The Chronic Oral Toxicity Testing of Siamese Crocodile *(Crocodylus siamensis)* Bile in Sprague Dawley Rats

Passaraporn Srimangkornkaew[1] Kittigan Suwannasaroj[1] Jindawan Siruntawineti[2] Win Chaeychomsri[2] and Sudawan Chaeychomsri[3]

[1]*National Laboratory Animal Center, Mahidol University, Nakhon Pathom 73170*

[2]*Department of Zoology, Faculty of Sciences, Kasetsart University, Bangkok 10900*

[3]*Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaen Saen Campus, Nakhon Pathom 73140, Thailand*

ABSTRACT In Thailand, Siamese crocodile *(Crocodylus siamensis)* is an economic animal and dried form of Siamese crocodile bile was used as traditional medicine and exported for a long time but safety information including toxicity testing is limited. For previous study, an oral acute and sub chronic toxicity study was conducted and the results showed that the Siamese Crocodile *(Crocodylus siamensis)* bile was classified in GHS category 4, the LD50 cut off at 500 mg/kg body weight and the no observed adverse effect level (NOAEL) of Siamese crocodile bile was considered to be 250 mg/kg body, respectively. Nevertheless, the study to provide information the possible major toxic effects, indicate target organs and the possibility of accumulation and estimation of a no-observed-adverse-effect level (NOAEL) for a long enough period to realize for chronic effects have not been carried out. Therefore, this study was done to support information as above for Siamese crocodile bile in Sprague Dawley rats in compliance with OECD Guidelines for the testing of chemicals 452, Chronic Toxicity Studies. The Siamese crocodile bile was not shown treatment related mortality and clinical signs of toxicity in Sprague Dawley rats including the treatment-relates changes were not observed in necropsy findings and histopathological finding in both sexes based on a result. The significantly different results of hematological tests and clinical biochemistry tests were not considered as a treatment-related toxicity effect but these results were recovered in recovery period (28 days). Thus, the NOAEL of Siamese crocodile bile in chronic oral toxicity studies were considered to be 250 mg/kg body weight per day for Sprague Dawley rats.

Keywords: Crocodile bile, Chronic oral toxicity, OECD Guidelines 452, *Crocodylus siamensis*

Corresponding author E-mail: fscijws@ku.ac.th

Received: 14 August 2020 *Revised: 14 January 2021* *Accepted: 5 February 2021*

Introduction

The crocodile product such as internal organ, blood, skin and hides can be sold at higher price than other animals that are allowed for commercial aquaculture farm.[1] Crocodile bile is by-product of the crocodile industry. In many countries such as China, Hong Kong and Taiwan *etc.*, people used crocodile bile for an ingredient of traditional medicine. Siamese crocodile bile was exported for a long time.[2] Crocodile bile can be used for drugs solvent and treatment of sepsis, hemorrhage and trauma.[3] The compositions of crocodile bile are 0.8% of Cholestanol, 68% of Coprocholic acid, 9% of Coprochenodeoxycholic acid and derivative includes; 10% of 3-oxo-7α, 12α-dihydroxy-5β-cholestanoic acid, 8% of 3α, 7α, 12α-trihydroxy-5βcholestanoic acid, 7-oxo-3α, 12α-dihydroxy-5β-cholestanoic acid, 3-oxo-7α, 12α-dihydroxy-5α-cholestanoic acid, chenodeoxycholic acid, 5α-cholestan-3α, 7α, 12α, 26-tertrol, 5β-cholestan-3α, 7α, 12α, 25-tertrol, Ursodeoxycholic acid and 5α-cholic acid.[4] Ursodeoxycholic acid (UDCA) was used to be useful for treating chronic hepatic and gallstones disease.[5] Moreover, bile acids are physiological detergents for intestinal nutrient absorption and biliary secretion of lipids, toxic metabolites, and xenobiotics.[6] And it has been implicated in the regulation of all the key enzymes involved in cholesterol homeostasis.[7] Toxic bile acids may cause inflammation, apoptosis, and cell death. Aqueous extracts from *Crocodylus siamensis* bile (AE-CB) had effect on cell growth, cell cycle and apoptosis of SMMC-7721 cells.[8] Bile extract from crocodile gallbladder had inhibited cells growth significantly, and the cell cycle was arrested in G1 phase. Bile extract induced QBC939 cell apoptosis. These results provide significant insight into the anticarcinogenic action of bile extract on cholangiocarcinoma cell.[9] For information of oral toxicity according to OECD Guidelines for the testing of chemicals 423[10], Siamese crocodile bile was classified in GHS category 4 or estimated the LD50 cut off at 500 mg/kg body weight in Sprague Dawley rats.[2, 11] However, long term study of oral toxicological of crocodile bile has been carried out of Siamese crocodile bile in Sprague Dawley rats in compliance with OECD Guidelines for the testing of chemicals 408, Repeated Dose 90-day Oral Toxicity Study in Rodents only.[12, 13] Therefore, the purpose of the present study is to provide information on the possible major toxic effects, indicate target organs and the possibility of accumulation and estimate of a no-observed-adverse-effect level (NOAEL) of Siamese crocodile bile in Sprague Dawley rats in compliance with OECD Guidelines for the testing of chemicals 452, Chronic Toxicity Studies.[14]

