Pakistan
Journal of Biological Sciences

Interference of Thai Traditional Medicine (Yahom Ampanthong) on Hepatic Cytochrome P450 Enzymes and Pentobarbital-Induced Sleeping in Mice

[1]Wanna Sirisangtrakul and [1,2]Bungorn Sripanidkulchai
[1]Faculty of Pharmaceutical Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Center for Research and Development of Herbal Health Products,
Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: Yahom Ampanthong, a Thai traditional medicine, is commonly used for treatment of nausea, vomiting and syncope. Its formula is composed of more than 10 medicinal plants. Currently, the herbal-drug interactions were reported among the case of co-administration of traditional and Western medicines, since cytochrome P450 enzymes involve in drug metabolism and affect the drug action. This study aimed to investigate the effects of Yahom extracts on hepatic cytochrome P450 enzymes and pentobarbital-induced sleeping in mice. Powder of Yahom Ampanthong was extracted with three different solvents, i.e., dichloromethane, methanol and distilled water. The activities of CYP1A1, CYP1A2, CYP2B, CYP2E1 and CYP3A4 were determined after the administration of Yahom extracts for 4 weeks. All three extracts significantly inhibited CYP1A1, CYP1A2, CYP2E1 activities. In contrast, only dichloromethane and methanol extracts enhanced CYP2B activity. However, all three extracts did not affect CYP3A4 activity. When compared to the control group, the dichloromethane extract-treated animals showed shorter pentobarbital-induced sleeping time after treatment for 1 and 4 weeks. In conclusion, Yahom Ampanthong extracts modulated hepatic microsomal cytochrome P450 activities and decreased the pentobarbital-induced sleeping time. Therefore, the concomitant administration of Yahom with certain drugs may give rise to the herbal-drug interaction, which may affect the clinical implication of drug actions.

Key words: Yahom Ampanthong, dichloromethane extract, cytochrome P450, microsome, pentobarbital-induced sleeping, herbal-drug interaction

INTRODUCTION

Yahom, a Thai traditional medicine, is one of the popularly used for treatment of fatigue, dizziness, nausea, vomiting and syncope, especially in the aged-population. There are more than 500 formulas of Yahom, each formula contains more than 10 medicinal plants. By name Yahom means aromatic medicine and has been used for modification of the life power to keep the balance of body elements. In general, Yahom has been claimed to be safe without acute and chronic toxicities (Chavalittumrong et al., 2009) and has no mutagenicity (Sripanidkulchai et al., 2007). Several types of Yahom have been observed to increase heart beat and blood flow (Suvitayavat et al., 2005a) and to change the sleeping time. Yahom Ampanthong is a powder dosage form, which was labeled to contain several principal ingredients including *Conioselinum univilatum* (selinum), *Syzygium aromatum* (clove), *Cinnamonum verum* (cinnamon), *Mesua ferrea* (Bun-nak), *Nelumbo nucifera* (lotus), *Glycyrrhiza glabra* (licorice), *Myristica fragrans* (mace),

Pogostemon cablin (phim-sen), *Viverricula indica* (civet). Some principal ingredients from medicinal plants in Yahom were reported to increase blood pressure (Suvitayavat et al., 2005a), whereas some traditional medicines such as *Lindera strychnifolia* (Shimomura et al., 2010), *Chamaemelum nobile* (Zeggwagh et al., 2009) and red mold dioscorea (Wu et al., 2009) were reported to decrease blood pressure. In contrast, licorice was reported to increase blood pressure (Miettinen et al., 2010). The various herbal medicines in most of Yahom recipe have both cardiovascular stimulatory and inhibitory effects. Some Yahom was reported to increase vascular muscle contraction and cerebral blood flow (Suvitayavat et al., 2005b; Jariyapongskul et al., 2006).

