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RESEARCH ARTICLE

Pomegranate Juice does not Affect the Bioavailability of Cyclosporine in Healthy Thai Volunteers

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Abstract: ***Background*:** It is still controversial whether pomegranate causes drug interactions. Pomegranate juice has been shown to inhibit CYP3A *in-vitro* and animal studies. The co-administration of pomegranate juice with cyclosporine, a narrow therapeutic drug that is the substrate of CYP3A, might lead to drug toxicity. The objective of this study is to investigate the effect of pomegranate juice on the pharmacokinetics of cyclosporine in healthy Thai volunteers.

***Methods*:** The study design was an open-label, randomized, single dose, crossover study with a 2-week washout period. Each fasting subject received 2 microemulsion tablets of 100 mg of cyclosporine with 500 ml of pomegranate juice (test) or 500 ml of water (control). Serial blood samples were collected up to 24 h after dosing, and blood samples were analyzed for cyclosporine concentrations by using chemiluminescent microparticle immunoassay. Fourteen healthy volunteers completed the study.

***Results*:** The 90% confidence intervals for the test/control ratio using logarithmically transformed data of area under the concentration-time curve (AUC) from time zero until the last measured concentration (AUC_{0-t}), AUC from time zero to infinity ($AUC_{0-\infty}$), and maximum concentration (C_{max}) were 91.6-105.6, 92.0-105.2 and 82.3-102.5, respectively. The results were within the accepted bioequivalence range for narrow therapeutic index drugs (90-111% for AUC and 80-125% for C_{max}). There were no differences in adverse event between the groups.

***Conclusion*:** Single dose administration of pomegranate juice with cyclosporine did not significantly affect the oral bioavailability of cyclosporine. However, further work is needed to thoroughly evaluate the effect of pomegranate on narrow therapeutic drugs.

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1. INTRODUCTION

Cyclosporine, a cyclical peptide of 11 amino acids, is a calcineurin inhibitor. The inhibition of calcineurin activity interferes with the activation of T-cell-specific transcription factor (NFAT) and prevents the translocation of NFAT to the nucleus [1]. All these will lead to the reduction of the synthesis of cytokines (*e.g.*, interleukin-2) that activates and proliferate T-cells [1, 2]. In other words, cyclosporine is an inhibitor of T-cell mediated immunity enabling its use as an immunosuppressant in both adults and children. In addition to the parent drugs, some metabolites (*e.g.*, AM1, AM19) have been found to have immunosuppressive action [2]. Due to the marked improvement in the rates of acute rejection and one year graft survival, cyclosporine has been the mainstay for use in solid organ transplantation [1, 3]. Additionally, the drug has been used in the treatment of bone marrow transplants [4], autoimmune diseases (*e.g.*, rheumatoid arthritis, psoriasis, Crohn's disease and ulcerative colitis) [5] and allergy/skin diseases (*e.g.*, chronic urticaria, atopic eczema) [6, 7].

The therapeutic window of cyclosporine between minimally immunosuppressive blood concentrations and concentrations associated with adverse events is relatively narrow. Cyclosporine is primarily eliminated *via* biotransformation by Cytochrome P450 (CYP) 3A in the gut wall and the liver [8]. Additionally, P-glycoprotein (P-gp), which is located in the gastrointestinal epithelium, can affect the blood concentration of cyclosporine after oral administration, presumably by counter transporting the drug back into the gastrointestinal lumen [9]. There have been numerous reports of clinically relevant drug-cyclosporine interactions [10, 11]. Dietary constituents, including grapefruit juice, can augment the blood cyclosporine concentration by inhibiting intestinal CYP3A [12]. Several flavonoids, such as naringin, quercetin [13] and furanocoumarins, including 6',7'-dihydroxybergamottin,

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have been isolated from grapefruit juice and identified as inhibitors of CYP3A4 *in vitro* [14-17].