Materials and Methods

Collection of *Crocodylus siamensis* gall bladder

Gall bladders of Siamese crocodile *(Crocodylus siamensis)* were collected from Ayutthaya crocodile farm, Tha Rua district, Phra Nakhon Si Ayutthaya, Thailand.

Preparation of dried *Crocodylus siamensis* bile

Process for dried Siamese crocodile bile production of Thai patent application No. 0901001231, Kasetsart University was performed as following. The gall bladders without fat tissue outside were surface sterile with 70% ethyl alcohol and the Siamese crocodile bile were collected in tray and freeze-drying using freeze dryer (Lyomaster, USA) for 36 hours. The freeze-dried bile was powdered by graining and stored in a sterilized and dry jar. The Siamese crocodile bile powder was kept at 4°C until further use.[15, 16]

Dose preparation

The 3 dose levels of Dried Siamese crocodile bile were 2.5, 25 and 250 mg/kg body weight and freshly mixed with distilled water prior to administration.

Preparation of animals

Healthy 6-8 weeks, 100 male and 100 female, Sprague Dawley rats of body weight range 200 g ± 20% were obtained from Office of Laboratory Animal Production, National Laboratory Animal Center, Mahidol University, Thailand. The animals were kept under standard conditions 12 hours light, 12 hours dark at 22±3 °C and 30-70% relative humidity. The animals were housed in stainless steel cages with feed and 5-7 ppm chlorinated water ad libitum. All the animals were acclimatized for at least 5 days prior to the study. Guidelines of "Guide for the care and use of laboratory animals" were strictly followed throughout the study.[17] The study was approved by National Laboratory Animal Center Animal Care and Use Committee (NLAC-ACUC), Mahidol University, Thailand, for all experimental protocols (RA2013-02).

Experimental design

The chronic oral toxicity studies were complied with OECD Guidelines for the testing of chemicals 452, Chronic Toxicity Studies.[14]

100 male and 100 female Sprague Dawley rats were used and randomized into 5 groups (20 animal per sexes per group). The 5 groups were consisted of group 1 – control group (vehicle control; distilled water), group 2 – low dose, group 3 – middle dose and group 4 – high dose (2.5, 25 and 250 mg/kg body weight of Siamese crocodile bile, respectively) and group 5 – satellite group (once daily at 250 mg/kg body weight of Siamese crocodile bile for 365 days and no administrated by gavage for the following 28 days). The Siamese crocodile bile was prepared with distilled water prior to administration. The dosage of administration by gavage for 365 days to each animal was calculated based on the body weight of animal prior to administrate at a constant volume not exceed 1 ml per 100 g body weight.

The animal health observations and mortality were observed outside the home cage at least once a day, at the same times. Animal body weights, feed and water consumption including signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, and change in skin and fur) were performed weekly.

Necropsy examination

On the last day, all animals were kept for overnight (15-18 hours) fasting (feed but not water) prior to blood sample collection. The animals were euthanized using CO_2 inhalation.[18] Blood samples were collected via cardiac puncture for hematological and clinical biochemistry analysis.

After blood samples collection, all animals were sacrificed. The positions, shapes, sizes and colours of internal organs were evaluated.[2] Liver, kidneys, lung, heart and spleen were removed and trimmed to visually detect gross lesions and weighed to determine relative organ's weights per 100 g body weight. Histopathological examinations were performed on liver, kidneys, lung, heart and spleen and compared between control and high dose group.

