



Pharmacokinetic interaction between *Kaempferia parviflora* extract and sildenafil in rats

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Abstract *Kaempferia parviflora* (KP) is a plant widely used in Southeast Asia. Its major compounds are 3,5,7,3',4'-pentamethoxyflavone (PMF), 5,7,4'-trimethoxyflavone (TMF), and 5,7-dimethoxyflavone (DMF). This study investigated the effect of KP extract on the blood levels and pharmacokinetics of sildenafil co-administration in rats. Rats were randomly assigned to four groups. Groups 1, 2, and 3 were given sildenafil 20 mg/kg daily for 9 days. On days 4–9 of each treatment period, the treated rats received KP extract (250 mg/kg) and vehicle (groups 2 and 3, respectively). Group 4 received KP extract only (250 mg/kg daily for 9 days). Daily blood concentrations of sildenafil, PMF, TMF, and DMF were determined by HPLC to evaluate the daily blood level interactions. Additional blood samples were collected at various times on the last day of treatment to evaluate the pharmacokinetic interactions. The KP extract decreased blood levels of sildenafil on the first day of co-administration by 95 % but the percentage reduction was insignificant on subsequent days. When co-administered with KP extract, the area under the curve (AUC), maximum concentration (C_{\max}), and half-life ($T_{1/2}$) of sildenafil were decreased by 60–65, 40–52, and 32–54 %, respectively, with the elimination rate constant (K_e) increased by 37–77 %. In addition, PMF, TMF, and DMF

concentrations and their AUC, C_{\max} , T_{\max} , K_e , and $T_{1/2}$ values were changed after co-administration of KP extract and sildenafil.

Keywords *Kaempferia parviflora* · Herbal–drug interaction · Pharmacokinetics · Sildenafil