Pomegranate (*Punica granatum* L.) is commonly consumed around the world, and it has been used in folk medicine for many therapeutic purposes [18]. Pomegranate is a rich source of crude fibers, pectin, sugars, and several tannins [19]. The major phytochemical component classes identified to date in pomegranate fruit are anthocyanins and hydrolyzable tannins, specifically ellagitannins, which release ellagic acid when hydrolyzed. Punicalagin, punicalin, gallagic acid, and ellagic acid were found to account for the majority of the ellagitannins in pomegranate juice [20]. Research has shown that the antioxidant activity of pomegranate juice is primarily attributable to the concentration of hydrolyzable tannins, whereas anthocyanins contribute very little to the *in vitro* antioxidant capacity [19]. In addition, beneficial health effects of pomegranate juice have been reported, such as the inhibition of low-density lipoprotein oxidation and decrease of the severity of cardiovascular diseases and breast cancer [21-24]. Based on these findings, the pomegranate has become increasingly popular as a supplementary health product. Higher pomegranate consumption allows for the increased possibility of pomegranate-drug interactions.

Some *in vitro* and animal studies have recently shown the effects of pomegranate juice on drug metabolism enzymes. Pomegranate juice inhibits the activity of cytochrome P450 and CYP3A *in vitro*. Pomegranate juice inhibits carbamazepine 10,11-epoxidation activity in human liver microsomes. In addition, a study using rat models has shown that pomegranate juice increases the Area Under the Concentration-time Curve (AUC) of carbamazepine by approximately 1.5-fold. Interestingly, pomegranate juice does not affect the elimination half-life of carbamazepine. These data suggest that the components of pomegranate juice impair the function of enteric but not hepatic CYP3A [25]. Some human studies were conducted to test the interference of pomegranate juice consumption on drugs. However, although pomegranate juice inhibited CYP3A *in vitro*, it did not affect the maximum concentration (C_{max}) and AUC of oral midazolam [26, 27] or simvastatin in healthy volunteers [28]. However, there was a report that the co-administration of pomegranate dietary products increased the concentration of the calcineurin inhibitor (tacrolimus) in a heart transplant patient [29]. Therefore, it is still controversial as to whether pomegranate juice causes drug interactions. To date, there has been no study of the interaction between pomegranate juice and cyclosporine, a calcineurin inhibitor, in humans. Therefore, our aim was to investigate the effect of pomegranate juice on the pharmacokinetics of cyclosporine in healthy volunteers.

2. MATERIALS AND METHODS

2.1. Subjects

Eighteen healthy Thai volunteers were enrolled in the study. The volunteers had to fulfill the following inclusion criteria: (1) Thai male aged between 20 and 45 years; (2) body mass index of 18-25 kg/m^2; and (3) in good health as judged by their medical history, physical examination, vital signs, such as heart rate and blood pressure, and laboratory tests, including blood urea nitrogen, creatinine, hematologic profiles (white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelets), liver function test (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin), blood sugar, and urine analysis.

The exclusion criteria were (1) a history of hypersensitivity or an allergic reaction to cyclosporine; (2) a history of gastrointestinal disease, hepatic disease, renal disease, and allergic disease; (3) a history of illicit drug or alcohol abuse; (4) a history of smoking; (5) a history of the use of drugs that are known to influence the absorption and disposition of cyclosporine; and (6) participation in any clinical study within 1 month. Consumption of beverages containing caffeine and certain foods that might affect drug metabolism, such as coffee and tea, were not allowed 2 weeks prior to and during the study period.

2.2. Study Design

The study was a single-dose, open-label, randomized, 2-period, 2-sequence, 2-treatment crossover design separated by a washout period of 2 weeks. Subjects were randomized into two sequences: administration of the drug with pomegranate juice (test) followed by the administration of the drug with water (control), or vice versa. During each period, subjects arrived at the hospital the day before the study. After an 8-hour overnight fast, subjects received 200 mg of microemulsion cyclosporine (Sandimmune soft gelatin capsules, Sandoz Pharmaceuticals Corp., Hanover, N.J.) with either 500 ml of pomegranate juice (test) or 500 ml of water (control). The pomegranates were bought from a local supermarket in Phitsanulok, Thailand, but their place of origin was unknown. Pomegranate juice was prepared by squeezing fresh fruit. Subjects received standardized meals 4 and 10 hours after the administration of cyclosporine. Venous blood samples (3 ml) were collected before drug administration (time 0) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours after drug administration. Blood was collected in Ethylenediaminetetraacetic Acid (EDTA) collection tubes, gently inverted several times to allow the mixing of the blood and EDTA, and stored at –80°C until analysis. The blood samples were analyzed within 4 weeks of collection by means of a chemiluminescent microparticle immunoassay (ARCHITECH *i* system, Abbott). The assay was performed according to the manufacturer's instructions for cyclosporine. The standards, controls, and samples were analysed together in each analytical run. The standard curve for cyclosporine was linear over a range of 30-1500 ng/ml. The Lower Limit Of Quantitation (LLOQ) was 20 ng/ml. Concentrations below the LLOQ were assigned a value of 0 ng/ml. Both the intra- and inter-assay bias (relative error) and the coefficient of variation were less than 15%.

