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Acute Oral Toxicity of Mixed *Crocodylus siamensis* Oil and *Kaempferia parviflora* Wall. Ex. Baker in Wistar Rats

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ABSTRACT *Crocodylus siamensis* oil and *Kaempferia parviflora* (black ginger) have traditionally been used as folk medicine to promote health. A new formulation, consisting of *C. siamensis* oil and black ginger extract, is developed as an alternative health product. Toxicological studies of this formulation have not been evaluated. This study was to determine acute oral toxicity of mixed *C. siamensis* oil and black ginger in Wistar rats. Our study was conducted in a stepwise procedure according to OECD Guidelines for the testing of chemicals 423, Acute Oral Toxicity-Acute Toxic Class Method (2001). After oral administration with 300 and 2,000 mg/kg body weight of mixed *C. siamensis* oil and black ginger, all animals were not shown any signs of toxic effects, moribund and mortality. The results indicated that mixed *C. siamensis* oil and black ginger was grouped in Globally Harmonized System of Classification and Labelling of Chemical as category 5 or unclassified and the LD_{50} is at 5,000-∞ mg/kg body weight. Our study suggested that the mixture of *C. siamensis* oil and black ginger is safe for oral administration and support its use as food supplements or other industrial applications.

Keywords: Acute Oral Toxicity, Crocodile Oil, *Crocodylus siamensis*, *Kaempferia parviflora* Wall. Ex. Baker

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Introduction

Siamese crocodile *(Crocodylus siamensis)* oil contains high levels of essential fatty acids omega-3, omega-6 and omega-9. It has been known that omega-3 fatty acid could support the brain and nervous system while omega-6 and-9 fatty acids could decrease level of high-density lipoprotein, increase level of low-density lipoprotein, reduce inflammation and have beneficial effects on human skin.[1, 2]

Kaempferia parviflora Wall. Ex. Baker (Black Ginger), commonly known as Krachai Dum, Thai Ginseng, Black Turmeric, and Black Galingale (Black Ginger), belongs to Zingiberaceae family. Quantitative analysis of *K. parviflora* using a gas chromatographic method revealed 11 flavonoid constituents of which 5, 7, 4´-trimethoxyflavone and 5,7-dimethoxyflavone were major constituents.[3] Additionally, analysis of methoxyflavones in *K. parviflora* ethanolic extract using high-performance liquid chromatography indicated that 3, 5, 7, 3´, 4´-pentamethoxyflavone, 5,7,4´- trimethoxyflavone and 5,7-dimethoxyflavone were major components.[4] Pharmacological activities of *K. parviflora* have been reported including anti-inflammatory, antioxidant, aphrodisiac, anti-gastric ulcer effect, anti-plasmodia, antifungal, and antibacterial activities.[5]

Owing to reports on pharmacological activities, a mixture of *C. siamensis* oil and black ginger could be a new food supplement. Safety data has not been conducted. Therefore, the present study was to investigate an acute oral toxicity of mixed *C. siamensis* oil and black ginger in Wistar rats in accordance with OECD Guidelines for the testing of chemicals, Acute Oral Toxicity-Acute Toxic Class Method (2001).[6]

Materials and Methods

C. siamensis Oil Extraction:

A total of 1 kilogram of Siamese crocodile *(C. siamensis)* fat were collected from *C. siamensis* at Thai crocodile farm, Sri-Ayuthaya Gold Medal Company Limited, Nong Khanak, Tha Ruea District, Phra Nakhon Si Ayutthaya Province, Thailand. The fat was thoroughly cleaned and freezed at -20°C.

A non-chemical method was used to extract Siamese crocodile oil using an extraction machine (Henny Penny BCS-10, MB Food Equipment, USA) and an oil press machine extractor (KDTQ, ZZKD, China).[1] After extraction, a liquid fraction of fat was segregated by centrifugation (Suprema 21, Tomy digital biology, Japan) at 6,000 g 4°C for 10 minutes. The oil was kept at 4°C until further use. Physical and chemical properties of oil were tested at Faculty of Sciences, Kasetsart University, Thailand. Microbial contamination was done by laboratory of National Laboratory Animal Center, Mahidol University, Thailand.

Extraction of *Kaempferia parviflora* Wall. Ex. Baker:

Black Ginger (*K. parviflora* Wall. Ex. Baker) was collected from Phetchabun province and its rhizome was clean, cut into small pieces and air-dried for 3 days. The dried rhizome was

ground and further extracted at SK Herb Company Limited, Krathum Baen District, Samut Sakhon Province, Thailand. Briefly, the dried powder was extracted with 70% ethanol at 1:40 w/v by the ultrasonic processor (UP100H, Hielscher, Germany) for 20 minutes and evaporated for further preparation. Physical and chemical properties of the extrac were tested at Faculty of Sciences, Kasetsart University, Thailand. Microbial contamination was done by laboratory of National Laboratory Animal Center, Mahidol University, Thailand.

Dose Preparation:

The mixed *C. siamensis* oil and black ginger was freshly prepared at doses of 300 and 2,000 mg/kg body weight prior to administration. The dose administration was not exceeded 1 ml per 100 g of animal body weight.

