Xenobiotica
the fate of foreign compounds in biological systems

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ixen20

# Effect of naringenin on the pharmacokinetics of metoprolol succinate in rats

Ravindra Babu Pingili, Sridhar Vemulapalli, Vijaya R. Dirisala, Surya Sandeep Mullapudi, Yamini Gullapalli & Naveen Babu Kilaru

**To cite this article:** Ravindra Babu Pingili, Sridhar Vemulapalli, Vijaya R. Dirisala, Surya Sandeep Mullapudi, Yamini Gullapalli & Naveen Babu Kilaru (2021) Effect of naringenin on the pharmacokinetics of metoprolol succinate in rats, Xenobiotica, 51:8, 926-932, DOI: 10.1080/00498254.2021.1942311

**To link to this article:** https://doi.org/10.1080/00498254.2021.1942311

Published online: 28 Jun 2021.

XENOBIOTICA
2021, VOL. 51, NO. 8, 926–932
https://doi.org/10.1080/00498254.2021.1942311

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE



# Effect of naringenin on the pharmacokinetics of metoprolol succinate in rats

Ravindra Babu Pingili[a], Sridhar Vemulapalli[b], Vijaya R. Dirisala[c], Surya Sandeep Mullapudi[d], Yamini Gullapalli[d] and Naveen Babu Kilaru[d]

[a]Faculty of Pharmacy, Vignan's Foundation for Science, Technology and Research (Deemed to be University), Vadlamudi, India; [b]Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE, USA; [c]Department of Biotechnology, Vignan's Foundation for Science, Technology and Research (Deemed to be University), Vadlamudi, India; [d]Department of Pharmaceutics and Pharmaceutical Biotechnology, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, India

**ABSTRACT**

1. The aim of the present study was to investigate the effect of naringenin (4,5,7-trihydroxy flavonone) on the pharmacokinetics of metoprolol, a substrate of Cytochrome P-450 3A4 (CYP3A4), CYP2C9, and CYP2D6 in rats.
2. Male Wistar rats were treated orally with metoprolol (30 mg/kg) alone and in combination with naringenin (25, 50, and 100 mg/kg) once daily for 15 consecutive days.
3. The plasma concentrations of metoprolol were determined using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) on the 1st day in single-dose pharmacokinetic (PK) study (SDS) and on the 15th day in multiple dosing PK studies (MDS).
4. Compared to the metoprolol control group, the $C_{max}$, AUC, and half-life ($T_{1/2}$) of metoprolol increased in rats pre-treated with naringenin, while there was no significant change in $T_{max}$. There is a significant decrease in clearance and volume of distribution.
5. The present study results revealed that naringenin significantly enhanced the $C_{max}$, AUC, MRT, $t_{1/2}$, and decreased the clearance of metoprolol possibly through the inhibition of CYP enzymes involved in the metabolism of metoprolol.

**ARTICLE HISTORY**
Received 28 April 2021
Revised 7 June 2021
Accepted 8 June 2021

**KEYWORDS**
Metoprolol; Naringenin; CYP2D6; CYP3A4; CYP2C9; bioavailability; pharmacokinetics

## Introduction

Globally, hypertension is the single most important risk factor for mortality and the third highest cause of morbidity (Ezzati et al. 2002). Hypertension is a major risk factor for coronary heart disease and stroke in the Indian population (Gupta et al. 2008). Metoprolol, a $\beta_1$-selective (cardioselective) adrenoceptor antagonist, is widely used in the treatment of mild to moderate hypertension and angina pectoris. Blockade of the $\beta_1$ receptor reduces heart rate, myocardial contractility, and cardiac output. It also reduces plasma renin activity (Benfield et al. 1986; Kendall et al. 1991). Intestinal absorption of metoprolol is rapid and almost complete and a significant beta-blockade effect occurs within 60 min of administration. However, due to extensive first-pass metabolism, the bioavailability of metoprolol is only approximately 50%. It is metabolized predominantly by CYP2D6 but that CYP3A4, CYP2B6, and CYP2C9 are also involved in the three major pathways of metoprolol metabolism. CYP3A4, 2B6, and 2C9 catalyse α-hydroxylation, O-demethylation, and N-dealkylation of metoprolol metabolism, respectively (Benjamin et al. 2018). These enzymes can be inhibited by several drugs or compounds. Therefore, concomitant use of CYP3A4, CYP2B6, CYP2C9, and CYP2D6 inhibitors will increase blood levels of metoprolol several-folds.

Flavonoids are polyphenolic plant secondary metabolites ubiquitous in foods of plant origin. They occur naturally as glycosides and consist of flavones, flavonols, flavanones, and isoflavones (Rice et al. 1996). Naringenin (4,5,7-trihydroxy flavonone) is a plant bioflavonoid found in grape fruit, tomato, and citrus fruits. It has been pharmacologically evaluated as neuroprotective (Raza et al. 2013), hepatoprotective (Renugadevi et al. 2010), antiatherogenic (Lee et al. 2001), nephroprotective (Badary et al. 2005), antioxidant (Ting et al. 2011), anticancer (Gao et al. 2006), and hypocholesterolemic (Jeon et al. 2007) activities. Worldwide, naringenin is widely used in medicine, food and other fields. Several previous experiments (Burkina et al. 2016; Liu et al. 2019) have indicated that naringenin is an inhibitor of CYP3A4 and CYP2C9.

