

การทดสอบความเป็นพิษเฉียบพลันทางปากของตารับยาแก้ลมอุทชั่งคามาواتาในหมูกระทะสายพันธุ์ Sprague Dawley

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บทคัดย่อ

จากที่มีการใช้ยาสมุนไพรปริมาณมากในปัจจุบัน การศึกษาเกี่ยวกับประสิทธิภาพและความปลอดภัยของพืชสมุนไพรและตารับยาสมุนไพรจึงเพิ่มขึ้นตามไปด้วย โดยเฉพาะในประเทศไทย การทดสอบความเป็นพิษเฉียบพลันทางปากเป็นการทดสอบที่ใช้กันมากที่สุดสำหรับการประเมินความเป็นพิษในยาสมุนไพร ในการศึกษานี้ได้ทดสอบความเป็นพิษเฉียบพลันทางปากของยาแก้ลมอุทชั่งคามาواتา โดยดำเนินการทดสอบความเป็นพิษเฉียบพลันทางปากด้วยวิธี Class Method ตาม Guideline No. 423 ใช้หมูกระทะสายพันธุ์ Sprague Dawley เพศเมีย 3 ตัวต่อขั้นตอนการทดสอบ ก่อนให้ตัวอย่างยาแก้ลมอุทชั่งคามาواتา สัตว์ทุกตัวอดอาหารเป็นเวลาประมาณ 16 ชั่วโมง ขนาดยาเริ่มต้นคือ 2,000 มก./กgr./น้ำหนักตัว โดยการป้อนทางปาก หลังจากนั้นติดตามดู การอยู่รอดของสัตว์ การตายและการเจ็บป่วย สังเกตอาการทางคลินิกที่ 30 นาที 1, 2, 3, 4 ชั่วโมง และทุกวันเป็นเวลา 14 วัน ผลการวิจัยพบว่าสัตว์ในทั้งสองขั้นตอนการทดสอบ ที่ได้รับขนาดยา 2,000 มก./กgr. น้ำหนักตัวมีอาการทางคลินิกปกติ ไม่มีการตายหรือการเจ็บป่วยจนกว่าจะยุติ ความเป็นพิษเฉียบพลันทางปากของยาแก้ลมอุทชั่งคามาواتา จึงขอยกยื่นในประเภทที่ 5 หรือไม่สามารถจัดกลุ่มได้ตามระบบ GHS ดังนั้น ค่า LD₅₀ จึงมากกว่า 5,000 มก./กgr. ของน้ำหนักตัวภายใต้เงื่อนไขของการศึกษานี้

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Acute Oral Toxicity Testing of Thai Traditional Medicine “Ya-Kae-Lom-Utthang-Ka-Ma-Va-Ta” Formula in Sprague Dawley Rat

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Abstract

Since herbal medicines are being used on large scale, the studies looking at the efficacy and safety profile of medicinal plants and herbal medicine formulary have increased especially in Thailand. Acute oral toxicity tests are the most commonly performed tests for toxicity assessment in herbal medicine. In this study, acute oral toxicity of Ya-Kae-Lom-Utthang-Ka-Ma-Va-Ta remedy (UKMVT) was determined by oral acute toxicity class method according to OECD guidelines No.423. Three female Sprague Dawley rats per step were used in the procedure. Before administering the sample, all animals fasted for approximately 16 hours. The starting dose was 2,000 mg/kg/body weight by gavage. After administration, animal survival, mortality and morbidity were confirmed. Clinical signs were observed at 30 minutes, 1, 2, 3, 4 hours, and every day for 14 days. The results showed that the animals in both steps with a dose 2,000 mg/kg body weight had normal clinical signs, with no mortality or morbidity, until termination. The acute oral toxicity of UKMVT is classified as category 5, or unclassified, according to the Globally Harmonized System (GHS) of classification and labeling of chemicals. Therefore, The LD₅₀ was greater than 5,000 mg/kg body weight under the conditions of this study.

Keywords: Acute toxicity, Thai traditional medicine, Herbal, Ya-Kae-Lom

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Introduction

The World Health Organization (WHO) estimated that 70–80% of the global population used herbal and traditional medicine¹⁻³. In Thailand, herbal medicine has become increasingly in health care for the treatment of various diseases and health promotion^{4,5}. Since herbal medicines are being used on large scale, the studies looking at the efficacy and safety profile of medicinal plants and herbal medicine formulary have been increased⁶⁻⁸. Toxicity of herbal remedies remains a huge challenge that limits their use despite the general public belief that they are safe and devoid of potential toxicities⁹. Acute oral toxicity tests are the most commonly performed tests for toxicity assessment in herbal medicine¹⁰⁻¹⁴. Moreover, Thailand became the first and only nation in South East Asia that legalized medicinal cannabis. Cannabis have been used but there are very few scientific data for Thai traditional medicine available to support the benefits and toxicity. UKMVT from national Thai traditional medicine formulary is a formulation that uses cannabis as an ingredient used for imbalance of upper wind flow that is the cause of paralysis following theory of Thai traditional medicine. The preparation is composed of twelve herbs and two compounds including *Piper nigrum*, *Euphorbia antiquorum*,

Ligusticum striatum, *Mansonia gagei*, *Piper retrofractum*, *Zanthoxylum rhetsa*, *Terminalia arjuna*, *Cannabis sativa*, *Syzygium aromaticum*, *Myristica fragrans*, *Ardisia polycephala*, *Atractylodes lancea*, asafoetida, and camphor. However, there is a lack of acute toxicity study of this remedy. Therefore, the present study aimed to investigate UKMVT acute oral toxicity in Sprague-Dawley rats. This is the first report on the toxicity profile of UKMVT at a preclinical level.