Tolerability was assessed by monitoring for adverse events after drug administration and at every blood sampling time. The second phase was identical to the first phase, with the exception that subjects received the alternative fluid. At the end of the study, blood samples were collected for serum creatinine analysis.

2.3. Pharmacokinetic Analysis

The pharmacokinetic parameters of individual subjects were calculated by use of noncompartmental pharmacokinetic methods using PKSolver. The AUC from time zero until the last measured concentration (AUC_{0-t}) was calculated by the log-linear trapezoidal method and subsequent extrapolation to infinity ($AUC_{0-\infty}$). The C_{max} and the time at the maximum concentration (t_{max}) were directly obtained from the concentration-time profile. The elimination rate constant (k_e) of cyclosporine was calculated by log-linear regression. The apparent half-life ($t_{1/2}$) was estimated using the following equation: $t_{1/2} = {}^{0.693}/_{k_e}$

2.4. Statistical Analysis

The pharmacokinetic parameters, including t_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, and k_e, are expressed as the mean ±SD. Log-transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were analyzed by Analysis of Variance (ANOVA) for comparison between pomegranate juice and water. The 90% confidence intervals (CIs) of the differences of log-transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were calculated. The accepted bioequivalence range as defined in the European Medicines Agency guidelines for narrow therapeutic index drugs is 90-111% for AUC and 80-125% for C_{max}. Untransformed t_{max} was compared using the Wilcoxon singed ranks test. The statistical package SPSS version 11.5 (SPSS Inc., Chicago, IL) was used for the statistical analyses.

3. RESULTS

Fourteen of the 18 subjects (age range, 23-29 years; weight range, 50-76 kg; height range, 1.60-1.85 m; body mass index, 18.2-25.4) completed the study. Four subjects were excluded from the study on the admission date due to a history of smoking. Note that the data from one subject were excluded because we were unable to draw blood from the subject 3 and 6 hours after drug administration in period 2 because of blood clotting and too small veins. Therefore, the data from this subject were not included in the analysis. The omission of data from five subjects did not adversely affect the power of the study, which remained higher than 95% based on 13 subjects, and the conclusions based on this smaller sample size are still valid. All subjects received either the drug with pomegranate juice or the drug with water in each study period. Seven subjects were administered the drug with pomegranate juice in the first study period and then with water in the second study period. Six subjects administered the drug with water in the first study period, and then with pomegranate juice in the second period.

The results presented here are from sample analysis of 13 subjects in both periods. The mean cyclosporine whole blood concentration versus time profile is shown in Fig. (**1**). The calculated pharmacokinetic parameters are listed in Table **1**.

