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MODURATORY EFFECT OF THAI TRADITIONAL MEDICINE (YAHOM TULTAVAI) ON HEPATIC CYTOCHROME P450 ENZYMES AND PENTOBARBITAL-INDUCED SLEEPING IN MICE

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Abstract

Yahom Tultavai is a Thai traditional medicine that has been widely used for the treatment of nausea, vomiting, dizziness and weakness in aged-people, especially. Its formula contains several medicinal plants, and one of them is *Kaempferia galanga* L., which has ethyl-p-methoxycinnamate (EPMC) as its major compound. Recently, several herbs and traditional medicines have been reported to demonstrate herbal-drug interaction with conventional medicines. This study aims to investigate the effect of Yahom Tultavai extracts on hepatic cytochrome P450 enzymes and pentobarbital-induced sleeping in mice. Three extracts of Yahom Tultavai, using dichloromethane, methanol and distilled water as solvents were orally administered for 28 days prior to determine CYP1A1, CYP1A2, CYP2B, CYP2E1 and CYP3A4 activities. All three extracts significantly inhibited CYP1A1, CYP1A2 and CYP 2E1 activities, but only dichloromethane extract enhanced CYP2B activity. In addition, all three extracts had no effect on CYP3A4 activity. As an indicator for metabolic drug interaction, pentobarbital-induced sleeping time was decreased in connection with the induction of CYP2B activity between 7 and 28 days of dichloromethane extract and EPMC-treated animals when compared to control. In conclusion, Yahom Tultavai extracts affected hepatic microsomal CYP enzyme activities and reduced pentobarbital-induced sleeping time in mice. The results suggest that Yahom Tultavai may potentially cause herbal and conventional drug interaction, which can affect the clinical implication of drug action. Therefore, the co-administration of Yahom Tultavai with certain drugs should be carefully considered.

Key words: Yahom Tultavai, Thai traditional medicine, cytochrome P450, pentobarbital-induced sleeping, herbal-drug interaction

Introduction

Yahom is a common name that refers to Thai traditional medicine which usually contains many medicinal plants. There are many formulas of Yahom, some of which have been found to be safe and have no mutagenicity (Chavalittumrong et Al., 2009; Sripanidkulchai et al., 2007). Yahom has been widely used among the aged-population for the treatment of nausea, vomiting, dizziness, weakness and fainting symptom. Yahom Tultavai contains more than 10 powdered medicinal ingredients including *Kaempferia galanga* L. (Proh Hom in Thai). This plant belongs to the Zingiberaceae family, and is widely found in South India and Southeast Asia. It is commonly used as a flavouring plant in Yahom, and its major constituent is ethyl-*p*-methoxycinnamate (EPMC) (Teawtrakul et al., 2005). Ethno-pharmacologically, this herb has several pharmacological activities including anti-bacterial and antifungal (Teawtrakul et al., 2005; Parvez et al, 2005), anti-gastric ulcer (Wanajak, 1999), vasorelaxant and anti-inflammatory (Mustafa et al., 1996; Othman et al., 2006), antinociceptive (Ridtitid et al, 2008), and sedative activities (Huang et al., 2008). Given its many potential utilisations, it may create drug interactions.

Drug metabolism via the cytochrome P450 (CYP) enzymes has emerged as an important role in the occurrence of herbal-drug or herbal-herbal interactions, which can result in drug or herbal toxicities (Gonzalez, 1990; Ogu and Maxa, 2000). CYP is a superfamily of isozymes, which was reported to be induced or inhibited, resulting in clinical significant implications (Cupp and Tracy, 1998). CYP enzymes from mouse have shown similarity to human counterparts. The most relevant CYP enzymes involved in the metabolism of clinically significant drugs are CYP1, CYP2 and CYP3, which account for about 70% of hepatic microsome CYPs and are active in a wide variety of xenobiotic metabolism (Randic and DiCarlo, 1997). Modification of these CYPs enzymes by herbal medicines was also reported such as andrographolide from *Andrographis paniculata* (Jarukamjorn et al., 2010: 2006; Chatuphonprasert et al., 2009) and *Pueraria candollei* (Udomsuk et al., 2010).

