± 10.152 µg/mL/h at the dose of hesperetin 25, 50, 100 mg/kg, respectively. The $AUC_{0-\infty}$ of metoprolol was increased from 35.526 ± 4.647 to 116.648 ± 9.599 and 35.526 ± 4.647 to 177.606 ± 12.354

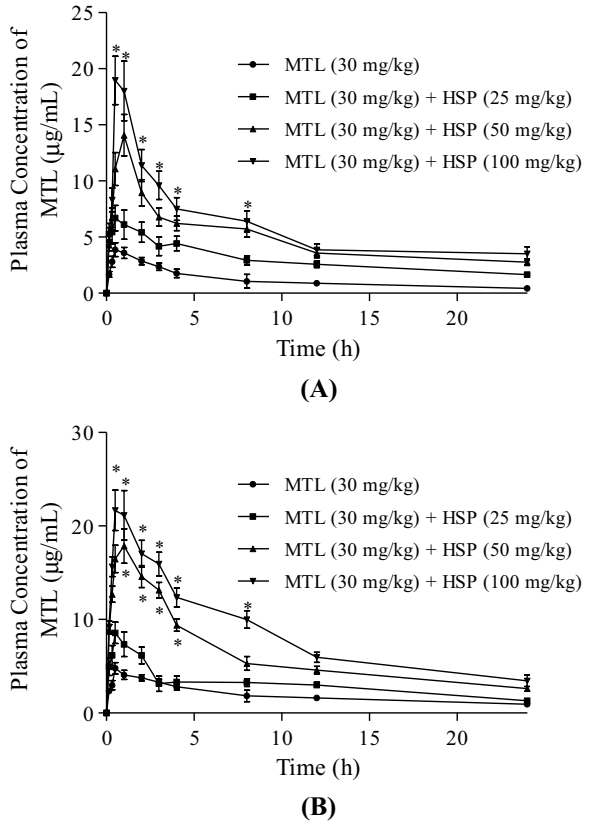


Fig. 2 Metoprolol mean plasma concentration–time curves after oral administration of 30 mg/kg metoprolol with or without hesperetin **A** on day 1; **B** on day 15. *$p < 0.001$ compared to metoprolol

and 35.526 ± 4.647 to 207.415 ± 16.955 µg/h/mL at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The $t_{1/2}$ of metoprolol was increased from 12.128 ± 3.477 to 19.261 ± 4.120 and 12.128 ± 3.477 to 15.811 ± 3.266 and 12.128 ± 3.477 to 16.495 ± 2.547 h at a dose of hesperetin 25, 50, 100 mg/kg, respectively. There is no significant change in T_{max}. The MRT of metoprolol was increased from 15.126 ± 3.771 to 26.104 ± 3.545 and 15.126 ± 3.771 to 22.545 ± 3.251 and 15.126 ± 3.771 to 20.377 ± 3.488 h at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The clearance of metoprolol was reduced from 0.059 ± 0.005 to 0.030 ± 0.006 and 0.059 ± 0.005 to 0.039 ± 0.007 and 0.059 ± 0.005 to 0.027 ± 0.007 mL/h/kg at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The volume of distribution of metoprolol was decreased from 1.600 ± 0.071 to 0.783 ± 0.068 and 1.600 ± 0.071 to 0.888 ± 0.075 and 1.600 ± 0.071 to 1.175 ± 0.358 mL/kg at a dose of hesperetin 25, 50, 100 mg/kg, respectively.

Effect of Hesperetin on the PK of Metoprolol in RDPK

Figure 2 depicts the plasma concentrations of metoprolol versus time curves in RDPK after oral dose of metoprolol alone and pre-treatment with hesperetin 25, 50, and 100 mg/kg. The mean PK parameters are summarized in Table 2. Hesperetin enhanced the C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, $t_{1/2}$, and MRT of metoprolol and lowered the clearance and volume of distribution of metoprolol in the current study ($p < 0.001$). The C_{max} of metoprolol was increased from 4.783 ± 1.585 to 8.569 ± 2.441 and 4.783 ± 1.585 to 17.863 ± 3.874 and 4.783 ± 1.585 to 21.664 ± 5.695 µg/mL at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The AUC_{0-24} of metoprolol was increased from 44.947 ± 6.112 to 71.013 ± 6.254 and 44.947 ± 6.112 to 103.087 ± 7.265 and 44.947 ± 6.112 to 210.293 ± 15.141 µg/h/mL at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The $AUC_{0-\infty}$ of metoprolol was increased from 67.739 ± 5.455 to 93.318 ± 7.635 and 67.739 ± 5.455 to 309.381 ± 15.622 and 67.739 ± 5.455 to 298.582 ± 21.845 µg/h/mL at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The $t_{1/2}$ of metoprolol was increased from 14.637 ± 3.444 to 17.704 ± 2.845 and 14.637 ± 3.444 to 18.192 ± 4.787