Some people may consume Yahom in combination with traditional and Western medicines, which may raise the possibility of herbal-drug or herbal-herbal interactions. Drug metabolism via the cytochrome P450 (CYP) enzymes has emerged as one important mechanism in the occurrence of herbal-drug or herbal-herbal

---

Corresponding Author: Bungorn Sripanidkulchai, Center for Research and Development of Herbal Health Products,
Khon Kaen University, Khon Kaen 40002, Thailand

interactions, which can result in drug or herbal toxicities (Gonzalez, 1990; Ogu and Maxa, 2000). CYP is a superfamily of isozymes. Several different CYP isozymes were reported to be induced or inhibited and can cause clinical significant implications (Cupp and Tracy, 1998). CYPs from mouse have shown similarity to human counterparts. The most relevant CYP enzymes involved in the metabolism of clinical significant drugs are CYP1, CYP2 and CYP3, which account for about 70% of hepatic microsome CYPs (Randic and Di-Carlo, 1997). There were reports of the herbal modification of CYP enzymes such as andrographolide from *Andrographis paniculata* induced CYP1A1 activity and mRNA expression (Jarukamjorn *et al.*, 2010, 2006; Chatuphonprasert *et al.*, 2009) and *Pueraria candollei* induced CYP2B9 activity and mRNA expression (Udomsuk *et al.*, 2010). Plant affecting central nervous system such as *Helietta apiculata* was reported to inhibit CYP2B activity and change pentobarbital-induced sleeping time (Goloubkova *et al.*, 1998).

Pentobarbital-induced sleeping was used as a model for investigation of drug-action as barbiturates are metabolized by several CYPs (Tsuji *et al.*, 1996; Golan *et al.*, 2008). The aim of this study was to investigate the effects of Yahom Ampanthong extract on cytochome P450 isozymes and pentobarbital-induced sleeping in mice.

MATERIALS AND METHODS

The project was conducted during August 2008-May 2010 at the Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.

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, 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, Ltd. (Italy). Ethanol and methanol were supplied by BDH (England). All other chemicals were analytical grade.

Extraction of Yahom Ampanthong: Yahom Ampanthong (Prasart-Thong Osoth Co., Ltd., Lot number 01, registration number G212/27, manufacturing date 22/1/07)

was purchased from drug stores in Khon Kaen provinces, Thailand. The main ingredients of Yahom Ampunthong as labeled on the product were *Conioselinum univilatum* (selinum) (7.45%), *Syzygium aromatum* (clove) (7.45%), *Cinnamonum verum* (cinnamon) (7.45%), *Mesua ferrea* (Bun-nak) (7.45%), *Nelumbo nucifera* (lotus) (7.45%), *Glycyrrhiza glabra* (licorice) (7.45%), *Myristica fragrans* (mace) (0.74%), *Pogostemon cablin* (phim-sen) (1.49%) and *Viverricula indica* (0.09%).

Dried powder of Yahom was separately extracted with dichloromethane and methanol at ratio of 70g/700 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 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. The dichloromethane and methanol extracts gave dark brown gum appearance with % yield of 3.8 and 10.2, respectively. Whereas the aqueous extract gave pale brown powder at % yield of 18.7. Finger print of the extracts was analyzed by 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: 20 mM NaH_2PO_4 (30:40:30), which was used for dichloromethane and methanol extracts. Mobile phase B was 25 μM ammonium phosphate buffer pH 7.5: methanol (95:5), which was used for aqueous extract. The flow rate was of 1 mL min^{-1}. A ThermoHypersyl-Keystone ODS HYPERSYL; 5 μm, 4.6×250 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°C with 12 h of dark-light cycle. In this study, a total of 144 animals were used where each group was composed of 6-16 animals. Some of the animals in the pentobarbital-induced sleeping experiment were excluded because they did not sleep within the time setting. For each experiment, the number of animals used were specified in each table and figure. These animals were provided with pellet diet (CP. Mice feed: 082, S. WT. CO., LTD., Thailand) and *ad libitum* water.

