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Modulatory effects of *Kaempferia parviflora* extract on mouse hepatic cytochrome P450 enzymes

Catheleeya Mekjaruskul[a,c], Michael Jay[b], Bungorn Sripanidkulchai[a,c,*]

[a] *Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, NC 27599, USA*
[c] *Center for Research and Development of Herbal Health Products, Khon Kaen University, Khon Kaen 40002, Thailand*

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ABSTRACT

Ethnopharmacological relevance: Kaempferia parviflora is a herbal plant, the extracts of which are commonly used as alternative medicines. It widely uses as aphrodisiac, anti-inflammation, anti-microbacterial, and anti-peptic ulcer.
Aim of the study: In order to obtain an effective utilization and safety of the herb, the influence of *Kaempferia parviflora* on hepatic CYP450 metabolizing enzymes including CYP1A1, CYP1A2, CYP2B, CYP2E1, and CYP3A was investigated.
Materials and methods: The impact of *Kaempferia parviflora* on CYP450 both *in vitro* and *in vivo* was examined by using ethoxyresorufin *O*-dealkylation, methoxyresorufin *O*-dealkylation, pentoxyresorufin *O*-dealkylation, *p*-nitrophenol hydroxylation, and erythromycin *N*-demethylation assays, respectively.
Results: In vitro studies using non-induced mouse hepatic microsomes in the presence or absence of *Kaempferia parviflora* extract showed that *Kaempferia parviflora* extract altered CYP1A1, CYP1A2, CYP2B, and CYP2E1 activities by non-competitive, mixed-competitive, competitive, and uncompetitive mechanisms, respectively. Among these enzymes, CYP1A2 was affected by *Kaempferia parviflora* based on the highest value of V_{max} (15.276 ± 0.206 nmol/min) and lowest of K_i value (0.008 ± 0.002 μg/ml). In addition, the plant extract also modulated CYP2B activity based on the low K_m value (1.599 ± 0.147 pmol). For *in vivo* studies, mice were orally treated with 250 mg/kg of *Kaempferia parviflora* extract for 7, 14, and 21 days. The results demonstrated that *Kaempferia parviflora* extract significantly induced CYP1A1, CYP1A2 enzyme activities following short-term treatment. CYP2B enzyme activities were markedly increased all *Kaempferia parviflora* extract treatment timepoints, whereas *Kaempferia parviflora* extract significantly enhanced CYP2E1 activity only after long-term treatment. However, *Kaempferia parviflora* extract did not affect the CYP3A enzyme activity.
Conclusions: Kaempferia parviflora extract modulated several CYP450 enzyme activities, thus, its utilization with drugs or other herbs should raise concern for potential drug–herb interactions.

1. Introduction

Kaempferia parviflora, a herbal plant belonging to the family Zingiberaceae, is found primarily in the North and Northeast of Thailand. Its rhizome has been widely used as a traditional medicine for centuries and is claimed to have many pharmacological activities. These include use as anti-peptic ulcer (Rujjanawate et al., 2005), anti-inflammation (Tuchida et al., 2002), anti-allergy (Tewtrakul et al., 2008), anti-mutagenic (Sripanidkulchai et al., 2004; Azuma et al., 2011), antibacterial (Kummee et al., 2008), anti-fungal, anti-mycobacterium, anti-plasmodium (Yenjai et al., 2004), anti-viral (Phurimask and Leardkamolkarn, 2005), and anti-depression agents (Wattanathorn et al., 2007). Other reported activities include anti-cholinesterase activity for Alzheimer's disease (Sawasdee et al., 2009), cardioprotective effects (Malakul et al., 2011), aphrodisiac effects (Sudwan et al., 2006; Wattanapitayakul et al., 2007; Chaturapanich et al., 2008) and anti-tumor activity (Banjerdpongchai et al., 2008, 2009; Leardkamolkarn et al., 2009). The major components of *Kaempferia parviflora* that demonstrated pharmacological effects are methoxyflavones, in particular 5,7-dimethoxyflavone (DMF), 5,7,4′-trimethoxyflavone (TMF), and 3,5,7,3′,4′-pentamethoxyflavone (PMF) (Sutthanut et al., 2007). DMF is an effective chemoprotectant in chemical-induced liver cancer (Wen et al., 2005) while DMF and PMF exhibited inhibition of multidrug resistance associated-protein (Patanasethanont et al.,

* Corresponding author at: Center for Research and Development of Herbal Health Products, Khon Kaen University, Khon Kaen 40002, Thailand. Tel.: +66 43202378; fax: +66 43202379.
E-mail addresses: catheleeya1@hotmail.com (C. Mekjaruskul), mjay@email.unc.edu (M. Jay), bungorn@kku.ac.th (B. Sripanidkulchai).