Introduction

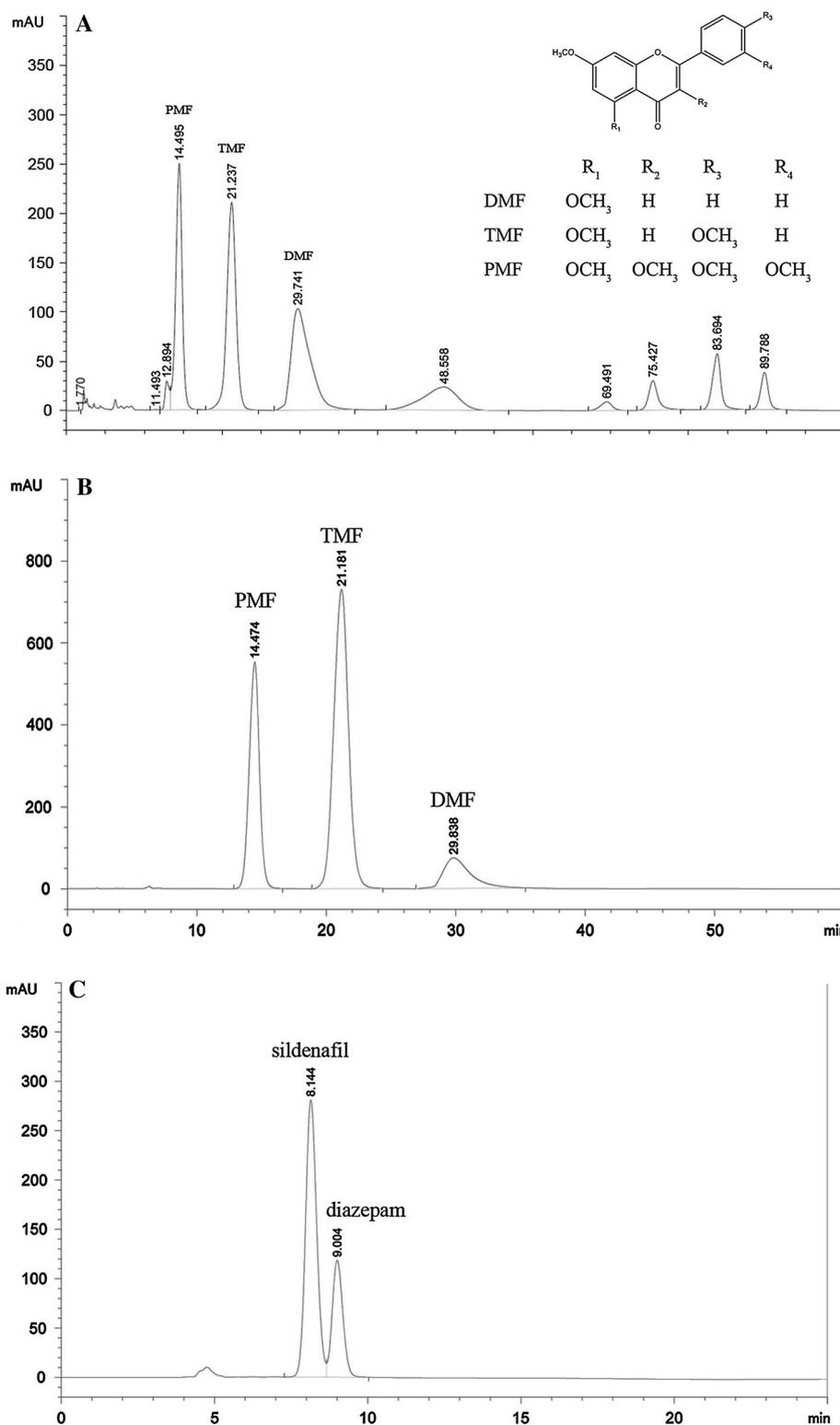
Sildenafil (Viagra[®]) is a cGMP phosphodiesterase type V inhibitor that has the potential to treat penile erectile dysfunction. Sildenafil is rapidly absorbed after oral administration, with bioavailability approximately 41 %. T_{\max} (time to maximum concentration) of the drug is about 0.5–1 h. Previous reports indicated that consumption of food would slow the rate of its absorption, delaying T_{\max} by 1 h and decreasing C_{\max} (maximum concentration) by 29 %. Sildenafil has a high level of plasma protein binding, at about 96 %. Its half-life ($T_{1/2}$) is about 4 h [1]. The drug undergoes hepatic first-pass and intestinal first-pass metabolism [2], resulting in low oral absorption. *N*-Demethylation is the major metabolic event of sildenafil biochemistry, and involves several cytochrome P450 (CYP) enzymes such as CYP3A4 (79 %), CYP2C19 (20 %), CYP2C9, and CYP2D6 (<2 %) [3, 4]. Warrington et al. [5] reported that CYP2C11 was a major enzyme involved in metabolizing sildenafil.

Kaempferia parviflora (KP), a plant in the family Zingiberaceae, is widely distributed in the northern and north-eastern regions of Thailand. The major plant secondary compounds of KP are 3,5,7,3',4'-pentamethoxyflavone (PMF, PubChem CID: 97332), 4',5,7-trimethoxyflavone (TMF, PubChem CID: 79730), and 5,7-dimethoxyflavone (DMF, PubChem CID: 88881), as shown in Fig. 1A [6].

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Fig. 1 HPLC chromatogram of KP extract (1 mg/ml) (A), a mixture of PMF (0.5 mg/ml), TMF (0.64 mg/ml), and DMF (0.34 mg/ml) (B), and sildenafil (0.06 mg/ml) and the internal standard diazepam (0.05 mg/ml) (C)



The rhizome of KP has been used for treatment of sexual dysfunction for centuries and has already been proven for this action [7–9]. In addition, KP extract and its major constituents are also reported to have anti-peptic ulcer [10], anti-inflammatory [11], anti-allergy [12], anti-mutagenicity

[13], anti-depression [14], antimicrobial [15], anti-cholinesterase [14, 16], anti-cancer [17, 18], cardioprotection [19, 20], and anti-obesity activities [21]. Accordingly, KP extract has increasingly been taken as an herbal medicine in Southeast Asia.

Pharmacokinetic studies of KP extract noted its low oral bioavailability, about 1–4 %. Methoxyflavones achieved the maximum concentration within 1–2 h after oral administration, and their $T_{1/2}$ is 3–6 h. The major compounds of KP extract were mainly excreted through urine in the form of demethylated, sulfated, and glucuronidated metabolites and as demethylated metabolites in the feces [22]. In addition, it was found that KP extracts induced several CYP450 enzyme activities; for instance, CYP1A1, CYP1A2, CYP2B, and CYP2E1 [23].