Drug–drug interactions (DDIs), which can produce unrelated, synergistic, additive, and antagonistic results, are increasingly recognized as important clinical events (Zhang et al. 2016; Zhou et al. 2019). However, people are becoming increasingly aware of the importance of DDIs. In the last few years, several studies on the interactions between flavonoids and drugs have been reported (Alnaqeeb et al. 2019; Zhao et al. 2019). Recently, naringenin was reported as antihypertensive through down regulation of kidney injury molecule 1, mineralocorticoid receptor and angiotensin converting enzyme (Ademola et al. 2020). Given the potential

CONTACT Naveen Babu Kilaru naveenbabukvsr@gmail.com KVSR Siddhartha College of Pharmaceutical Sciences, Polyclinic road, Siddhartha Nagar, Vijayawada, Andhra Pradesh 520010, India

antihypertensive effect of naringenin, it is often used together with metoprolol for the treatment of hypertension. However, the herb–drug interaction between naringenin and metoprolol is still unknown. The aim of the present study was to investigate the effects of naringenin on the pharmacokinetics of metoprolol in Wistar rats. The pharmacokinetic parameters of metoprolol in rats with or without naringenin pre-treatment were analysed using a sensitive and reliable reverse phase-high-performance liquid chromatography (RP-HPLC) system.

## Materials and methods

### Drugs and chemicals

Naringenin was purchased from Sigma Chemical Co. (St. Louis, MO). Metoprolol and quinidine were obtained as gift samples from Orchid Health Care, Chennai, India, and Sipra Labs Ltd., Hyderabad, India, respectively. HPLC-grade acetonitrile, water, sodium carboxymethyl cellulose (SCMC), and dimethyl sulphoxide purchased from Finar chemicals Ltd., Ahmadabad, India. Distilled water prepared from deionized water, was used throughout the study. All other chemicals and reagents used were of analytical grade.

### Experimental animals

Animal experiments were performed according to the institutional guidelines for the care and use of laboratory animals, and approved by the animal ethics committee of KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, India (993/a/06/CPCSEA). Male Wistar rats (180–220 g) were procured from Mahaveer Enterprises, Hyderabad, India. Animals were housed, six per cage, and given free access to food (Hindustan Lever, Mumbai, India) and water in an animal house at the KVSR Siddhartha College of Pharmaceutical Sciences. Before starting the experiments, animals were kept under standard laboratory conditions (12/12 h light/darkness, 20 °C, and 50–60% humidity) for at least 1 week.

### Study design

This study consisted of two experiments (i.e. single-dose pharmacokinetic study and multiple-dose pharmacokinetic study) as it has been described previously by Challa et al., (2013). Ingestion of 150–900 mg doses of naringenin is safe in healthy adults (Candida et al. 2020). The maximum dose of metoprolol is 50–400 mg/day. The following formula was used to calculate the doses of metoprolol and naringenin.

$$\text{HED}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{Animal dose}\left(\frac{\text{mg}}{\text{kg}}\right)\text{X Animal Km/Human Km}$$

### Single-dose pharmacokinetic (PK) study

In the single-dose PK study (SDS), Wistar rats were randomly divided into four groups of six animals in each group. Metoprolol and naringenin was individually suspended in 1% SCMC for their administration to rats orally.

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

Group II: naringenin (25 mg/kg) and metoprolol (30 mg/kg)

Group III: naringenin (50 mg/kg) and metoprolol (30 mg/kg)

Group IV: naringenin (100 mg/kg) and metoprolol (30 mg/kg).

After administration, 100 μL blood samples were collected from retro-orbital plexus at different times (0.16, 0.33, 0.50, 1, 2, 3, 4, 8, 12, and 24 h) under slight ether anaesthesia. The plasma samples were separated by centrifugation (Remi, R4C Compact model, Mumbai, India) at 6000 rpm for 6 min and stored at −20 °C until analysis.

### Multiple dosing PK study in rats

In the multiple dosing PK study (MDS), the rats were treated with same drugs once daily for 15 consecutive days. After administration, 100 μL blood samples were collected from retro-orbital plexus at different times (0.16, 0.33, 0.50, 1, 2, 3, 4, 8, 12, and 24 h) under slight ether anaesthesia on the 15th day. The plasma samples were separated by centrifugation (Remi, R-4C Compact model) at 6000 rpm for 6 min and stored at −20 °C until analysis.