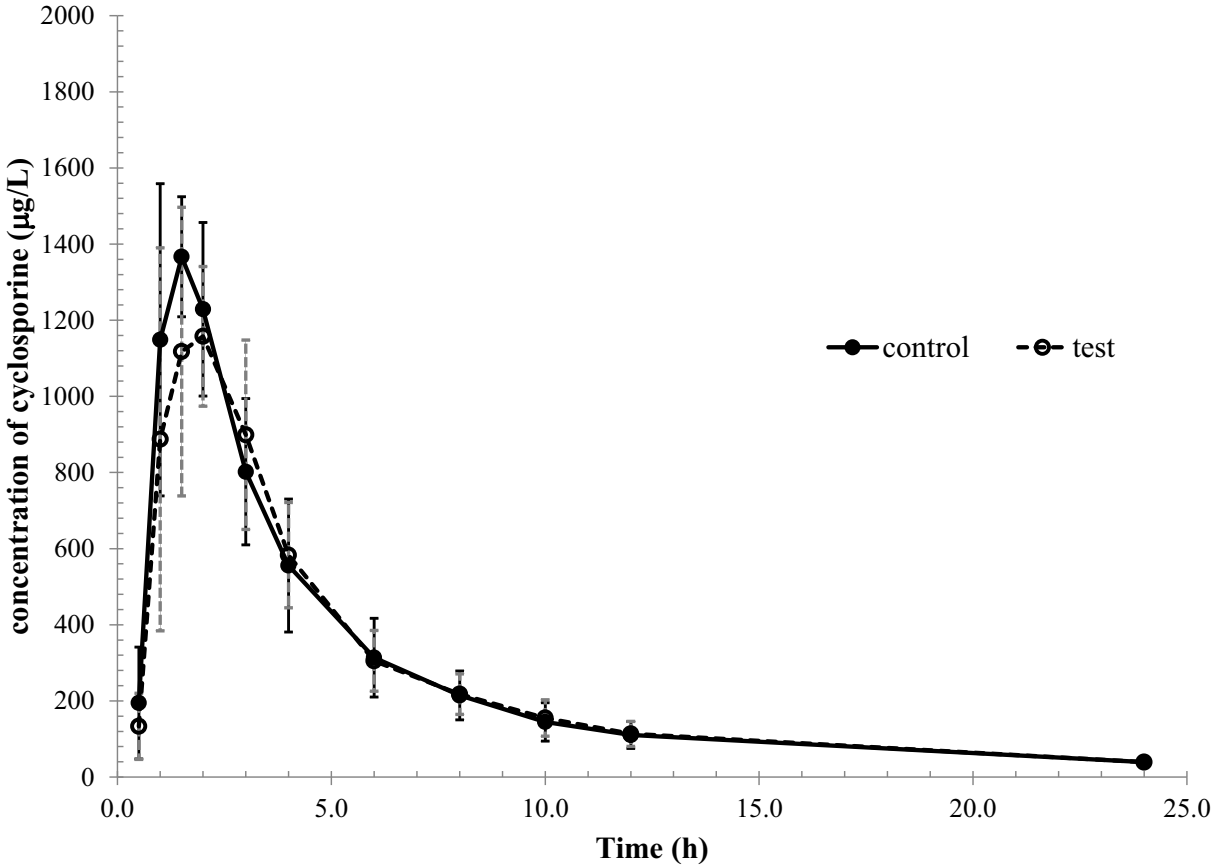

Fig. (1). Mean(±SD) cyclosporine whole blood concentration *versus* time after oral administration of 200 mg dose with water (control) or pomegranate juice (test) to 13 subjects.

The apparent elimination phase was well characterized by sampling up to 24 hours, and the %extrapolation of the AUC was below 20% for all the subjects.

The ANOVA results of the 13 subjects are presented in Table **2**. The results of our present study show that pomegranate juice has no effect on the pharmacokinetics of cyclosporine in healthy Thai volunteers. The 90% confidence intervals for the test/control ratio using logarithmically transformed data of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were 91.6-105.6, 92.0-105.2 and 82.3-102.5, respectively. These results were within the accepted bioequivalence range as defined in the European Medicines Agency guidelines for narrow therapeutic index drugs (90-111% for AUC and 80-125% for C_{max}).

Table 1. Pharmacokinetics of cyclosporine administered with water or pomegranate juice in 13 healthy Thai volunteers.

Parameters	Pomegranate Juice	Water
t_{max}(h)	1.82 ± 0.65	1.43 ± 0.33
C_{max}(µg/l)	1337.9 ± 208.7	1464.4 ± 219.3
AUC_{0-t}(µg.h/l)	5978.4 ± 1191.6	6215.0 ± 1369.7
$AUC_{0-\infty}$(µg.h/l)	6374.4 ± 1260.9	6628.5 ± 1460.6
%AUC extrapolation	6.3 ± 2	6.3 ± 2
$t_{1/2}$(h)	6.42 ± 1.45	7.04 ± 0.74

values were expressed as mean±SD, sample size (n) =13. SD: Standard deviation. t_{max}: time to maximum blood concentration, C_{max}: maximum blood concentration, AUC_{0-t}: Area under curve from time 0 to last measurable concentration, $AUC_{0-\infty}$: area under the curve from time 0 to infinity, t½: apparent the first-order terminal elimination half-life.

Table 2. Statistical comparison of selected pharmacokinetic parameters of cyclosporine between pomegranate juice (test) versus water (control).