Currently, patients afflicted with male sexual dysfunction are increasingly seeking drugs or herbal dietary supplements for treatment. Sildenafil has been approved as a treatment for erectile dysfunction. As KP extract has traditionally been taken to treat male sexual dysfunction, a combination of sildenafil and KP extract may be used to help achieve the target effect. To the best of our knowledge, no data are available concerning the interaction between KP extract and sildenafil. Therefore, the aim of the present study was to investigate the impact of KP extract on the blood levels and pharmacokinetics of sildenafil (and vice versa), in order to evaluate the herbal–drug interaction of the concurrent consumption of sildenafil and KP extract.

Materials and methods

Chemicals

Sildenafil citrate was obtained from Raksit Drugs Pvt. Ltd., India. Diazepam was purchased from the Govt. Pharm. Org., Thailand. All chemicals for HPLC analysis were purchased as HPLC grade. Other chemical reagents were purchased as analytical grade.

KP extract preparation

Romkaou strain KP rhizomes were obtained from Loei Province, Thailand and the voucher specimen (No. KP-BS-2010) was deposited at the Center for Research and Development of Herbal Health Products, Khon Kaen University, Thailand. The ethanolic extract was prepared by maceration techniques following our previous study [23], providing a yield of 5.71 %. The standardized extract contained 23.32 mg of PMF/g of the extract, 31.06 mg of TMF/g of the extract, and 21.10 mg of DMF/g of the extract.

Preparation of dosing formulations

As KP extract has low aqueous solubility, a cosolvent should be used to prepare dosing formulations. The KP

extract was dissolved in a vehicle containing propylene glycol (28 %), polyethylene glycol 400 (35 %), ethanol (2 %), and water (35 %) to be the solution. Sildenafil was dissolved in water to make the sildenafil formulation for rats. These formulations were prepared once for the entire study and kept in the refrigerator (4 °C) throughout the study. Sildenafil and three methoxyflavones in the formulations were analyzed to control the stability in the formulation throughout the study by the method described below.

Animals

Male Wistar rats were purchased from the National Laboratory Animal Center, Mahidol University, Thailand. The rats (6–8 weeks old and 270–310 g body weight) were housed in standard rat cages on a 12-h light/dark cycle at an ambient temperature of 22 ± 2 °C and had free access to food and ad libitum water throughout the entire experiment. The experiments were done under the approval of the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (Approval Number AE.KKU. 41/2553).

Experimental design

Rats were randomly divided into four groups ($n = 7$): group 1 were orally administered sildenafil alone (20 mg/kg) for 9 consecutive days, groups 2 and 3 were orally given sildenafil (20 mg/kg) for 3 consecutive days and on days 4–9 co-administered KP extract (250 mg/kg) and vehicle of KP extract (propylene glycol, polyethylene glycol 400, ethanol, and water), respectively, by two separate gavages 5 min apart. Group 4 orally received KP extract alone (250 mg/kg) for 9 consecutive days. After treatment, blood samples were drawn as described below. At the end of the experiment, the rats were killed and their organs were examined for any abnormality.

Effect of single-dose KP extract on sildenafil concentration

On the fourth day of the experiment, on which KP extract was co-administered with sildenafil for the first time, blood samples from the tail vein were drawn at 17 min after sildenafil administration (T_{\max} of sildenafil) [2] and at 1 h after KP extract administration (T_{\max} of methoxyflavones of KP) into heparinized tubes [22].

Effect of multiple-dose KP extract on sildenafil concentration and pharmacokinetic interaction

On days 5–9 of the experiment, blood samples were drawn from the tail vein 17 min after sildenafil administration

(T_{\max} of sildenafil) [2] and 1 h after KP extract administration (T_{\max} of methoxyflavones of KP) into heparinized tubes in order to analyze the effect of multiple co-administration [22]. On the last day of treatment, blood samples were taken at 0, 10, 30, 60, 90, and 120 min for evaluation of pharmacokinetic interactions. Blood concentrations were plotted versus times, and C_{\max} and T_{\max} were obtained from the plots. The area under the blood concentration and time profile (AUC) was calculated by the linear trapezoid rule from the concentration–time profile [24]. The elimination rate constant (K_e) was estimated by log-linear regression using at least three data points judged to be in the terminal phase. The $T_{1/2}$ was calculated by dividing 0.693 by K_e .

