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Sub-chronic Oral Toxicity of *Crocodylus siamensis* Bile in Sprague Dawley Rats

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Abstract Siamese crocodile *(Crocodylus siamensis)* bile has been widely used for traditional medicine in many countries including Thailand. However, safety evaluation has been limited. This study aimed to determine sub-chronic toxicity of dried *C. siamensis* bile in male and female Sprague Dawley rats at doses of 2.5, 25 and 250 mg/kg body weight for 90 days. The results revealed no clinical signs of toxicity at all doses. Body weights, feed and water intake, and relative organ's weights of all groups treated showed no significant difference from control groups. No significant changes on hematological parameters were observed at any concentrations. In comparison with the control groups, significant increase in levels of ALT, AST and ALP at doses of 25 and 250 mg/kg body weight was demonstrated, however, all values were not significantly different after 14 days of recovery. Cholesterol levels in both males and females were significantly lower than the controls. No treatment-related changes were macroscopically and microscopically found. Our study could suggest that oral administration of dried *C. siamensis* bile as high as 250 mg/kg body weight for 90 days showed no systematic toxicity in male and female Sprague Dawley rats.

Keywords: crocodile bile, sub-chronic oral toxicity, *Crocodylus siamensis*

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Crocodylus siamensis (siamese crocodile) bile is consisted of cholestanol, coprocholic acid, coprocheno- deoxycholic acid and other derivatives including 3-oxo-7α, 12α-dihydroxy-5β-cholestanoic acid, 3α, 7α, 12α-trihydroxy-5β cholestanoic acid, 7-oxo-3α, 12α-dihydroxy-5β-cholestanoic acid, 3-oxo-7α, 12α-dihydroxy-5α-cholestanoic acid, chenodeoxy-cholic acid, 5α-cholestan-3α, 7α, 12α, 26-tertrol, 5β-cholestan-3α, 7α, 12α, 25-tertrol, ursodeoxycholic acid and 5α-cholic acid.[1] Many cultures have used crocodile bile as treatment for sepsis, hemorrhage, trauma and lung diseases[2] in their indigenous traditional medicine. Less scientific studies have, however, been investigated for its toxic effects.

The acute oral toxicity test revealed that siamese crocodile bile classified in GHS (*Globally Harmonized System* of Classification and Labelling of Chemicals) category 4 (300 mg/kg < LD50 < 2,000 mg/kg)[3,4] Further toxicity study on a long-term use is essential. Therefore, this study was to investigate sub-chronic oral toxicity of dried *C. siamensis* bile.

Materials and Methods

Preparation of dried *Crocodylus siamensis* bile:

Bile of *C. siamensis* was obtained from gallbladders of 100 siamese crocodiles at Ayutthaya crocodile farm, Tha Rua district, Phra Nakhon Si Ayutthaya, Thailand. Dried bile was processed according to the patented production of Thai patent application No. 0901001231, Kasetsart University. In brief, the bile was collected after removal of fat tissues and further 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[5,6].

Preparation of Animals:

Fifty male and fifty female Sprague Dawley rats with body weights of 200±20 grams were obtained from National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand. The animals were housed in stainless steel cages with standard diet (Perfect Companion Group Co., Ltd., Thailand) and hyperchlorinated water (5-7 ppm). The standard environmental conditions were temperature at 22±3°C, 30-70% relative humidity and 12 hours dark-light cycle. The study was approved by National Laboratory Animal Center Animal Care and Use Committee (NLAC-ACUC), Mahidol University; Thailand. (IACUC Approval No. RA2013-02, 2013 October 28).

Sub-chronic Toxicity Test

The sub-chronic toxicity was conducted using OECD 408 with modification[7]. The rats were randomized and divided into 5 groups, consisting of 10 female and 10 male for each group.

According to our acute oral toxicity study, the doses of dried bile used in this study were 2.5, 25 and 250 milligrams/kilograms body weight (mg/kg BW). The 5 groups were as followed; group 1: vehicle control (distilled water), group 2: low dose (2.5 mg/kg body weight), group 3: medium dose (25 mg/kg body weight), group 4: high dose (250 mg/kg body weight) and group 5: satellite group (250 mg/kg body weight).

The dried bile was dissolved in distilled water and orally given to each group of rats daily for 90 days while the control group received distilled water. All groups were daily observed for any signs of toxicity, mortality and changes in the body weights. Food and water consumption were recorded weekly. After 90 days, group 5 was continuously observed for a total of 14 days without administration of the dried bile.

