*nigrum, Euphorbia antiquorum, Ligusticum striatum, Mansonia gagei, Piper retrofractum, Zanthoxylum rhetsa, Terminalia arjuna, Cannabis sativa, Syzygium aromaticum, Myristica fragrans, Ardisia polycephala, 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±3˚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 $CO_2$ 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 $LD_{50}$ 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 ± 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 containning *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 acitvities,

**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<br>2<br>3 | No toxic signs | 0 |
| 2 | 2,000 | 4<br>5<br>6 | No toxic signs | 0 |

**Table 3.** Body weight record

| Step | Dose (mg/kg bw) | Animal No. | Body weight (g) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Arrival day | [a]Day 0 | Day 7 | Day 14 | Body weight change (day 0-14) |
| 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 |

[a] Body weight recorded shortly before dosing

**Table 4.** Food and water intake

| Date | Animal Identification No. | Food (g) | | | | Water (ml) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 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 digestion[16]. 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 phos-phatase, aspartateand alanine amino-transferases, and lactate dehydrogenase activities[19]. 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 extract[20].

## 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 $LD_{50}$ 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
$LD_{50}$ = lethal dose 50
SEM = standard error of mean

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