Preparation of Animals:

Female Wistar rats (*Rattus norvegicus* species, Mlac: WR) with weighs ranging from 189 to 209 gram (g) were obtained from National Laboratory Animal Center, Mahidol University, Thailand. The animals were kept under standard conditions of 12 hours light, 12 hours dark at 22±3 °C and 30-70% relative humidity. All were housed in stainless steel cages with food (082, Perfect Companions, Thailand), 5-7 ppm chlorinated water *ad libitum* and environmental enrichment. They were acclimatized for 5 days prior to study.[7]

The study was approved by National Laboratory Animal Center Animal Care and Use Committee (NLAC-ACUC), Mahidol University; Thailand, code RA2018-37.

Dose Administration:

The acute oral toxicity test was conducted using OECD Guidelines for the testing of chemicals 423, Acute Oral Toxicity-Acute Toxic Class Method (2001) (Figure 1). It is a stepwise procedure with the use of 3 female Wistar rats for each step. Doses of 300 and 2,000 mg/kg body weight of mixed *C. siamensis* oil and black ginger were used for this study. All animals were fasted for 15-18 hours prior to administration and further 3-4 hours after administration. All animals were observed for toxic effects after administration at the first 30 minutes, 4, 6 and 24 hours. Changes in skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and daily behavioral pattern were observed for 14 days. Additionally, observations of tremors, convulsions, gasping, cyanosis, vocalization, salivation, diarrhea, lethargy, sleep and coma were determined. Survived animals were generally clinical observed once daily for a total of 14 days.[6] Body weights, feed and drinking water consumptions of the survivals were recorded.

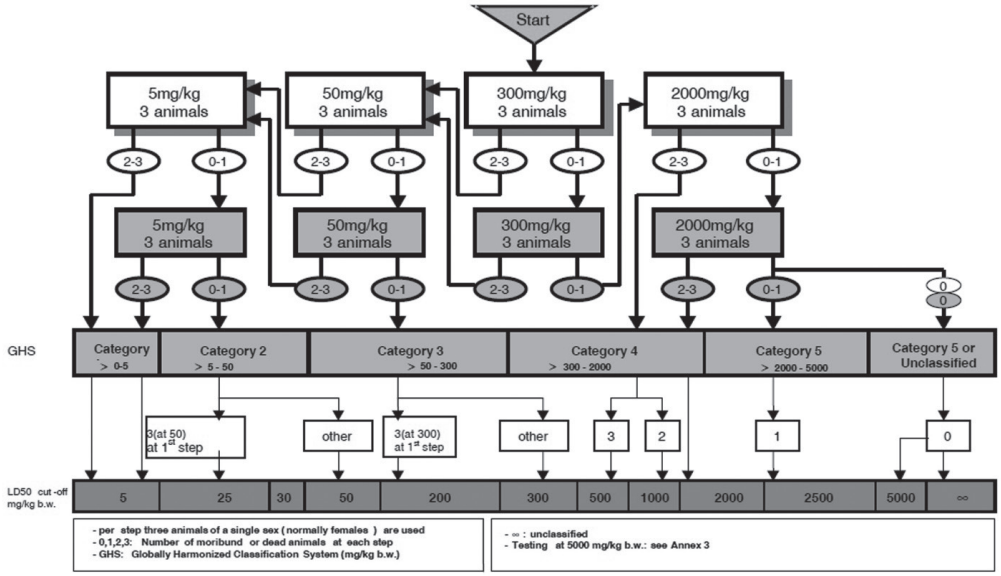

Fig.1 OECD Guidelines for the testing of chemicals 423, Acute Oral Toxicity–Acute Toxic Class Method (2001)

Necropsy Examination:

After 14 days, all survived animals were euthanized using CO_2 inhalation.[8] Gross pathological changes including positions, shapes, sizes and colours of internal organs were examined.[9]

Results

Characteristics of *C. siamensis* oil and *K. parviflora* Extracts:

The extract of crocodile oil was light yellow color with pH 5.9. Its major components were Palmitic acid, Oleic acid and Linoleic acid. Microbial examinations of the extract showed a total plate count was <100 colony forming unit/gram (CFU/g) and was negative for *Staphylococcus aureus*, *Salmonella* spp., *Clostridium* spp. and *Escherichia coli* (data not shown).

The extract of *K. parviflora* was dark brown color with pH 6.0. Its major component was 5,7-dimethoxyflavone. A total plate count of the extract was <100 CFU/g. In addition, yeast and mold was < 100 CFU/g (data not shown).