Parameters	Ratio of Means	90% CI (Test/Control)	Power (%)
C_{max}(µg/l)	91.9	82.3 – 102.5	95.53
AUC_{0-t} (µg.h/l)	98.4	91.6 – 105.6	99.87
$AUC_{0-\infty}$ (µg.h/l)	98.4	92.0 – 105.2	99.91

ANOVA was performed on the natural log-transformed pharmacokinetic parameters. sample size (n) =13. CI: confidence interval, C_{max}: maximum blood concentration, AUC_{0-t}: Area under the curve from time 0 to last measurable concentration, $AUC_{0-\infty}$: area under the curve from time 0 to infinity.

Table 3. Statistical comparison of t_{max} of cyclosporine between pomegranate juice versus water by wilcoxon signed rank test.

	Product	Median t_{max} (range) (h)	*P*
Cyclosporine	Pomegranate	1.50 (1.00-3.10)	0.068
	Water	1.50 (1.00-2.00)	

Sample size (n) =13, t_{max}: time to maximum blood concentration, P<0.05: considered significantly.

Table 4. Adverse events during clinical study after patients taking cyclosporine with pomegranate juice and the drug with water (sample size =13).

	Pomegranate n(%)	Water n(%)
Blood pressure ≥ 90/140 mmHg	3(23)	2(23)
Flushing	6(46)	7(54)
Nausea	1(8)	2(15)
Vomiting	1(8)	1(8)
Fainting	2(15)	0(0)
Dizziness	2(15)	0(0)
Headache	1(8)	1(8)
Serious adverse events	0(0)	0(0)

The t_{max} values are shown in Table **3**. The median t_{max} was not different from that of pomegranate juice. In addition, no significant (p-Value > 0.1) in sequence and period effect for AUC_{0-t}, $AUC_{0-\infty,}$ and C_{max} were observed in the ANOVA analysis (data not shown).

Adverse events are reported in Table **4**. The most common adverse events in this study included high blood pressure and flushing. All of the adverse events spontaneously resolved, and all of the adverse events resolved without sequelae. No abnormal value of serum creatinine was detected at the end of the study to suggest a treatment effect.

4. DISCUSSION

The results of our present study show that pomegranate juice has no effect on the pharmacokinetics of cyclosporine in healthy Thai volunteers. The 90% confidence intervals for the test/control ratio using logarithmically transformed data of AUC_{0-t}, $AUC_{0-\infty,}$ and C_{max} were 91.6-105.6, 92.0-105.2 and 82.3-102.5, respectively. These results were within the accepted bioequivalence range as defined in the European Medicines Agency guidelines for narrow therapeutic index drugs (90-111% for AUC and 80-125% for C_{max}).

It has been reported that pomegranate juice has an inhibitory effect comparable to that of grapefruit juice on CYP3A in human microsomes [25]. Results from animal studies are in agreement with the results of the human microsome study. Pomegranate juice significantly increased AUC by approximately 1.5-fold after oral administration of carbamazepine in rats, suggesting that pomegranate inhibits CYP3A-mediated carbamazepine metabolism [25]. Pomegranate juice increased the bioavailability of buspirone in rabbits [30]. Pomegranate juice also significantly increased the area under the concentration-time curve and peak plasma concentration of nitrendipine by 2.03- and 2-fold, respectively, in rabbits [31].

In contrast to the results of animal studies, ingestion of a single bolus of pomegranate juice did not alter the clearance of orally and intravenously administered midazolam, an *in vivo* CYP3A probe, in healthy male volunteers [26]. Farkas *et al*. explained that this result might be because of the lower intake volume per kilogram of pomegranate juice used in the human study (3.2 ml/kg) compared to that used in the rat study (6.7 ml/kg). However, the intake volume of 7.1 ml/kg of pomegranate juice used in our study did not affect the bioavailability of the drug substrate. Moreover, repeated consumption of pomegranate juice did not significantly alter the pharmacokinetic profile of midazolam in humans [27]. Another human study showed that drinking pomegranate juice did not affect the oral bioavailability of simvastatin [32]. However, an increase in the level of tacrolimus during the consumption of popsicles containing pomegranate juice concentrate was reported in a heart transplant patient [29].

This study confirmed the results of previous human studies [26-28, 32], suggesting that pomegranate juice does not alter the activity of hepatic or intestinal CYP3A in humans. These results contrast with *in vitro* experiments and *in vivo* studies on rats and rabbits [25, 30, 33]. The lack of a clinically significant interaction in humans could be due to the species differences in drug metabolism. Cyclosporine is metabolized in the liver, small intestine, and kidneys in both species, but the metabolism of cyclosporine is carried out by different CYP3A isoforms. In humans, cyclosporine is primarily metabolized by CYP3A4 and CYP3A5, whereas in rats, cyclosporine is primarily metabolized by CYP3A2 [34]. The difference between humans and rats may be the susceptibility of enzymes to inhibition.

