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Original Article

Chronic Toxicity of *Thunbergia laurifolia* Lindl. Extract

Songpol Chivapat*,#
Pranee Chavalittumrong*
Aimmanas Attawish*
Jaree Bansiddhi*
Songpol Padungpat*

Abstract

***Thunbergia laurifolia* Lindl., or *rang chuet,* is a medicinal plant that is used for the treatment of poisoning with toxic substances. However, the toxicological data for this plant are not complete. Therefore, a chronic toxicity study of *rang chuet* extract (TLE) was undertaken in six groups of Wistar rats. The control group (group 1) was given 10 ml/kg of distilled water per day orally. The five experimental groups (groups 2-6) were orally administered TLE at doses of 20, 200, 1,000, 2,000 and 2,000 mg/kg/day for six months, respectively, which were equivalent to 1, 10, 50, and 100 times the therapeutic dose. Group 6 (2000R) was added to the study recovery group after 14 days of TLE discontinuation. The results revealed that TLE did not affect the body weight, food consumption, behavior or general health of the animals. TLE did not produce cumulative toxic signs and fatal effects. Male rats receiving TLE at the dose of 2,000 mg/kg/day and the 2000R group had a significant decrease in RBCs, including a significant difference in red cell indices, but these were within the normal range. Female rats receiving TLE at doses of 1,000 and 2,000 mg/kg/day showed significant increases in WBCs. Both sexes of the rats treated with 2,000 mg/kg of TLE had a significant increase in bilirubin levels; however, these were within the normal range. TLE did not produce any histological alterations of the visceral organs in any group of rats. However, the results suggest that hematological and clinical chemistry values should be monitored during prolonged use of TLE.**

***Key words:* *Thunbergia laurifolia*, chronic toxicity**

Introduction

Thunbergia laurifolia Lindl. is a large woody climber belonging to the Family Acanthaceae[1] including Thunbergiaceae.[2] Its leaves are ovate or oblong-lanceolate, almost entire repand-toothed, more or less glossy. Inflorescences as a raceme are pendulous in the axil of leaves. The flowers are large, about 5-8 cm in diameter, and pale blue in color. Capsules are 1.5 cm wide, with beaks up to 3 cm long.[1] The plants are common in tropical and mixed forest throughout the country, often cultivated as an ornamental plant or trained over walls or trellises. This plant has many local names in Thai such as *rang chuet*, *rang yen* or *nam nong*.[3] The leaves and stems of *T. laurifolia* are used as a detoxifying agent in cases of poisoning and also as an antipyretic in Thai traditional medicine.[4] Phytochemical constituents of *T. laurifolia* leaves are phenolic, carotenoid and chlorophyll compounds, besides caffeic acid and apigenin are found to be major

*Medicinal Plant Research Institute, Department of Medical Sciences, Tiwanont Road, Muaeng District, Nonthaburi Province, Thailand 11000.
Corresponding author. Tel: (662) 589-9850-9; Fax (662) 589-9866.
E-mail address: schivapat@yahoo.com

constituents in *T. laurifolia* aqueous extract.[5]

The water extract of *T. laurifolia* has been demonstrated to reduce cholinergic effects and decrease mortality in rats treated with folidol, an organophosphate insecticide.[6] Tea from the leaves of this plant has also been shown to effectively reduce insecticide residue levels in the blood circulation of agriculturists.[7] Pramyoththin *et al*. reported that the aqueous extract of *T. laurifolia* leaves was found to possess hepatoprotective activity against ethanol-induced liver injury both *in vitro* and *in vivo*.[8] The above-mentioned reports suggest that *rang chuet* has the potential to be used as an herbal detoxifying agent. However, little toxicological data, especially in laboratory animals, have been reported. A 28-day repeated dose toxicity study of the *T. laurifolia* aqueous extract at an oral dose of 500mg/kg revealed that the extract did not cause any histological changes of the organs; however, it may affect some hematological values and kidney weights.[9] In this study, we investigated the chronic toxicity of *T. laurifolia* water extract in rats in order to obtain more toxicological information to assure the safety of this plant.

Materials and Methods

Preparation of *Thunbergia laurifolia* extract

Leaves of *Thunbergia laurifolia* Lindl. (Acanthaceae) were collected from a cultivated area in Pitsanulok Province, Thailand. A voucher specimen (Bansiddhi 45-11) was deposited at the Botanical Laboratory, Medicinal Plant Research Institute, Nonthaburi Province. The botanical identification was determined by following the description of Backer and Bakhuizen[1] and compared with authentic specimens (Lakshnakara 1368) at the Bangkok herbarium (BK), Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

The leaves of *Thunbergia laurifolia* were first washed, then dried in a hot-air oven at 40°C and pulverized into coarse powder. Three hundred grams of *T. laurifolia* powder were extracted with 300 ml of distilled water by reflux method twice, each for 2 hours. The filtrate was evaporated at low pressure using a rotary evaporator to obtain dried extract at a yield of 23.283 percent (w/w). Prior to dosing in animals, the dried extract of *T. laurifolia* (TLE) was then dissolved in distilled water and adjusted to the desired concentrations for toxicity study.

Laboratory animals

Ninety male Wistar rats weighing 200-220 g and an equal number of female rats weighing 170-190 g were purchased from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom Province and used in this study. The animals were housed in a hygienic conventional animal room of the laboratory animal center, Department of Medical Sciences, where the environment of the room was maintained at 25±1°C with 60 percent humidity and 12 hours of a light-dark cycle. Prior to the chronic toxicity study, the animals were acclimatized in that environment for two weeks and they were reared on a commercially pelleted diet and clean water provided ad lib. This study was approved by the Institutional Animal Care and Use Committee, Department of Medical Sciences.