For hematology and clinical biochemistry analysis, all animals were kept fasted for 15-18 hours prior to blood sample collection. The animals were euthanized on the last day of each experimental group using CO_2 inhalation. Blood samples were collected via cardiac puncture. Hematology and clinical biochemistry analysis were performed using automation analyzer (CELL-DYN 3700, Abbott Laboratory, USA and Cobas c111 automate blood analyzer, Roche, Switzerland).

The positions, shapes, sizes and colours of internal organs were observed for gross lesions. Liver, kidneys, lung, adrenals, testes (male), epididymides (male), uterus (female), ovaries (female), heart, spleen, brain and thymus were trimmed and weighed to determine relative organ's weights. Histopathological examinations were performed on liver, kidneys, lung, heart and spleen[8].

Statistical analysis:

Quantitative results were expressed as mean ± standard deviation. Data was statistically analyzed by Kolmogorov-Smirnov[a] and Levene's test for normality and homogeneity of variances. Statistical significance between the control group and each treatment group was determined by ANOVA and Mann Whitney U test using SPSS® Statistic software version 18.0.0. P values ≤ 0.05 were considered significant.

Results

The 90-day study of orally given dried *C. siamensis* bile at all doses did not reveal any changes related to mortality and clinical signs of toxicity (data not shown). The body weights and relative organ weights (g/100g BW) of both sexes of each experimental group were not significantly different from the control groups, as shown in Tables 1 and 2.

Hematological parameters of male and female rats of all tested groups were not significantly changed as compared with the controls (Tables 3 and 4). For blood chemistry analysis, significant elevation of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) levels were found in male and female rats orally gavage with

the dried bile at the doses of 250 mg/kg BW. The AST level was also significantly higher in both sexes of rats given 25 mg/kg BW of the dried bile than the controls. Cholesterol level of male group receiving the dried bile at concentration of 250 mg/kg BW was significantly lower than the control group, while significant reduction of cholesterol was shown in female groups orally administration with 25, 250 mg/kg BW of dried bile and the satellite group (Tables 5 and 6). In addition, all groups did not show significant histopathological changes (data not shown).

Table 1 Body Weights of rats receiving dried *C.siamensis* bile for 90 days

Sex	Dose of *C.siamensis* Bile (mg/kg BW/day)	Body Weight (g)*					
		Day 0	Day 7	Day 14	Day 28	Day 60	Day 90
Male	Control	217.90±10.55	267.70±11.82	316±16.30	385.70±19.08	459.40±29.21	516.70±35.14
	2.5	213.20±7.42	261.10±8.31	310.80±9.45	376.20±11.58	453.30±12.91	510.50±16.23
	25	215.60±8.29	268.30±13.58	317.00±14.41	382.30±16.61	455.20±20.99	507.60±20.24
	250	221.10±8.69	262.50±14.28	305.40±34.60	370.20±37.61	447.00±21.04	492.56±24.53
	250 (Satellite)	220.40±8.11	265.40±10.77	319.00±10.59	379.50±11.71	448.60±19.49	503.80±24.86
Female	Control	190.80±6.63	206.20±7.79	221.30±9.49	243.20±10.75	272.30±12.47	284.50±11.52
	2.5	188.10±6.24	205.40±6.77	224.00±6.91	246.60±7.60	272.20±14.37	284.50±19.11
	25	194.50±5.04	210.00±7.13	229.60±6.92	253.00±8.01	277.11±11.98	295.33±15.86
	250	190.60±5.87	203.20±6.03	223.30±10.57	243.00±12.94	269.40±15.46	283.20±13.62
	250 (Satellite)	191.80±10.76	203.60±11.94	223.00±12.63	240.30±15.64	261.20±15.49	273.20±17.29

*mean ± standard deviation

Table 2 Relative organ's weights of rats receiving dried *C.siamensis* bile for 90 days