Effect of Yahom extracts on hepatic cytochrome P450 enzymes: As the recommended daily dose of Yahom for human consumption is 1.5 g and the % yield of Yahom extracts were 3.8, 10.2 and 18.7, the doses used in this study were 1.2, 2.5 and 5 mg kg^{-1} for dichloromethane, methanol and aqueous extracts, respectively. The animals were divided into 8 groups, each group contained six mice. Group 1 received olive oil vehicle, acted as a negative control. Groups 2-4 received dichloromethane extract (1.2 mg kg^{-1}), methanol extract (2.5 mg kg^{-1}) and aqueous extract (5 mg kg^{-1}), respectively. All extracts were administered orally by giving daily for 4 weeks. Groups 5-8 were positive control groups which received each typical CYP inducer as follows: groups 5-6 were subcutaneously administered with 3-MC (100 mg kg^{-1}) and PCN (50 mg kg^{-1}) for 5 days; group 7 was intraperitoneally injected with PB (100 mg kg^{-1}) 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 -80°C for microsomal preparation.

Preparation of hepatic microsome: Hepatic microsome was prepared by modification of a previous method of Jagow *et al.* (1965). Livers were thawed and minced and then homogenized 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 homogenizing vessel in the ice bath. The crude homogenate was centrifuged at 9,000 g, 4°C for 20 min. The free lipid layer of supernatant was further centrifuged at 100,000 g, 4°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°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^{-1}.

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 pmol/min/mg protein.

CYP2E1 activity was determined by measurement of p-nitrophenol hydroxylase activity (Elbarbry *et al.*, 2006; Mischin *et al.*, 1996). The reaction was assessed in microsomal fraction and the end product, 4-nitrocatechol, was separated by using reverse phase-HPLC and monitoring at wavelength of 345 nm. The enzyme 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, 6 β-hydroxytestosterone was separated by liquid-liquid extraction of ethyl acetate and reverse phase-HPLC, then monitored at wavelength of 254 nm. The CYP 3A4 activity was expressed as μmole/min/mg protein

Effect of dichloromethane extract of Yahom on pentobarbital-induced sleeping: The animals were divided into 6 groups of sixteen animals each and treated as follows: groups 1-2 were orally received vehicle; groups 3-4 were orally treated with Yahom extract (2 g kg^{-1}), daily for a period of 1 and 4 weeks, respectively. Groups 5-6 were the positive control groups, which were intraperitoneally injected with PB (100 mg/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^{-1}, for 1 and 4 weeks 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 characterized by righting of animal was noted. The sleeping time was defined as the time taken for the animal to regain spontaneous movements. Twenty-six animals that failed to fall asleep within 15 min after pentobarbital administration were excluded from the experiment. Therefore, the number animals used in each group were 6-16.

Statistical analysis: The data were expressed as Mean±SE. The analysis of variance (ANOVA) was used for the statistical significance of difference between groups and followed by Student's t-test. A significant difference was set at p-value lower than 0.05.

RESULTS

HPLC chromatogram of Yahom Ampanthong extracts: The HPLC chromatograms of three extracts of Yahom Ampanthong are shown in Fig. 1. By using mobile phase
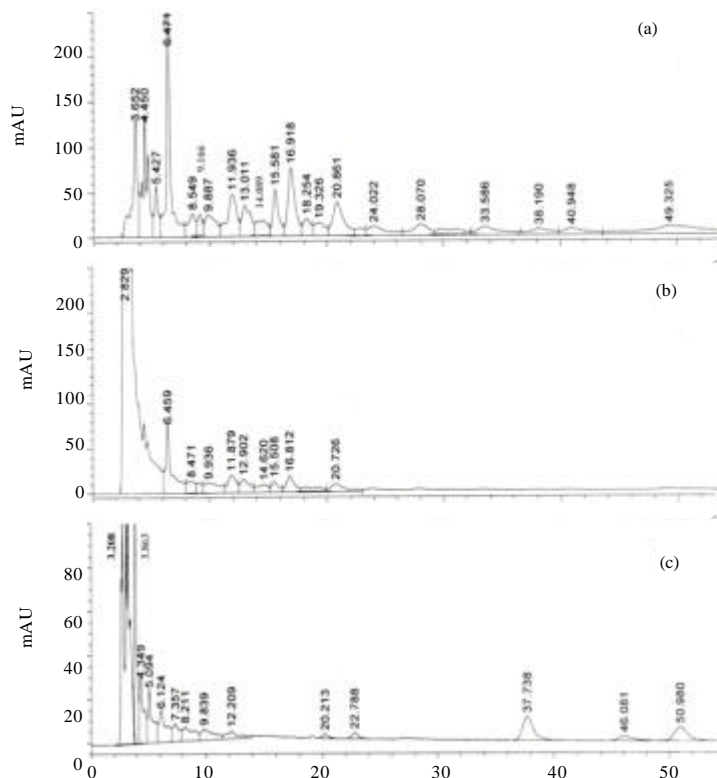
Fig. 1: HPLC chromatograms of three Yamon Ampanthong extracts: dichloromethane extract (a) methanol extract (b) using mobile phase containing acetronitrile: methanol 20 mM NaH_2PO_4 (30: 40: 30) and detection at 270 nm; aqueous extract and (c) using mobile phase containing 25 μm ammonium phosphate buffer pH 7.5: methanol (95:5) and detection at 250 nm

