

Effects of Lemon and Seville Orange Juices on the Pharmacokinetic Properties of Sildenafil in Healthy Subjects

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Abstract

Purpose Several severe drug interactions have been reported when sildenafil, a potent drug for the treatment of erectile dysfunction, is co-administered with drugs or herbal remedies that inhibit cytochrome P450 (CYP) 3A4. This study evaluates the effects of two citrus fruit juices, lemon and Seville orange, on the pharmacokinetics of sildenafil in male healthy subjects following a single oral dose.

Methods We conducted an open-label, three-way cross-over study in nine healthy male volunteers. Participants received a single oral dose of sildenafil (50 mg) after pretreatment with 250 mL of either water (control), undiluted lemon juice, or Seville orange juice for 3 consecutive days. All subjects were monitored for adverse effects during the study period. Plasma samples were collected for 12 h after dosing and analyzed for sildenafil concentration. **Results** Compared with pretreatment with water, Seville orange juice significantly increased the area under the plasma concentration-time curve from time zero to infinity and the peak plasma concentration of sildenafil by 44 % (90 % confidence interval [CI] 30–60) and 18 % (90 % CI 108–129), respectively, without affecting the time to reach peak plasma concentration. Additionally, Seville orange juice significantly reduced the apparent oral clearance of sildenafil by 30 % (90 % CI 63–75) without affecting its

elimination half-life. In contrast, lemon juice did not cause any significant alterations in the pharmacokinetics of sildenafil. There was no significant treatment-related adverse effects reported during the study.

Conclusions Although it is considered as a moderate CYP3A4 inhibitor, Seville orange only caused a mild increase in exposure to sildenafil after a single oral dose, without manifestation of any adverse effects. The enhanced bioavailability of sildenafil by Seville orange may be attributed to inhibition of its intestinal first-pass effect (CYP3A4 and or p-glycoprotein). Lemon juice, in contrast, had no effects on the pharmacokinetics of sildenafil.

Key Points

Consumption of Seville orange juice for 3 consecutive days enhances bioavailability of a single oral administration of sildenafil, without causing any noticeable adverse effects.

The effect of Seville orange juice may be attributed to inhibition of the intestinal first-pass effect of sildenafil that is mediated by cytochrome P450 3A4 and efflux transporters.

Consumption of lemon juice did not alter the pharmacokinetics of sildenafil.

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1 Introduction

The discovery of phosphodiesterase type 5 inhibitors in the late 1990s has revolutionized erectile dysfunction treatment. Sildenafil is the first available phosphodiesterase

type 5 inhibitor used by more than 30 million men worldwide for the treatment of erectile dysfunction [1]. Sildenafil is a substrate for the intestinal and hepatic cytochrome P450 (CYP) 3A4, which accounts for the extensive first-pass effect and an oral bioavailability of only 40 % [2]. Owing to the significant contribution of CYP3A4 in the biotransformation of sildenafil, the concomitant intake of drugs or herbal remedies that inhibit CYP3A4 may cause serious drug interactions.

Consumption of citrus fruit and their juices is very common worldwide owing to their presumed health-promoting effects, mainly anti-oxidative and anti-proliferative activities. Since the discovery of the potential for grapefruit juice to significantly impact the pharmacokinetics, and hence, the pharmacological actions of CYP3A4 substrates, great attention has been drawn to fruit juice–drug interactions in clinical practice [3]. It has been well established in the published literature that modulation of CYP3A4 activity could impact the pharmacokinetics of sildenafil and lead to significant drug–drug or drug–food interactions. However, the outcome of these interactions has been inconsistently reported. For example, grapefruit juice increases the bioavailability and area under the concentration–time curve (AUC) of sildenafil by 23 % without affecting its maximum plasma concentration (C_{max}) [4]. Additionally, co-administration of ciprofloxacin and clarithromycin significantly increases sildenafil bioavailability [5]. In contrast, pummelo juice, a potent CYP3A4 inhibitor, reduces the rate and extent of sildenafil absorption to around 60 % [6].

Seville orange, one of the common varieties of the *Citrus aurantium* species, previously called *Fructus aurantii*, is also the botanical name of a plant commonly known as bitter orange, sour orange, or green orange [7]. Seville orange has been used as an ingredient of dietary supplements marketed for weight loss aid owing to its claimed effects on metabolism, increasing the basal metabolic rate and lipolysis, and also as an appetite suppressant [8]. Lemon (*Citrus lemon*) is a yellow fruit used for both culinary and cleaning purposes primarily for its juice [9]. Many physicians in the Middle East recommend the use of lemon juice with sildenafil to enhance its effects. However, to our knowledge, there is no scientific evidence supporting these claims.