Barbiturate-induced anaesthesia is widely used as a model of pharmacological or toxicological study. Barbiturates are metabolised by several CYP such as CYP2B6, CYP2D6, CYP3A4, CYP3A5 and CYP3A7 (Tsuji et al., 1996; Golan et al., 2008). The length of pentobarbital-induced sleeping time is inversely related to the rate of drug metabolism. Its duration of action can be altered by inducers or inhibitors (Lovell, 1986). Phenobarbital pre-treatment is known to shorten the sleeping time induced by pentobarbital. In contrast, inhibition of CYP by pre-treatment with beta-diethyl diphenylpropylacetate increases the duration of sleep in rats (Piel et al., 1969). Plants affecting central nervous system such as *Helietta apiculata* were reported to inhibit CYP2B activity and to change pentobarbital-induced sleeping time (Goloubkova et al., 1998). Our

previous study reported that EPMC from *K. galanga* L. induced mouse microsomal CYP2B activity (Sirisangtragul and Sripanidkulchai, 2011). The goal of this study is to investigate the effects of Yahom Tultavai on cytochrome P450 isozymes and pentobarbital-induced sleeping in mice, to understand if there exists drug interaction.

Materials and Methods

Chemicals

Pregnenolone 16α-carbonitrile (PCN), potassium chloride, tris(hydroxymethyl)-aminomethane, glycerol, reduced nicotinamide adenine dinucleotide phosphate (NADPH), ethoxyresorufin (ER), methoxyresorufin (MR), penthoxyresorufin (PR), standard resorufin, *p*-nitrophenol, 4-nitrocatechol, testosterone, and 6-β-hydroxytestosterone were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 3-methylchloranthene (3-MC) was supplied by Eastman (Rochester, USA). Phenobarbital (PB) was a product of Aventis (USA). Pentobarbital was provided by Abbott S.pa. Campoverde, Lt, (Italy) Ethanol and methanol were supplied by BDH (England). All other chemicals were analytical grade. Standard EPMC was kindly provided by Associate Professor Chavi Yenjai, Faculty of Sciences, Khon Kaen University.

Extraction of Yahom Tultavai

Yahom Tultavai was purchased from drug stores in Khon Kaen provinces, Thailand. Dried powder of Yahom Tultavai was separately macerated with dichloromethane and methanol at a ratio of 100g/1,000 mL for 7 days. Then, the mixtures were passed through Whatman No.1 filter paper and the solvents were removed by rotary evaporator (Eyela, SB-1000, Japan). For aqueous extract, 70 g of Yahom Tultavai was stirred in 1000 mL of hot water for 10 min before centrifugation at 2,000 g for 20 min. Supernatant was collected and dried by freeze-drier. Both dichloromethane and methanol extracts have a dark brown gum appearance with % yield of 4.8 and 12.1, respectively. The aqueous extract gave a pale brown powder at % yield of 19.1. Finger print of the extracts was analysed using HPLC. The assay was conducted with an Agilent 1100 series and UV-VWD detector using two mobile phase systems. Mobile phase A was an isocratic solvent containing acetronitrile: methanol: 20mM NaH_2PO_4 (30:40:30), which was used for dichloromethane and methanol extracts. Mobile phase B was 25uM ammonium phosphate buffer pH 7.5: methanol (95:5), which was used for the aqueous extract. The flow rate was of 1 mL/minute. A ThermoHypersyl-Keystone ODS HYPERSYL; 5µm, 4.6x250 mm (Agilent, Germany) and guard column, µBondpack 10 µm C18(Water, U.S.A.) were used. For animal administration, the dichloromethane and methanol extracts were suspended in olive oil vehicle, whereas the aqueous extract was suspended in distilled water.

Animals

ICR male mice at 8 weeks of age were obtained from the National Laboratory Animal Centre, Salaya Mahidol University, Nakorn Pathom, Thailand. The animal care was conducted under the National Institute of Health Guide for Laboratory Animals (NIH Publication No 80-23) revised 1996. The animal room was maintained at 25+3^{o}C with 12 hr of dark-light cycle. The animal was provided with pellet diet and *ad libitum* water.