Safety evaluation of acute oral toxicity:

After oral administration with 300 and 2,000 mg/kg body weight of mixed *C. siamensis* oil and black ginger, all animals showed no signs of toxicity, no moribund and no mortality. The body weights of all animals were continued to gain throughout the study (Table 1). Animal feed and drinking water consumptions were normal (Table 2). The necropsy results of all animals were not found any lesions on external and internal organs (data not shown). Taken together, the mixed *C. siamensis* oil and black ginger was categorized in GHS category: category 5 or unclassified and the LD_{50} is 5,000-∞ mg/kg body weight.[10]

Table 1 Animal Body Weights (g)

Dose Level (mg/kg body weight)	No.	Body Weight (g)				Body Weight Change (%)
		Day 0	Day 7	Day 14	Terminate	
300	1	198	216	225	232.12	17.23
	2	200	214	216	222.88	11.44
	3	208	217	218	224.52	7.94
300	4	198	215	224	232.93	17.64
	5	198	208	221	222.39	12.32
	6	207	216	232	237.06	14.52
2,000	7	194	210	219	211.81	9.18
	8	201	218	225	228.49	13.68
	9	208	219	225	229.41	10.29
2,000	10	189	200	212	218.44	15.58
	11	202	210	221	229.60	13.66
	12	209	219	222	232.12	11.06

Table 2 Animal Feed and Drinking Water Consumptions (g)

Dose Level (mg/kg body weight)	No.	Feed Consumption (g)		Water Consumption (g)	
		Week 1	Week 2	Week 1	Week 2
300	1	12	15	35	47
	2	15	12	31	36
	3	12	11	24	28
300	4	14	13	33	40
	5	14	13	32	31
	6	16	11	30	24
2,000	7	11	12	26	41
	8	12	11	30	24
	9	12	12	25	28
2,000	10	13	13	23	22
	11	11	16	23	30
	12	13	11	27	26

Discussion

Results on acute oral toxicity studies indicated that neither Siamese crocodile oil nor black ginger demonstrated any signs of toxic effects to animals.[11,12] A mixture of Siamese crocodile oil and black ginger (*K. parviflora* Wall. Ex. Baker) extract was developed as a new formulation in order to boost human health. Therefore, it was necessary to assess its safety data prior to given to human.

Our findings showed no remarkable lesions in all external and internal organs when orally given the mixture at the doses of 300 and 2,000 mg/kg body weight to the Wistar rats. No signs of toxicity were demonstrated. Feed and drinking water consumptions were normal. In regards to OECD Guidelines for the testing of chemicals 423, Acute Oral Toxicity-Acute Toxic Class Method (2001), it could suggest that *C. siamensis* oil and *K. parviflora* mixture was safe.

Conclusion

Safety evaluation of mixed *C. siamensis* oil and *K. parviflora* administered orally at doses of 300 and 2,000 mg/kg body weight did not reveal acute toxicity in Wistar rats. The mixture of *C. siamensis* oil and *K. parviflora* was, therefore, classified in GHS category 5 or unclassified with the LD_{50} was 5,000-∞ mg/kg body weight.

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ความเป็นพิษเฉียบพลันทางปากของน้ำมันจระเข้ *(Crocodylus siamensis)* ผสมกระชายดำ (*Kaempferia parviflora* Wall. Ex. Baker) ในหนูแรทสายพันธุ์ Wistar

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บทคัดย่อ น้ำมันจระเข้เป็นน้ำมันจากสัตว์ที่นิยมใช้เพื่อดูแลสุขภาพ ส่วนกระชายดำถูกนำมาใช้เป็นส่วนผสมในยาแผนโบราณของไทย สูตรผสมน้ำมันจระเข้และกระชายดำได้ถูกพัฒนาขึ้นเพื่อใช้ดูแลสุขภาพ แต่ยังไม่มีรายงานการศึกษาพิษวิทยาของสารผสมดังกล่าว จึงได้ทำการประเมินความเป็นพิษเฉียบพลันทางปากของสารผสมน้ำมันจระเข้และกระชายดำในหนูแรทสายพันธุ์ Wistar ในขนาดของสารผสมน้ำมันจระเข้และกระชายดำที่ 300 และ 2,000 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง ตามวิธีการมาตรฐาน OECD Guideline หมายเลข 423 หลังจากได้รับสารผสมน้ำมันจระเข้และกระชายดำที่ 300 และ 2,000 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง พบว่าสัตว์ทดลองไม่แสดงอาการพิษ ไม่แสดงความผิดปกติ และไม่ทำให้เกิดการตาย ผลการศึกษาแสดงให้เห็นว่าสารผสมน้ำมันจระเข้และกระชายดำจัดอยู่ในระบบการจัดกลุ่มสารเคมี การติดฉลาก และการแสดงรายละเอียดบนเอกสารข้อมูลความปลอดภัยสากลหมวดที่ 5 หรือไม่ถูกจัดหมวด และมี LD_{50} ที่ 5,000–∞ มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง ผลจากการศึกษานี้แสดงให้เห็นว่าสารผสมน้ำมันจระเข้และกระชายดำมีความปลอดภัยในการบริโภค และอาจพัฒนาเป็นผลิตภัณฑ์เสริมอาหาร หรือการประยุกต์ใช้ในอุตสาหกรรมอื่น ๆ ได้

คำสำคัญ: กระชายดำ, ความเป็นพิษเฉียบพลันทางปาก, น้ำมันจระเข้