Both Seville orange and lemon are safe for daily intake [10]. Chemotaxonomically, they belong to the same genus as grapefruit and share many common constituents, especially furanocoumarins. Theoretically, these citrus juices may have similar potential for causing food–drug interactions by inhibition of intestinal CYP3A4. Medications metabolized by the intestinal CYP3A4 enzyme (potent first-pass effect) such as sildenafil are required to be evaluated for potential interaction with juices affecting

intestinal CYP3A4 enzyme in clinical research [11]. Therefore, the aim of this study is to evaluate the effects of Seville orange and lemon juices on the pharmacokinetics of sildenafil in healthy volunteers after a single 50-mg oral dose.

2 Materials and Methods

2.1 Subjects

Nine healthy male volunteers were included in the study. The average age of the volunteers was 37 years (range 22–45 years) and the average weight was 78 kg (range 65–95 years). The study protocol was approved by the Ethical Committee of Tanta University and was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent before participation. All subjects were ascertained to be healthy by medical history, physical examination, and routine laboratory tests.

2.2 Chemicals

Sildenafil powder was obtained from Medical Union Pharmaceuticals (Ismailia, Egypt), and propyl paraben was obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Acetonitrile, methanol, and ammonium dihydrogen phosphate and phosphoric acids were purchased from (Riedel-De Haen, Seelze, Germany). All solvents were high-performance liquid chromatography (HPLC) grade. Diethyl ether of analytical grade was obtained from Honil Ltd (London, UK). The sildenafil 50-mg tablet (Viagra[®] 50 mg; Pfizer, Giza, Egypt) was purchased from the local market.

2.3 Citrus Fruit Juices

Fresh lemon and Seville orange fruits were purchased from local markets and concentrated juices were made fresh. According to species varieties, 250 mL of concentrated Seville orange juice contains approximately 32, 5, and 36 $\mu\text{mol/L}$ of the major furanocoumarins bergapten, bergamottin, and 6',7'-dihydroxybergamottin, respectively. In contrast, 250 mL of concentrated lemon juice contains approximately 4.0 $\mu\text{mol/L}$ bergamottin [12].

2.4 Evaluation of Safety

Adverse events were monitored throughout the study. All observed adverse events were recorded by the principal investigator, including severity and potential relationship to sildenafil. Safety was also assessed by physical examination, clinical laboratory tests, and monitoring of vital signs before and during blood sampling.

2.5 Study Design

A random three-period, intra-individual, crossover, single-dose study was employed to investigate the effects of consumption of 250 mL of citrus juice (Seville orange or lemon) once daily for 3 consecutive days on the pharmacokinetics of a single oral dose (50 mg) of sildenafil. Participants were asked to abstain from taking any drug and herbal remedies including fruit juices and smoking for at least 3 days before and until the end of the study period. The subjects ingested 250 mL of an assigned drink of water, 250 mL of Seville orange juice (fresh extract of 250 g *C. aurantium* fruits), or 250 mL of *C. lemon* juice (fresh extract of 250 g *C. lemon* fruits) for 2 days. On day 3, after subjects had fasted overnight for 8 h, each volunteer received sildenafil 50 mg with 250 mL of the assigned liquid. All volunteers had a standardized meal 3 h after administration of sildenafil. At least a 1-week washout period was required between each intervention (Fig. 1). A 1-week washout period is sufficient for complete elimination of sildenafil [4].

2.6 Sampling

In each study period, 3-mL venous blood samples were collected from an indwelling catheter placed in an antecubital vein, or by direct venipuncture, into heparinized

tubes at pre-dose (0 h) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 10, and 12 h after sildenafil administration. Plasma was obtained by centrifugation (EBA 12 Centrifugator; Hettich, Kirchlegern, Germany) and stored at -20 °C until analysis.

2.7 Analytical High-Performance Liquid Chromatography Methods

Plasma samples were analyzed for sildenafil using a previously published method with little modification [5]. A clean test tube was spiked with 50 µL of the internal standard solution (5 µg/mL propyl paraben in methanol) and the methanol was left to evaporate in a water bath adjusted at 50 °C. To this tube, 0.5 mL of the collected plasma sample was added and the tube contents were vortex mixed for 3 min. The plasma samples already spiked with the internal standard were extracted with 4 mL of ether followed by vortex mixing for 3 min. After centrifugation for 10 min, the ether layer was transferred to a clean test tube and was evaporated in a water bath at 50 °C. The residue was dissolved in 150 µL of the mobile phase and 50 µL of the resulting solution was injected onto the HPLC. The mobile phase consisted of acetonitrile and 50 mM ammonium dihydrogen phosphate buffer (pH adjusted at 3.5 with phosphoric acid) at a ratio of 35:65. Separation was achieved at ambient temperature using