Although there is still controversy regarding the effects of CYP3A variants on cyclosporine exposure, there is plenty of evidence that suggests genetic polymorphisms in CYP3A genes [35-37]. Regarding the metabolism of cyclosporine, the CYP3A5 variant contributed more than the CYP3A4 variant [35]. Compared to the *CYP3A5*1/*1* carriers, the *CYP3A5*3* carriers have significantly lower expression of *CYP3A5* mRNA in the intestine and liver. The association of *CYP3A5*3/*3* genotype with decreased clearance of cyclosporine was reported. The last meta-analysis showed that the *CYP3A5*3* carriers had higher cyclosporine dose-adjusted trough level, and lower cyclosporine daily dosage requirements than the *CYP3A5*1/*1* carriers [35]. The frequency of the *CYP3A5*3* allele was different according to ethnicity (55% for African-Americans, 33% for Asians and 10-40% for Caucasians) [35]. In addition to ethnicity, gender and age have been shown to have a significant effect on the pharmacokinetics of cyclosporine [36]. The Study of Eng *et al*. showed that male and female patients had significant differences in cyclosporine dose requirement and the ratio of trough concentration and dose. Female patients needed higher doses of cyclosporine to achieve the target concentration [36]. The lower bioavailability and higher clearance of cyclosporine in children than in adults were reported [38]. In younger ages, there is an association between *POR*28* allele and *CYP3A5*3* allele with higher cyclosporine dose requirements and lower concentration/dose ratio [39]. Considering the effect of differences in polymorphism, race, gender and age, the extrapolation of the result from this study that conducted in adult Asian males with untested polymorphism to other populations should be done with caution, since it is possible that the difference in pharmacokinetics of the drug in each population might yield a different outcome of pharmacokinetic interaction between cyclosporine and pomegranate juice.

Some limitations of this study should be noted. Natural products are known to vary in their composition. The relationship between the chemical constituents of pomegranate and their effect on drugs is not clear. Among the variety of chemical components present in pomegranate, anthocyanins, ellagic acid, ellagitannins, and flavonoids seem to be responsible for the inhibition activity. During juice processing, the whole fruit is pressed, and ellagitannins are extracted into pomegranate juice in significant quantities [19]. The use of arils alone or the whole fruit to make juice has an enormous impact on the polyphenol content. Additionally, access to pomegranates and the geographical region, harvesting, and season can alter the fruit composition and, consequently, the juice composition [40]. In addition, the present study addressed only the effect of acute consumption of typical amounts of pomegranate juice on cyclosporine. Finally, the chronic effects of cyclosporine on the metabolic enzyme and

the efflux transporter need to be considered. Transplant patients who were undergoing maintenance therapy with cyclosporine demonstrated the induction of intestinal CYP3A4 and inhibition of hepatic and intestinal P-glycoprotein activity. The observed differences were reproduced in healthy controls who received 8 mg/kg of cyclosporine 12 hours and 2 hours before testing. Accordingly, chronic cyclosporine therapy may result in differential responses to the ingestion of dietary constituents [41]. Therefore, additional studies are needed to more fully determine the interaction potential of pomegranate juice on cyclosporine disposition.

CONCLUSION

In this study, healthy volunteers received a single 200-mg oral dose of cyclosporine and a single 500-ml volume of pomegranate juice, no significant alteration in the pharmacokinetic profile of cyclosporine compared with that of the control was observed. However, further work is needed to thoroughly evaluate the effect of pomegranates from different sources or different doses of pomegranate juice on drug substrates.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in this study involving human subjects were approved by the ethical standards of the Human Ethics Committee of Naresuan University Phitsanulok, Thailand, (Ethic Number 5403010002). Written informed consent was obtained from all subjects. Before participation, all subjects underwent medical screening that included their medical history, a physical examination, and routine laboratory investigations.

HUMAN AND ANIMAL RIGHTS

No animals were involved in the study. All procedures performed in this study involving human subjects were in accordance with the ethical standards of the Helsinki Declaration and its later amendments or comparable ethics standards.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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