### Analytical methods

The plasma concentrations of metoprolol were quantitated using a previously described method by Kallem et al. (2013) with modifications. A Shimadzu HPLC system (Shimadzu, Tokyo, Japan) consisted of a pump (LC-20AT VP), C18 column (Kromasil, (Brewster, NY) 1504.6 mm, 5 m particle size) and a dual wavelength ultraviolet (UV)–visible detector (SPD-10A VP). LC solution software (Tokyo, Japan) was used to collect and process the data. The mobile phase consisted of 0.2% formic acid in acetonitrile and water (80:20 v/v). The prepared mobile phase was filtered through 0.45 mm membrane filter and ultrasonically degassed before use. The injection volume was 20 μL, and the effluent was monitored at 222 nm with a UV detector at a flow rate of 1 mL/min. The total chromatographic run time was 5.0 min, and the elution of metoprolol was occurred at 3.35 min (Figure 1). The analysis was carried out at ambient temperature.

### Extraction of metoprolol from plasma

Liquid–liquid extraction method was used to extract the metoprolol from rat plasma (Kallem et al. 2013). To an aliquot of 50 μL plasma, 1.2 mL *tert*-butyl methyl ether was added, vortex mixed for 5 min and centrifuged at 6000 rpm for 5 min. The supernatant (1 mL) was dried under gentle stream of nitrogen at 40 °C. The dried residue was reconstituted with 200 μL of mobile phase and 20 μL was injected into RP-HPLC system for analysis.

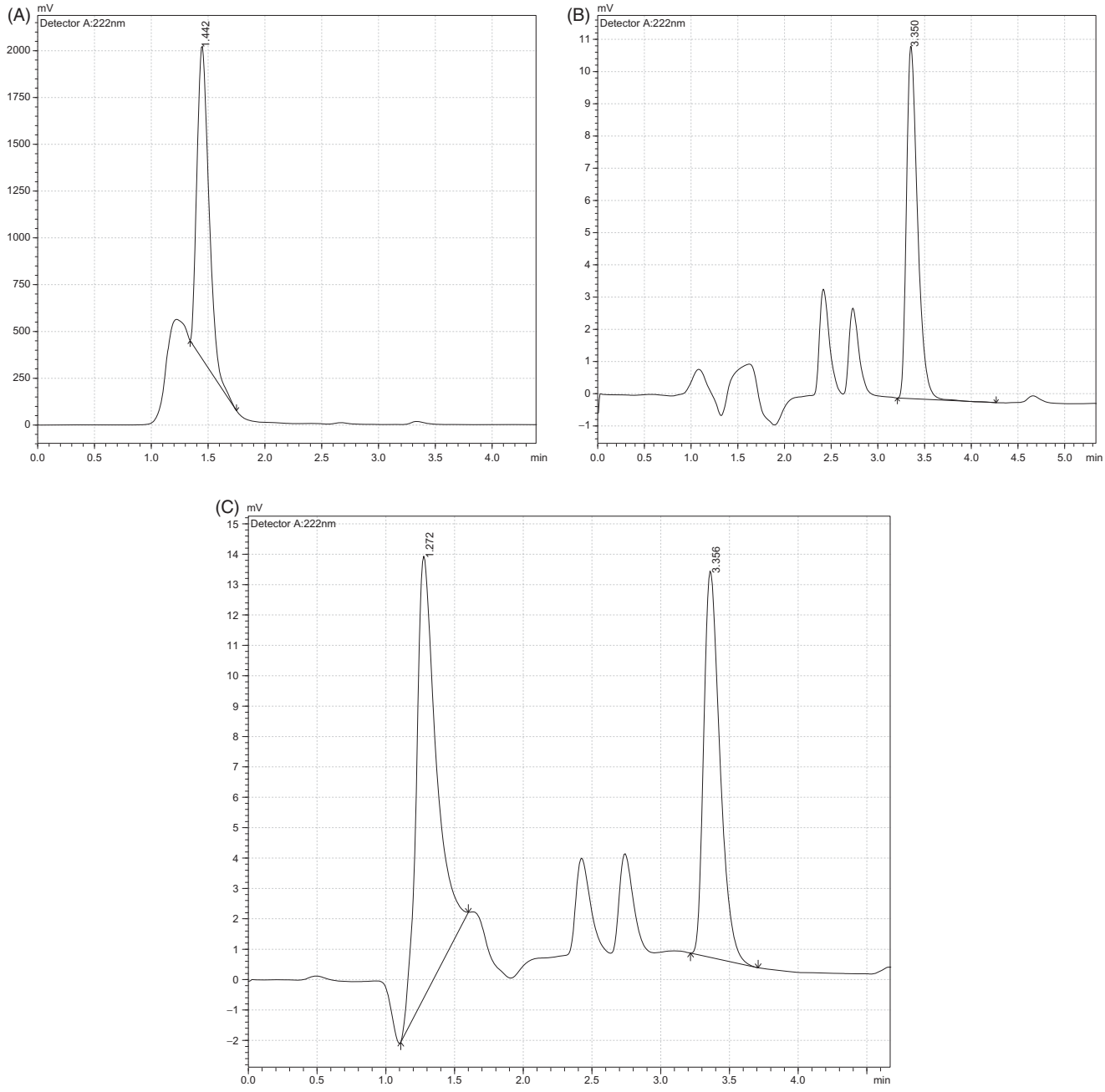


Figure 1. Representative chromatograms of (A) blank plasma; (B) metoprolol succinate (4 μg/mL); (C) plasma + metoprolol succinate (4 μg/mL). *X*-axis is the time (min) and *Y*-axis is the intensity of absorbance (mV).