Statistical analysis

Quantitative results were expressed as mean ± standard deviation. The obtained data were statistically analyzed by Kolmogorov-Smirnov and Levene's test[19] for normality and homogeneity of variances. For parametric statistics, all data were compared between the vehicle control group and each treatment group by 2-sided Dunnett or Dunnett's T3 test, using ANOVA analysis. For non-parametric statistics, the data were compared between the vehicle control group and each treatment group by Mann Whitney U test. A significantly differences were considered at 0.05 levels with SPSS® Statistic software version 18.0.0.

Results

The Siamese crocodile bile were not shown treatment related mortality and clinical signs of toxicity in Sprague Dawley rats. The feed and water consumptions including body weights of all animals that were measured throughout the study, were showed normal. The data of body weight was presented in Fig 1 and Fig 2 for male and female animal, respectively. Besides that, the clinical signs in their skin and fur, eyes and mucous membranes, respiratory and behavior pattern were not found in control and treatment groups of animals. The other observations as tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were not showed too. The result of hematological tests that shown in Table 1, at high dose group (250 mg/kg body weight of Siamese crocodile bile) have the means of platelet higher than the control group for both sexes within normal range for Sprague Dawley rats of National Laboratory Animal Center (NLAC) (1,069.0±224.1). For clinical biochemistry test that presented in Table 2, at high dose group were higher than the control group for both sexes where male animals have high the mean of ALT (alanine amino transferase), AST (aspartate amino transferase) and ALP (alkaline phosphatase) but female animals have only high the mean of ALT (alanine amino transferase) and AST (aspartate amino transferase). Nevertheless, these effects of hematological and clinical biochemistry tests were able to recovery in 14 days.

The organ's weight was calculated in the relative weights per 100 g animal body weight that showed the relative weight was not related with dose level what used in this study, represented in Table 3.

For pathology in this study can divide two sections: gross/microscopic finding and macroscopic finding. In first section, the observation of male animal was found as watery diarrhea without any lesions of intestine, colon gas distension, thymus petechial hemorrhage, lung consolidation, hydronephrosis, simple cortical cyst contained with clearly fluid and proteinaceous plug. The observation of female animal was found gas distension in small intestine, subcutaneous mass at axillary area, petechial hemorrhage, calcification deposit a focal in renal medullar and hydrometra. In second section, the lesions of kidney, heart, liver, spleen and lung were observed in control and high dose group.

Kidney: glomerulosclerosis, focal inflammation, hyaline cast, tubular regenerative, tubular dilation, single to few glomerulonephritis, single to few glomerulo necrosis, focal tubular necrosis, predominate focal calcification and chronic progressive nephropathy (CPN).

Heart: various stage of cardiomyopathy and degenerative to necrosis.

Liver: various distribution of macrovesicle, micro vesicle fatty change, hepatocyte atrophy, giant cell infiltration, hepatocyte hypertrophy and focus eosinophilia.

Spleen: mesothelial hyperplasia and mild amyloidosis.

Lung: eosinophil accumulation frequency occur in perivascular and peribronchiole, histiocytosis, arterial calcification, foreign bodies, perivascular necrosis and osseous metaplasia. These lesions were noted incidental finding, congenital defect, spontaneous, ageing lesion and the effect of carbon dioxide euthanization.[20]