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2007a). TMF was shown to possess anti-cholangiocarcinoma action (Leardkamolkarn et al., 2009), and PMF was also demonstrated to inhibit P-glycoprotein function (Patanasethanont et al., 2007b). Due to these broad pharmacological activities, *Kaempferia parviflora* has been increasingly used as an alternative medicine. Because many patients ingest a wide range of herbal health products in combination with conventional drugs, the potential number of incidences of herbal–drug or herbal–herbal interactions is increased.

Herbal products may alter the metabolism of certain drugs and, likewise, the metabolism of components of herbal products may be altered by some drugs, specifically those that affect cytochrome P450 enzymes (CYP). These can bring about an alteration of the pharmacokinetics and pharmacological activity of drug or herbal product components leading to adverse reactions following their co-administration (Gonzalez, 1990; Ogu and Maxa, 2000). CYP is a superfamily of isozymes in which CYP1, CYP2, and CYP3 play major roles in the metabolism of many drugs (Randic and DiCarlo, 1997). The induction or inhibition of some of these isoforms can lead to clinically significant manifestations (Cupp and Tracy, 1998). While drug candidates are routinely evaluated for their effect on drug-metabolizing enzymes during the development process, this is not the case for many herbal medicines. Recently, some herbal medicines were administered for the purpose of measuring the inhibition or induction of hepatic CYP enzymes. For example, andrographalide from *Andrographis paniculata* was shown to alter CYP1A1 activity and mRNA expression in mice hepatic microsomes (Jarukamjorn et al., 2006, 2010; Chatuphonprasert et al., 2009). *Pueraria candollei* induced CYP2B9 activity and mRNA expression in mouse liver (Udomsuk et al., 2010), while *Curcuma comosa* extracts increased CYP1A1 and CYP2B1/2B2 activities in rat liver (Kittichanun et al., 2010). In addition, Yahom Ampanthong (combination of traditional medicines) inhibited CYP1A1, CYP1A2, and CYP2E1 activities in mice (Sirisangtrakul and Sripanidkulchai, 2011). Nevertheless, there are no reports regarding the hepatic metabolism of *Kaempferia parviflora* extract, neither the contribution of CYP450 enzymes to its metabolism nor the effect of the extract on various liver CYP450 isoforms. Therefore, the aim of this study was to investigate the effects of *Kaempferia parviflora* extract on the *in vitro* and *in vivo* activity of pivotal isoforms of mouse hepatic CYP enzymes involved in drug metabolism including CYP1A1, CYP1A2, CYP2B, CYP2E1, and CYP3A. This is expected to raise awareness of possible metabolic interactions with concomitant administered traditional and herbal medicines.

2. Materials and methods

2.1. Chemicals

4-Nitrocatechol, 3-methylchrolanthrene, β-naphthoflavone, dexamethasone, erythromycin, resorufin ethyl ether, resorufin methyl ether, resorufin pentyl ether, resorufin, and NADPH were purchased from Sigma–Aldrich (St. Louis, MO, USA). *p*-Nitrophenol, folin reagent, and phenobarbital were purchased from Carlo Erba Reagenti Spa (Milano, Italy), Merck (Munchen, Germany), and Sanofi Aventis (Paris, France), respectively. Formic acid and acetonitrile (HPLC-grade solvents) were purchased from Fisher® Scientific (Loughborough, England) and LabScan® Asia Co., Ltd. (Bangkok, Thailand), respectively. All other chemicals were analytical grade.