### Linearity

Six calibration samples (1, 2, 4, 6, 8, and 10 μg/mL) were prepared by spiking metoprolol with appropriate volumes of the working solutions. The calibration curve was constructed by plotting a graph of peak mean area versus concentration. The linearity of calibration curve was evaluated using linear regression analysis.

### Precision

Intra-day precision was defined by the percentage of relative standard deviation (RSD) of six standards at six different concentrations analysed on the same day. Inter-day precision was estimated from the analysis of the six standards on six separate days during method validation. The RSD values for intra- and inter-day assays of calibration samples did not exceed 2%, thus, indicating the good precision of the method.

### Accuracy

The accuracy of method was assessed by recovery studies on calibration samples. The percent recovery by the assay of calibration samples ranged from 97.21% to 102.36%. The good recovery values for accuracy study ascertain that method is accurate.

### Calculation of PK parameters

The plasma concentrations versus time data obtained from each rat were submitted to a non-compartmental PK analysis

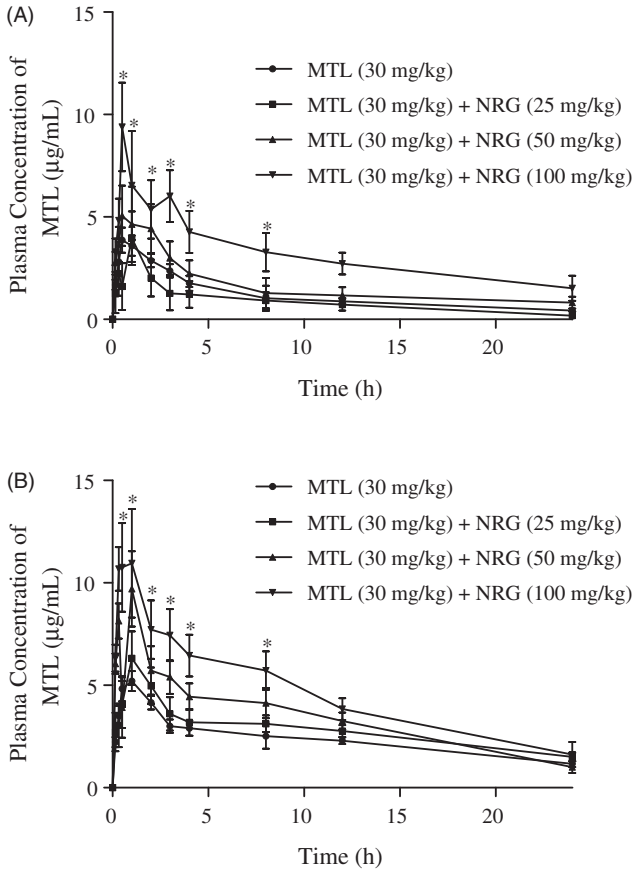


Figure 2. Mean plasma concentration–time curves of metoprolol following an oral administration of metoprolol (30 mg/kg) to rats with or without naringenin (A) on day 1; (B) on day 15. (●) MTL (30 mg/kg), (■) with 25 mg/kg NRG, (▲) with 50 mg/kg NRG, (▼) with 100 mg/kg NRG. MTL, Metoprolol; NRG, Naringenin. *$p < 0.001$ compared to Metoprolol alone group (two-way ANOVA followed by Bonferroni post-tests to compare to each column to column). Each point represents the mean ± S.D. of 6 rats.

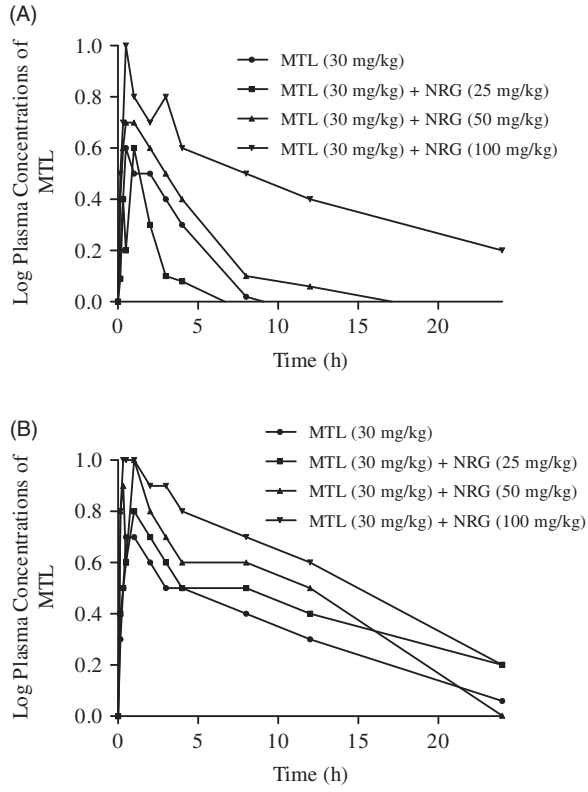


Figure 3. Log plasma concentration–time curves of metoprolol following an oral administration of metoprolol (30 mg/kg) to rats with or without naringenin (A) on day 1; (B) on day 15. (●) MTL (30 mg/kg), (■) with 25 mg/kg NRG, (▲) with 50 mg/kg NRG, (▼) with 100 mg/kg NRG. MTL: Metoprolol; NRG: Naringenin.

using Thermo Kinetica (Version 5.1, Thermo Electron Corporation, Waltham, MA). The maximum plasma concentrations ($C_{max}$) and times to achieve maximum plasma concentrations ($T_{max}$) were obtained directly from the individual plasma concentration–time curves. Other PK parameters were calculated using Thermo Kinetica.