Table 1 Pharmacokinetic parameters of metoprolol succinate in the presence or absence of hesperetin (25, 50, and 100 mg/kg) on the 1st day (n = 6)

PK Parameter	MTL 30 mg/kg	MTL + HSP 25 mg/kg	MTL + HSP 50 mg/kg	MTL + HSP 100 mg/kg
C_{max} (µg/mL)	3.871 ± 0.856	6.696 ± 2.544***	14.086 ± 4.362***	18.962 ± 4.115***
AUC_{0-24} (µg/mL/h)	27.876 ± 3.685	70.797 ± 6.955***	114.615 ± 9.690***	138.743 ± 10.152***
$AUC_{0-\infty}$ (µg/mL/h)	35.526 ± 4.647	116.648 ± 9.599***	177.606 ± 12.354***	207.415 ± 16.955***
$t_{1/2}$ (h)	12.128 ± 3.477	19.261 ± 4.120**	15.811 ± 3.266*	16.495 ± 2.547**
T_{max} (h)	0.5 ± 0.1	0.5 ± 0.15	1.0 ± 0.2*	0.5 ± 0.15
MRT (h)	15.126 ± 3.771	26.104 ± 3.545**	22.545 ± 3.251*	20.377 ± 3.488*
CL/F (mL/h/Kg)	0.059 ± 0.005	0.030 ± 0.006**	0.039 ± 0.007**	0.027 ± 0.007**
V_z/F (mL/Kg)	1.600 ± 0.071	0.783 ± 0.068**	0.888 ± 0.075*	1.175 ± 0.358*

MTL, Metoprolol, *HSP*, Hesperetin

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

Table 2 Pharmacokinetic parameters of metoprolol succinate in the presence or absence of hesperetin (25, 50, and 100 mg/kg) on the 15th day (n = 6)

PK Parameter	MTL 30 mg/kg	MTL + HSP 25 mg/kg	MTL + HSP 50 mg/kg	MTL + HSP 100 mg/kg
C_{max} (μg/mL)	4.783 ± 1.585	8.569 ± 2.441**	17.863 ± 3.874***	21.664 ± 5.695***
AUC_{0-24} (μg/mL/h)	44.947 ± 6.112	71.013 ± 6.254**	103.087 ± 7.265***	210.293 ± 15.141***
$AUC_{0-\infty}$ (μg/mL/h)	67.739 ± 5.455	93.318 ± 7.635*	309.381 ± 15.622***	298.582 ± 21.845***
$t_{1/2}$ (h)	14.637 ± 3.444	17.704 ± 2.845*	18.192 ± 4.787*	20.243 ± 2.480**
T_{max}	0.5 ± 0.12	0.5 ± 0.12	1.0 ± 0.25	0.5 ± 0.15
MRT (h)	22.193 ± 3.890	26.817 ± 3.877	38.678 ± 3.515**	28.331 ± 2.588*
CL/F (L/h/Kg)	10.333 ± 2.515	3.750 ± 0.858**	2.262 ± 0.577***	4.688 ± 1.220**
V_z/F (mL/Kg)	1.248 ± 0.355	0.630 ± 0.245**	0.875 ± 0.362*	0.859 ± 0.284*

MTL, Metoprolol, *HSP*, Hesperetin

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

and 14.637 ± 3.444 to 20.243 ± 2.480 h at a dose of hesperetin 25, 50, 100 mg/kg, respectively. There is no significant change in T_{max}. The MRT of metoprolol was increased from 22.193 ± 3.890 to 26.817 ± 3.877 and 22.193 ± 3.890 to 38.678 ± 3.515 and 22.193 ± 3.890 to 28.331 ± 2.588 h at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The clearance of metoprolol was reduced from 10.333 ± 2.515 to 3.750 ± 0.858 and 10.333 ± 2.515 to 2.262 ± 0.577 and 10.333 ± 2.515 to 4.688 ± 1.220 L/h/kg at a dose of hesperetin 25, 50, 100 mg/kg, respectively. The volume of distribution of metoprolol was decreased from 1.248 ± 0.355 to 0.630 ± 0.245 and 1.248 ± 0.355 to 0.875 ± 0.362 and 1.248 ± 0.355 to 0.859 ± 0.284 mL/kg at a dose of hesperetin 25, 50, 100 mg/kg, respectively.

