

hydroxymidazolam, was carried out using high performance liquid chromatography (HPLC) as previously described with minor modifications.²¹ HPLC separation were performed on the C₁₈ HPLC analytical column (4.60 × 100 mm i.d.; 3.5 µm particle size) with an isocratic elution system. The mobile phase consisted of 10 mM sodium acetate with pH 4.0 and acetonitrile (55:45, v/v). The flow rate was 0.80 mL/min, at 30 °C and the absorbance of eluent was monitored at 220 nm.

Real time RT-PCR analysis

Hepatic and intestinal RNA was isolated from tissue samples snap frozen in liquid nitrogen using TRIzol reagent (Life Sciences), according to the manufacturer's instructions. Total RNA was transcribed to cDNA using ImProm-II™ Reverse Transcription System (Promega). The mRNAs encoding for *Mdr1a*, *Mdr1b* and *Slc21a5* were determined by TaqMan gene expression assays (Applied Biosystems, Carlsbad, CA). Expression of the interested genes normalized to housekeeping gene glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was calculated by $2^{-\Delta\Delta C_t}$ using StepOnePlus™ software.

Pharmacokinetic analysis

The maximum blood concentration (C_{max}) and time to maximum concentration (t_{max}) of simvastatin and simvastatin acid were obtained directly from the concentration-time profiles. The area under the plasma concentration-time curve from time zero to the last quantifiable concentration (AUC_{0-t}) was calculated using the trapezoidal rule. The elimination rate constant (k_{el}) for simvastatin and simvastatin acid were calculated by linear regression analysis of the terminal log-linear part of the concentration-time profiles. The $AUC_{t-\infty}$ was determined by the equation: $AUC_{t-\infty} = C_{last}/k_{el}$. The elimination half-life ($t_{1/2}$) was calculated by the equation: $t_{1/2} = \ln 2/k_{el}$.²²

Statistical analysis

An unpaired two-tailed Student's t-test was used for statistical comparison of all pharmacokinetic parameters except t_{max} which was analyzed by the Wilcoxon rank test. Differences in parameters of mRNA expression and CYP3a2 activity analysis also employed by the unpaired two-tailed Student's t-test. For each of these tests, differences were considered significant when * $p < 0.05$ and ** $p < 0.01$.

Results

Total flavonoid content in pomelo juice

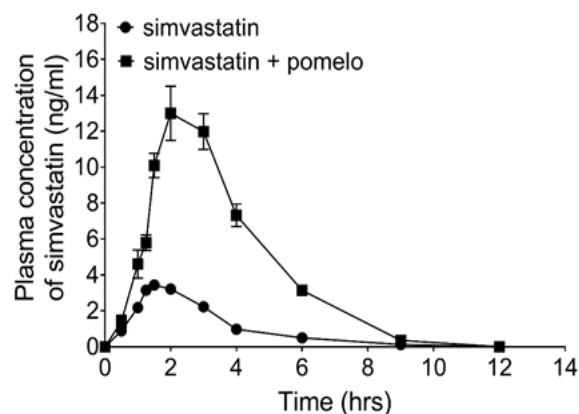
Using chromogenic quantitative assay, we found that total flavonoid content present in the Thong-dee pomelo juice was in the range of 9,169.37-12,782.62 µg/100 g of edible portion of pomelo from 8 duplicate analyses.

Area under the plasma drug concentration time curve of simvastatin and simvastatin acid

We then investigated the effect of pomelo juice on pharmacokinetic profiles of simvastatin and simvastatin

acid, its primary active metabolite. The mean plasma concentration-time profiles of simvastatin and simvastatin acid after oral administration of simvastatin alone (20 mg/kg) and simvastatin in concomitant with pomelo juice (1 mL/kg) are shown in Figure 1 and the pharmacokinetics parameters of simvastatin and simvastatin acid are illustrated in Table 1. As shown in Figure 1A, pomelo juice significantly affected pharmacokinetic profile of simvastatin. $AUC_{0-\infty}$ and C_{max} of simvastatin in rats receiving simvastatin combined with pomelo juice were significantly increased by 4-fold ($p < 0.01$) and 3.9-fold ($p < 0.01$), respectively, when compared with those in rats receiving simvastatin alone. Despite notable changes in $AUC_{0-\infty}$ and C_{max} , pomelo juice did not significantly alter t_{max} , $t_{1/2}$ and k_{el} of simvastatin. In a manner similar to simvastatin, pomelo juice significantly increased the $AUC_{0-\infty}$ and C_{max} of simvastatin acid by a factor of 3 ($p < 0.01$) and 3.6 ($p < 0.01$), respectively (Figure 1B). There were no differences in t_{max} , $t_{1/2}$ and k_{el} of simvastatin acid between rats treated with simvastatin alone and rats treated with simvastatin combined with pomelo juice.

A



B

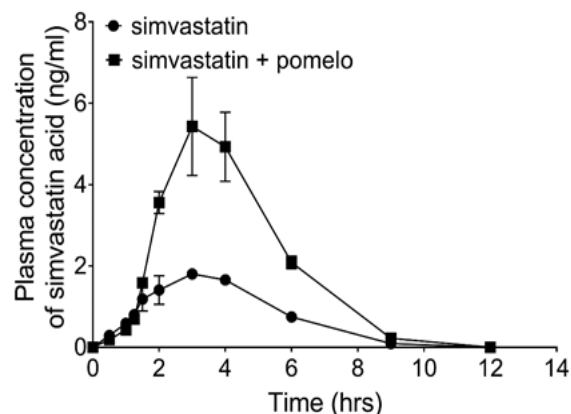


Figure 1. Mean plasma concentration-time profiles of (A) simvastatin and (B) simvastatin acid in rats after oral administration of 20 mg/kg simvastatin alone or concomitant with 1 mg/kg pomelo juice twice daily for 7 days and 30 minutes before simvastatin ingestion at day 8.