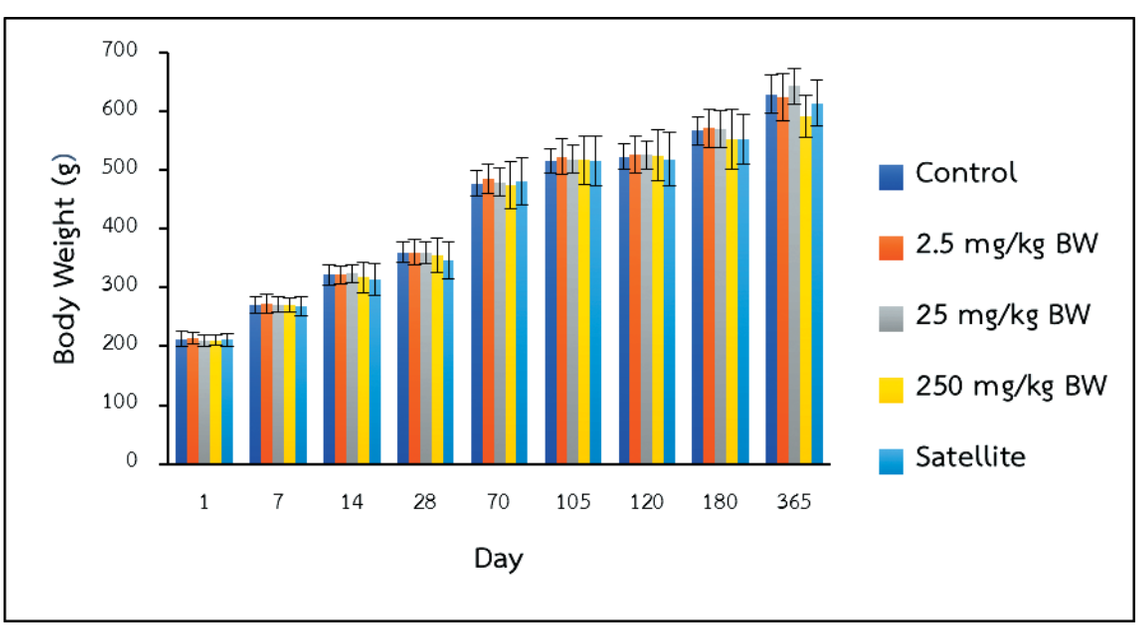

Figure 1 The body weight of male Sprague Dawley rats

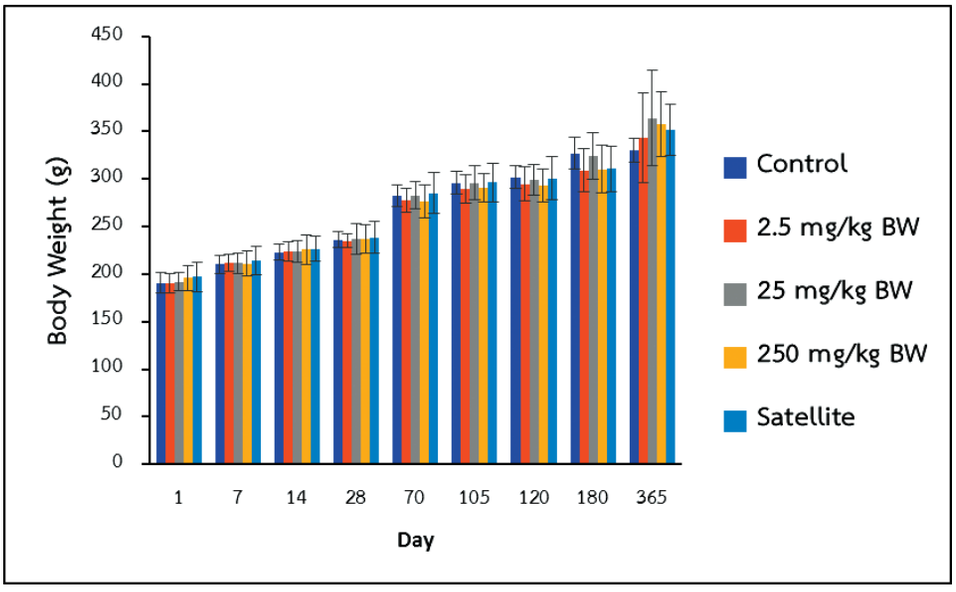

Figure 2 The body weight of female Sprague Dawley rats

Table 1 Hematological analysis of Sprague Dawley rats

Sex	Parameters	Mean of parameters ± standard deviation				
		Control	2.5 mg/kg BW	25 mg/kg BW	250 mg/kg BW	Satellite
Male	RBC (10^6/µl)	8.41±0.45	8.27±0.26	8.32±0.28	8.30±0.31	8.32±0.55
	HBG (g/dl)	15.9±0.79	15.6±0.55	15.7±0.43	15.4±0.55	15.6±1.23
	HCT (%)	49.6±2.44	49.1±1.44	49.2±1.59	48.9±1.55	48.5±2.63
	MCV (fl)	59.0±1.07	59.4±1.07	59.2±1.70	58.9±1.56	58.3±2.18
	MCH (pg)	19.0±1.35	18.9±0.54	18.9±0.34	18.6±0.62	18.8±0.80
	MCHC (g/dl)	32.2±2.00	31.8±0.81	31.9±1.03	31.6±0.37	32.2±1.24
	PLT (10^3/µl)	890±109.20	918±95.96	967±104.99	1067±126.04*	1012±78.59*
	WBC (106/µl)	6.43±1.32	7.12±1.90	5.34±1.31	6.18±1.63	7.01±1.42
Female	RBC (10^6/µl)	8.10±0.35	7.93±0.48	8.04±0.32	8.06±0.29	7.96±0.35
	HBG (g/dl)	16.0±0.56	15.6±0.76	15.8±0.59	15.9±0.53	15.8±1.01
	HCT (%)	50.2±1.73	49.3±2.32	49.2±1.70	48.7±1.52	48.2±2.11
	MCV (fl)	62.0±0.91	61.8±1.22	61.3±1.96	60.5±2.38	60.6±2.02
	MCH (pg)	19.7±0.72	19.6±0.50	19.7±0.65	19.7±0.41	19.8±1.01
	MCHC (g/dl)	31.9±0.77	31.7±0.52	32.2±0.97	32.6±1.21	32.8±2.11
	PLT (10^3/µl)	772±71.38	811±106.12	819±63.27	840±61.01*	799±91.49
	WBC (10^6/µl)	5.07±1.39	6.04±1.93	4.49±1.31	4.93±0.96	5.01±1.09

