The Thai Journal of Pharmaceutical Sciences

Volume 36
Issue 0 *supplement issue*

Article 15

1-1-2012

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KEYWORDS: *Anaxagorea luzonensis*, Kam-lang-wua-thalueng, *Ziziphus. Attopensis*, Kam-lang-seuakhrong, Acute oral toxicity, Rats

INTRODUCTION

The plant *Anaxagorea luzonensis* A. Grey (Annonaceae) is a tree indigenous to Philippines, Sri Langa, Myanmar, India, Laos, Indonesia and Thailand. In Thailand, *A. luzonensis* (AL) has been known as Kam-lang-wua-thalueng. Chemical investigations have demonstrated that the extract of AL contains flavones, flavonones, flavonols and xanthones (1). Akiyama and his colleagues reported that prenylflavonoids obtained from this plant had an estrogenic activity. The prenylflavonoids are reported to possess a wide rang of biological activities (2). AL has been widely used in Thai traditional medicine as a health promoting herb. The extract of AL has several pharmacological effects including antipyretic, stomachic, blood tonic, antioxidant, anti-tyrosinase activity, antihistamine, vasorelaxant effect, antihypertensive agents and for treatment of muscular pain (3).

Ziziphus is a genus of about 40 species of spiny shrubs and small trees in the Rhamnaceae family, distributed in the warm-temperate and subtropical regions throughout the world. *Z. attopensis* is one of the plants in this genus and most commonly found in sparse forests, thickets, up to 1,500 m in Laos and Thailand. In Thailand *Z. attopensis* (ZA) has been known as Kam-lang-seuakhrong. Several pharmacological activities of plants in *Ziziphus* genus have been shown in many scientific literatures, such as anti-ulcerogenic of *Z. lotus* extract (4), antiproliferation of melanoma cells by *Z. jujube* (5), and antiplasmodial and antimycobacterial activities of *Z. mauritiana* (6). According to Thai traditional folk medicine, the decoction of bark and wood plant of *Z. attopensis* has been commonly used as tonic, carminative, appetizer, and muscle analgesic. However, previous study on oral toxicity of the ethanolic extract of these plants still have no found in Thailand. Thus, the objective of this study is to determine the safety of 95% ethanolic extract of *A. luzonensis* and *Z. attopensis* in rats.

MATERIAL AND METHOD

Animals: Male (250 ± 20 g) and Female (230 ± 20 g) Wistar rats were obtained from National Laboratory Animal Centre, Mahidol University, Salaya, Nakornpathom. They were kept in cages with sterilized wood shavings as bedding at 24 ± 2°C in 12 h light/dark cycle and feed with standard diets and tap water *ad libitum*. All rats were acclimatized for 7 days prior to the experiments.

Method: Acute oral toxicity test was carried out following the "Guideline No. 423: Acute oral toxicity-Acute toxic class method of the OECD Guidelines for Testing of Chemicals (7)". In brief, animals were divided into five groups and each group contains five rats of both sexes. Group 1 was served as a negative control which was received 0.5% CMC in equivolume to the test group. Group 2-3 were served as treatment groups which were received Kam-lang-wua-thalueng extract at dose of 2,000 mg/kg and 7,500 mg/kg, respectively. Group 4-5 were served as treatment groups which were received Kam-lang-seuakhrong extract at dose of 2,000 mg/kg and 15,000 mg/kg, respectively. The rats were fasted for 16 hrs prior to dosing the test sample while drinking water was available *ad libitum*. And food was withheld for a further 3-4 hrs. Any toxic signs were immediately observed at ½, 1 and 3 hrs. The special care should be considered to animals that obviously showed toxic signs during the first 4 hrs after dosing and observed once daily thereafter for 14 days. Body weight was recorded weekly and at the end of the test. All survivors were euthanized by CO_2 asphyxiation and then performed necropsy finding. The mean of body weight gain of the animals in the test groups was calculated in comparison to the rats of the control group using Student's *t*-Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

As shown in Table 1 and 2. All groups of treated rats (2,000 mg/kg, 7,500 mg/kg and 15,000 mg/kg) did not show any toxic sings and death through the observation period. The body weight gain of the rats showed no difference from the control group. Necropsy findings exhibited normal appearance and no macroscopic pathological lesions of visceral organ. Thus, LD_{50} (lethal dose) were estimated over than 7,500 mg/kg (Kam-lang-wua-thalueng extract) and 15,000 mg/kg (Kam-lang-seuakhrong extract).

Table1: Summary of mortality rate and gross pathology of control and treated rats.

