
Acute Oral Toxicity of *Crocodylus siamensis* Bile in Sprague Dawley Rats

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Abstract Crocodile bile is consisted of coprocholic acid, coprochenodeoxycholic acid, cholestanol, and other derivatives. In many countries, bile of animals has been used as an ingredient of traditional medicine. Although usage of *Crocodylus siamensis* (Siamese crocodile) bile has been used for treatment of various symptoms, toxicological studies of crocodile bile have not been evaluated. This study was to determine acute oral toxicity of *C. siamensis* bile in Sprague Dawley rats. Our study was conducted in a stepwise procedure and used fixed dose of *C. siamensis* bile at 300 and 2,000 mg/kg body weight according to OECD Guidelines for the testing of chemicals 420, Acute Oral Toxicity-Fixed Dose Procedure. The results showed that no mortality of rats orally given dried *C. siamensis* bile at the dose of 300 mg/kg body weight. In contrast, the dose of 2,000 mg/kg body weight demonstrated mortality within 24 hours after testing. The results suggested that *C. siamensis* bile is classified in Globally Harmonized System for Classification and Labeling of Chemicals as category 4 (300 mg/kg <LD50 ≤2,000 mg/kg).

Keyword: Crocodile Bile, Acute Oral Toxicity Testing, *Crocodylus siamensis*

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Introduction

Crocodile bile is consisted of 68% of Coprocholic acid, 9% of Coprochenodeoxycholic acid, 0.8% of Cholestanol, and other derivatives including 10% of 3-oxo-7 α , 12 α -dihydroxy-5 β -cholestanoic acid, 8% of 3 α , 7 α , 12 α -trihydroxy-5 β cholestanoic acid, 7-oxo-3 α , 12 α -dihydroxy-5 β -cholestanoic acid, 3-oxo-7 α , 12 α -dihydroxy-5 α -cholestanoic acid, chenodeoxy cholic acid, 5 α -cholestan-3 α , 7 α , 12 α , 26-tertrol, 5 β -cholestan-3 α , 7 α , 12 α , 25-tertrol, Ursodeoxycholic acid and 5 α -cholic acid.⁽¹⁾ Bile acids are steroidal amphipathic molecules derived from catabolism of cholesterol, predominantly found in bile of mammals.⁽²⁾ Bile acids are physiological detergents that facilitate excretion, absorption and transportation of fats and sterols in the intestine and liver.⁽³⁾

Bile of *Crocodylus siamensis* is reported to be used as drugs solvent and for treatment of sepsis, hemorrhage, trauma and lung diseases.⁽⁴⁾ Toxicological studies of crocodile bile have, however, not been carried out. The purpose of this study was, therefore, to determine the acute oral toxicity of *C. siamensis* bile in Sprague Dawley rats by fixed dose procedure according to Test No. 420: OECD Guidelines for the testing of chemicals, Acute Oral Toxicity-Fixed Dose Procedure.⁽⁵⁾

Materials and Methods

Collection of *Crocodylus siamensis* gallbladder:

A total of 10 gallbladders were collected from *C. siamensis* at Ayutthaya crocodile farm, Tha Rua district, Phra Nakhon Si Ayutthaya, Thailand.

Preparation of dried *Crocodylus siamensis* bile:

The process for dried bile production has been patented with Thai patent application No. 0901001231, Kasetsart University. Briefly, fat tissues covered the gallbladders were removed and the gallbladders were sterilized with 70% ethyl alcohol. The bile was collected and freeze-dried using a freeze dryer (Lyomaster, USA) for 36 hours. The freeze-dried bile was powdered by graining and stored in a sterilized and dry jar. The bile powder was kept at 4°C until further use.^(6,7)

Dose Preparation:

The dried *C. siamensis* bile at concentrations of 300 and 2,000 mg/kg body weight was freshly prepared with distilled water prior to administration. The dose administration was not exceeded 1 ml per 100 g of animal body weight.

Preparation of Animals:

Female Sprague Dawley rats strain (*Rattus Norvegicus* species, Mlac:SD) weighing 179 -190 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 \pm 3°C and

30–70% relative humidity. The animals were housed in stainless steel cages with food (082, Perfect Companions, Thailand) and 5–7 ppm chlorinated water ad libitum. All animals were acclimatized for at least 5 days prior to the study.⁽⁸⁾ The study was approved by National Laboratory Animal Center Animal Care and Use Committee (NLAC-ACUC), Mahidol University; Thailand, code RA2013-02.