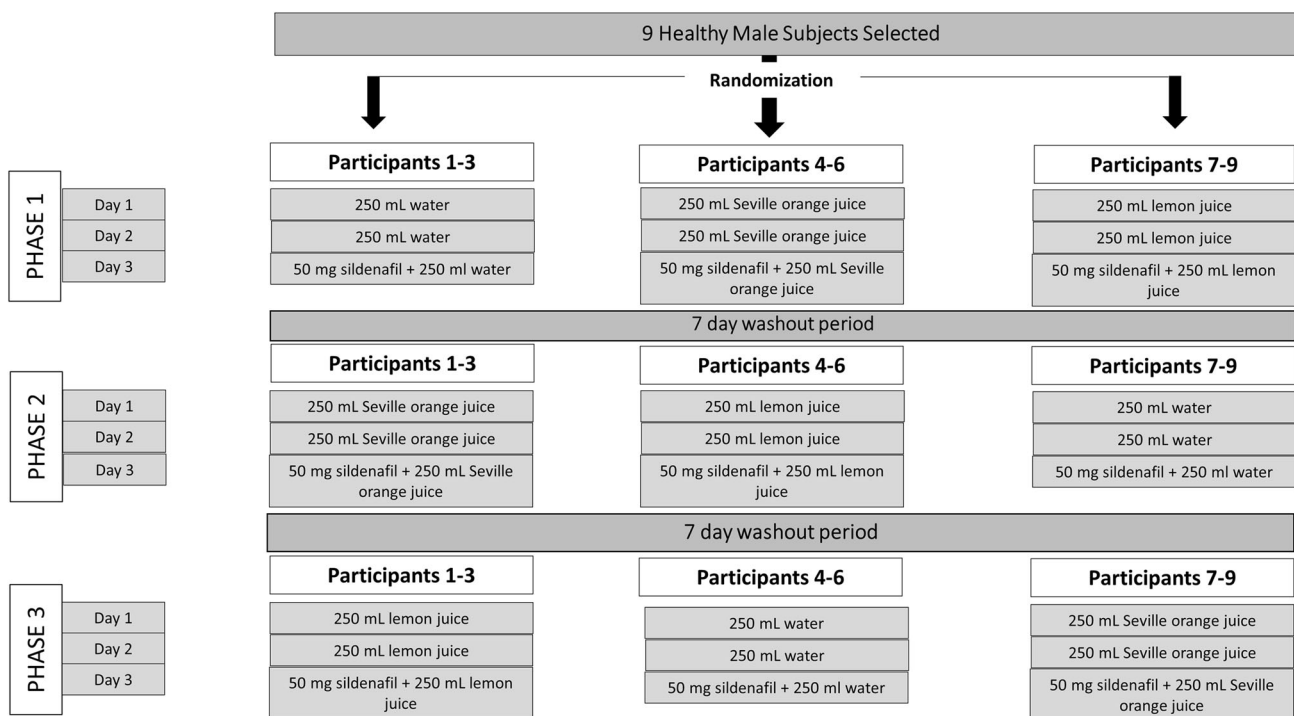


Fig. 1 Schematic of the study design

column C_{18} at a flow rate of 1.5 mL/min. The column effluent was monitored by an ultraviolet detector at 220 nm. The limit of quantification for sildenafil was 3.0 $\mu\text{g/L}$ and the method was linear in the range of 3.0–1000 $\mu\text{g/L}$. The method was validated using blank human plasma and was shown to be accurate (96–116 %) and precise (% coefficient of variation less than 16 %) in quantifying all standards and quality control samples.

2.8 Pharmacokinetic and Statistical Analysis

The plasma concentration–time profiles of sildenafil of each subject were analyzed by a non-compartmental method using WinNonlin[®] software (v 6.1; Pharsight Corporation, Mountain View, CA, USA). Pharmacokinetic values for C_{max} and time to C_{max} (T_{max}) were taken directly from the observed data. Individual concentration vs. time profiles were plotted, and the terminal elimination rate constant (k_e) was determined by the log–linear regression of at least three data points judged to be in the terminal phase. The elimination half-life ($t_{1/2}$) was determined from the elimination rate constant using the equation $(0.693/k_e)$. The AUC from time zero to infinity ($\text{AUC}_{0-\infty}$) was determined by the trapezoidal method for the detected values and subsequent extrapolation to infinity. The apparent oral clearance (CL/F) was obtained as $\text{dose}/\text{AUC}_{0-\infty}$.