Effect of Yahom Tultavai extracts on hepatic cytochrome P450 enzymes

As the recommended daily dose of Yahom Tultavai for human consumption is 1.5 g and the obtained yield of Yahom Tultavai extracts were 4.8, 12.1 and 19.1 %, the doses used in this study were 1.2, 3 and 5 mg/kg for dichloromethane, methanol and aqueous extracts respectively. The animals were divided into 8 groups. Group 1 received olive oil vehicle, groups 2-4 received dichloromethane extract (1.2 mg/kg), methanol extract (3 mg/kg) and aqueous extract (5 mg/kg) respectively. All extracts were administered orally by gavaging daily for 4 weeks. The other (positive control) four groups each received typical CYP inducer as follows: groups 5-7 were subcutaneously administered with 3-MC (100mg/kg); PCN (50mg/kg) intraperitoneally injected with PB (100mg/kg) for 5 days. Group 8 received 10% of ethanol via drinking water for 2 weeks, for the induction of CYP1A1, CYP1A2, CYP3A4, CYP2B and CYP2E1, respectively. The animals were sacrificed by decapitation 24 h after the last treatment and then livers were removed, weighted, and immediately frozen in liquid nitrogen and stored at -70 ^{o}C for microsomal preparation.

Preparation of hepatic microsome

Livers were thawed and minced, and then homogenised in 3 volumes of ice-cold 1.15% (W/V) KCl in 0.1 M potassium phosphate buffer pH 7.4 by a motor-driven Teflon pestle in a glass homogenisation vessel in the ice bath. The crude homogenate was centrifuged at 9,000 g, 4^{o}C for 20 min. The lipid free layer of supernatant was further centrifuged at 100,000g, 4^{o}C for 1 h. The microsomal fraction was obtained by suspending of the sediment with 0.1 M of potassium phosphate pH 7.4 containing 20% glycerol (v/v), 1 mM EDTA and 0.1 mM dithiotrietol. Aliquots of microsomal fraction were kept at -70^{0}C until further analysis. The microsomal protein concentration was determined under instruction of protein assay (BioRad laboratory) with bovine serum albumin as a standard. The total amount of protein in final volume was 10-20 mg/ml.

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Measurement of CYP activities

Microsomal CYP1A1, CYP1A2, and CYP2B enzymes were performed by measuring ethoxyresorufin *O*-dealkylase (EROD), methoxyresorufin *O*-dealkylase (MROD), and penthoxyresorufin *O*-dealkylase (PROD) activities, respectively (Sakuma et al, 1999; Jarukamjorn et al, 1999). The formation of the resorufin product was continuously measured by spectrofluorometric method with excitation and emission wavelength at 530 and 580 nm, respectively. The enzyme activity was expressed as *p*mol/min/mg protein.

CYP2E1 activity was determined by measurement of *p*-nitrophenol hydroxylase activity (Elbarbry et al, 2006). The reaction was assessed in microsomal fraction and the end product, 4-nitrocatechol, was separated by using reverse phase-HPLC (HP 1100 series, Agilent, Japan) and monitoring at wavelength of 345 nm. A Thermo Hypersyl-Keystone, ODS Hypersyl 5 um, 4.6 mm x 25 cm column (Agilent, Germany) was used. Samples were isocratically eluted with a mixture of 30% of acetronitrile containing 0.1% of trifluoro acetic acid at a flow rate of 1 ml/minute. The activity was expressed as µmole/in/mg protein.

CYP3A4 activity was examined, using testosterone as a catalytic marker (Baltes et al., 1998). Testosterone hydroxylation and its metabolite, 6β-hydroxytestosterone, were separated by liquid-liquid extraction of ethyl acetate and reverse phase-HPLC **(**HP 1100 series, Agilent, Japan), using a Thermo Hypersyl-Keystone, ODS Hypersyl 5 um (4.6 mm x 25 cm) column (Agilent, Germany). The mobile phase contained a mixture of 100 µM phosphate buffer pH. 6.0, methanol, and acetronitrile (50:38.5:11.5 v/v/v) which was used at a flow rate of 1 ml/minute, and then monitored at wavelength of 254 nm. The CYP 3A4 activity was expressed as µmole/min/mg protein.

Effect of dichloromethane extract of Yahom Tultavai on pentobarbital-induced sleeping and CYP2B activity

The animals were divided into 8 groups and treated as follows: groups 1-2 (controls) received vehicle; groups 3-6 were orally treated with EPMC (80 mg/kg) and dichloromethane extract of Yahom Tultavai (2 g/kg, equivalence to 80 mg EPMC) daily for a period of 7 and 28 days, respectively. Groups 7-8 were the positive control groups, which were intraperitoneally injected with PB (100mg/kg/day) for 4 days. After the last treatment, pentobarbital-induced sleeping was performed as described by Ma et al., (2007). Pentobarbital sodium was intraperitoneally administered to each mouse to induce sleep at doses of 60 and 100 mg/kg, for 7 and 28 days treatment, respectively. All experiments were carried out between 10 am and 1 pm. The animals that stayed immobile for more than 3 min and lost its righting reflex were judged to be asleep. The animals were observed constantly, and the time of awakening as characterised by righting of animal was noted. The sleeping time was defined as the time taken for the animal to regain spontaneous movements. Animals that failed to fall asleep within 15 min after pentobarbital administration were excluded from the experiment. Therefore, the number of animals used in each group were 6- 14. At 24 h after the experiment, the animals were decapitated, and then the livers were removed. The microsomal fraction was prepared and used for the determination of CYP2B activity as previously described.