Dose Administration:

OECD Test Guidelines 420 (Figures 1 and 2) was used to perform single dose toxicity studies. For the sighting study: the acute toxicity of dried *C. siamensis* bile was carried out using 2 concentrations, i.e., 300 and 2,000 mg/kg BW. One female rat was orally administered in a single dose using a stainless steel stomach tube. The animal was fasted over-night (15–18 hours) prior to administration and further 3–4 hours after administration of dried *C. siamensis* bile. The animals were observed for severe toxic effects or death within the first 24 hours. After evaluation of toxic effects under the sighting study, the main study was carried out using 1 female rat from the sighting study and 4 additional female rats and those were administered with 300 mg/kg BW. Attention given during the first 4 hours, periodically during the first 24 hours. The observations were included changes in skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and daily behavioral pattern for 14 days. Additionally, observations of tremors, convulsions, gasping, cyanosis, vocalization, salivation, diarrhea, lethargy, sleep and coma were determined.⁽⁵⁾ Body weights, feed and drinking water consumptions of the survivals were measured and recorded on day 1, day 7 and day 14 after dosing.

Necropsy Examination:

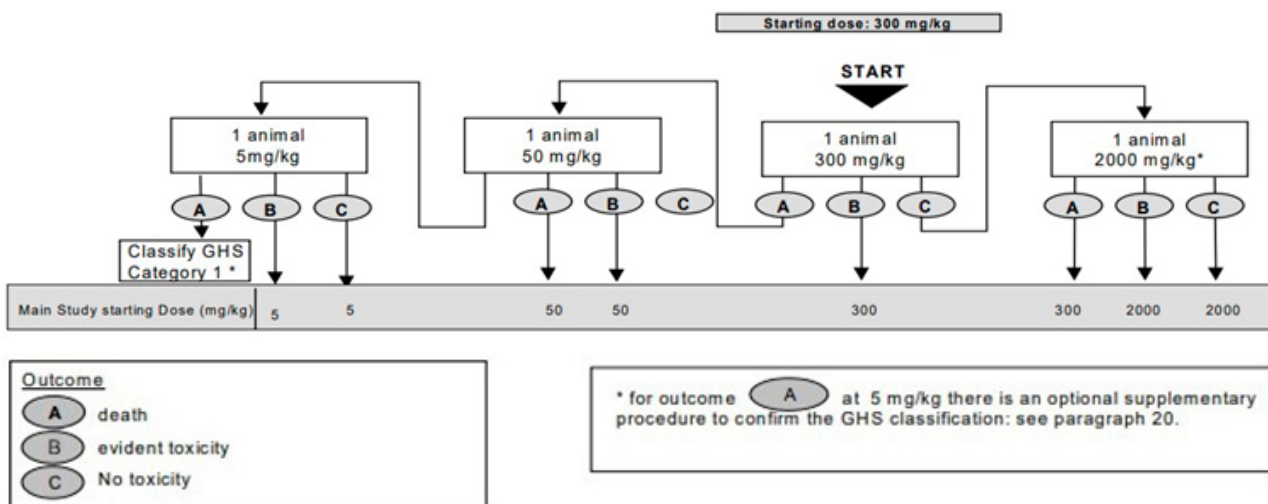


Fig.1 OECD/OCDE, OECD Guidelines for the testing of chemicals 420, Sighting Study⁽⁵⁾