Sex	Dose of *C.siamensis* Bile (mg/kg BW/day)	Relative Organ's Weight per 100 g Body Weight (g)*					
		Liver	Kidney Lt.	Kidney Rt.	Heart	Lung	Spleen
Male	Control	2.82±0.21	0.34±0.02	0.34±0.02	0.32±0.02	0.32±0.03	0.19±0.01
	2.5	2.80±0.18	0.32±0.01	0.33±0.02	0.30±0.02	0.31±0.02	0.18±0.01
	25	2.84±0.20	0.33±0.03	0.34±0.02	0.32±0.03	0.31±0.02	0.18±0.01
	250	2.83±0.07	0.34±0.02	0.34±0.02	0.31±0.02	0.31±0.02	0.19±0.01
	250 (Satellite)	2.82±0.17	0.35±0.03	0.35±0.01	0.32±0.02	0.33±0.02	0.19±0.02
Female	Control	2.38±0.10	0.32±0.02	0.32±0.02	0.35±0.02	0.47±0.04	0.23±0.01
	2.5	2.32±0.15	0.31±0..03	0.31±0.02	0.35±0.02	0.45±0.06	0.23±0.03
	25	2.38±0.16	0.31±0.01	0.32±0.02	0.35±0.01	0.45±0.05	0.22±0.01
	250	2.35±0.16	0.32±0.01	0.33±0.01	0.35±0.01	0.46±0.04	0.25±0.02
	250 (Satellite)	2.32±0.14	0.33±0.02	0.33±0.02	0.35±0.02	0.46±0.06	0.25±0.02

*mean ± standard deviation

Table 3 Effect of dried *C. siamensis* bile on hematological values of male rats

Parameters	Dose of *C. siamensis* Bile (mg/kg BW/day)*				
	Control	2.5	25	250	250 (Satellite)
RBC (10^6/µl)	8.80±0.26	8.81±0.41	9.01±0.26	8.97±0.27	8.78±0.36
Hemoglobin (g/dl)	14.90±0.37	15.00±0.59	15.20±0.44	15.1±0.56	14.6±0.49
Hematocrit (%)	49.30±1.25	49.30±1.77	50.00±1.42	50.50±1.85	48.00±1.36
MCV (fl)	56.00±0.72	56.10±1.08	55.80±0.72	55.80±0.72	55.10±1.28
MCH (pg)	16.90±0.30	17.00±0.28	16.90±0.32	16.80±0.31	16.80±0.34
MCHC (g/dl)	30.20±0.24	30.40±0.27	30.50±0.26	30.40±0.27	30.30±0.46
PLT (10^3/µl)	853.00±50.86	885.00±50.75	919.00±56.43	916.00±88.65	912.00±26.33
WBC (10^6/µl)	7.50±0.94	7.51±1.69	8.29±1.49	8.00±0.86	8.02±1.02

*mean ± standard deviation

Table 4 Effect of dried *C. siamensis* bile on hematological values of female rats

Parameters	Dose of *C. siamensis* Bile (mg/kg BW/day)*				
	Control	2.5	25	250	250 (Satellite)
RBC (10^6/µl)	8.36±0.47	8.49±0.36	8.22±0.21	8.45±0.32	8.56±0.33
Hemoglobin (g/dl)	14.90±0.62	15.00±0.68	14.60±0.34	15.00±0.52	14.80±0.23
Hematocrit (%)	49.20±1.74	49.50±2.01	48.20±1.16	49.20±1.56	49.20±0.88
MCV (fl)	58.90±1.60	58.30±0.74	58.60±0.43	58.30±0.81	58.50±1.23
MCH (pg)	17.80±0.35	17.70±0.38	17.80±0.16	17.80±0.24	17.50±0.50
MCHC (g/dl)	30.30±0.35	30.30±0.44	30.40±0.11	30.40±0.23	30.30±0.36
PLT (10^3/µl)	839.00±43.15	835.00±91.00	843.00±67.06	852.00±35.19	849.00±85.66
WBC (10^6/µl)	6.39±0.90	6.45±0.30	6.48±1.97	6.48±0.82	6.47±0.52

*mean ± standard deviation

Table 5 Effect of dried *C. siamensis* bile on biochemical values of male rats

Parameters	Dose of *C. siamensis* Bile (mg/kg BW/day)*				
	Control	2.5	25	250	250 (Satellite)
Total protein (g/dl)	8.20±0.63	8.20±1.13	8.30±0.84	8.40±0.24	8.00±0.34
Cholesterol (mg/dl)	131.00±8.26	126.00±8.65	124.00±6.62	121.00±8.51**	118.00±8.93
Triglyceride (mg/dl)	86.00±6.55	86.00±4.76	83.00±5.93	80.00±7.46	85.00±5.44
ALT (U/l)	45.00±6.73	46.00±6.46	53.00±8.69	60.00±6.83**	44.00±3.09
AST (U/l)	72.00±3.59	73.00±4.16	87.00±7.01**	91.00±3.81**	69.00±6.64
ALP (U/l)	69.00±7.48	68.00±7.86	73.00±6.97	88.00±5.55**	67.00±3.28
Total bilirubin (mg/dl)	0.09±0.01	0.10±0.02	0.10±0.02	0.10±0.01	0.09±0.02
Albumin (g/dl)	5.40±0.39	5.40±0.54	5.40±0.37	5.40±0.34	5.30±0.16
Globulin (g/dl)	2.80±0.26	2.80±0.64	2.90±0.49	2.90±0.12	2.90±0.28
Creatinine (mg/dl)	0.30±0.05	0.30±0.04	0.30±0.05	0.30±0.05	0.30±0.03
BUN (mg/dl)	20.60±2.42	20.60±2.08	20.70±1.12	20.60±0.34	20.60±1.25
Uric acid (mg/dl)	2.80±0.30	2.70±0.22	2.40±0.73	2.60±0.60	2.60±0.43
Glucose (mg/dl)	203.00±20.74	195.00±38.11	190.00±48.77	185.00±25.37	188.00±39.03