Statistical analysis

The analysis of variance (ANOVA) or Student's *t-test* was employed. The results were expressed as mean±S.E. and statistical significant was set at p-value lower than 0.05.

Results

HPLC chromatogram of Yahom Tultavai extracts

The HPLC chromatograms of three extracts of Yahom Tultavai were shown in Figure 1. By using mobile phase A, dichloromethane extract showed the most numbers of constituents, which was more than 17 peaks of different retention times with the prominent peak of EPMC at 4 mg% concentration (Figure 1A and 1B). Methanol extract demonstrated similar peaks as observed in dichloromethane extract, however with lower number of peaks and peak area, it contained 0.6 mg% of EPMC (Figure 1C). In contrast, the aqueous extract cannot be separated by mobile phase A, but when using mobile phase B it demonstrated different HPLC chromatogram from both dichloromethane and methanol extracts (Figure 1D).

Body and organ weight

After 28 days of treatment with three extracts of Yahom Tultavai, there was no animal death. In general, the animals gained weight, but the final body and liver weights were not significantly different between the control and treated groups as shown in Table 1.

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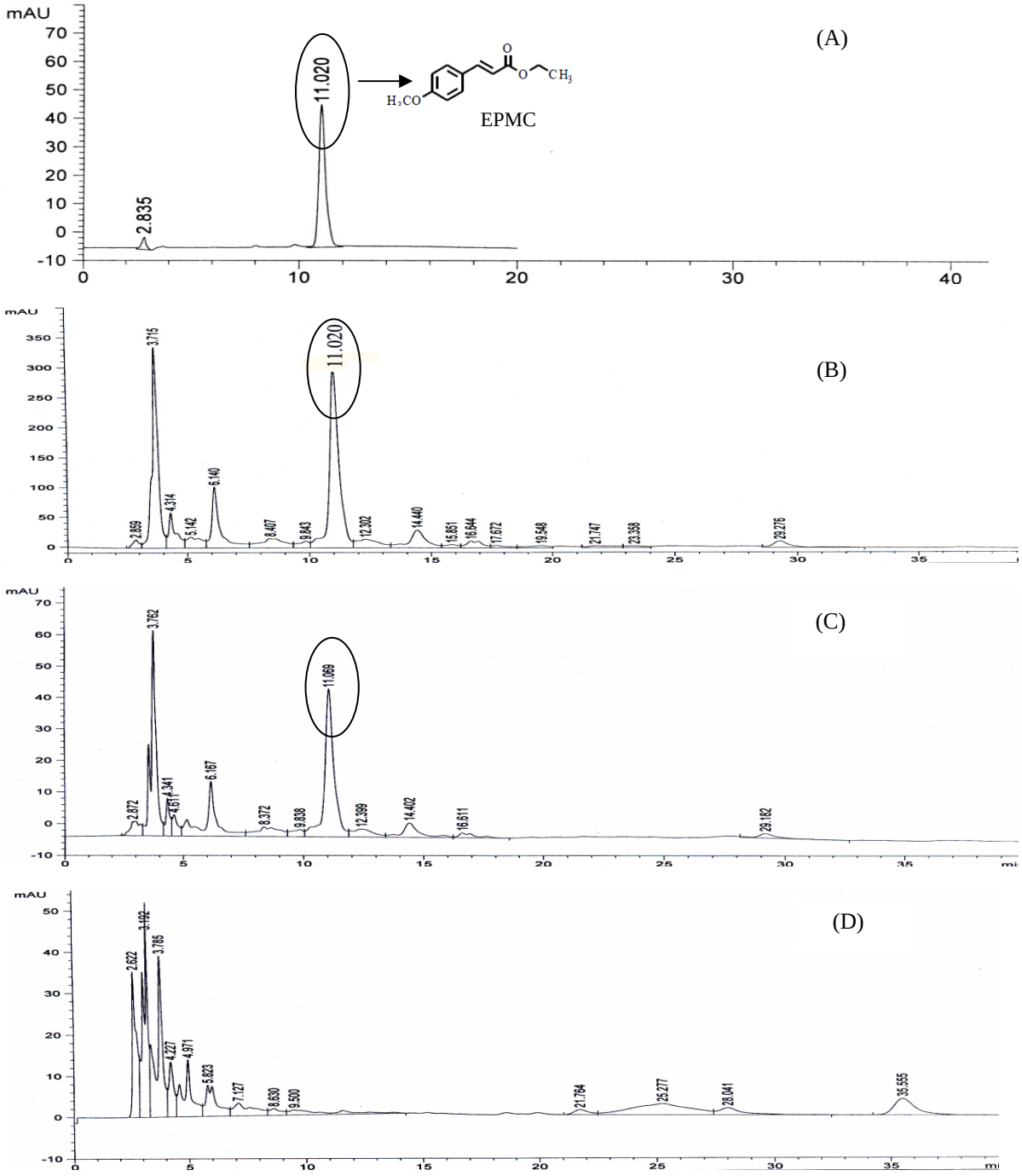