Methoxyflavone assay

Methoxyflavone concentrations in blood samples and in KP extract were determined using HPLC. Whole blood samples were extracted by a liquid–liquid extraction technique using acetonitrile as a solvent. The collected supernatant was evaporated and the precipitate was reconstituted with methanol before analysis by HPLC. The KP extract solution and the standard mixture of methoxyflavones were separately prepared by dissolving in methanol and filtered via a 0.45- μm syringe filter before HPLC analysis. HPLC–UV was performed with an Agilent® 1200 series, VWD detector, quaternary pump, and autosampler (Germany). The methoxyflavones in KP were separated using an Agilent Hypersil ODS column (C18, 5 μm , 4.6 \times 250 mm) and maintained at 55 °C. The mobile phase was composed of methanol and 0.2 % orthophosphoric acid in water and carried on a gradient system starting from 60 % methanol at a flow rate of 1.2 ml/min at 0 min, 53 % methanol at a flow rate of 1 ml/min at 5 min, 40 % methanol at a flow rate of 0.7 ml/min at 40 min, and 56 % methanol at a flow rate of 1 ml/min at 80 min. The injection sample volume was 20 μl and the UV detection wavelength was set at 254 nm. Total run time was 100 min.

Sildenafil assay

Sildenafil concentration was quantified by a modification of a previously described method [25]. The whole blood was extracted using liquid–liquid extraction with methanol as a solvent to quantify sildenafil concentration. Diazepam (0.05 mg/ml) was used as an internal standard and was added to blood samples before extraction. The sample was injected into an HPLC–UV (Agilent® 1200 series VWD detector with a quaternary pump, Germany) using an Eclipse XDB-CN C18 column (5 μm , 4.6 \times 150 mm) maintained at 20 °C. Methanol with 10 mM ammonium acetate pH 7.0 was the mobile phase. Total run time was 20 min on a gradient

system beginning with 85 % methanol at a flow rate of 0.3 ml/min and gradually changing to 10 % methanol at 8 min of run time at a flow rate of 0.2 ml/min.

Statistical analysis

The results were presented as mean \pm SD. Statistical comparisons of the results were performed using independent *t* tests and analysis of variance (ANOVA) followed by post-hoc tests (least squares mean) for two-group and three-group comparisons, respectively. Analyses were done using SPSS version 17.0. $P < 0.05$ was considered statistically significant.

Results

Analytical method validation

The analytical methods for determination of sildenafil and methoxyflavones in rat blood samples were successful; the percentage yields of the extractions for sildenafil and methoxyflavones were 97.28 ± 0.89 and 91.63 ± 0.23 %, respectively. The calibration curve for the three methoxyflavones was sufficiently linear ($R^2 > 0.993$) over a range of 0.25 to 640 $\mu\text{g/ml}$. The limit of detection (LOD) and limit of quantitation (LOQ) of methoxyflavones ranged from 0.02 to 0.04 and 0.05 to 0.07 $\mu\text{g/ml}$, respectively. The HPLC chromatograms of KP extract and the three methoxyflavones, PMF, TMF, and DMF, are shown in Fig. 1A and B, respectively.

The standard curve developed to quantify the sildenafil concentration in rat blood was linear ($R^2 = 0.9999$) over a range of concentrations 3.88–62.00 $\mu\text{g/ml}$. Intra- and inter-day assay precision (%RSD) of sildenafil was in the range 0.05–0.94 and 3.23–5.72 %, respectively, over the concentration range of 15.50–62.00 $\mu\text{g/ml}$. The accuracy was higher than 94 %. LOQ was 2.18 $\mu\text{g/ml}$. LOD was 0.73 $\mu\text{g/ml}$. Figure 1C shows the HPLC chromatograms of sildenafil (0.06 mg/ml) and diazepam (0.05 mg/ml), the internal standard, at the retention times of 8.1 and 9.0 min, respectively.