Descriptive statistics, including the geometric mean and associated 90 % confidence interval, were used to summarize the pharmacokinetic parameters of sildenafil. For $t_{1/2}$ and T_{max} , the arithmetic mean and median are presented, respectively. Statistical comparisons of the estimated pharmacokinetic values were performed using analysis of variance with Tukey test. P values <0.05 were considered significant.

3 Results

There was no significant difference in vital signs among the treatment groups during the study period (data not shown). The nine subjects completed the study without reporting any adverse effect from sildenafil. Plasma concentrations of sildenafil were determined after a single oral administration with and without consumption of lemon or Seville orange juice for 3 consecutive days prior to sildenafil administration using a validated HPLC–ultraviolet method. The retention times for sildenafil and the internal standard were 6.5 min and 10.0 min, respectively, as shown in Fig. 2. The mean plasma concentration–time curves of a single 50-mg dose of sildenafil after 3 consecutive days of pretreatment with water, lemon, or Seville orange juices are shown in Fig. 3.

3.1 Effect of Seville Orange Juice on the Pharmacokinetics of Sildenafil

Following a single oral administration of 50 mg, sildenafil experienced a rapid absorption phase reaching a maximum plasma concentration in about 1 h. There was no observed effect on the rate of absorption of sildenafil following administration of Seville orange juice compared with pretreatment with water as indicated by non-significant changes in T_{max} . Relative to the control, administration of Seville orange juice resulted in a significant increase in the absorption of sildenafil as demonstrated by a 44 % and an 8 % increase in $\text{AUC}_{0-\infty}$ and C_{max} , respectively. Additionally, Seville orange juice significantly reduced the CL/F of sildenafil by 30 %, without any significant alterations in its $t_{1/2}$. These results indicate that consumption of Seville orange juice enhances the exposure to sildenafil after a single oral administration. However, this interaction was mild (less than 2-fold increase in AUC) and resulted in no reported adverse effects. Table 1 summarizes the geometric mean ratios and related 90 % confidence interval for the pharmacokinetic parameters of sildenafil with and without administration of Seville orange juice.

3.2 Effect of Lemon Juice on the Pharmacokinetics of Sildenafil

Relative to the control, sildenafil plasma concentration vs. time curves appeared remarkably similar after administration of lemon juice as shown in Fig. 3. Similar to the impact of Seville orange juice, pre-treatment with lemon juice for 3 days did not alter the rate of absorption of sildenafil as indicated by non-significant changes in T_{max} . Additionally, consumption of lemon juice resulted in non-significant alterations in mean values of $\text{AUC}_{0-\infty}$, C_{max} , $t_{1/2}$, and CL/F compared with water. These results indicate that consumption of lemon juice does not alter the

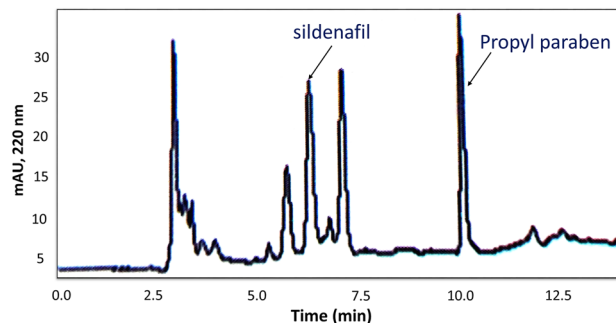


Fig. 2 Representative high-performance liquid chromatogram of a human plasma sample containing sildenafil and the internal standard propyl paraben, obtained following a single oral administration of a 50-mg sildenafil tablet. Retention times for sildenafil and propyl paraben are 6.3 and 10.2 min, respectively

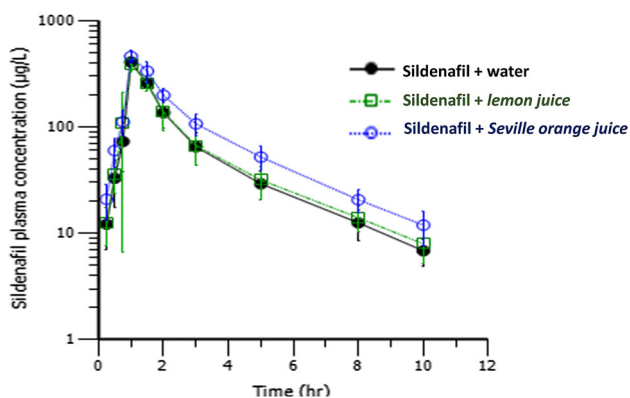


Fig. 3 Mean plasma concentration of sildenafil after a single 50-mg oral administration of sildenafil with water (closed circles), or 250 mL lemon juice (open squares), or 250 mL Seville orange juice (open circles) once daily for 3 days. Mean \pm standard deviation plasma concentration-time profile of sildenafil following a single 50-mg oral dose and pretreatment with water, lemon juice, or Seville orange juice once daily for 3 days