Figure 1: HPLC chromatograms of standard ethyl-p-methoxycinnamate (EPMC) and three Yahom Tultavai extracts: (A) EPMC, (B) dichloromethane extract, (C) methanol extract , using mobile phase A containing acetronitrile: methanol: 20mM NaH_2PO_4 (30:40:30) and detection at 270 nm; (D) aqueous extract using mobile phase B containing 25uM ammonium phosphate buffer pH 7.5: methanol (95:5) and detection at 250 nm.

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Table 1: Body and liver weights of mice treated with three Yahom Tultavai extracts for 28 days

Treatments	Body weight (g)		Liver weight
	Initial weight	Final weight	(g/100 g body weight)
Control	33.83±0.68	43.38±0.67*	7.19±0.49
Dichloromethane extract (1.2 mg/kg)	39.05±0.66	43.28±1.06*	7.14±0.28
Methanol extract (3 mg/kg)	34.42±2.29	40.75±0.80*	7.93±0.91
Aqueous extract (5 mg/kg)	38.38±0.49	41.30±0.69*	7.29±0.44

Values are expressed as mean±S.E (n = 6). * Represents significant differences from the initial body weight at $p < 0.05$

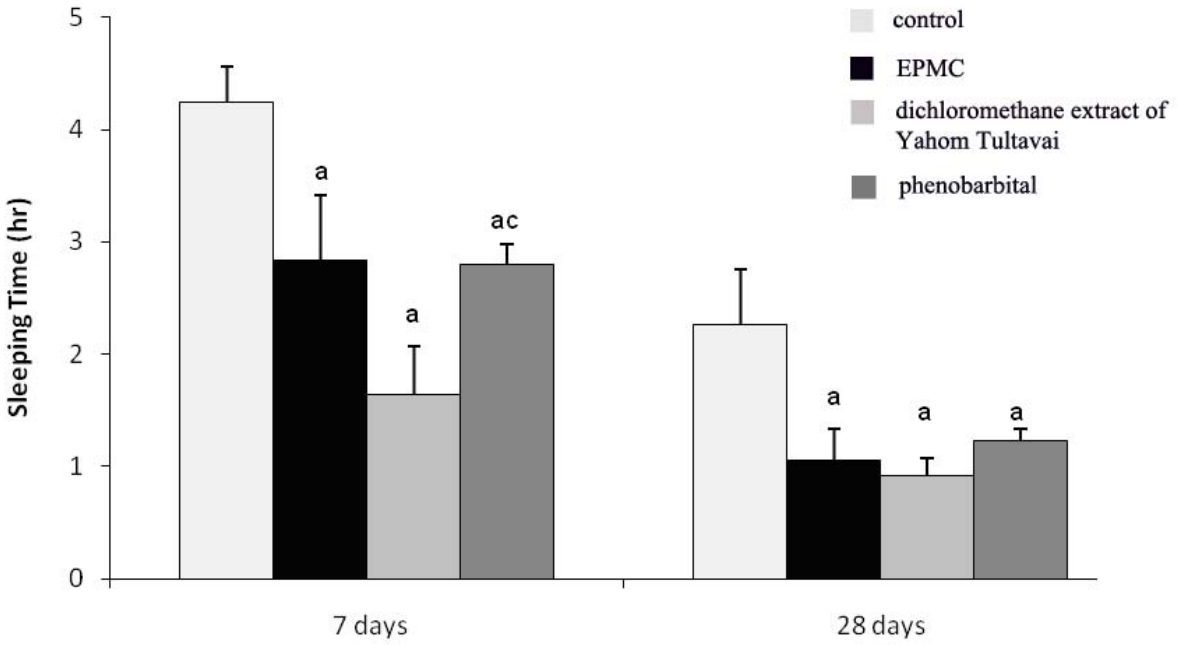

Figure 2: Effect of Ethyl-*p*-methoxycinnamate (EPMC) and dichloromethane extract of Yahom Tultavai on sleeping time in phentobarbital-induced mice. Values are expressed as mean±S.E, the numbers of animals used is specified in Table 3. [a,b,c] represents significant differences to control, EPMC and dichloromethane extract of Yahom Tultavai treated groups at the same duration, respectively at $p < 0.05$

Effect of Yahom Tultavai extracts on hepatic microsomal cytochrome P450 enzymes

The alkoxyresorufin *O*-dealkylation (AROD) activities have been utilised as activity-probes for selective measurement of P450 isoforms. After 28 days of treatment, the dichloromethane extract of Yahom Tultavai significantly reduced CYP1A activity. Both methanol and aqueous extracts of Yahom Tultavai significantly reduced CYP1A2 activity to 73% and 61%, respectively, whereas the dichloromethane extract increased CYP2B activity. It is interesting to observe that both dichloromethane and aqueous extract decreased CYP2E1 activity, but the statistical significance was observed for only the aqueous extract. Moreover, only the aqueous extract induced CYP3A4 activity. The specific inducer of each CYP enzyme demonstrated the positive control results of our experimental animals (Table 2).

Effect of EPMC and dichloromethane extracts on pentobarbital-induced sleeping and CYP2B activity

After 7 days of treatment, the animal gained weight but there were no significant differences among the control and treated groups. On the other hand, the liver weights significantly increased in animals treated with EPMC and dichloromethane extract of Yahom Tultavai when compared to animals in the control group. At 28 consecutive days of treatment, animals treated with all extracts had significantly increased in their body weights. However, animals' liver weight had significant difference only in animals treated with dichloromethane extract of Yahom Tultavai when compared to animals in the control group (Table 3). Furthermore, the duration of sleep caused by the administration of pentobarbital to

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Yahom Tultavai-treated group was significantly shorter than that of the negative control and the positive control (or PB-treated) groups, at both 7 and 28 days of experiments. Similarly, EPMC treated group showed shorter sleeping time than the control group at both 7 and 28 days of treatment. Moreover, the sleeping times of all animals at 28 days treatment were significantly shorter than that of 7 days treatment (Fig 2). Both EPMC and the dichloromethane extract induced CYP2B activity at both 7 and 28 days of treatment. However, the induction was lower than that of Phenobarbital treatment (Fig 3).