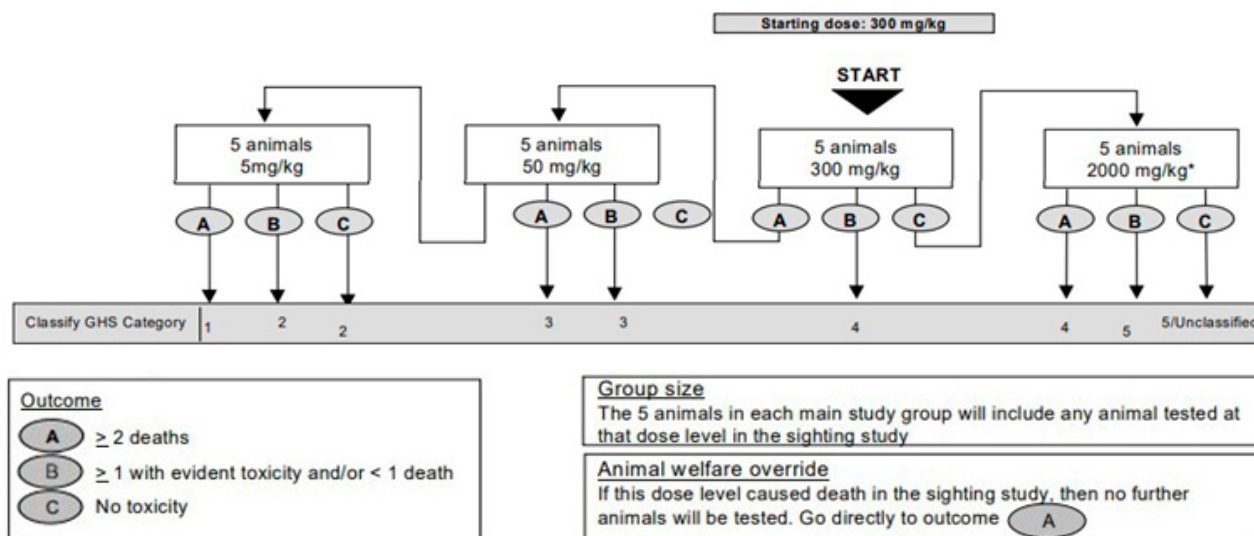


Fig.2 OECD/OCDE, OECD Guidelines for the testing of chemicals 420, Main Study⁽⁵⁾

After 14 days, all survived animals were euthanized using CO₂ inhalation.⁽⁹⁾ Gross pathological changes including positions, shapes, sizes and colours of internal organs were examined.⁽¹⁰⁾

Results

Sighting Study:

No signs of toxic effects showed after oral administration of dried *C. siamensis* bile at the dose of 300 mg/kg BW. On the contrary, dosing with 2,000 mg/kg BW of dried *C. siamensis* bile, mortality was observed within 24 hours. Gross pathology of the rat orally given 2,000 mg/kg BW revealed abnormal findings of stomach which were severe and diffused red mucosal stomach, mucosal erosion/hemorrhage, redness and mucosal thinning (Figure 3). Moreover, the duodenum was found redness and thin mucosal (Figure 4). According to the results of sighting study, the starting dose of main study was, therefore, 300 mg/kg BW of dried crocodile bile.

Main Study:

The main study was conducted with the dose of 300 mg/kg BW. As shown in Tables 2 and 3, body weights of all tested animals were increased throughout the study. Feed intake and water consumption of each animal were not different. No changes in skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern were observed (data not shown). There were no signs of tremors, convulsions, gasping, cyanosis, vocalization, salivation, diarrhoea, lethargy, sleep and coma. As for the gross examination of internal organs, slightly red intestinal mucosa especially at duodenum of all rats was demonstrated.

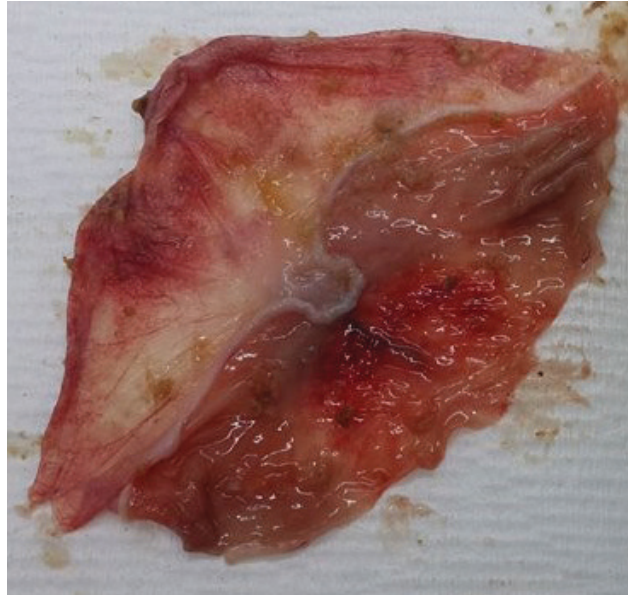


Fig.3 Severe diffuse red mucosal of stomach



Fig.4 Redness and thin mucosal of duodenum

Table 1 Survival and Mortality Results (Number of Survived/All Tested Animals)

Time After Administration	Sighting Study		Main Study
	300 mg/kg BW	2,000 mg/kg BW	300 mg/kg BW
30 minutes	1/1	1/1	4/4
1 hour	1/1	1/1	4/4
2 hours	1/1	1/1	4/4
3 hours	1/1	1/1	4/4
4 hours	1/1	1/1	4/4
1 Day	1/1	0/1	4/4
2 Days	1/1	0/1	4/4
7 Days	1/1	0/1	4/4
14 Days	1/1	0/1	4/4

Table 2 Body weights of animals

Dose Level (mg/kg BW)	Study	Animal No.	Body Weight (g)			Body Weight
			Day 1	Day 7	Terminated	Change (%)
300	Sighting	1	186	198	226	21.51
2,000	Sighting	2	190	-	161(Carcass)	-18.01
300	Main	3	179	202	221	23.46
	Main	4	187	198	221	18.18
	Main	5	184	203	230	25.00
	Main	6	184	208	225	22.28