pharmacokinetics of a single oral administration of sildenafil, and may suggest that no food–drug interaction is expected when sildenafil and lemon juice are co-administered. Table 1 summarizes the geometric mean ratios and related 90 % confidence interval for the pharmacokinetic parameters of sildenafil with and without administration of lemon juice. As illustrated in Fig. 4, the inter-individual variation within control and treatment groups were similar and did not exceed 2-fold in any group.

4 Discussion

This is the first clinical study that investigates the effects of two commonly consumed citrus fruit juices on the pharmacokinetics of sildenafil following a single oral 50-mg dose in healthy subjects. Consumption of Seville orange

juice, but not lemon juice, significantly enhanced the extent of absorption of sildenafil. This increase in the systemic exposure of sildenafil was not associated with any adverse effects. Additionally, neither of these citrus fruit juices altered the rate of absorption as C_{max} was reached at approximately 1 h in all groups. Therefore, we do not anticipate any delay in the onset of action from sildenafil after drinking lemon or Seville orange juices.

Previous animal studies were in agreement with our results. Bioavailability of amiodarone, a CYP3A4 substrate, was increased dramatically in rats pre-treated with Seville orange extract [13]. Both grapefruit and Seville orange revealed a similar significant increase in felodipine pharmacokinetics by increasing AUC of felodipine by 76 and 93 %, respectively, through inactivation of intestinal CYP3A4 [12]. Although very few human studies are available, they report conflicting conclusions regarding the effect of Seville orange on the pharmacokinetics of CYP3A4 substrates. Di Marco et al. revealed that the bioavailability of dextromethorphan was increased significantly with Seville orange juice and the effects lasted 3 days, suggesting an irreversible inhibition of gut CYP3A4 [14]. In another study in healthy subjects, the AUC of cyclosporine was increased only with grapefruit juice but not with Seville orange juice [15]. Unexpectedly, both the rate and the extent of absorption of colchicine, a CYP3A4 substrate, were significantly impaired by Seville orange juice in healthy volunteers as reflected by a 1-h delay in T_{max} with a 24 and 20 % decrease in C_{max} and AUC, respectively [16].

Few studies report the inhibitory effects of *C. lemon* on CYP3A4 enzymes. In 2001, Baltes et al. reported the first study for a potential interaction of lemon juice with hydroxytestosterone through inhibition of CYP3A4 activity. Lemon juice significantly inhibited 6-hydroxytestosterone production in an in-vitro model of human

Table 1 Pharmacokinetic parameters of sildenafil after a single oral dose (50 mg) with and without co-administration of Seville orange juice or lemon juice to nine healthy male subjects

Pharmacokinetic parameters	Water phase	Seville orange juice phase		Lemon juice phase	
	GM (CV %)	GM (CV %)	GMR (90 % CI)	GM (CV %)	GMR (90 % CI)
$AUC_{0-\infty}$ ($\mu\text{g h/L}$)	628 (12)	898 (14.9)	1.44 (1.30–1.60)	647 (8.6)	1.03 (0.92–1.15)
C_{max} ($\mu\text{g/L}$)	408 (10)	480 (14)	1.18 (1.08–1.29)	401 (10.5)	0.98 (0.89–1.08)
CL/F (L/h)	77.5 (12.8)	53.6 (14.8)	0.70 (0.63–0.75)	74.9 (8.6)	0.97 (0.92–1.03)
T_{max} (h) ^a	1.0	1 (1.0–1.5)		1 (0.75–1.0)	
$t_{1/2}$ (h) ^b	2.4 (9.5)	2.1 (14.6)		2.5 (17)	

$AUC_{0-\infty}$ area under the concentration-time curve from time zero to infinity, CI confidence interval, CL/F apparent oral clearance, C_{max} maximum plasma concentration, CV % coefficient of variation, GM geometric mean, GMR geometric mean ratio (citrus juice/water), T_{max} time required to achieve C_{max} , $t_{1/2}$ elimination half-life

^a Median (range)

^b Arithmetic mean (CV %)