Table 1: Body and liver weights of mice treated with Yahom Ampanthong extracts for 4 weeks

Treatments	Body weight (g) Initial weight	Final weight	Liver weight (g)/100 g body weight
Control	35.32±0.75	41.99±0.86*	8.09±0.45
Dichloromethane extract (1.2 mg kg^{-1})	38.02±0.68	41.18±0.68*	8.25±0.45
Methanol extract (2.5 mg kg^{-1})	36.39±0.38	42.87±0.88*	8.25±0.35
Aqueous extract (5 mg kg^{-1})	41.42±0.65	43.47±0.53*	7.88±0.38

Values are expressed as Mean±SE. (n = 6). *Represents the significant difference from the initial body weight at $p<0.05$

A, dichloromethane extract showed the most numbers of constituents, which was more than 20 peaks of different retention times (Fig. 1a). Methanol extract demonstrated some similar peaks as observed in the HPLC chromatogram of dichloromethane extract, however with lower number of peaks and peak area (Fig. 1b). 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 (Fig. 1c).

Body and organ weight: In general, after 4 weeks of treatment with three extracts of Yahom Ampanthong, the animals did not show any behavioral change. The animal body and liver weights were not significantly different among the control or treated groups as shown in Table 1.

Effect of Yahom extracts on hepatic microsomal cytochrome P450 enzymes: The alkoxyresorufin O-dealkylation (AROD) activities have been utilized as activity-probes for selective measurement of P450 isoforms. After 4 weeks of treatment, all extracts of Yahom Ampanthong significantly reduced CYP1A activity. Both methanol and water extracts of Yahom Ampanthong significantly reduced CYP1A2 activity to 40 and 59%, respectively, whereas dichloromethane and methanol extracts increased CYP2B activity when compared to control group. It is interesting that all three extracts also caused significant reduction of CYP2E1 activity, whereas CYP3A4 activity was not altered in all treated groups. The specific inducer of each CYP enzyme demonstrated

Table 2: Effect of yahom ampanthong extracts on heptic microsome cytochrome P450 enzymes

Treatment (dose/duration)	CYP1A1	CYP1A2	CYP2B	CYP2E1	CYP3A4
Control (4 weeks)	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^{-1}/4 weeks)	4.88±0.35[a]	4.39±0.87	1.16±0.14[a]	1.61±0.06[a]	7.09±0.83
Methanol extract (2.5 mg kg^{-1}/4 weeks)	5.10±0.42[a]	2.27±0.16[a,b]	1.11±0.11[a]	1.72±0.09[a]	6.09±0.33
Aqueous extract (5 mg kg^{-1}/4 weeks)	4.45±0.29[a]	3.32±0.18[a,c]	0.60±0.09[b,c]	1.78±0.10[a]	7.07±0.36
3-MC (100 mg kg^{-1}/ 5days)	38.25±7.46*	38.37±5.30*	ND	ND	ND
PB (100 mg kg^{-1}/5 days)	ND	ND	20.26±2.98*	ND	ND
PCN (50 mg kg^{-1}/ 5 days)	ND	ND	ND	ND	11.50±0.59*
EtOH (10%v/v/2 weeks)	ND	ND	ND	4.77±0.50*	ND