Effect of KP extract on blood levels of sildenafil

Sildenafil concentrations in rat blood samples are listed in Table 1. On day 4, the blood sildenafil concentration of rats receiving sildenafil plus KP extract was significantly lower than those of the control groups receiving sildenafil plus vehicle or sildenafil alone ($P < 0.01$). After this point (days 5–9), the blood sildenafil concentrations of rats in the co-administration group were not significantly lower than those of the two control groups ($P > 0.05$).

Table 1 Blood sildenafil concentration in rats after oral administration of sildenafil with and without KP extract for 9 days

Day of treatment	Sildenafil citrate concentration ($\mu\text{g/ml}$)		
	Sildenafil plus KP extract	Sildenafil plus vehicle	Sildenafil
Day 4	8.68 \pm 1.04 ^a	189.52 \pm 21.20	170.61 \pm 66.37
Day 5	6.49 \pm 1.36	6.46 \pm 0.69	4.89 \pm 0.57
Day 6	21.18 \pm 2.39	18.08 \pm 4.56	18.32 \pm 0.71
Day 7	20.06 \pm 3.91	20.90 \pm 2.71	20.43 \pm 6.31
Day 8	21.48 \pm 1.71	15.73 \pm 3.92	15.98 \pm 1.52
Day 9	16.88 \pm 3.80	16.99 \pm 4.49	24.48 \pm 1.70

Values are expressed as mean \pm SD ($n = 7$)

KP *Kaempferia parviflora*

^a Significant difference from other groups at $P < 0.01$

Effect of sildenafil on blood levels of methoxyflavones

The blood concentration of PMF in the co-treated group (sildenafil plus KP extract) was significantly higher than those in the KP extract alone group during days 4–9 ($P < 0.01$) (Fig. 2). The mean TMF concentration in the co-administration group was significantly higher than that of the KP extract alone group only on day 5 of treatment, whereas its concentration at day 8 was lower than that of the KP extract alone group ($P < 0.05$). DMF concentrations in the co-administration of sildenafil plus KP extract group on days 4–5 of the treatment were markedly higher than those obtained in the KP extract alone group ($P < 0.05$); subsequently, the differences were not significant.

Pharmacokinetic interaction between KP extract and sildenafil

The pharmacokinetic parameters for sildenafil are shown in Table 2. Sildenafil was rapidly absorbed into the bloodstream after the three administrative applications (sildenafil plus KP extract, sildenafil plus vehicle, and sildenafil alone), and reached its maximum concentration after approximately 30–42 min. When compared to the control groups (sildenafil plus vehicle and sildenafil alone) the co-administration of sildenafil plus KP extract significantly decreased C_{max} (0.29 \pm 0.07 vs. 0.50 \pm 0.14 and 0.61 \pm 0.13 mg/ml, respectively; $P < 0.05$) and AUC (14.05 \pm 7.13 vs. 40.76 \pm 3.68 and 37.09 \pm 0.60 mg min/ml, respectively; $P < 0.01$). K_e of sildenafil was significantly increased when compared to the control groups (sildenafil plus vehicle and sildenafil alone) (0.04 \pm 0.02 vs. 0.01 \pm 0.00 and 0.03 \pm 0.00 per min, respectively; $P < 0.05$). Meanwhile, T_{max} of sildenafil was similar both with or without the administration of KP extract.

The pharmacokinetic parameters of these major methoxyflavones after co-administration of KP extract plus sildenafil are listed in Table 3. C_{max} values of the three methoxyflavones (0.53–2.03 $\mu\text{g/ml}$) were obtained 70–75 min after administration of KP extract plus sildenafil, with a K_e of 0.01–0.02 per min and a blood half-life of 34.70–76.07 min. The AUC values of the methoxyflavones ranged from 43.11 to 93.36 $\mu\text{g min/ml}$.