Note: * The mean difference is significant at the 0.05 levels of control group

Table 2 Clinical biochemistry analysis of Sprague Dawley rats

Sex	Parameters	Mean of parameters ± standard deviation				
		Control	2.5 mg/kg BW	25 mg/kg BW	250 mg/kg BW	Satellite
Male	TP (g/dl)	7.6±0.85	7.7±1.34	7.9±0.63	7.6±1.02	7.4±0.60
	CHOL (mg/dl)	174±56.04	145±44.94	150±32.74	146±36.89	169±29.63
	TRIGL (mg/dl)	111±43.92	102±32.19	99±20.72	96±24.09	112±30.65
	ALT (U/l)	60±16.54	59±11.38	73±20.06	119±72.84*	85±40.76
	AST (U/l)	74±13.84	83±19.60	81±21.96	98±29.66*	86±33.58
	ALP (U/l)	66±14.90	61±19.06	83±16.99	116±24.78*	92±35.23
	ALB (g/dl)	4.9±0.45	5.0±0.84	4.7±0.59	4.5±0.44	4.4±0.50
	GLO (g/dl)	2.8±0.59	2.7±0.68	3.1±0.33	3.1±0.92	3.0±0.72
	CREA (mg/dl)	0.3±0.05	0.3±0.06	0.4±0.05	0.3±0.08	0.4±0.06
	BUN (mg/dl)	18.7±3.31	18.0±3.36	18.9±1.82	18.6±1.96	18.6±4.19
	UA (mg/dl)	2.8±1.13	2.8±0.89	2.7±1.04	2.8±0.65	2.7±0.73
	GLU (mg/dl)	185±50.74	188±45.29	179±36.93	178±31.04	178±40.80
Female	TP (g/dl)	8.0±0.68	7.5±0.77	7.9±0.80	8.0±0.91	7.5±0.69
	CHOL (mg/dl)	138±39.24	124±13.36	117±22.39	117±16.49	132±25.47
	TRIGL (mg/dl)	92±41.38	72±21.97	71±19.54	74±21.39	92±46.53
	ALT (U/l)	54±5.47	58±7.04	59±6.10	60±4.10*	54±7.68
	AST (U/l)	81±9.02	89±7.64	91±4.15	94±3.72*	80±8.72
	ALP (U/l)	53±7.17	57±4.97	57±3.97	60±6.39	54±3.99
	ALB (g/dl)	5.7±0.86	5.6±0.47	5.6±0.49	5.7±0.62	5.6±0.36
	GLO (g/dl)	2.3±0.33	2.3±0.31	2.2±0.40	2.3±0.40	2.2±0.91
	CREA (mg/dl)	0.4±0.08	0.3±0.05	0.4±0.06	0.3±0.05	0.3±0.05
	BUN (mg/dl)	16.9±1.72	16.5±2.17	16.1±2.22	16.2±2.17	16.3±1.90
	UA (mg/dl)	2.8±0.89	2.3±0.35	2.3±0.69	2.3±0.59	2.8±1.07
	GLU (mg/dl)	184±35.01	165±16.54	164±33.51	163±29.10	185±60.32