Materials and Methods

Regulatory guidelines

The study was conducted according to OECD Guidelines for Testing of Chemicals No. 423; Acute Oral Toxicity-Acute Toxic Class Method and standard operating procedures (SOPs)¹⁵. The result shows LD₅₀ cut off and allows the acute toxicity of the test item to be ranked according to the Globally Harmonized System for the classification of chemicals. The study was approved by animal's ethics committee of Thailand institute of scientific and technological research (TISTR), approval number TS-65028.

Preparation of sample and dose administration

UKMVT was prepared from powder dried herbals including *Piper*

nigrum, *Euphorbia antiquorum*, *Ligusticum striatum*, *Mansonia gagei*, *Piper retrofractum*, *Zanthoxylum rhetsa*, *Terminalia arjuna*, *Cannabis sativa*, *Syzygium aromaticum*, *Myristica fragrans*, *Ardisia polyccephala*, *Atractylodes lancea*, asafetida, and camphor. The sample was dissolved in RO water at a concentration 20% w/v and administered at dose 2,000 mg/kg body weight within 1 hour for both steps. In the first step, the dose was 2,000 mg/kg body weight to confirm the survival rate observed in the first step, three further animals received the same dose, following the guidelines in OECD 423.

Preparation of experimental animals

Sprague Dawley rats were obtained and approved by Thailand Institute of Scientific and Technological Research (TISTR) in Pathumthani Province, Thailand (TSRI-ID-TS-65028). For husbandry, the animals were housed in the social housing in a group of three at approximate 12 hours/12 hours of light and dark cycle, temperature at $22\pm3^{\circ}\text{C}$, and at relative humidity of 30-70%. Standard pellet food and reverse osmosis water were provided ad libitum.

Group assignment, six eight-week-old healthy female Sprague Dawley rats weighting 189-206 g at the receiving day were employed and acclimatized to the

laboratory environment for at least 5 days prior to experimentation. The animals were randomly assigned into 2 groups (3 animals/group for each step). All animals were fasted for approximately 16 h before administration, but with free access to water. Each animal was weighted shortly before administration.

Acute toxicity study in rats

A stepwise procedure was used in this study. All animals were fasted for approximately 16 h before administration, but with free access to water. Each animal was weighted shortly before administration. In the first step, the test item was administrated to each of the first 3 animals at 2,000 mg/kg body weight by gavage. Thereafter they were observed every day of any signs of toxicity. The second, confirmatory step was performed following the same procedures as the first step. Each animal was observed and recorded for mortality, morbidity, and clinical signs at 30 min, 1 h, 2 h, 3 h and 4 h after dosing and daily thereafter, for 14 days. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations include changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavior pattern. Attention should be directed to observations of tremors,

convulsions, salivation, diarrhea, lethargy, sleep and coma. If no mortality and morbidity was observed, the next step was started based on the scientific judgment of study director. All survival animals were euthanized by CO₂ and necropsied after 14 days of observation, any gross pathologies were recorded for each animal. The test item was categorized according to Globally Harmonized System (GHS) and LD₅₀ cut-off range determination based on number of moribund and mortality of animals at each step.

Statistical analysis

The data is presented as the Mean \pm SEM (standard error of mean) and *T*-test was used to show significant differences between days 0 and 14. Statistical significance was considered at *p*-value < 0.05 .

Results and Discussion

Dosing volume of test item for both step as shown in Table 1. The animals in both steps showed normal clinical signs observations include changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity, behavior pattern, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma, with no mortality or morbidity, until termination, as

shown in Table 2. The body weight was recorded when the animals arrived, then at day 0 (starting experiment), day 7 and day 14 after treatment, as shown in Table 3. Body weight of the animals did not decrease during the study and normal of food and water intake in Table 4. And all of the rats appeared normal in gross pathological assessment. The histopathological evaluation of the vital organs showed that the structures were normal and that no microscopic changes occurred in kidney and liver. These results are summarized in Table 5.