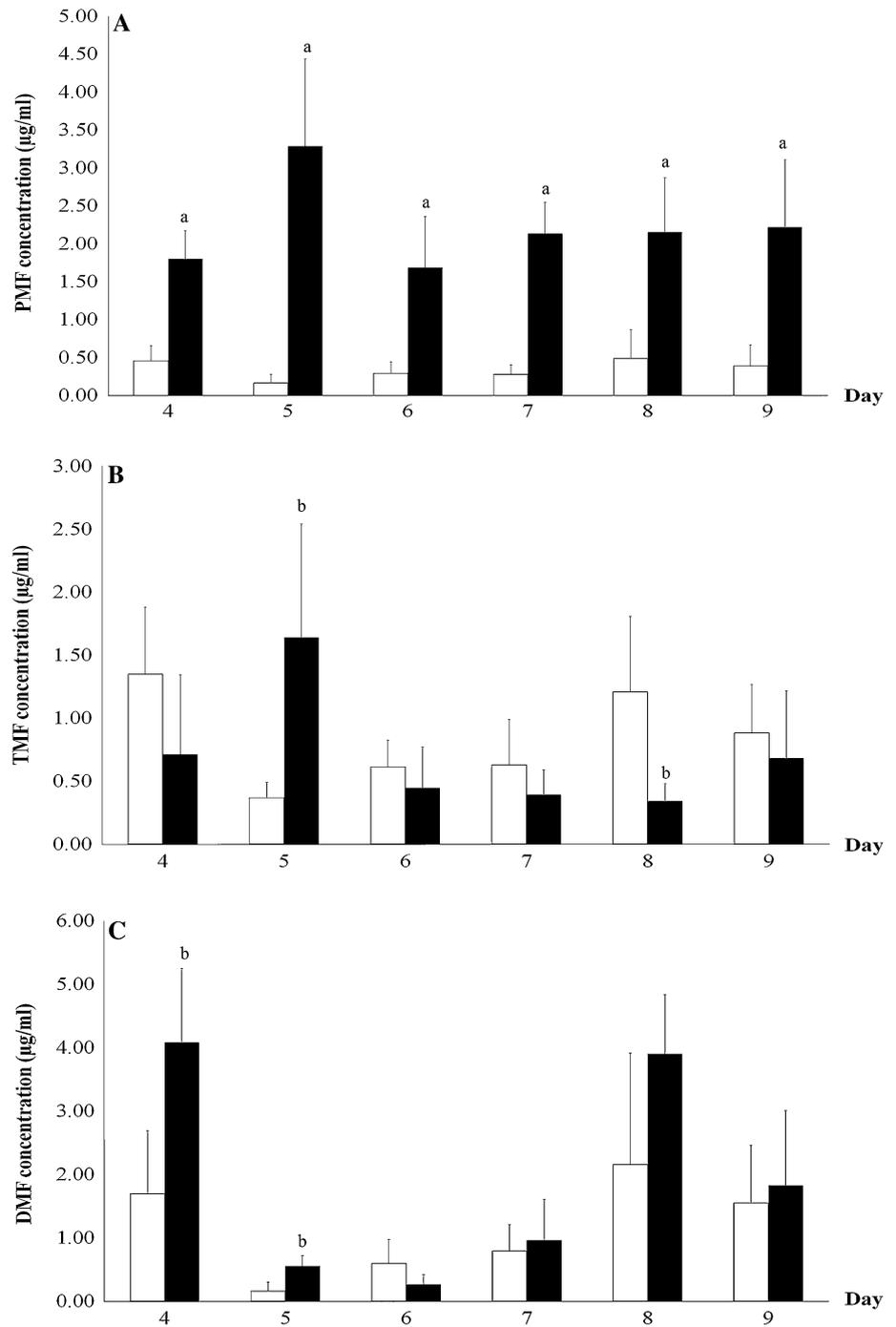
Discussion

KP, the plant, and sildenafil, a synthetic medicine, are taken for a similar purpose, male sexual dysfunction. To achieve the optimum therapeutic effect, a combination of Western and traditional medicines may be used for maximum efficiency. In such an approach, data on herbal–drug interactions are important. This study thus evaluated the effect of KP extract on blood levels and pharmacokinetic parameters of sildenafil in rats.