### Data analysis

All statistics were calculated using GraphPad Prism 5.0 software (San Diego, CA). PK parameter values for groups were compared using one-way analysis of variance (ANOVA) followed by Tukey–Kramer post-test for multiple comparisons. Plasma concentrations of metoprolol analysed using two-way ANOVA followed by Bonferroni post-tests to compare to each column to column. The $p$ value less than 0.05 were considered significant.

## Results

### Effect of naringenin on the PK of metoprolol in SDS

Figure 2 shows metoprolol plasma concentrations versus time curves after oral administration of metoprolol alone and in combination with naringenin 25, 50, and 100 mg/kg in SDS and MDS. Log plasma concentration–time curves of metoprolol following an oral administration of metoprolol (30 mg/kg) to rats with or without naringenin are shown in Figure 3. PK parameters except $T_{max}$ were logarithmically transformed and compared by one-way ANOVA followed by Dunnett's test for multiple comparisons. Mean plasma PK parameters are summarized in Tables 1 and 2. In both studies, naringenin significantly ($p < 0.001$) increased the $C_{max}$ and $AUC_{0-24}$ of metoprolol in dose-dependent manner. The $C_{max}$ of metoprolol was increased 1.30-folds (at 50 mg/kg), and 2.422-folds at 100 mg/kg of naringenin. The $AUC_{0-24}$ of metoprolol was increased 1.37-folds (at 50 mg/kg), and 2.91-folds at 100 mg/kg of naringenin. The $AUC_{total}$ of metoprolol was increased 1.90-folds (at 50 mg/kg), and 4.0-folds at 100 mg/kg of naringenin. The $t_{1/2}$ of metoprolol was significantly increased 1.21-folds (at 50 mg/kg), and 1.55-folds at 100 mg/kg of naringenin. The MRT of metoprolol was significantly increased 2.03-folds at (50 mg/kg), and 1.74-folds at 100 mg/kg of naringenin. The CL/F of metoprolol was significantly decreased 1.19-folds (at 25 mg/kg), 1.85-folds (at 50 mg/kg), and 1.94-folds at 100 mg/kg of naringenin. The $V_z/F$ of metoprolol was significantly decreased 1.69-folds (at 50 mg/kg), and 02.52-folds at 100 mg/kg of naringenin.

### Effect of naringenin on the PK of metoprolol in MDS

The $C_{max}$ of metoprolol was significantly increased 1.74-folds (at 50 mg/kg), and 1.95-folds at 100 mg/kg of naringenin. The

Table 1. Pharmacokinetic parameters of metoprolol succinate after the oral administration of metoprolol succinate (30 mg/kg) to rats in the presence or absence of naringenin (25, 50, and 100 mg/kg) on the 1st day ($n = 6$).

| Parameter | Metoprolol (30 mg/kg) | Metoprolol + Naringenin (25 mg/kg) | Metoprolol + Naringenin (50 mg/kg) | Metoprolol + Naringenin (100 mg/kg) |
|---|---|---|---|---|
| $C_{max}$ (µg/mL) | 3.9 ± 1.0 | 3.9 ± 1.3 | 5.1 ± 1.8*** | 9.4 ± 2.1*** |
| $AUC_{0-24}$ (µg /mL/h) | 27.9 ± 3.5 | 30.0 ± 4.6 | 38.2 ± 4.6** | 81.2 ± 6.6*** |
| $AUC_{0-\infty}$ (µg/mL/h) | 35.6 ± 4.3 | 41.8 ± 3.2 | 67.6 ± 5.4** | 142.2 ± 8.2*** |
| $t_{1/2}$ (h) | 12.1 ± 2.2 | 13.7 ± 1.5 | 14.7 ± 2.5 | 18.8 ± 2.3* |
| $T_{max}$ (h) | 0.5 ± 0.1 | 1.0 ± 0.2 | 0.5 ± 0.1 | 0.5 ± 0.1 |
| MRT (h) | 15.1 ± 2.8 | 20.6 ± 2.1 | 30.7 ± 3.3** | 26.4 ± 3.7* |
| CL/F (L/h/Kg) | 0.190 ± 0.05 | 0.160 ± 0.05 | 0.103 ± 0.05* | 0.098 ± 0.05** |
| $V_z/F$ (L/Kg) | 0.300 ± 0.04 | 0.234 ± 0.05** | 0.182 ± 0.02** | 0.119 ± 0.05 |

$AUC_{0-24}$: area under the plasma concentration–time curve from 0 h to 24 h; $AUC_{0-\infty}$: area under the plasma concentration–time curve from 0 h to infinity; $C_{max}$: peak plasma concentration; $T_{max}$: time to reach peak plasma concentration; $t_{1/2}$: terminal half-life; MRT: mean residence time; CL/F: apparent total body clearance or oral clearance; $V_z/F$: apparent volume of distribution. All values are mean ± SD. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ when compared to metoprolol alone group (one-way ANOVA followed by the Tukey–Kramer post-test to compare to each column to column).