Note: *The mean difference is significant at the 0.05 levels of control group

Table 3 Organ's weight of Sprague Dawley rats

Sex	Parameters	Mean of parameters ± standard deviation				
		Control	2.5 mg/kg BW	25 mg/kg BW	250 mg/kg BW	Satellite
Male	Liver	2.74±0.19	2.85±0.22	2.77±0.27	3.03±0.27*	3.01±0.26*
	Kidney Rt.	0.34±0.02	0.34±0.02	0.34±0.03	0.37±0.02*	0.38±0.03*
	Kidney Lt.	0.34±0.02	0.34±0.02	0.34±0.03	0.38±0.03*	0.38±0.03*
	Heart	0.31±0.02	0.30±0.02	0.30±0.02	0.31±0.01	0.32±0.03
	Lung	0.31±0.03	0.32±0.05	0.31±0.03	0.32±0.02	0.35±0.09
	Spleen	0.18±0.02	0.18±0.02	0.19±0.02	0.21±0.02*	0.20±0.02
Female	Liver	2.28±0.15	2.43±0.25	2.22±0.16	2.49±0.21*	2.51±0.21*
	Kidney Rt.	0.32±0.02	0.34±0.03	0.32±0.03	0.34±0.02	0.34±0.03
	Kidney Lt.	0.31±0.02	0.33±0.02	0.31±0.02	0.33±0.02	0.34±0.03
	Heart	0.34±0.02	0.37±0.03	0.34±0.03	0.36±0.03	0.34±0.03
	Lung	0.45±0.05	0.46±0.07	0.44±0.06	0.45±0.05	0.42±0.07
	Spleen	0.21±0.02	0.23±0.03	0.21±0.02	0.24±0.02*	0.22±0.03

Discussion

The present study carried out the toxicity level assessment of Siamese crocodile bile in the dose levels 2.5, 25, and 250 mg/kg body weight were no mortality and clinical signs cause of toxicity in animals. The general clinical observation results of all animals including body weights, Feed and water consumption were normal.

The pathological evaluation concluded that, there were no treatment-related change in both sexes. Simple kidney cysts are fluid-filled sacs that form in the renal cortex which do not enlarge the kidneys, replace their normal structure, or cause reduced kidney function like other cyst type that the causes for simple kidney cysts are more common as age[21] or obstruction of tubules, or deficiency of blood supply to the kidneys may play a role, diverticula sacs that form on the tubules may detach and become simple kidney cysts. The role of genetic factors in the development of simple kidney cysts has not been studied.[22] Proteinaceous plugs are commonly noted as postmortem change resulting from agonal secretion of accessory sex gland fluids during euthanasia[23] The treatment-relates changes were not observed in necropsy findings and histopathological finding in both sexes based on a result. The significantly different results of hematological tests and clinical biochemistry tests were not considered as a treatment-related toxicity effect because that can recovered in recovery period (28 days).

From the issue of Herbal and Dietary Supplements related hepatotoxicity is a growing concern particularly with the evidence in both the United States and Europe that the use of these products appears to be increasing.[24] Herbal and Dietary Supplements have been responsible for causing liver injury and the major implicated agents include anabolic steroids, green tea extract, and multi-ingredient nutritional supplements (MINS).[25, 26] Based on the results as declared above, there were no pathology-related effects from any dose of the Siamese crocodile bile in Sprague Dawley rats as the Siamese crocodile bile was administered once a day by oral gavage for 365 days with 28 days for recovery period.

Conclusion

In summary, the Siamese crocodile bile doses not produce any toxic signs or obvious symptoms of chronic toxicity. Thus, the no observed adverse effect level (NOAEL) of Siamese crocodile bile was considered to be 250 mg/kg body weight per day for Sprague Dawley rats.

Acknowledgement

The study was supported from National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand for equipment and facility all over this research. The authors would like to acknowledge Faculty of Science, Kasetsart University for providing knowledge and experience during the course of this research.

References

1. Praduptong A, Siruntawineti J, Chaeychomsri S, Srimangkornkaew P, Chaeychomsri W. Acute oral toxicity testing of siamese crocodile *(Crocodylus siamensis)* oil in wistar rats. Biosci Discov 2018; 9(3): 409-15.
2. Srimangkornkaew P, Praduptong A, Siruntawineti J, Chaeychomsri S, Chaeychomsri W. The evaluation of acute oral toxicity testing of siamese crocodile (Crocodylus siamensis) bile in Sprague Dawley rats in compliance with OECD guideline 423. Biosci Discov 2018; 9(4): 469-73.
3. Picheinsutthorn C, Jeerawong, V. Animal medicinal materials. In: Hand out of Thai medicinal pharmacy. 3rd ed. Bangkok, Thailand: Amarin Press; 2003. p. 150-167.
4. Pharmacopoeia Commission of PRC, editors. Pharmacopoeia of the People's republic of China. Beijing, China: Chemical Industry Press; 1997.
5. Maddison JE, Page SW, Church DB, editors. Small animal clinical pharmacology. In: Alexander JG, Jill EM, Grant G, editors. Gastrointestinal drugs. 2nd ed. London, United Kingdom: Saunders Ltd.; 2008. p. 469-497.
6. Chiang JY. Bile acid metabolism and signaling. Compr Physiol 2013; 3(3): 1191-212.
7. The Metabolomics Innovation Centre (TMIC). Showing metabocard for Coprocholic acid (HMDB0000601). [online]. 2005; [cited 2018 Nov 26]; [12 screens]. Available from: URL: https://hmdb.ca/metabolites/HMDB0000601.