A previous report showed that KP at 250 mg/kg had neuropharmacological activities [14], and three methoxyflavones (DMF, TMF, and PMF) in KP at this dose were quantitated by the HPLC method. In the case of sildenafil, the effective dose for treatment of erectile dysfunction in rats was 20 mg/kg [26]. Therefore, 250 mg/kg of KP extract and 20 mg/kg of sildenafil were selected for this study. After the treatment, no treated animals showed signs of toxicity from the treatments. There were no abnormalities in the visual appearance of the rats' internal organs.

In the current study, sildenafil levels were markedly decreased (by approximately 95 %) on the first day of concurrent administration with KP extract, after which the level returned to normal compared to the control groups (sildenafil plus vehicle and sildenafil alone). This observation can be explained by KP extract interfering with absorption or first-pass metabolism of sildenafil in liver or intestine, or a combination of these effects. Compounds in KP extract may competitively bind with or affect transporters of sildenafil such as ATP-binding cassette transporters (ABCs) or organic anion transporting polypeptides (OATPs); this needs further study. When sildenafil was repeatedly co-administered with KP extract, it could apparently overcome this effect on the binding of transporters. This resulted in there being no significant differences in sildenafil concentration between the co-administration group (sildenafil plus KP extract) and control groups in repeated doses. A similar result has been previously reported, e.g., pommel juice decreased sildenafil levels when co-administered with sildenafil [27]. The phenomenon was explained by the competition for transporters of the juice and sildenafil. Nevertheless, the high levels of sildenafil concentration on day 4 in the sildenafil plus vehicle and sildenafil alone groups, which were

Fig. 2 Rat blood concentrations of PMF (A), TMF (B), and DMF (C) after oral administration of sildenafil plus KP extract (*black bars*) and KP extract alone (*white bars*) on days 4–9 of the treatments. Data are expressed as mean \pm SD ($n = 7$). *KP Kaempferia parviflora*, *a* and *b* significant differences from other groups at $P < 0.001$ and $P < 0.01$, respectively



different from those on other days in the same groups, were still dubious. It may be that the repeated administration of sildenafil gives the highest level on day 4 and then decreases; however, this needs further study.

In the case of methoxyflavone concentrations after co-administration of sildenafil and KP extract, sildenafil markedly increased methoxyflavone concentrations in the blood in the early stage of the co-administration (1–2 days). This observation may be explained by competition for

transporters or channels of drug absorption or by competition between methoxyflavones and sildenafil in the first-pass metabolism in the liver or intestine. Sildenafil was reported to inhibit efflux transporters, including ABCB1 and ABCG2 [28]. This implies that the methoxyflavones might involve the same efflux transporters or channels for their absorption or first-pass metabolism. In accordance with this, previous studies have indicated that the major components in KP inhibit P-glycoprotein [29] and multidrug resistance-

Table 2 Pharmacokinetic parameters of sildenafil following treatment with sildenafil alone or with KP extract or with vehicle control in rats

Pharmacokinetic parameters	Sildenafil plus KP extract	Sildenafil plus vehicle	Sildenafil
AUC (mg min/ml)	14.05 ± 7.13 ^a	40.76 ± 3.68	37.09 ± 0.60
C_{\max} (mg/ml)	0.29 ± 0.07 ^b	0.50 ± 0.14	0.61 ± 0.13
T_{\max} (min)	42.00 ± 16.43	30.00 ± 0.00	30.00 ± 0.00
K_e (min ⁻¹)	0.04 ± 0.02 ^b	0.01 ± 0.00	0.03 ± 0.00
$T_{1/2}$ (min)	19.22 ± 6.49 ^b	41.55 ± 37.76	28.44 ± 3.92

Data are expressed as mean ± SD ($n = 7$)

KP *Kaempferia parviflora*, AUC area under the curve, C_{\max} maximum concentration, T_{\max} time to maximum concentration, K_e elimination rate constant, $T_{1/2}$ half-life

^{a,b} Significant differences from other groups at $P < 0.01$ and $P < 0.05$, respectively

Table 3 Pharmacokinetic parameters of three methoxyflavones (PMF, TMF, and DMF) following administration of sildenafil together with KP extract in rats