Table 2. Pharmacokinetic parameters of metoprolol succinate after the oral administration of metoprolol succinate (30 mg/kg) to rats in the presence or absence of naringenin (25, 50, and 100 mg/kg) on the 15th day ($n = 6$).

| PK parameter | Metoprolol (30 mg/kg) | Metoprolol + Naringenin (25 mg/kg) | Metoprolol + Naringenin (50 mg/kg) | Metoprolol + Naringenin (100 mg/kg) |
|---|---|---|---|---|
| $C_{max}$ (µg/mL) | 5.6 ± 1.6 | 6.3 ± 1.4 | 9.1 ± 2.1*** | 10.9 ± 2.1*** |
| $AUC_{0-24}$ (µg /mL/h) | 55.5 ± 6.4 | 68.4 ± 4.2* | 86.7 ± 5.7*** | 120.9 ± 6.9*** |
| $AUC_{0-\infty}$ (µg/mL/h) | 81.7 ± 7.6 | 99.7 ± 6.4* | 132.2 ± 10.6*** | 221.7 ± 15.6*** |
| $t_{1/2}$ (h) | 13.3 ± 3.6 | 14.5 ± 3.5 | 15.7 ± 3.1 | 19.3 ± 2.9* |
| $T_{max}$ (h) | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 |
| MRT (h) | 20.5 ± 1.2 | 21.8 ± 3.5 | 22.4 ± 2.6 | 28.9 ± 3.8** |
| CL/F (L/h/Kg) | 0.564 ± 0.3 | 0.508 ± 0.2 | 0.393 ± 0.1* | 0.315 ± 0.1 |
| $V_z/F$ (L/Kg) | 0.483 ± 0.1 | 0.329 ± 0.06* | 0.285 ± 0.05** | 0.221 ± 0.04** |

$AUC_{0-24}$: area under the plasma concentration–time curve from 0 h to 24 h; $AUC_{0-\infty}$: area under the plasma concentration–time curve from 0 h to infinity; $C_{max}$: peak plasma concentration; $T_{max}$: time to reach peak plasma concentration; $t_{1/2}$: terminal half-life; MRT: mean residence time; CL/F: apparent total body clearance or oral clearance; $V_z/F$: apparent volume of distribution. All values are mean ± SD. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ when compared to metoprolol alone group (one-way ANOVA followed by the Tukey–Kramer post-test to compare to each column to column).

$AUC_{0-24}$ of metoprolol was significantly increased 1.56-folds (at 50 mg/kg), and 2.18-folds at 100 mg/kg of naringenin. The $AUC_{total}$ of metoprolol was increased 1.62-folds (at 50 mg/kg), and 2.71-folds at 100 mg/kg of naringenin. The $t_{1/2}$ of metoprolol was increased 1.45-folds at 100 mg/kg of naringenin. The $T_{max}$ of metoprolol is remained constant for 25, 50, and 100 mg/kg doses of naringenin. The MRT of metoprolol was increased 1.42-folds at 100 mg/kg of naringenin. The CL/F of metoprolol was decreased 1.44-folds at (50 mg/kg), and 1.79-folds at 100 mg/kg of naringenin. The $V_z/F$ of metoprolol was increased 1.69-folds (at 50 mg/kg), and 2.18-folds at 100 mg/kg of naringenin.

## Discussion

The results of this study indicated that naringenin inhibited the metabolism of metoprolol in rats. When orally administered, naringenin significantly increased the $C_{max}$, AUC, prolong the $t_{1/2}$, and decreased the CL/F of metoprolol. Several previous studies reported that naringenin was a potent inhibitor of CYP enzymes including CYP3A4 and CYP2C9 (Wenjie et al. 2011; Burkina et al. 2016; Liu et al. 2019). The present study results are consistent with these previous studies.

Ravindra et al. (2016) reported that naringenin is the inhibitor of CYP enzymes, thereby increased the $C_{max}$ of rasagiline from 8.25 ± 2.33 to 15.81 ± 3.01 ng/mL (at 12.5 mg/kg) and 23.55 ± 6.12 ng/mL (at 25 mg/kg) in rats. The $AUC_{0-\infty}$ of rasagiline also increased from 26.23 ± 4.22 to 63.10 ± 7.58 (at 12.5 mg/kg) and 140.08 ± 25.74 ng h/mL (at 25 mg/kg) in rats. The $t_{1/2}$ of rasagiline was increased from 2 ± 0.45 to 3.47 ± 1.55 and 10.13 ± 3.46 h at the dose of 12.5 and 25 mg/kg of naringenin, respectively. In addition, naringenin decreased the CL/F and $V_z$/F of rasagiline in rats.