8. Song W, Li SS, Qiu PP, Shen DY, Tian L, Zhang QY, et al. Apoptosis induced by aqueous extracts of crocodile bile in human heptacarcinoma SMMC-7721. Appl Biochem Biotechnol 2013; 170(1): 15-24.
9. Kang JH, Zhang WQ, Song W, Shen DY, Li SS, Tian L, et al. Apoptosis mechanism of human cholangiocarcinoma cells induced by bile extract from crocodile. Appl Biochem Biotechnol 2012; 166(4): 942-51.
10. Organisation for Economic Co-operation and Development (OECD). OECD guidelines for the testing of chemicals, test No. 423: acute oral toxicity – acute toxic class method. [online]. 2001; [cited 2019 Oct 20]; [14 screens]. Available from: URL: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf.
11. 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.
12. Organisation for Economic Co-operation and Development (OECD). OECD guidelines for the testing of chemicals, test No. 408: repeated dose 90-day oral toxicity study in rodents. [online]. 1998; [cited 2019 Oct 20]; [10 screens]. Available from: URL: https://www.oecd.org/env/ehs/testing/E408_1998.PDF.
13. Srimangkornkaew P, Praduptong A, Siruntawineti J, Chaeychomsri S, Chaeychomsri W. Sub-chronic oral toxicity of Crocodylus siamensis bile in sprague dawley rats. Bull Dept Med Sci 2020; 62(2): 50-8.
14. Organisation for Economic Co-operation and Development (OECD). OECD guidelines for the testing of chemicals, test No. 452: chronic toxicity studies. [online]. 2009; [cited 2019 Oct 20]; [16 screens]. Available from: URL: https://www.oecd.org/env/ehs/testing/E452_2009.pdf.
15. 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.
16. Chaeychomsri W, Siruntawineti J. Process for preparation of crocodile blood powder and its product, Thai Petty Patent No. 5074. Bangkok, Thailand: Kasetsart University; 2006.
17. National Research Council. Guide for the care and use of laboratory animals. 8th ed. Washington, DC: The National Academies Press; 2011.
18. Institutional Animal Care and Use Committee. Guidelines for euthanasia of rodent fetuses and neonates. [online]. 2013; [cited 2019 Jun 18]; [3 screens]. Available from: URL: https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b4_rodent_euthanasia_pup.pdf.
19. Mooi E, Sarstedt M. Hypothesis testing & ANOVA. In: A concise guide to market research. 3th Edition. Berlin, Heidelberg: Springer; 2011. p. 113-160.
20. Tanaka H, Suzuki S, Ninomiya F, Matsubara K, Hakoi K. Spontaneous thymoma in a 10-week-old sprague-dawley rat. J Toxicol Pathol 2012; 25(1): 37-40.

21. Torres V, Grantham J, editors. Cystic diseases of the kidney. In: Brenner B, editor. The Kidney. 6th ed. Philadelphia, United States: Saunders Elsevier 2008; p. 1451-1453.
22. National Kidney and Urologic Diseases Information Clearinghouse (NKUDIC). Simple kidney cysts. [online] 2012; [Cited 2019 (Jun 18]; [4 screens]. Available from: URL: https://www.niddk.nih.gov/-/media/Files/Kidney-Disease/Simple_Kidney_Cysts_508.pdf.
23. National Toxicity Program. Urinary bladder-proteinaceous plug. [online]. 2016; [cited 2019 Oct 18]; [2 screens] Available from: URL: https://ntp.niehs.nih.gov/nnl/urinary/urinary_bladder/protplug/.
24. Navarro VJ, Lucena MI. Hepatotoxicity induced by herbal and dietary supplements. Semin Liver Dis 2014; 34(2): 172-93.
25. Bailey RL, Gahche JJ, Lentino CV, Dwyer JT, Engel JS, Thomas PR. Dietary supplement use in the United States, 2003-2006. J Nutr 2011; 141(2): 261-6.
26. Navarro VJ, Khan I, Björnsson E, Seeff LB, Serrano J, Hoofnagle JH. Liver injury from herbal and dietary supplements. Hepatology 2017; 65(1): 363-73.