Effect of hesperetin on the P-gp mediated transport of metoprolol using everted rat gut sacs

P-gp in the intestine acts as a barrier, preventing xenobiotics and medicines from reaching the intraluminal space. This has a major impact on P-gp substrate bioavailability and, as a result, therapeutic potential. The intestinal absorption of metoprolol was measured from the mucosal compartment to the serosal compartment using everted gut sacs. The addition of hesperetin to metoprolol improved its absorption in a concentration-dependent manner (Table 3). At a concentration of 50 μg/mL, the amount of metoprolol transported alone was found to be 12.562 ± 1.725 μg/mL at 60 min. The amount of metoprolol transport was increased from 12.562 ± 1.725 to 14.684 ± 1.853 and 12.562 ± 1.725 to 18.362 ± 2.515 and 12.562 ± 1.725 to 20.655 ± 3.687 μg/mL in the presence of hesperetin at concentrations of 25, 50, 100 μg/mL at 60 min. To further establish the P-gp role in metoprolol transport, similar assays were performed in the presence of 50 μg/mL quinidine, a P-gp inhibitor. In the presence of quinidine, the amount of metoprolol transport was increased from 12.562 ± 1.725 to 19.514 ± 3.561 μg/mL at a concentration of 50 μg/mL at 60 min. These study results indicated that hesperetin and quinidine enhanced the absorption of metoprolol due to the inhibition of P-gp.

Discussion

CYP enzymes and P-gp play a significant role in the first-pass metabolism of several orally administered drugs. The oral bioavailability of metoprolol is 50% due to its extensive first-pass metabolism. In the present SDPK and RDPK study, hesperetin which is known to be a has inhibitory effects on CYP3A4 [16], CYP2C9 [17], and CYP2B6 [18] was co-administered with metoprolol.

Table 3 Effect of hesperetin on the absorption of metoprolol

Time (Min)	MTL (50 μg/mL)	MTL + QDN (50 μg/mL)	MTL + HSP (25 μg/mL)	MTL + HSP (50 μg/mL)	MTL + HSP (100 μg/mL)
10	4.525 ± 1.084	6.524 ± 1.423*	5.688 ± 1.353^{NS}	7.466 ± 1.220*	8.584 ± 1.235**
20	6.858 ± 1.240	8.878 ± 1.685**	6.256 ± 1.402^{NS}	9.658 ± 1.062**	10.685 ± 1.508**
30	7.128 ± 1.536	11.581 ± 2.458**	8.633 ± 1.355^{NS}	10.763 ± 2.284**	12.362 ± 1.685**
40	8.365 ± 2.121	14.251 ± 2.120***	9.745 ± 1.834^{NS}	12.654 ± 1.665**	15.236 ± 2.263***
50	10.968 ± 1.489	17.260 ± 2.285***	12.636 ± 2.108*	14.759 ± 2.352**	18.896 ± 2.585***
60	12.562 ± 1.725	19.514 ± 3.561***	14.684 ± 1.853*	18.362 ± 2.515**	20.655 ± 3.687***

MTL, metoprolol; *QDN*, quinidine; and *HSP*, hesperetin

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $^{NS}p > 0.05$

Hesperetin co-administration significantly increased the plasma concentration and systemic exposure of metoprolol in rats. In the SDPK, hesperetin enhanced the C_{max} of metoprolol by 1.75-, 3.5-, and 4.75-fold, AUC_{0-24}, by 2.5-, 4.1-, and 4.9-fold, $AUC_{0-\infty}$ by 3.28-, 5.0-, and 5.8-fold, $t_{1/2}$ by 1.6-, 1.3-, and 0.85-fold, MRT by 1.7-, 1.5-, and 1.34-fold in a dose-dependent manner when pre-treated with hesperetin 25, 50, 100 mg/kg, while hesperetin treatment lowered the clearance and volume of distribution of metoprolol in the current study ($p < 0.001$).