Values are expressed as Mean±SE (n = 6). CYP1A1, CYP1A2 and CYP2B activities were in term of pmol/min/mg protein, whereas CYP2E1 and CYP3A4 activities were in term of μmol/min/mg protein. [a, b, c]Different letters represents significant differences to control, dichloromethane and methanol extracts-treated-groups, respectively at p<0.05. *Represents significant difference to all other groups at p<0.05, ND: Not determined

Table 3: Effect of yahom ampanthong extract on pentobarbital-induced sleeping in mice

			Body weight (g)		
Treatments	Duration (weeks)	No. of animals	Initial wt.	Final wt.	Liver weight (g)
Control	1	14	40.05±0.39	40.05±0.37	6.84±0.13 (n = 14)
	4	6	39.20±0.38	41.83±0.75*	8.14±0.51 (n = 6)
Phenobarbital (100 mg kg^{-1})	1	12	40.50±0.40	39.82±0.46	7.89±0.39[a] (n = 12)
	4	13	39.17±0.15	41.63±0.23*	8.54±0.43 (n = 13)
Dichloromethane extract (2 g kg^{-1})	1	9	39.86±0.27	38.5±1.06	8.82±0.68[a] (n = 9)
	4	16	39.32±0.30	40.84±0.54	7.63±0.43 (n = 16)

Values are expressed as Mean±SE, *Represents the significant difference from the initial body weight at p<0.05, [a]Represents the significant difference from the control group at 1 week duration at p<0.05
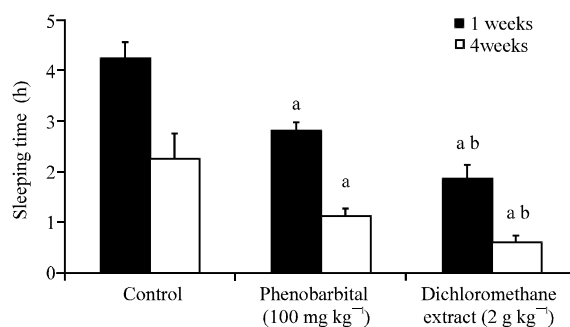

Fig. 2: Effects of dichloromethane extract of Yahom Ampanthong on sleeping time in pentobarbital-induced mice. Values are expressed as Mean±SE and the numbers of animals used were specified in Table 3. [a, b]Different letters represent the significant differences to control and phenobarbital-treated group of the same duration of treatment, respectively at p<0.05

the positive control results of our experimental animals (Table 2). Moreover, all animals did not show any differences in their general behaviors.

Effect of dichloromethane extracts on pentobarbital-induced sleeping: In general, the animals gained weight after 4 weeks of treatment. When compared to the control group, oral administration of dichloromethane extract in mice at 1 and 4 weeks did not cause significant changes of body and liver weights of the animals (Table 3). But the

duration of sleeping caused by administration of pentobarbital to Yahom-treated group was significantly shorter than that of the negative control and the positive control or PB-treated groups, at both 1 and 4 weeks of experiments. Moreover, the sleeping times of all animals at 4 weeks treatment were significantly shorter than that of 1 week treatment (Fig. 2).

DISCUSSION

In an attempt to investigate the effect of Thai traditional medicine for potential of herbal-herbal or herbal-drug interaction, we observed that Yahom Ampanthong significantly affected the hepatic microsomal CYP enzyme activities and pentobarbital-induced sleeping in mice. All three extracts of Yahom Ampanthong, by using dichloromethane, methanol and aqueous, did not affect the mouse body and liver weights when they were administered for 4 weeks. But these extracts caused significant inhibition of CYP1A1, CYP1A2 and CYP2E1 activities. Only dichloromethane and methanol extracts can induce CYP2B activity. However, the effects of these extracts were lower than that of phenobarbital, the specific inducer of CYP2B enzyme. Our results are concordance with the previous report that medicinal plants, such as pomegranate juice and Triphala, a polyherbal of Ayurveda showed inhibitory effects on cytochrome P450 (Faria *et al.*, 2007; Ponnusankar *et al.*, 2011). In contrast *andrographis paniculata* induced CYP450 enzyme activity (Jarukanjorn *et al.*, 2006, 2010;

Chatuphonprasert *et al.*, 2009). This reflects the different plants having different chemical constituents which results in differential effects on CYP activities. Therefore, it is suggested that the continuously intake of Yahom affects the CYP450 enzymes. In the case of the inhibition of CYP enzyme, the drug substrate may be less metabolized and may affect the drug action. The certain compounds or drugs metabolized by CYP1A1, CYP1A2 and CYP2E1 such as caffeine, warfarin, acetaminophen and ethanol should be cautiously given when co-administered with this Yahom.