การทดสอบความเป็นพิษเรื้อรังทางปากของน้ำดีจระเข้สายพันธุ์ไทย *(Crocodylus siamensis)* ในหนูแรทสายพันธุ์ Sprague Dawley

ภัสสราภรณ์ ศรีมังกรแก้ว[1] กฤติกาญจน์ สุวรรณสโรช[1] จินดาวรรณ สิรันทวิเนติ[2] วิน เชยชมศรี[2] และสุดาวรรณ เชยชมศรี[3]

[1]*ศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล จังหวัดนครปฐม 73170*

[2]*ภาควิชาสัตววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กรุงเทพฯ 10900*

[3]*ศูนย์ปฏิบัติการวิจัยและเรือนปลูกพืชทดลอง มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน นครปฐม 73140*

บทคัดย่อ จระเข้สายพันธุ์ไทยเป็นสัตว์เศรษฐกิจที่สำคัญของประเทศ น้ำดีจระเข้สายพันธุ์ไทยถูกนำมาใช้เป็นยาแผนโบราณและส่งขายต่างประเทศมาเป็นเวลานาน แต่การศึกษาด้านความปลอดภัยรวมถึงการทดสอบความเป็นพิษนั้นมีจำกัด สำหรับการศึกษาก่อนหน้านี้ได้มีการทดสอบความเป็นพิษเฉียบพลันและเป็นพิษกึ่งเรื้อรังทางปาก และจากผลการทดสอบพบว่าน้ำดีจระเข้สายพันธุ์ไทย จัดอยู่ในระบบการจัดกลุ่มสารเคมี การติดฉลาก และการแสดงรายละเอียดบนเอกสารข้อมูลความปลอดภัยสากลหมวดที่ 4 มี LD50 ที่ 500 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง และระดับความปลอดภัยที่ไม่เกิดผลกระทบในการใช้น้ำดีจระเข้สายพันธุ์ไทยในหนูแรทสายพันธุ์ Sprague Dawley คือ 250 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัวสัตว์ทดลอง ตามลำดับ อย่างไรก็ตาม การศึกษาเพื่อให้ข้อมูลเกี่ยวกับความเป็นพิษ อวัยวะเป้าหมาย การสะสมและประเมินระดับความปลอดภัยในระยะเวลาที่นานพอที่จะทำให้เกิดผลกระทบแบบเรื้อรังนั้นไม่เคยมีการศึกษามาก่อน ดังนั้น การศึกษานี้จึงจัดทำขึ้นเพื่อสนับสนุนข้อมูลข้างต้นสำหรับน้ำดีจระเข้สายพันธุ์ไทยในหนูแรทสายพันธุ์ Sprague Dawley ตามวิธีการมาตรฐาน OECD Guideline หมายเลข 452 โดยผลการทดสอบพบว่า น้ำดีจระเข้สายพันธุ์ไทยไม่ก่อให้เกิดความเป็นพิษในสัตว์ทดลอง รวมทั้งไม่พบการเปลี่ยนแปลงทางพยาธิวิทยาที่มีความแตกต่างกันระหว่างกลุ่มควบคุมและกลุ่มทดสอบที่ระดับ 250 มิลลิกรัม/กิโลกรัมน้ำหนักตัวในสัตว์ทดลองทั้ง 2 เพศ สำหรับผลการวิเคราะห์เลือดทางโลหิตวิทยา และเคมีคลินิก พบว่าตัวแปรที่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ แต่สามารถกลับสู่ภาวะปกติได้ภายในระยะเวลา 28 วันหลังหยุดให้สารทดสอบ จากผลการศึกษาทั้งหมดจึงสามารถสรุปได้ว่า น้ำดีจระเข้สายพันธุ์ไทยไม่มีความเป็นพิษต่อสัตว์ทดลองที่ระดับ 250 มิลลิกรัม/กิโลกรัมน้ำหนักตัว เมื่อได้รับต่อเนื่องเป็นระยะเวลา 365 วัน

คำสำคัญ: น้ำดีจระเข้, การทดสอบความเป็นพิษเรื้อรัง, OECD Guidelines หมายเลข 452, *Crocodylus siamensis*