Similarly, hesperetin enhanced the C_{max} of metoprolol by 1.79-, 3.73-, and 4.52-fold, AUC_{0-24}, by 1.57-, 2.29-, and 4.67-fold, $AUC_{0-\infty}$ by 1.37-, 4.56-, and 4.4-fold, $t_{1/2}$ by 1.2-, 1.2-, and 1.38-fold, MRT by 1.2-, 1.74-, and 1.27-fold in a dose-dependent manner when pre-treated with hesperetin 25, 50, 100 mg/kg in RDPK, while hesperetin treatment lowered the clearance and volume of distribution of metoprolol in the current study ($p < 0.001$). Similarly, the intestinal absorption of metoprolol was measured from the mucosal compartment to the serosal compartment using everted gut sacs and the intestinal absorption increased by 1.16-, 1.46-, and 1.6-fold when pre-treated with hesperetin at concentrations of 25, 50, 100 µg/mL establishing that P-gp suppression by hesperetin improved the intestinal absorption of metoprolol. These results are consistent with previous study reports. In human liver microsomes, hesperetin inhibited the CYP2C9-mediated conversion of diclofenac to 4'-hydroxydiclofenac. The clinical relevance here is that CYP2C9 is responsible for the biotransformation of drugs with a narrow therapeutic index [17]. This indicates that the concomitant administration of hesperetin with diclofenac results in enhanced bioavailability due to the inhibition of CYP2C9 by hesperetin. In another study, hesperetin inhibited the CYP3A4-mediated metabolism of felodipine in rats, thereby increasing its systemic exposure and suggesting the role of hesperetin as a CYP3A4 inhibitor [16]. Another study has shown the role of hesperetin as a weak inhibitory activity on CYP2B6 [18].

Concomitant administration of duloxetine and metoprolol orally in rat models results in increased the systemic exposure and plasma concentration of metoprolol in rats due to inhibition of CYP2D6 and P-gp by duloxetine [23]. In another study, felodipine, which is a substrate of CYP3A4, significantly increased the C_{max} and AUC_{0-12} of metoprolol increased in healthy male volunteers [24]. Similarly, co-administration of pyronaridine and artesunate (PA) increased the peak concentration of metoprolol by 47.93% and the AUC_{0-t} by 25.60%, indicating that PA co-administration will likely increase exposure to CYP2D6 substrates [25].

Amiodarone (AM) is the most effective antiarrhythmic drug, and its principal metabolite desethylamiodarone (DEA) is similarly effective. Both AM and DEA inhibit CYP2D6, which converts metoprolol to alpha-hydroxymetoprolol. The combination of amiodarone/desethylamiodarone and metoprolol was investigated in another study. The results suggested that concentrations of amiodarone and desethylamiodarone were increased and the plasma concentration of metoprolol still increased even at the decreased dosage of metoprolol decreased due to inhibition of CYP2D6 [26]. Similarly, hesperetin significantly increased the plasma concentration and systemic exposure of rasagiline in rats due to inhibition of CYP and P-gp [27] in another study. The authors of previous studies are also followed encapsulated techniques to improve the bioavailability of encorafenib [28] and 5-fluorouracil [29]. These findings of the other studies further support that results of increased bioavailability of the metoprolol in the current study might be due to the inhibition of CYP2D6, CYP3A4, CYP2C9 by hesperetin and increased intestinal absorption might be due to the inhibition of P-gp by hesperetin.

Conclusion

The results of this study have shown that hesperetin significantly enhanced the C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, $t_{1/2}$, and MRT and decreased the clearance, and volume of distribution of metoprolol ($p < 0.001$) in a dose-dependent manner might be due to the inhibition of CYP-mediated metabolism according to the findings of the investigation. Similarly, hesperetin and quinidine significantly increased the absorption of metoprolol in in vitro intestinal absorption study due to P-gp inhibition.

Abbreviations

CYP3A4	Cytochrome P-450 3A4
CYP2B6	Cytochrome P-450 2B6
CYP2D6	Cytochrome P-450 2D6
CYP2C9	Cytochrome P-450 2C9
SDPK	Single-dose pharmacokinetic study
RDPK	Repeated-dose pharmacokinetic study
RP-HPLC	Reverse phase-high-performance liquid chromatography
P-gp	P-glycoprotein

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Author contributions

Dr. NBK and Dr. RBP designed the work and guided the students. Mr. SV, Mr. SSM, and Mr. HSC were involved in animal studies and drafted the rough manuscript. Dr. VRD analyzed the data and finalized the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data and material can be provided upon request.

Declarations

Ethics approval and consent to participate
The protocol was approved by the IAEC at KVSR Siddhartha College of Pharmaceutical Sciences (Regd. No: 993/PO/Re/S/06/CPCSEA). The protocol number was KVSRSCOPS/11-03-14-006.

Consent for publication
Not applicable.

Competing interests
The authors declare that there is no conflict of interest with anyone or any institute.

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