Treatment/Dose	[a]Mortality rate			Gross Pathology
	Male	Female	Total	
Control group 0.5% CMC equivolume to the treatment group	0/5	0/5	0/10	Normal
Treatment group "Kam-lang-wua-thalueng extract 2,000 mg/kg b.wt."	0/5	0/5	0/10	Normal
Treatment group "Kam-lang-wua-thalueng extract 7,500 mg/kg b.wt."	0/5	0/5	0/10	Normal
Treatment group "Kam-lang-seuakhrong extract 2,000 mg/kg b.wt."	0/5	0/5	0/10	Normal
Treatment group "Kam-lang-seuakhrong extract 15,000 mg/kg b.wt."	0/5	0/5	0/10	Normal

[a] Number of dead rats/number of rats tested

Table 2: Means of body weight gain of the control and treated rats recorded during experimentation and at termination

Sex	Treatment/Dose	*Mean of body weight gain (g)	
		Day 8	Day 15
Male	**Control group** 0.5% CMC	46.80 ± 2.32	79.40 ± 5.08
	Treatment group "Kam-lang-wua-thalueng extract 2,000 mg/kg b.wt."	42.00 ± 0.89	68.40 ± 2.83
	Treatment group "Kam-lang-wua-thalueng extract 7,500 mg/kg b.wt."	44.00 ± 4.81	69.20 ± 1.76
	Treatment group "Kam-lang-seua khrong extract 2,000 mg/kg b.wt."	40.60 ± 2.83	68.60 ± 4.78
	Treatment group "Kam-lang-seua khrong extract 15,000 mg/kg b.wt."	41.60 ± 1.77	68.40 ± 2.67
Female	**Control group** 0.5% CMC	28.20 ± 1.76	39.20 ± 1.85
	Treatment group "Kam-lang-wua-thalueng extract 2,000 mg/kg b.wt."	25.00 ± 2.38	38.80 ± 1.95
	Treatment group "Kam-lang-wua-thalueng extract 7,500 mg/kg b.wt."	25.60 ± 0.74	36.60 ± 1.16
	Treatment group "Kam-lang-seua khrong extract 2,000 mg/kg b.wt."	27.00 ± 2.04	39.00 ± 2.20
	Treatment group "Kam-lang-seua khrong extract 15,000 mg/kg b.wt."	25.20 ± 3.09	38.80 ± 4.27

* Data shown in the table are mean ± SEM

CONCLUSION

The LD_{50} of 95% ethanolic extract of Kam-lang-wua-thalueng in rats is greater than 7,500 mg/kg body weight and The LD_{50} of 95% ethanolic extract of Kam-lang-seuakhrong in rats is greater than 15,000 mg/kg body weight. Therefore, this study indicated that Kam-lang-wua-thalueng and Kam-lang-seuakhrong extracts may be safe in use as material source for herbal drug development. However, the repeated dose toxicity evaluation of these extracts are still necessary in further study.

ACKNOWLEDGEMENT

The author would like to express gratitude thanks to Thailand Institute of Scientific and Technological Research for their financial support.

REFERENCES

1.Gonda, R., T. Takeda and T. Akiyama. 2000. Studies on the constituents of *Anaxagorea luzonensis* A. Grey. *Chem. Pharm. Bull. (Tokyo),* 48: 1219-1222.

2. Tep-Areenan, P. and Sawasdee, P. 2011. The vasorelaxant Effect of *Anaxagorea luzonensis* A. Grey in the Rat Aorta. *Int. J. Pharmacol.*, 7 (1): 119-124.

3. Na Songkhla B., "Thai Medicinal Plants," 2nd Ed., Part 1, The Funny Publishing Ltd., Bangkok, 1982, pp. 76-77.

4. Wahida B, Abderrahman B, Nabil C. Antiulcerogenic activity of *Zizyphus lotus* (L.) extracts. *Journal of Ethnopharmacology*. 2007;112(2):228–231.

5.Hung C-F, Hsu B-Y, Chang S-C, Chen B-H. Antiproliferation of melanoma cells by polysaccharide isolated from *Zizyphus jujuba*. *Nutrition*. 2012;28(1):98–105.

6. Panseeta P, Lomchoey K, Prabpai S, et al. Antiplasmodial and antimycobacterial cyclopeptide alkaloids from the root of *Ziziphus mauritiana*. *Phytochemistry*. 2011;72(9):909–915.

7. Organization for Economic Co-operation and Development. 2001. OECD Guidelines for Testing of Chemicals, Volume 2, Section 4: Health Effects. Acute Oral Toxicity-Acute Toxic Class Method, Test Guideline No. 423.