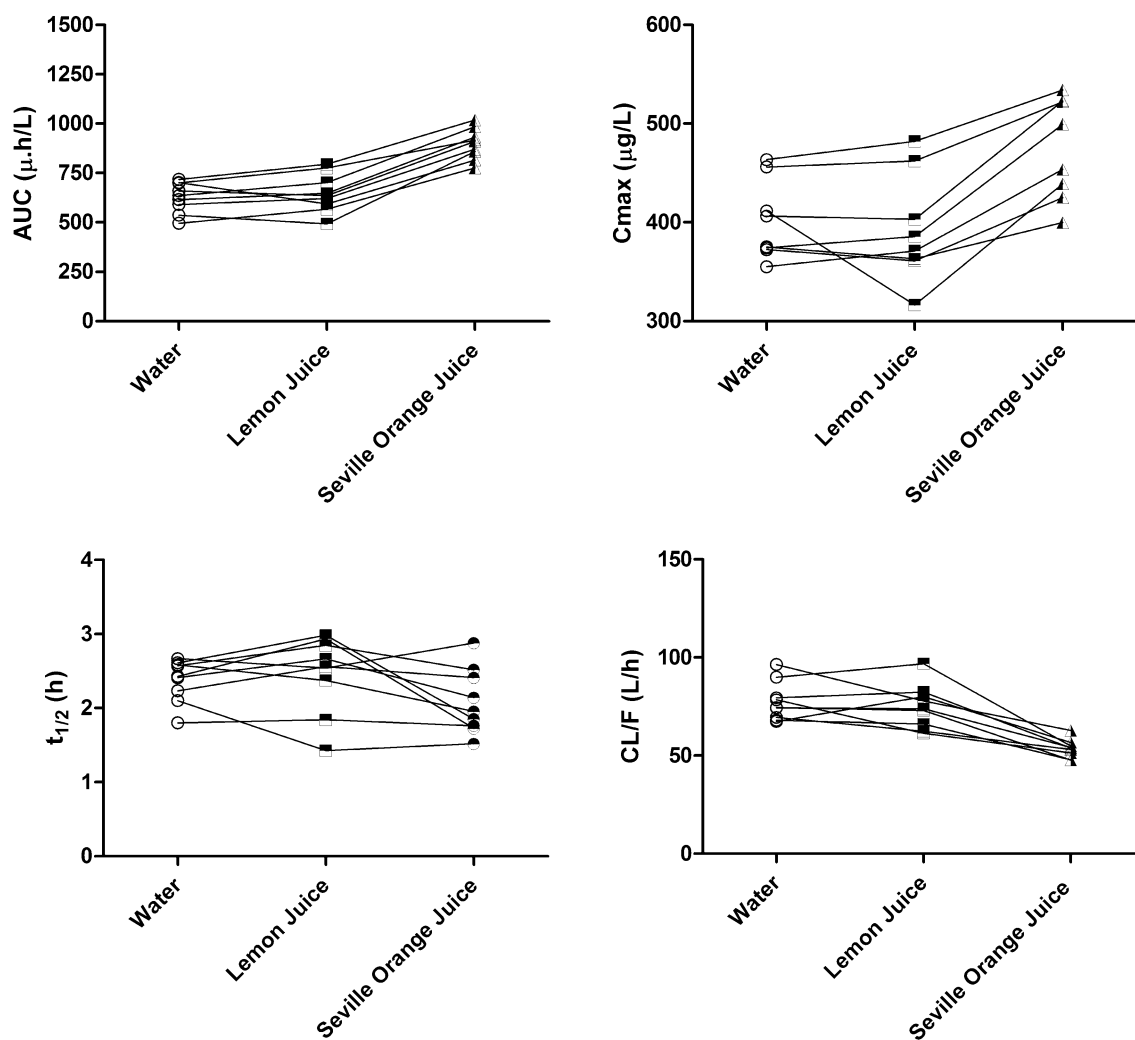


Fig. 4 Individual changes in the pharmacokinetic parameters AUC, C_{max} , $t_{1/2}$, and CL/F values during water, lemon juice, and Seville orange juice periods in nine healthy volunteers after oral

administration of a single 50-mg dose of sildenafil. AUC area under the concentration-time curve, CL/F apparent oral clearance, C_{max} maximum plasma concentration, $t_{1/2}$ elimination half-life

lymphoblastoid cell line (h3A4/OR)-expressing CYP3A4 activity [17]. In another in-vitro study, lemon juice inhibited CYP3A4-mediated hydroxylation of both testosterone and midazolam with IC_{50} values of 6.20 and 19.10 μ M, respectively [18]. Contrary to these published reports, when sildenafil was administered with lemon juice in the current study, no significant changes on the absorption or elimination pharmacokinetics of sildenafil were observed. To our knowledge, we report the first in-vivo or human study regarding the effects of lemon juice on the pharmacokinetics of sildenafil.