*mean ± standard deviation

**$p \leq 0.05$

Table 6 Effect of dried *C. siamensis* bile on biochemical values of male rats

Parameters	Dose of *C. siamensis* Bile (mg/kg BW/day)*				
	Control	2.5	25	250	250 (Satellite)
Total protein (g/dl)	8.60±1.14	8.50±0.34	8.50±0.97	8.60±0.97	8.40±0.32
Cholesterol (mg/dl)	151.00±8.27	143.00±8.05	137.00±6.47**	124.00±7.04**	130.00±9.29**
Triglyceride (mg/dl)	62.00±4.57	62.00±2.23	60.00±3.54	59.00±2.13	63.00±7.25
ALT (U/l)	54.00±6.90	54.00±5.66	61.00±3.44	70.00±5.19*	52.00±3.05
AST (U/l)	108.00±4.17	108.00±4.37	114.00±7.30**	116.00±3.16**	102.00±3.11
ALP (U/l)	54.00±7.69	54.00±3.83	63.00±9.03	65.00±6.02**	59.00±4.62
Total bilirubin (mg/dl)	0.13±0.02	0.12±0.02	0.14±0.04	0.14±0.02	0.13±0.02
Albumin (g/dl)	6.00±0.65	5.90±0.35	6.00±0.49	6.00±0.27	5.80±0.18
Globulin (g/dl)	2.60±0.58	2.60±0.53	2.50±0.47	2.60±0.29	2.50±0.30
Creatinine (mg/dl)	0.40±0.07	0.40±0.08	0.40±0.07	0.40±0.08	0.40±0.05
BUN (mg/dl)	22.90±2.69	22.80±1.66	22.80±0.98	22.80±1.57	22.80±0.47
Uric acid (mg/dl)	2.80±0.66	2.80±0.55	2.70±0.25	2.80±0.45	2.70±0.54
Glucose (mg/dl)	176.00±34.37	144.00±23.66	138.00±21.70	138.00±8.60	139.00±19.65

*mean ± standard deviation
**$p \leq 0.05$

Discussion

The present study was performed to assess the sub-chronic toxicity of dried *C. siamensis* bile in male and female Sprague Dawley rats. There were no significant difference in body weight, feed and drinking water consumptions between the control and treatment groups. The toxic outcomes on vital body organs of all doses given, which were liver, kidney, heart, lung and spleen, were not observed either macroscopic or microscopic examinations as compared with the control group. In addition, no significant variations were found in relative organ weights It could suggest that dried *C. siamensis* bile at concentrations used in this study did not have any effects on the growth of rats.

There were no changes to RBC, Hemoglobin, Hematocrit, MCV, MCH, MCHC, Platelet and WBC of all groups of animals tested. This study, therefore demonstrated that dried *C. siamensis* bile is not toxic to blood parameters.

Biochemistry results showed significant elevation in ALT and ALP levels at the dose of 250 mg/kg BW and AST levels at doses of 25 mg/kg BW and 250 mg/kg BW, however, those levels in the satellite groups were not significantly different from the controls. Moreover, histological findings did not demonstrate any damage in liver in all groups. Therefore, in this study, it could suggest that dried *C. siamensis* bile orally administration at the dose as high as 250 mg/kg BW for 90 days did not have permanent effects to the liver. Significant reduction of cholesterol levels in both male and female rats was observed. It might possibly be due to an effect on elimination of cholesterol, one of the bile functions[9].

Conclusion

Oral administration of dried *C.siamensis* bile for 90 days at the doses of 2.5, 25 and 250 mg/kg body weight revealed no toxicities in male and female rats. Thus, no observed adverse effect level (NOAEL) of Siamese crocodile bile for Sprague Dawley rats could be equal to 250 mg/kg body weight per day.