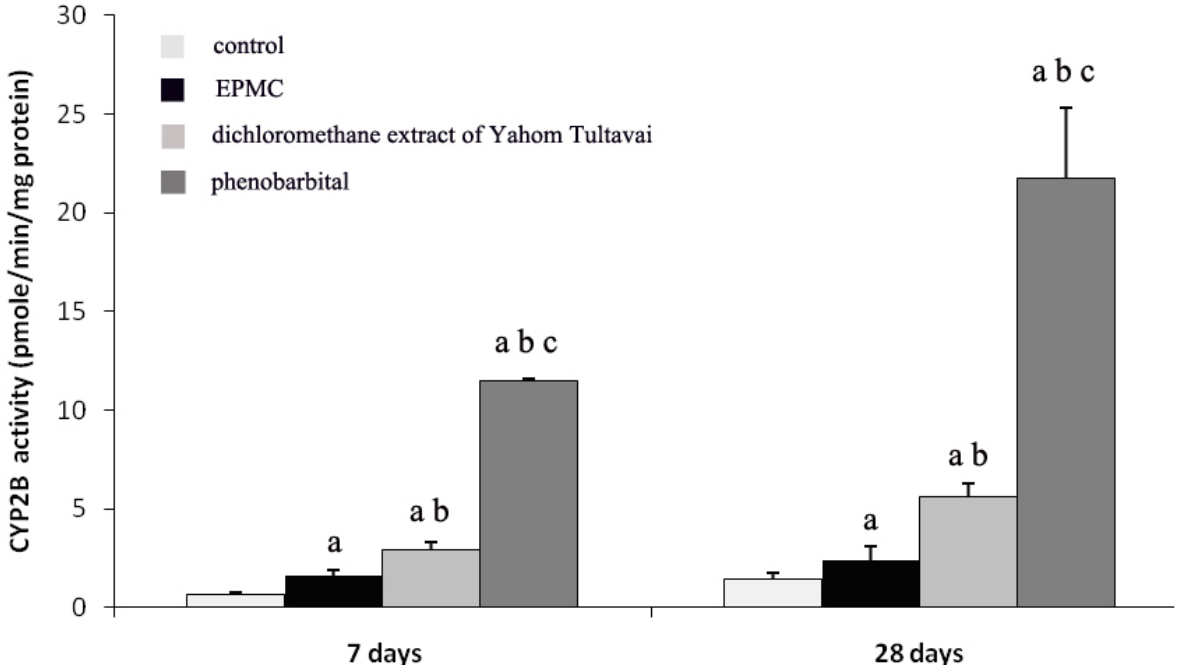

Fig 3: Effects of Ethyl-*p*-methoxycinnamate (EPMC) and dichloromethane extract of Yahom Tultavai on CYP2B activity in phentobarbital-induced mice. Results are expressed as mean ± S.E., (n=3).
[a,b,c] represents significant differences to control, EPMC and dichloromethane extract of Yahom Tultavai treated groups at the same duration, respectively at $p < 0.05$.

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Table 2: Effect of Yahom Tultavai extracts on hepatic microsome cytochrome P450 enzymes

Treatment (dose)	Duration (days)	CYP1A1	CYP1A2	CYP2B	CYP2E1	CYP3A4
Control	28	6.59±0.68	5.62±0.71	0.62±0.10	2.17±0.12	6.87±0.57
Dichloromethane extract (1.2 mg/kg)	28	4.66±0.41[a]	5.76±0.51	0.93±0.12	1.88±0.11	6.87±0.38
Methanol extract (3 mg/kg)	28	6.90±0.59	4.12±0.29[b]	0.55±0.09	2.34±0.19	8.05±0.61
Aqueous extract (5 mg/kg)	28	5.74±0.67	3.42±0.34[a, b]	0.32±0.13	1.69±0.11[a, c]	8.38±0.53[b]
3-MC (100 mg/kg)	5	38.25±7.46[*]	38.37±5.30[*]	ND	ND	ND
PB (100 gm/kg)	5	ND	ND	20.26±2.98[*]	ND	ND
PCN (50 mg/kg)	5	ND	ND	ND	ND	11.50±0.59[*]
EtOH (10% by vol)	14	ND	ND	ND	4.77±0.50[*]	ND

Values are expressed as mean ± S.E (n = 6). CYP1A, CYP1A2 and CYP2B activities were in term of pmol/min/mg protein; CYP2E1and CYP3A4 activities were in term of μmol/min/mg protein.