Like grapefruit, bergamottin and 6-,7-dihydroxybergamottin (the main furanocoumarins in Seville orange and lemon juice) are expected to be the primary compounds affecting CYP3A4 inhibition [12]. Other components such as naringin, the major flavonoid in Seville orange, also showed inhibitory effects in human liver microsomes in vitro. However, the amounts of these components in

administration of a single 50-mg dose of sildenafil. AUC area under the concentration-time curve, CL/F apparent oral clearance, C_{max} maximum plasma concentration, $t_{1/2}$ elimination half-life

grapefruit juice are too low to account for the inhibition of CYP3A4 by grapefruit [19].

It has been reported that naringin, the second major compound found in citrus fruits, is an inhibitor of p-glycoprotein (p-gp)-mediated efflux in the intestinal epithelium [20]. Both Seville orange and lemon contain similar concentrations of bergamottin to grapefruit [21]. However, and according to our results, pharmacokinetic interactions were only significant with Seville orange but not with lemon juice. These results suggest that bergamottin and 6-,7-dihydroxybergamottin are not the only compounds responsible for CYP3A4 inhibition and other compounds may also have a role in these pharmacokinetics alterations. In the case of Seville orange, the enhanced AUC and C_{max} could be attributed to inhibition of intestinal CYP3A4 and/or intestinal p-gp activity. A reduction in CL/F without any significant effect on $t_{1/2}$ may support the hypothesis that the enhanced increase in exposure to sildenafil by Seville

orange is attributed only to inhibition of the intestinal first-pass effect. The unexpected findings with lemon juice, although believed to have similar ingredients as grapefruit and Seville orange, may be owing to the difference in concentrations of these compounds, especially bergamottin, 6-,7-dihydroxybergamottin, and naringin.

Therefore, further investigations are required to study the effect of each compound on CYP3A4 and p-gp activities. A recent in-vitro study has shown that sildenafil is a substrate for both CYP3A4 and efflux transporters such as p-gp [22]. Therefore, it may be possible that both CYP3A4 and p-gp would contribute to the low oral bioavailability of sildenafil in a concerted manner, and the inhibitory effect of Seville orange juice on intestinal CYP3A4 and/or p-gp may explain the enhanced bioavailability of sildenafil in the current study.

Because sildenafil is commonly indicated in an on-demand basis, this study only examined the pharmacokinetics of a single oral dose of sildenafil in adult male volunteers. However, it should be noted that sildenafil is also indicated for pulmonary arterial hypertension and may be prescribed to male and female adults, children, and neonates. Owing to the potential sex- and age-related differences in CYP3A4 activity in humans, extrapolation of the results of our study to female individuals, children, and to chronic treatment conditions may require further investigation.

Although only healthy Egyptian subjects were included in this study, it has been reported that Egyptian and Middle East populations show similar incidences to Caucasians with regard to allelic frequencies of the tested variants of CYP3A4 and CYP3A5. Therefore, the effects of Seville orange and *C. lemon* juices are expected to be similar to that of European people [23].

5 Conclusions

We report that short-term consumption of Seville orange juice causes an increase in systemic exposure of sildenafil, without causing any adverse effects. In contrast, we report that short-term consumption of lemon juice did not appear to have any effect on sildenafil pharmacokinetics. Further investigation of the impact of each major constituent of the citrus fruits is necessary to understand their effects on CYP3A4 and drug transporters.

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Compliance with Ethical Standards

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Conflict of interest Drs. Abdelkawy, Donia, Turner, and Elbarbry declare no conflict of interest.