2.2. Preparation of Kaempferia parviflora ethanolic extract and HPLC analysis of flavonoids

Kaempferia parviflora rhizomes were collected from cultivable sources in the Loei province of Thailand during the 2007 harvest. The rhizomes were sliced, dried at 45 °C, and ground. The dried powders were extracted by maceration with 95% ethanol. After evaporation of ethanol, the residual solvent was removed by lyophilization to yield the solid extract. Major methoxyflavones were isolated using column chromatography following the method of Sutthanut et al. (2007). The contents of the major methoxyflavone components were quantified by HPLC (Agilent® 1200 series, Germany) with VWD detector at 335 nm and quaternary pump. The analytical column was an Agilent® Hypersil BDS-C8 column (4.0 mm × 250 mm, 5 μm) controlled at 40 °C. The mobile phase was acetonitrile and 0.5% formic acid in water using the following gradient: 0–10 min: isocratic at 29% acetonitrile/71% of 0.5% formic acid in water at a flow rate 1.0 ml/min; 10–20 min: gradient to 54% acetonitrile/46% of 0.5% formic acid in water at a flow rate 0.8 ml/min; 20–40 min: isocratic at 54% acetonitrile/46% of 0.5% formic acid in water at a flow rate 0.6 ml/min; 40–50 min: gradient to 29% acetonitrile/71% of 0.5% formic acid in water at a flow rate 0.8 ml/min; and 50–60 min: isocratic at 29% acetonitrile/71% of 0.5% formic acid in water at a flow rate 1.0 ml/min. The injection volume was 20 μl.

2.3. Experimental animals

Male mice at 6–8 weeks of age and 25–30 g of body weight were purchased from the National Laboratory Animal Center, Mahidol University, Saraya, NP, Thailand. Animals were housed at ambient temperature of 22 ± 2 °C with 12 h light/dark cycles and free access of food (C.P. Mice feed 082, S. WT Co Lid, Thailand) and *ad libitum* water. All animals were acclimated for seven days prior to the experiment. The protocols of animal housing and treatments used in this study were approved by the Khon Kaen University Ethics Committee for animal research (Approval no. AE.KKU.31/2007).

Mice were divided into 12 groups of 10 animals each. Groups 1–5 were positive controls. Group 1 orally received 100 mg/kg of 3-methylchrolanthrene in olive oil in order to induce CYP1A1 activity for 3 days (Jarukamjorn et al., 2006); group 2 was intraperitoneally (ip) administered with 80 mg/kg of β-naphthoflavone in distilled water for 7 days to induce CYP1A2 (Bray et al., 2002); group 3 received 100 mg/kg of phenobarbital in distilled water ip for 7 days to induce CYP2B (Jarukamjorn et al., 2006); group 4 was administered 2.5 g/kg of 35% ethanol in distilled water ip for 7 days to induce CYP2E1 (Wang and Cederbaum, 2007); and group 5 received 75 mg/kg of dexamethasone in distilled water ip for 7 days to induce CYP3A (Bray et al., 2002). Groups 6–8 were orally administered 250 mg/kg of *Kaempferia parviflora* extracts in 2% carboxymethyl cellulose for 7, 14, and 21 days, respectively. Groups 9–11 were vehicle controls that were orally administered with 2% carboxymethyl cellulose for 7, 14, and 21 days, respectively. Group 12 was negative control group (untreated). The mice were cervically decapitated 24 h after the last treatment and hepatic microsomes were harvested.

2.4. Preparation of hepatic microsome

Hepatic microsomes were prepared following the method of Dudda and Kulzel (2006). Briefly, a liver sample was homogenized at 2000 cpm and centrifuged at 10,000 × *g* at 4 °C for 10 min. The supernatant was further centrifuged at 104,000 × *g* at 4 °C for 60 min. The microsomal fraction was obtained by suspending the pellet in Tris-alkylresorufin buffer (pH 7.8) for the alkylresorufin *O*-dealkylation assay, phosphate buffer pH 6.8 for the *p*-nitrophenol hydroxylation assay, or phosphate buffer pH 7.4 for the erythromycin *N*-demethylation assay. In order to obtain the appropriate concentration of microsomal protein, the microsome was further diluted by using cool distilled water. Protein concentration in the microsome fraction was determined as previously