Table 3 Feed and drinking water consumptions of animals

Dose Level	Study	Animal No.	Feed Consumption (g)			Water Consumption (g)		
			Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
300	Sighting	1	19.0	17.0	12.0	34.0	33.0	32.0
2,000	Sighting	2	16.0	-	-	24.0	-	-
300	Main	3	17.0	16.0	15.5	38.0	36.0	38.0
	Main	4	17.0	16.0	15.5	38.0	36.0	38.0
	Main	5	16.5	16.0	15.0	42.0	31.0	30.0
	Main	6	16.5	16.0	15.0	42.0	31.0	30.0

Discussion

A variety of animals/parts of animals have been widely used in traditional medicine such as bear,^(11,12) snake,^(13,14) duck, chicken^(15,16) and crocodile. Bile of *Crocodylus siamensis* is reported to be used as drugs solvent and for treatment of sepsis, hemorrhage, trauma and lung diseases⁽⁴⁾. Safety evaluation studies in animals are, therefore, essential to provide data on toxicity assessment before the initiation of human studies.

In our study, OECD Guidelines for the testing of chemicals 420, Acute Oral Toxicity-Fixed Dose Procedure⁽⁵⁾ was used to investigate acute toxicity of dried *C. siamensis* bile. It was observed that dried *C. siamensis* bile at the dose of 300 mg/kg BW did not show its effects on feed and water intake as well as changes in body weight, suggesting normal functions of the body and behavior⁽¹⁷⁾. In addition, all tested animals were not shown signs of toxicity, moribund and mortality. Erythematous mucosa, an increase of blood flow into the mucosa, is an underlying condition or irritation, suggesting that slight redness in mucosal duodenum did not possibly effect on animal health.

According to Globally Harmonized System of Classification and Labeling of Chemicals (GHS),⁽¹⁸⁾ the dried *C. siamensis* bile can be classified as GHS category 4 (300 mg/kg <LD₅₀ ≤2,000 mg/kg).

Conclusion

Using OECD TG 420, the dried *Crocodylus siamensis* bile at 300 mg/kg BW showed no mortality and no toxicity effects to the treated rats, whereas mortality was shown at the dose of 2,000 mg/kg BW within 24 hours after testing. Thus, *Crocodylus siamensis* bile was classified in GHS category 4.

Acknowledgements

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การทดสอบความเป็นพิษเฉียบพลันของน้ำดีจระเข้ (*Crocodylus siamensis*) ในหนูแรท สายพันธุ์ Sprague Dawley

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บทคัดย่อ น้ำดีจระเข้ประกอบด้วย Cholestanol, Coprocholic acid, Coprochenodeoxycholic acid และอนุพันธ์อื่น ๆ ในหลายประเทศนำน้ำดีของสัตว์มาใช้เป็นส่วนผสมในตำรับยาแผนโบราณ น้ำดีจระเข้สายพันธุ์ไทยถูกใช้ และส่งออกมาเป็นเวลานาน ในขณะที่ยังไม่มีข้อมูลการทดสอบทางพิษวิทยาของน้ำดีจระเข้อย่างเพียงพอ การศึกษานี้ได้ทำการประเมินความเป็นพิษเฉียบพลันทางปากในรูปแบบขนาดคงที่ของน้ำดีจระเข้ในหนูแรทสายพันธุ์ Sprague Dawley ในการทดสอบใช้ขนาดของน้ำดีจระเข้ขนาดคงที่ที่ 300 และ 2,000 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง ตามวิธีการมาตรฐาน OECD Guideline หมายเลข 420 ผลการทดสอบพบว่า น้ำดีจระเข้ที่ขนาด 300 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลองไม่ก่อให้เกิดความเป็นพิษ และการตายในสัตว์ทดลอง แต่น้ำดีจระเข้ที่ขนาด 2,000 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง ทำให้สัตว์ทดลองตายภายใน 24 ชั่วโมงหลังการได้รับสารทดสอบ ผลการศึกษาแสดงให้เห็นว่า น้ำดีจระเข้สายพันธุ์ไทยจัดอยู่ในระบบการจัดกลุ่มสารเคมี การติดฉลาก และการแสดงรายละเอียดบนเอกสารข้อมูลความปลอดภัยสากลหมวดที่ 4 และอาจกล่าวได้ว่ามี LD₅₀ ที่ >300 และ ≤2,000 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง

คำสำคัญ: น้ำดีจระเข้, การทดสอบความเป็นพิษเฉียบพลัน, *Crocodylus siamensis*