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References

- Hatzimouratidis K. Sildenafil in the treatment of erectile dysfunction: an overview of the clinical evidence. *Clin Interv Aging*. 2006;1(4):403–14.
- Hyland R, Roe EG, Jones BC, Smith DA. Identification of the cytochrome P450 enzymes involved in the *N*-demethylation of sildenafil. *Br J Clin Pharmacol*. 2001;51(3):239–48.
- Hanley MJ, Cancalon P, Widmer WW, Greenblatt DJ. The effect of grapefruit juice on drug disposition. *Expert Opin Drug Metab Toxicol*. 2011;7(3):267–86.
- Jetter A, Kinzig-Schippers M, Walchner-Bonjean M, et al. Effects of grapefruit juice on the pharmacokinetics of sildenafil. *Clin Pharmacol Ther*. 2002;71(1):21–9.
- Hedaya MA, El-Afify DR, El-Maghraby GM. The effect of ciprofloxacin and clarithromycin on sildenafil oral bioavailability in human volunteers. *Biopharm Drug Dispos*. 2006;27(2):103–10.
- Al-Ghazawi MA, Tutunji MS, Aburuz SM. The effects of pumelo juice on pharmacokinetics of sildenafil in healthy adult male Jordanian volunteers. *Eur J Clin Pharmacol*. 2010;66(2):159–63.
- Haaz S, Fontaine KR, Cutter G, et al. Citrus aurantium and synephrine alkaloids in the treatment of overweight and obesity: an update. *Obes Rev*. 2006;7(1):79–88.
- Stohs SJ, Preuss HG, Bagchi D. Bitter orange, ephedra, and hydroxycitric acid: issues with the article “Dietary supplements for improving body composition and reducing body weight: where is the evidence?”. *Int J Sport Nutr Exerc Metab*. 2012;22(6):407–10.
- Morton JF. Mucilaginous plants and their uses in medicine. *J Ethnopharmacol*. 1990;29(3):245–66.
- Seifert JG, Nelson A, Devonish J, et al. Effect of acute administration of an herbal preparation on blood pressure and heart rate in humans. *Int J Med Sci*. 2011;8(3):192–7.
- Shin HS, Bae SK, Lee MG. Pharmacokinetics of sildenafil after intravenous and oral administration in rats: hepatic and intestinal first-pass effects. *Int J Pharm*. 2006;320(1–2):64–70.
- Malhotra S, Bailey DG, Paine MF, et al. Seville orange juice-felodipine interaction: comparison with dilute grapefruit juice and involvement of furocoumarins. *Clin Pharmacol Ther*. 2001;69(1):14–23.
- Rodrigues M, Alves G, Falcao A. Investigating herb-drug interactions: the effect of Citrus aurantium fruit extract on the pharmacokinetics of amiodarone in rats. *Food Chem Toxicol*. 2013;60:153–9.
- Di Marco MP, Edwards DJ, Wainer IW, Ducharme MP. The effect of grapefruit juice and Seville orange juice on the

- pharmacokinetics of dextromethorphan: the role of gut CYP3A and P-glycoprotein. *Life Sci.* 2002;71(10):1149–60.
15. Edwards DJ, Fitzsimmons ME, Schuetz EG, et al. 6',7'-Dihydroxybergamottin in grapefruit juice and Seville orange juice: effects on cyclosporine disposition, enterocyte CYP3A4, and P-glycoprotein. *Clin Pharmacol Ther.* 1999;65(3):237–44.
 16. Wason S, Digiacinto JL, Davis MW. Effects of grapefruit and Seville orange juices on the pharmacokinetic properties of colchicine in healthy subjects. *Clin Ther.* 2012;34(10):2161–73.
 17. Baltes MR, Dubois JG, Hanocq M. Application to drug-food interactions of living cells as in vitro model expressing cytochrome P450 activity: enzyme inhibition by lemon juice. *Talanta.* 2001;54(5):983–7.
 18. Han YL, Yu HL, Li D, et al. Inhibitory effects of limonin on six human cytochrome P450 enzymes and P-glycoprotein in vitro. *Toxicol In Vitro.* 2011;25(8):1828–33.
 19. Fukuda K, Ohta T, Yamazoe Y. Grapefruit component interacting with rat and human P450 CYP3A: possible involvement of non-flavonoid components in drug interaction. *Biol Pharm Bull.* 1997;20(5):560–4.
 20. Bailey DG. Fruit juice inhibition of uptake transport: a new type of food-drug interaction. *Br J Clin Pharmacol.* 2010;70(5):645–55.
 21. Ishihara M, Toda H, Sunagane N, Ohta T. Furanocoumarins contents and cytochrome P450 3A (CYP3A) inhibitory activities of various processed fruit peel products: outflow of 6',7'-dihydroxybergamottin during processing treatment of peel. *Yakugaku Zasshi.* 2011;131(5):679–84.
 22. Choi MK, Song IS. Characterization of efflux transport of the PDE5 inhibitors, vardenafil and sildenafil. *J Pharm Pharmacol.* 2012;64(8):1074–83.
 23. Yousef AM, Bulatova NR, Newman W, et al. Allele and genotype frequencies of the polymorphic cytochrome P450 genes (CYP1A1, CYP3A4, CYP3A5, CYP2C9 and CYP2C19) in the Jordanian population. *Mol Biol Rep.* 2012;39(10):9423–33.