[a, b, c,] * Represent significant differences to control, dichloromethane, methanol treated and all other groups, respectively at $p < 0.05$. ND = Not determined.

Table 3: Body and organ weights of mice treated with Ethyl-*p*-methoxycinnamate (EPMC) and dichloromethane extract of Yahom Tultavai (DYT) for 7 and 28 days

Treatments	Duration (days)	Body weight (g)		Liver weight
		Initial weight	Final weight	(g/100 g body weight)
Control	7	40.05 ± 0.39	40.65 ± 0.37	6.84 ± 0.13 (n=14)
	28	39.20 ± 0.38	41.83 ± 0.75[*]	7.54 ± 0.42 (n=6)
EPMC(80 mg/kg)	7	40.01 ± 0.41	40.97 ± 0.35	9.42 ± 0.30 [a](n=13)
	28	39.50 ± 0.42	43.16 ± 0.51[*]	8.13 ± 0.26 (n=12)
DYT(2g/kg)	7	40.18 ± 0.39	40.31 ± 0.63	7.74 ± 0.28 [a, b] (n=10)
	28	39.45 ± 0.31	41.74 ± 0.41[*]	9.54 ± 0.34 [a, b] (n=14)
Phenobarbital (100mg/kg)	7	40.50 ± 0.40	39.82 ± 0.46	7.90 ± 0.30 [a, b] (n=12)
	28	39.17 ± 0.15	41.63 ± 0.23[*]	8.22 ± 0.36 [c] (n=13)

Values are expressed as mean ± S.E. (n=number of animal)
[*] represent significant differences from the initial body weight at the same duration time, respectively at $p < 0.05$.
[a,b,c] represent significant differences from control, EPMC and dichloromethane extract of Yahom Tultavai treated groups at the same duration time, respectively at $p < 0.05$.