In another study by Keech et al. (1986), verapamil significantly increased the $C_{max}$ and AUC of metoprolol in patients with coronary artery disease due to inhibition of CYP enzymes. Similarly Ibrutinib is an inhibitor of Bruton's tyrosine kinase, which is prescribed to treat chronic lymphocytic leukaemia and was mainly metabolized by CYP3A4. Naringenin significantly affected the pharmacokinetics of ibrutinib, including prolonging its half-life, increase the area under the concentration–time curve and reducing its clearance time due to inhibition of CYP3A4 (Liu et al. 2019). Surya et al.'s study shows that at a dose of naringenin at 100 mg/kg increased the P.K. properties of felodipine like $C_{max}$, $AUC_{total}$ increased significantly in both SDS and MDSv, respectively, due to P-gp and CYP3A4 inhibition by naringenin.

Combination use of Naringenin and tofacitinib for the treatment of rheumatoid arthritis is practiced in Chinese clinics. Wang et al. (2020) reported that due to the inhibition CYP3A4 by naringenine the $AUC_{0-24}$ of tofacitinib increased from 1222.81 ± 222.07 to 2016.27 ± 481.62 ng/mL/h, the $T_{max}$ increased from 0.75 ± 0.29 to 3.00 ± 0.00 h ($p < 0.05$), and the MRT(0–24) w increased from 4.90 ± 0.51 to 6.57 ± 0.66 h ($p < 0.05$), but the clearance was decreased from 4.10 ± 0.72 to 2.42 ± 0.70 L/h/kg ($p < 0.05$) in rats due to inhibition of CYP3A4.

In addition to the above studies conducted by different groups, the current shows the inhibitory effect of naringenin on the metabolism by CYP3A4 enzymes. The current study focus on and improving the bioavailability of metoprolol by inhibiting the CYP3A4 enzymes using a flavonoid naringenin. A SDS study and MDS conducted to identify the short term and long term effects of naringenin on the PK parameters of metaprolol.

In SDS and MDS studies of metoprolol in combination with naringenin significantly ($p < 0.001$) increased several PK parameters of metoprolol in a dose-dependent manner from 25, 50, and 100 mg/kg of naringenin. These parameter include the $C_{max}$, $AUC_{0-24}$, $AUC_{total}$, $t_{1/2}$, $T_{max}$, and MRT. The CL/F and $V_z/F$ decreased in a dose dependent manner in both the studies. All the observed parameters indicate that the availability of the metoprolol increased with the combination treatment of naringenin. It indeed indicates that naringenin acts as a potent inhibitor of metaprolol metabolising enzymes namely CYP3A4 and CYP2C9 in this study.

## Conclusion

The present study results revealed that naringenin significantly enhanced the $C_{max}$, AUC, MRT, and prolonged $t_{1/2}$ and decreased the clearance of metoprolol in rats, possibly through the inhibition of CYP enzymes involved the metabolism of metoprolol. The liver participates in the first-pass metabolism of metoprolol, leading to significantly reduced bioavailability. Such interaction would increase the bioavailability of naringenin, and metoprolol was concomitantly administered. It is suggested that concurrent use of naringenin or naringenin glycoside-containing herbs or dietary supplements with metoprolol increases metoprolol's pharmacological actions or serious adverse effects. The dose of metoprolol might be adjusted. We conclude that naringenin significantly affects the metabolism of metoprolol by inhibiting CYPs involved in its metabolism. However, further studies are required to confirm this interaction more specifically.

## Acknowledgements

The authors are grateful to Vignan's Foundation for Science, Technology, and Research (Deemed to be University), Vadlamudi, and KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, for providing necessary facilities and their encouragement in completing this work.

## Disclosure statement

The authors declare that this research does not have any conflict of interest with anyone or any Institute.

## References

Ademola AO, Temidayo OO, Olumuyiwa AA. 2020. Antihypertensive power of Naringenin is mediated via attenuation of mineralocorticoid receptor (MCR)/angiotensin converting enzyme (ACE)/kidney injury molecule (Kim-1) signaling pathway. Eur J Pharmacol. 880:173142.

Alnaqeeb M, Mansor KA, Mallah EM, Ghanim BY, Idkaidek N, Qinna NA. 2019. Critical pharmacokinetic and pharmacodynamic drug-herb interactions in rats between warfarin and pomegranate peel or guava leaves extracts. BMC Complement Altern Med. 19(1):29.

Badary OA, Maksoud SA, Ahmed WA, Owieda GH. 2005. Naringenin attenuates cisplatin nephrotoxicity in rats. Life Sci. 76(18):2125–2135.

Benfield P, Clissold SP, Brogden RN. 1986. Metoprolol. An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in hypertension, ischaemic heart disease and related cardiovascular disorders. Drugs. 31(5):376–429.

Benjamin B, Fabio B, Urs D, Stephan K, Manuel H. 2018. Cytochrome P450 enzymes involved in metoprolol metabolism and use of metoprolol as a CYP2D6 phenotyping probe drug. Front Pharmacol. 24:774.