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References

1. China Pharmacopoeia Commission. Pharmacopoeia of the People′s Republic of China. Beijing, China: Chemical Industry Press; 1997.

2. Picheinsutthorn C, Jeerawong V. Animal medicinal materials. In: Hand out of Thai medicinal pharmacy Vol. III. Bangkok: Amarin Press; 2003. p. 150-167.
3. Organization for Economic Co-operation and Development (OECD). OECD guidelines for the testing of chemicals No. 420: acute oral toxicity – fixed dose procedure [online]. 2001; [cited 2020 Apr 26]; [14 screens]. Available from: URL: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl420.pdf.
4. Srimangkornkaew P, Praduptong A, Siruntawineti J, Chaeychomsri S, Chaeychomsri W. Acute oral toxicity of Crocodylus siamensis bile in sprague dawley rats. Bull Dept Med Sci 2020; 62(1): 16-25.
5. Chaeychomsri W, Siruntawineti J, Osotsila I. Process suitable for the preservation of crocodile gall bladder for adding value to product. In: Proceeding of the 35th congress on science and technology of Thailand; 2009 Oct 15–17; Chonburi, Thailand. Bangkok, Thailand: The Science Society of Thailand Under the Patronage of His Majesty the King; 2009. p. 569-576.
6. Chaeychomsri W, Siruntawineti J. Process for preparation of crocodile blood powder and its product, Thai Petty Patent No. 5074. Bangkok, Thailand: Kasetsart University; 2006.
7. Organization for Economic Co-operation and Development (OECD). OECD guidelines for the testing of chemicals test No. 408: repeated dose 90-days oral toxicity study in rodents [online]. 2018; [cited 2019 Oct 20]; [16 screens]. Available from: URL: https://www.oecd-ilibrary.org/docserver/9789264070707-en.pdf?expires=1589271810&id=id&accname=guest&checksum=8BF6058D341C0664D65F9A5B948F0A99.
8. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 8th ed. London, England: Elsevier; 2018.
9. Hofmann AF. The continuing importance of bile acids in liver and intestinal disease. Arch Intern Med 1999; 159(22): 2647-58.

พิษกึ่งเรื้อรังของน้ำดีจระเข้สายพันธุ์ไทย *(Crocodylus siamensis)* ในหนูแรทสายพันธุ์ Sprague Dawley

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บทคัดย่อ น้ำดีจระเข้สายพันธุ์ไทยถูกใช้เป็นส่วนผสมในยาแผนโบราณมาเป็นเวลานาน แต่การศึกษาความปลอดภัยค่อนข้างจำกัด การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาความเป็นพิษกึ่งเรื้อรังของน้ำดีจระเข้สายพันธุ์ไทยในหนูแรท สายพันธุ์ Sprague Dawley โดยทำการป้อนสารที่ความเข้มข้น 2.5, 25 และ 250 มิลลิกรัม/กิโลกรัมน้ำหนักตัว ทุกวันต่อเนื่องกันเป็นเวลา 90 วัน ผลการทดสอบพบว่าน้ำดีจระเข้สายพันธุ์ไทย ไม่พบการเปลี่ยนแปลงของน้ำหนักตัว การบริโภคอาหารและน้ำดื่ม รวมถึงน้ำหนักสัมพัทธ์ของอวัยวะสำคัญ ค่าโลหิตวิทยาไม่มีความแตกต่างที่สัมพันธ์กับขนาดสารทดสอบทุกความเข้มข้น จากการทดสอบค่าเคมีคลินิกในเลือดพบว่าค่า ALT AST และ ALP ของหนูแรทที่ป้อนน้ำดีจระเข้ที่ความเข้มข้น 25 และ 250 มิลลิกรัม/กิโลกรัมน้ำหนักตัว เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญภายหลังหยุดให้น้ำดีจระเข้เป็นเวลา 14 วัน ขณะที่ค่า cholesterol ลดลง ส่วนการตรวจสอบทางพยาธิวิทยาไม่พบความเปลี่ยนแปลงในหนูทั้ง 2 เพศ ผลการศึกษาแสดงว่าน้ำดีจระเข้สายพันธุ์ไทยที่ความเข้มข้น 250 มิลลิกรัม/กิโลกรัมน้ำหนักตัวเมื่อป้อนให้หนูแรทสายพันธุ์ Sprague Dawley นั้นมีความปลอดภัย

คำสำคัญ: น้ำดีจระเข้, การทดสอบความเป็นพิษกึ่งเรื้อรัง, *Crocodylus siamensis*