Discussion

The recent increase in global use of herbal medicines in the last few decades raises concerns about the adverse effect of herbal-drug interactions. One of the main causes of this interaction is the modulation of drug-metabolising enzymes. The changes of drug metabolism can be deleterious on patients who have concomitantly used drugs with herb or traditional medicine. Thus, investigation of either metabolic inhibition or activation is important for assessment of possible herb-drug interaction. This study demonstrated that Yahom Tultavai affected hepatic microsomal CYP enzyme activities and pentobarbital-induced sleeping in mice. All three extracts of Yahom Tultavai (dichloromethane, methanol and aqueous) did not affect the mouse body and liver weights when they were orally administrated for 28 days. However, these extracts significantly altered CYP1A1, CYP1A2 and CYP2E1 activities. Inhibition of CYP1A1 was observed in animals treated with dichloromethane extract. While CYP1A2 activity was inhibited by methanol and aqueous extracts, only aqueous extract can decrease CYP2E1 activity. These results demonstrated that different extracts could affect CYP isoforms to different extents. The inhibition of CYP enzymes may lead to elevation of administered drug concentration in tissues and may increase drug action. Our findings are in concordance with previous reports that medicinal plants affect CYP activities, such as inductive effect of *Evodia rutacarpa* and *Andrographis paniculata* (Ueng et al., 2002; Jarukamjorn et al., 2006) or inhibitory effect of *Piper nigrum* and *Hypericum perforatum* (Delgoda and Westlake, 2004). Interaction of these medicinal plants with CYP activities result in clinically relevant drug adversities. These results suggest that the continuous intake of Yahom Tultavai may affect the metabolism of certain drugs that use the affected CYP enzymes such as caffeine, imipramine, theophylline and ethanol. Therefore, co-administration of Yahom Tultavai with herbal products, or conventional drugs should be carefully considered.

Furthermore, the induction of CYP2B activity that responds to barbiturate metabolism was observed in dichloromethane extract. However, the induction was lower than that of Phenobarbital, the specific CYP2B enzyme inducer. Since dichloromethane extract contained more constituents than methanol and aqueous extracts, and also had high content of EPMC. Therefore, the dichloromethane extract and EPMC were selected to test for the pentobarbital-induced sleeping. According to our previous studies, low dose of this Yahom Tultavai extracts did not affect pentobarbital-induce sleeping (data not shown). Moreover, with no evidence of Yahom Tultavai toxicity and LD_{50} of several marketed Yahom extracts were more than 5g/kg (Thongparditchote et al, 1999), a high dose of dichloromethane extract of Yahom Tultavai (2g/kg) was used in this study. Both Yahom Tultavai extract and EPMC shortened pentobarbital-induced sleeping time as Phenobarbital did. The antagonistic effect was associated with an increase in hepatic CYP2B activity as time-dependent manner. This result indicated that Yahom Tultavai extract and EPMC mediated the reduction of pentobarbital-induced sleeping due to the induction of CYP2B enzyme activity. Our finding is consistent with the report on some traditional medicine such as Tonica, an aqueous herbal haematinic, or Sho-saiko-to and Saiko-keisi-to which decreased pentobarbital-induced sleeping and enhanced CYPs activity (Martey et al., 2009; Nose et al., 2003). However, the induction of CYP2B by dichloromethane extract is lower than that of its specific inducer Phenobarbital. Their durations of pentobarbital-induced sleeping were shortest, suggesting that other mechanisms may be involved in this phenomenon, needing further investigation.

Conclusion

This study demonstrated the risk assessment of herbal-drug interaction of Yahom Tultavai containing *K. galanga* L., and its potential influence on CYP activity, especially CYP2B and pentobarbital-induced sleeping time. Since *K. galanga* L. is a common ingredient in Thai traditional medicines usually prescribed for long term use (Ridtitid et al, 2008), concomitant administration of Yahom Tultavai with herbs or certain drugs should therefore be carefully considered. The clinical implication of these interactions needs further investigation.

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