Phenobarbital is a substrate of CYP2B, both dichloromethane and methanol extracts of Yahom induced CYP2B activity (Table 2). Since dichloromethane extract contained more constituents than methanol extract, the dichloromethane extract was selected to test for the effect on pentobarbital-induced sleeping. In our preliminary experiment, we failed to detect the effect of the lower dose of dichloromethane extract (1.2-5 mg kg^{-1}) on the sleeping time. Although there was no evidence on the toxicity of Yahom Ampanthong, Thongpraditchote *et al.* (1999) reported that LD$_{50}$ of several marketed Yahom extracts were more than 5 g kg^{-1}. Therefore, the high dose of dichloromethane extract (2 g kg^{-1}) was used to test for its effect on pentobarbital-induced sleeping. Moreover, the low dose of pentobarbital sodium of 60 mg kg^{-1} administered to the animals in 1 week study can induce sleep more effectively than those of 4 weeks study. This suggests the tolerance to pentobarbital of the animals. Therefore, pentobarbital sodium at dose of 100 mg kg^{-1} was given to the animals in the 4 weeks study.

The results of the present study on pentobarbital-induced sleeping indicated that Yahom extract shortened the sleeping time as phenobarbital did. However, our finding is opposite to the report on some traditional medicines such as *Zizyphi spinosae* semen, magnolol and obovatol from *Magnolia obovata* which enhanced pentobarbital-induced sleeping (Ma *et al.*, 2008, 2009a, b; Wang *et al.*, 2008, 2010). Since herbal products are commonly used in combination with various medications in the older adults (Gonzalez-Stuart, 2011), the risk to experience some sort of herbal-drug interaction is important to be aware of the possibilities of health complication. In this case, the herbal-drug interaction caused by Yahom extract should require careful consideration in the condition that concomitant administration of other certain drugs or herbs. For safety use, the monitoring of drug serum level will help to maintain effective and appropriate dose adjustment. The clinical implication of these interactions required further investigations.

CONCLUSION

In conclusion, this study demonstrated the possible herbal drug interactions caused by consumption of a Thai traditional medicine, Yahom Ampanthong, via the changes of hepatic microsomal cytochrome P450 enzyme activities. The administration of three extracts of Yahom Ampanthong to the mice for 4 weeks did not affect the animal body and liver weights. However, the extracts significantly changed the hepatic microsomal CYP1A1, CYP1A2, CYP2B and CYP2E1 activities. Moreover, the high dose of dichloromethane extract of Yahom Ampanthong increase CYP2B activity and decreased the pentobarbital-induced sleeping time.

REFERENCES

Baltes, M.R.H., J.G. Dubois and M. Hanocq, 1998. Ethyl acetate extraction procedure and isocratic high-performance liquid chromatography assay for testosterone metabolites in cell microsomes. J. Chromatogr. B Biomed. Sci. Appl., 706: 201-207.

Chatuphonprasert, W., K. Jarukamjorn, S. Kondo and N. Nemoto, 2009. Synergistic increases of metabolism and oxidation-reduction genes on their expression after combined treatment with a CYP1A inducer and andrographolide. Chem. Biol. Interact., 182: 233-238.

Chavalittumrong, P., S. Chivapat, A. Attawish and N. Soonthornchareonnon, 2009. Chronic toxicity of Yahom Navagoth extract. J. Trad. Thai. Alt. Med., 7: 134-145.

Cupp, M.J. and T.S. Tracy, 1998. Cytochrome P450 new nomenclature and chemical implications. Am. Fam. Physician., 57: 107-116.

Elbarbry, F., K. Wilby and J. Alcom, 2006. Validation of HPLC method for the determination of ñ-nitrophenol hydroxylase activity in rat hepatic microsome. J. Chromatogr. B., 834: 199-203.