Ya-Kae-Lom-Utthang-Ka-Ma-Va-Ta (UKMVT) formulation containing *P. nigrum*, *E. antiquorum*, *L. striatum*, *M. gagei*, *P. retrofractum*, *Z. rhetsa*, *T. arjuna*, *C. sativa*, *S. aromaticum*, *M. fragrans*, *A. polycephala*, *A. lancea*, asafoetida, and camphor have been traditionally used for imbalance of upper wind flow that is the cause of paralysis. The *P. nigrum* or black pepper is the main ingredient. The pharmacological property is the presence of specific phenolic components such as alkaloids (piperine), flavonoids, carotenoids, terpenoids. Many studies showed that piperine possesses several therapeutic properties such as antioxidant, anti-inflammatory, anticancer, antidiabetic antimicrobial, and antidepressant activities,

Table 1. Dosing volume

Step	Animal No.	Dosing volume (ml)
1	1	2.30
	2	2.30
	3	2.10
2	4	2.50
	5	2.50
	6	2.50

Table 2. Mortality, morbidity and clinical observation

Step	Dose (mg/kg bw)	Animal No.	Toxic signs	Mortality and morbidity
1	2,000	1		
		2	No toxic signs	0
		3		
2	2,000	4		
		5	No toxic signs	0
		6		

Table 3. Body weight record

Step	Dose (mg/kg bw)	Animal No.	Arrival day	Body weight (g)			Body weight change (day 0-14)
				^a Day 0	Day 7	Day 14	
1	2,000	1	206	227	253	280	53
		2	205	230	266	289	59
		3	189	208	255	267	59
Mean ±SEM			200.00±5.51	221.67±6.89	258.00±4.04	278.67±6.39	57.00±2.00
2	2,000	4	202	251	277	284	33
		5	198	254	281	290	36
		6	205	250	278	285	35
Mean ±SEM			201.67±2.03	251.67±1.20	278.67±1.20	286.33±1.86	34.67±0.88

^aBody weight recorded shortly before dosing

Table 4. Food and water intake

Animal		Food (g)				Water (ml)			
Date	Identification No.	Provided	Left over	Total food intake/cage	Total food intake/animal/day	Provided	Left over	Total water intake/cage	Total water intake/animal/day
15-20/02/2022	1,2,3 (3 animals)	400	145	255	14.17	700	400	300	16.67
21-28/02/2022		600	218	382	15.92	1,750	810	940	39.17
1-9/03/2022		600	135	465	17.22	2,100	955	1145	42.41
Total food intake/animal/day				15.77	Total water intake/animal/day				32.75
15-20/02/2022	4,5,6 (3 animals)	400	142	258	14.33	700	400	300	16.67
21-28/02/2022		600	177	423	17.63	1,750	620	1130	47.08
1-9/03/2022		800	299	501	11.93	2,800	610	2190	52.14
Total food intake/animal/day				14.63	Total water intake/animal/day				38.63

Table 5. Results of gross pathology

Step	Dose (mg/kg bw)	Animal no.	Gross pathological finding
1	2,000	1	Normal
		2	Normal
		3	Normal
2	2,000	4	Normal
		5	Normal
		6	Normal

enhance the bioavailability, and help to aid digestion¹⁶. From previous studies showed that single oral administration of water extract from the dried fruits of *Piper nigrum* L. at a dose of 5,000 mg/kg body weight in 5 males, 5 female rats did not produce signs of toxicity, behavioral changes, mortality, changes on gross appearance or histopathological changes of internal organs^{17,18}. Which is consistent with this study that found no toxicity of UKMVT at dose 2,000 mg/kg/body weight. There was no obvious sign of toxicity or mortality of *Myristica fragrans* in acute toxicity test after 24 h of extracts administration up to 5000 mg/kg body weight but the n-hexane extract at 1000 mg/kg body weight consistent with hepatotoxicity. Further-more, in rats fed extracts were significant ($p < 0.5$) elevations in urea, total bilirubin and creatinine concentrations, alkaline phosphatase, aspartate and alanine aminotransferases, and lactate dehydrogenase

activities¹⁹. Cannabis has been used in this remedy but there is no scientific information about traditional drug development such as therapeutic and toxicity effects. This research showed that use of cannabis leaves in this drug is safe, which does not cause toxicity or adverse effects in rats. According to the toxicological reports of cannabis it was found mortality in rats apparently resulted from severe hypothermia and other central effects from Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-THC, and cannabis extract²⁰.

Conclusion

Acute toxicity of UKMVT was studied in female Sprague Dawley rats by single dose of UKMVT at a dose of 2000 mg/kg body weight. The results showed that the animals in both steps with dose 2,000 mg/kg body weight had normal clinical signs, with no mortality or morbidity and no signs of acute toxicity were observed until termination. It was

estimated that the acute oral toxicity of UKMVT Remedy is classified as category 5, or unclassified, according to Globally Harmonized System (GHS) of Classification and Labeling of Chemicals. Therefore, The LD₅₀ was greater than 5,000 mg/kg body weight under the conditions of this study.

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Conflict of interest

The authors have no conflicts of interest to declare.

Abbreviation

mg = miligram

g = gram

kg = kilogram

bw = body weight

%w/v = %weight per volume

°C = degree of celsius

h = hour

LD₅₀ = lethal dose 50

SEM = standard error of mean

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