Burkina V, Zlabek V, Halsne R, Ropstad E, Zamaratskaia G. 2016. In vitro effects of the Citrus flavonoids diosmin, naringenin and naringin on the hepatic drug-metabolizing CYP3A enzyme in human, pig, mouse and fish. Biochem Pharmacol. 110-111:109–116.

Candida JR, Robbie AB, Juan JLL, Frank LG, Eric R, David MR, Alexander P, Brandon JK, Hector FC, Shawn RC, et al. 2020. Safety and pharmacokinetics of naringenin: a randomized, controlled, single-ascending-dose clinical trial. Diabetes Obes Metab. 22:91–98.

Challa VR, Babu PR, Challa SR, Johnson B, Maheswari C. 2013. Pharmacokinetic interaction study between quercetin and valsartan in rats and in vitro models. Drug Dev Ind Pharm. 39(6):865–872.

Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. 2002. Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. Lancet. 360(9343):1347–1360.

Gao K, Henning SM, Niu Y, Youssefian AA, Seeram NP, Xu A, Heber D. 2006. The citrus flavonoid naringenin stimulates DNA repair in prostate cancer cells. J Nutr Biochem. 17(2):89–95.

Gupta R, Joshi P, Mohan V, Reddy KS, Yusuf S. 2008. Epidemiology and causation of coronary heart disease and stroke in India. Heart. 94(1):16–26.

Jeon S-M, Kim HK, Kim H-J, Do G-M, Jeong T-S, Park YB, Choi M-S. 2007. Hypocholesterolemic and antioxidative effects of naringenin and its two metabolites in high-cholesterol fed rats. Transl Res. 149(1):15–21.

Kallem RR, Ramesh M, Seshagirirao JVLN. 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 Chromatogr. 27(6):784–791.

Keech AC, Harper RW, Harrison PM, Pitt A, McLean AJ. 1986. Verapamil appears to increase the bioavailability of metoprolol. Amencan Journal of Cardiology. 58(6):551–552.

Kendall MJ, Maxwell SR, Sandberg A, Westergren G. 1991. Controlled release metoprolol. Clinical pharmacokinetic and therapeutic implications. Clin Pharmacokinet. 21(5):319–330.

Lee C-H, Jeong T-S, Choi Y-K, Hyun B-H, Oh G-T, Kim E-H, Kim J-R, Han J-I, Bok S-H. 2001. Antiatherogenic effect of citrus flavonoid, naringin and naringenin associated with hepatic ACAT and acrostic VCAM-1 and MCP-1 in high cholesterol fed rabbits. Biochem Biophys Res Commun. 284(3):681–688.

Liu J, Liu H, Zeng Q. 2019. The effect of naringenin on the pharmacokinetics of ibrutinib in rat: a drug–drug interaction study. Biomed Chromatogr. 33(5):e4507.

Ravindra BP, Sridhar V, Surya SM, Siddhartha N, Sivaprasad P, Naveen BK. 2016. Pharmacokinetic interaction study between flavanones (hesperetin, naringenin) and rasagiline mesylate in Wistar rats. Drug Dev Ind Pharm. 42:1110–1117.

Raza SS, Khan MM, Ahmad A, Ashafaq M, Islam F, Wagner AP, Safhi MM, Islam F. 2013. Neuroprotective effect of naringenin is mediated through suppression of NF-κB signaling pathway in experimental stroke . Neuroscience. 230:157–171.

Renugadevi J, Milton Prabu S. 2010. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol. 62(2): 171–181.

Rice EC, Miller NG, Paganga G. 1996. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med. 20: 833–956.

Surya SM, Sridhar V, Puneeth Y, Ravindra BP, Naveen BK. 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(10):1371–1377.

Ting S, Yeh HS, Lien TF. 2011. Effects of supplemental levels of hesperetin and naringenin on egg quality, serum traits and antioxidant activity of laying hens. Anim Feed Sci Tech. 163:59–66.

Wang B, Shen J, Zhou Q, Meng D, He Y, Chen F, Wang S, Ji W. 2020. Effects of naringenin on the pharmacokinetics of tofacitinib in rats. Pharm Biol. 58(1):225–230.

Wenjie JL, Valentina F, Cong X, David AF, Salvatore C. 2011. Enantiomers of Naringenin as pleiotropic, stereoselective inhibitors of cytochrome P450 isoforms. Chirality. 23:891–896.

Zhang Y, Li J, Lei X, Zhang T, Liu G, Yang M, Liu M. 2016. Influence of verapamil on pharmacokinetics of triptolide in rats. Eur J Drug Metab Pharmacokinet. 41(4):449–456.

Zhao Q, Wei J, Zhang H. 2019. Effects of quercetin on the pharmacokinetics of losartan and its metabolite EXP3174 in rats. Xenobiotica. 49(5):563–568.

Zhou Y, Song X, Dong G. 2019. Effects of verapamil on the pharmacokinetics of puerarin in rats. Xenobiotica. 49(10):1178–1182.