Faria, A., R. Monteiro, I. Azevedo and C. Calhan, 2007. Pomegranate juice effects on cytochrome P450s expression: *In vivo* studies. J. Med. Food, 10: 643-649.

Golan, D.E., A.H.Jr. Tashijian, E.J. Armstrong and A.W. Armstrong, 2008. Pharmacology of GABAergic and Glutamatergic Neurotransmission. In: Principle of Pharmacology: The Pathophysiologic Basis of Drug Therapy, Golan, D.E. (Ed.). 2nd Edn. Lippincott Williams and Wilkins, Baltimore, MD, USA., pp: 173.

Goloubkova, T.D., E. Heckler, S.M.K. Rates, J.A.P. Henriques and A.T. Henriques, 1998. Inhibition fo cytochrome P450-dependent monooxygnases by an alkaloid fraction from *Helietta apiculata* markedly potentiate the hypnotic action of pentobarbital. J. Ethnopharmacol., 60: 141-148.

Gonzalez, F.J., 1990. Molecular genetic of the P-450 superfamily. Pharmacol. Therapeutics, 45: 1-38.

Gonzalez-Stuart, A., 2011. Herbal product use by older adults. Maturitas, 68: 52-55.

Jagow, R.V., H. Kampffmeyer and M. Kiese, 1965. The preparation of microsome. Naunyn-Schmiedebergs Arch. Exp. Path. U. Pharmak., 251: 73-87.

Jariyapongskul, A., S. Pathumraj and H. Nimi, 2006. Effects of Yahom on the regional cerebral blood flow in rat using videomicroscopy. Clin. Hemorheol. Microcirc., 34: 139-144.

Jarukamjorn, K., T. Sakuma, J. Miyaura and N. Nemoto, 1999. Different regulation of the expression of mouse hepatic cytochrome P450 2B enzymes by glucocorticoid and phenobarbital. Arch. Biochem. Biophys., 369: 89-99.

Jarukamjorn, K., K. Don-in, C. Makejaruskul, T. Laha, S. Daodee, P. Pearaksa and B. Sripanidkulchai, 2006. Impact of *Andrographis paniculata* crude extract on mouse hepatic cytochrome P450 enzymes. J. Ethnopharmacol., 105: 464-467.

Jarukamjorn, K., S. Kondo, W. Chatuphonprasert, T. Sakuma, Y. Kawasaki and N. Nemoto, 2010. Gender-associated modulation of inducible CYP1A1 expression by andrographolide in mouse liver. Eur. J. Pharm. Sci., 39: 394-401.

Ma, Y., H. Han, J.S. Eun, H. Kim, J. Hong and K. Oh, 2007. Sanjoinine a isolated from *Zizyphi spinosi* semen augments pentobarbital-induced sleeping behaviors through the modification of gaba-ergic system. Biol. Pharm. Bull., 30: 1748-1753.

Ma, Y., H. Han, S.Y. Nam, Y.B. Kim, J.T. Hong, Y.P. Yun and K.W. Oh, 2008. Cyclopeptide alkaloid fraction from *Zizyphi spinosis* semen enhances pentobarbital-induced sleeping behaviors. J. Ethnopharmacol., 117: 318-324.

Ma, H., C.S. Kim, Y. Ma, S.Y. Nam and D.S. Kim *et al.*, 2009a. Magnolol enhances pentobarbital-induced sleeping behaviors: Possible involvement of GABAergic systems. Phytother. Res., 23: 1340-1344.

Ma, H., Y.J. Jo, Y. Ma, J.T. Hong, B.M. Kwon and K.W. Oh, 2009b. Obovatol isolated from *Magnolia obovata* enhances pentobarbital-induced sleeping time: Possible involvement of GABAA receptors/chloride channel activation. Phytomedicine, 16: 308-313.

Miettinen, H.E., K. Piippo, T. Hannila-Handelberg, K. Paukku and T.P. Hiltunen *et al.*, 2010. Licorice-induced hypertension and common variants of genes regulating renal sodium reabsorption. Ann. Med., 42: 465-474.