Pharmacokinetic parameters	PMF	TMF	DMF
AUC (μg min/ml)	75.73 ± 31.07	43.11 ± 12.30	93.36 ± 13.81
C_{\max} (μg/ml)	0.53 ± 0.40	0.70 ± 0.26	2.03 ± 0.03
T_{\max} (min)	70.00 ± 17.32	75.00 ± 21.21	75.00 ± 21.21
K_e (min ⁻¹)	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
$T_{1/2}$ (min)	34.70 ± 9.46	75.68 ± 27.18	76.07 ± 23.09

Data are expressed as mean ± SD ($n = 7$)

KP *Kaempferia parviflora*, AUC area under the curve, C_{\max} maximum concentration, T_{\max} time to maximum concentration, K_e elimination rate constant, $T_{1/2}$ half-life

associated protein [30], both of which are drug transporters. However, the effect of the methoxyflavones on protein transporters or channels should be determined by further study.

With regard to pharmacokinetic interactions, KP extract significantly decreased AUC, which indicates the extent of drug absorption, and C_{\max} , which indicates the intensity of therapeutic response, of sildenafil by approximately 60–65 and 40–52 %, respectively. K_e of sildenafil was also enhanced by 37–77 % when co-administered with KP extract. These results imply that the compounds in KP extract may affect sildenafil metabolism, especially CYP450-metabolizing enzymes. A previous report indicated the induction of CYP450 enzymes by KP extract [23]. In addition, the reduction of AUC and/or C_{\max} was also reported in interaction studies of sildenafil and bosentan [31] and sildenafil and *Epimedium sagittatum* [32]. Both bosentan and *E. sagittatum* may induce CYP450-

metabolizing enzymes and elimination of sildenafil, resulting in the reduction of AUC and C_{\max} of sildenafil. In addition, recent studies have found that KP extract did not affect the T_{\max} value of sildenafil. Similarly, T_{\max} of acetaminophen was also unchanged when co-administered with KP extract [33]. These phenomena imply that the components in KP extract have no effect on the peristalsis of the gastrointestinal tract or gastric emptying time.

The present study investigated the pharmacokinetic parameters of three methoxyflavones (DMF, TMF, and PMF) in KP extract when co-administered with sildenafil. When the values obtained are compared to the pharmacokinetic parameters of KP extract administration alone from our previous study [22], sildenafil decreased the AUC of PMF by 50 % (75.7 vs. 150.5 μg min/ml), TMF by 90 % (43.1 vs. 415.2 μg min/ml), and DMF by 73 % (93.4 vs. 351.5 μg min/ml). Moreover, sildenafil also reduced C_{\max} of PMF by 70 % (0.53 vs. 1.76 μg/ml) and TMF by 49 % (0.70 vs. 1.38 μg/ml). In addition, sildenafil enhanced K_e values of PMF, TMF, and DMF by 90 % (0.021 vs. 0.002 per min), 80 % (0.010 vs. 0.002 per min), and 80 % (0.010 vs. 0.002 per min), respectively. These observations indicate that sildenafil probably affects metabolism and/or elimination of methoxyflavones. Sildenafil is metabolized via several CYP450 enzymes, especially CYP3A4 2C9, 2C19, and 2C11 [3, 5], and KP extract also involved CYP1A1, 1A2, 2B, and 2E1 [23]. Mekjaruskul et al. [22] reported the elimination of methoxyflavones in KP extract via demethylation and conjugation via glucuronide and sulfate conjugations. Therefore, the reduction in AUC and C_{\max} and the increase in K_e of the methoxyflavones in KP extract caused by sildenafil may imply that, beside the reported enzymes, the metabolism of the methoxyflavones may involve other CYP450 enzymes such as CYP2C9, 2C19, and 2C11.

Conclusion

There was a significant interaction between KP extracts and sildenafil, resulting in the reduction in the AUC and C_{\max} values of sildenafil by 60–65 and 40–52 %, respectively. The herbal–drug interaction of KP extracts and sildenafil should be taken into consideration in clinical practice in order to prevent possible therapeutic failure of the treatment of sexual dysfunction.

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Conflict of interest The authors declare that there are no conflicts of interest.

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