Mischin, V.M., T. Kovisto and C.S. Lieber, 1996. The determination of cytpchrome P450 2E1-dependent ñ-nitrophenol hydroxylation by high performance liquid chromatography with electrochemical detection. Anal. Biochem., 233: 212-215.

Ogu, C.C. and J.L. Maxa, 2000. Drug interactions due to cytochem P450. Baylor Univ. Med. Center Proceed., 13: 421-423.

Ponnusankar, S., S. Pandit, R. Babu, A. Bandyopadhyay and P.K. Mukherjee, 2011. Cytochrome P450 inhibitory potential of triphala-A rasayana from ayurveda. J. Ethnopharmacol., 133: 120-125.

Rendic, S. and F.J. Di-Carlo, 1997. Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers and inhibitors. Drug Metab. Rev., 29: 413-580.

Sakuma, T., M. Ohtake, Y. Katsurayama, K. Jarukamjorn and N. Nemoto, 1999. Induction of CYP1A2 by Phenobarbital in the livers of aryl hydrocarbon-responsive and-nonresponsive mice. Drug. Metab. Dispos., 27: 379-384.

Shimomura, M., H. Ushikoshi, A. Hattori, I. Murata and Y. Ohno *et al.*, 2010. Treatment with *Lindera strychnifolia* reduces blood pressure by decreasing sympathetic nerve activity in spontaneously hypertensive rats. Am. J. Chin. Med., 38: 561-568.

Sripanidkulchai, B., N. Fangkratok, P. Saralamp and N. Soonthorchareonnon, 2007. Mutagenicity and antimutagenicity tests of extract from Thai traditional medicines. KKU. Res. J., 12: 492-498.

Suvitayavat, W., S. Tunlert, S.S. Thirawarapan and N. Bunyapraphatsara, 2005a. Effect of Yahom on blood pressure in rats. J. Ethnopharmacol., 97: 503-508.

Suvitayavat, W., S. Tunlert, S.S. Thirawarapan, C. Kitpati and N. Bunyapraphatsara, 2005b. Actions of Ya-hom, a herbal drug combination, on isolated rat aortic ring and atrial contractions. Phytomedicine, 12: 516-569.

Thongpraditchote, S., Y. Wongkrajang, W. Suvitayavat, C. Kitpati and K. Atisuk, 1999. A toxicity of Yahom. Thai J. Phytopharm, 6: 1-10.

Tsuji, R., K. Isobe, Y. Kurita, K. Hanai, K. Yabusaki and H. Kawasaki, 1996. Species difference in the inhibition of pentobarbital metabolism by empenthrin. Environ. Toxicol. Pharmacol., 2: 331-337.

Udomsuk, L., K. Churikawit, W. Putalun and K. Jarukamjorn, 2010. Impact of *Pueraria candollei* root cultures on cytochrome P450 2B9 enzyme and lipid peroxidation in mice. J. Health. Sci., 56: 182-187.

Wang, L.E., X.Y. Cui, S.Y. Cui, J.X. Cao and J. Zhang *et al.*, 2010. Potentiating effect of spinosin, a C-glycoside flavonoid of semen *Ziziphi spinosae*, on pentobarbital-induced sleep may be related to postsynaptic 5-HT1A receptors. Phytomedicine, 17: 404-409.

Wang, L.E., Y.J. Bai, X.R. Shi, X.Y. Cui and S.Y. Cui *et al.*, 2008. Spinosin, a C-glycoside flavonoid from semen *Zizhiphi spinozae*, potentiated pentobarbital-induced sleep via the serotonergic system. Pharmacol. Biochem. Behav., 90: 399-403.

Wu, C.L., C.L. Lee and T.M. Pan, 2009. Red mold dioscorea has a greater antihypertensive effect than traditional red mold rice in spontaneously hypertensive rats. J. Agric. Food Chem., 57: 5035-5941.

Zeggwagh, N.A., A. Moufid, J.B. Michel and M. Eddouks, 2009. Hypotensive effect of *Chamaemelum nobile* aqueous extract in spontaneously hypertensive rats. Clin. Exp. Hypertens., 31: 440-450.