Chronic toxicity study

Wistar rats were randomly divided into five groups of 15 animals/sex each. ***Group 1*** received 10 ml/kg per day of distilled water. ***Groups 2* to 6** were orally administered a solution of TLE at doses of 20, 200, 1000, 2000 and 2000 mg/kg/day respectively, for six months, which were equivalent to 1, 10, 50 and 100 times the therapeutic dose (5 grams of dried *T. laurifolia* powder/day/person). After the six-month period of TLE treatment, only **Group 6** (2000R) was reared for two more weeks without TLE administration in order to observe the recovery or delayed effects of the extract. During the period of the experiment, body weight and food consumption were recorded weekly and the rats were closely observed for general appearance, behavior and signs of abnormality. At the end of the 180-day treatment period, the animals were fasted for 16 hours before being sacrificed with diethyl ether inhalation. Laparotomy was performed and blood samples were collected from the

inferior vena cava of each animal for analyzing hematological and clinical chemistry values by using the automatic hematological analyzer Cell Dyn®3500 and the automatic chemistry analyzer Hitachi®912, respectively. The hematological parameters examined were hematocrit (Hct), erythrocytes (RBCs), hemoglobin, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cells (WBC), neutrophils, eosinophils, lymphocytes, monocytes, basophils and platelets. The clinical chemistry parameters measured were alkaline phosphatase (ALP), alanine transminase (ALT), aspartate transminase (AST), total protein, albumin, bilirubin, blood urea nitrogen (BUN), creatinine, glucose, uric acid, triglyceride, cholesterol, sodium, potassium and chloride ion. Necropsy was then performed to determine the gross lesions of various visceral organs. Brain, heart, lung, liver, kidney, stomach, spleen, testis, uterus and adrenal glands were weighed by using a Mettler Toledo® PB 153 balance. The organ weights were calculated into relative organ weights (g/100g body weight). The visceral organs were then preserved in 10 percent buffered formalin and subsequently subjected to histological process for preparing tissue slides stained with hematoxylin and eosin (H&E). Tissue slides were histopathologically examined by a veterinary pathologist.

Statistical data analysis

Body weight, food intake, relative organ weight, hematological and clinical chemistry values were statistically analyzed using the SPSS program (version 11.0). One-way ANOVA was performed and the data were tested for homogeneity of variance by Levene test. Bonferroni test was used in cases of equal variance, whereas Dunnette's T3 test was applied for unequal variance in multiple comparisons. The incidence of histopathological lesions was analyzed by Fisher's exact test and the statistical significance of all data was set at $p < 0.05$.

Results

Effects on body weight, food consumption and physical appearance

The average body weight and food consump-

Table 1 Body weight (g) and percentage relative organ weight of male rats receiving *Thunbergia laurifolia* extract for 6 months

Parameters	Dose of *Thunbergia laurifolia* extract (mg/kg/day)					
	Control N= 15	20 N = 15	200 N = 15	1000 N = 15	2000 N = 15	2000R N = 15
Initial body weight	222.48 ±14.77	221.19 ± 9.65	222.93 ±15.33	223.29 ±14.03	226.95 ±16.40	223.90 ±11.28
Final body weight	576.89 ±73.73	562.50 ±50.93	554.41 ±51.48	559.43 ±62.27	568.29 ±67.96	553.25 ±46.03
Brain	0.38 ± 0.05	0.39 ± 0.03	0.39 ± 0.02	0.39 ± 0.04	0.38 ± 0.04	0.38 ± 0.03
Heart	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.23 ± 0.03	0.24 ± 0.03
Lung	0.28 ± 0.03	0.30 ± 0.04	0.29 ± 0.02	0.29 ± 0.03	0.28 ± 0.03	0.30 ± 0.03
Liver	2.23 ± 0.14	2.30 ± 0.20	2.25 ± 0.17	2.31 ± 0.17	2.41 ± 0.16	2.35 ± 0.19
Stomach	0.37 ± 0.04	0.39 ± 0.04	0.37 ± 0.03	0.38 ± 0.04	0.39 ± 0.04	0.37 ± 0.04
Spleen	0.16 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.18 ± 0.02*	0.17 ± 0.01
Right kidney	0.21 ± 0.02	0.21 ± 0.01	0.22 ± 0.02	0.23 ± 0.01	0.24 ± 0.01*	0.23 ± 0.02
Left kidney	0.20 ± 0.02	0.20 ± 0.01	0.21 ± 0.02	0.22 ± 0.02	0.23 ± 0.01*	0.22 ± 0.02*
Right testis	0.52 ± 0.04	0.54 ± 0.07	0.51 ± 0.10	0.51 ± 0.12	0.49 ± 0.06	0.53 ± 0.06
Left testis	0.53 ± 0.04	0.54 ± 0.07	0.52 ± 0.09	0.50 ± 0.14	0.51 ± 0.08	0.53 ± 0.06
Right adrenal	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.006 ± 0.001
Left adrenal	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
Bladder	0.036 ± 0.013	0.036 ± 0.007	0.037 ± 0.011	0.031 ± 0.003	0.033 ± 0.006	0.029 ± 0.006

Note : The values are expressed as mean ± SD.

*Significantly different from the control group ($p<0.05$).

Table 2 Body weight (g) and percentage relative organ weight of female rats receiving *Thunbergia laurifolia* extract for 6 months

Parameters	Dose of *Thunbergia laurifolia* extract (mg/kg /day)					
	Control N = 15	20 N = 15	200 N = 15	1000 N = 15	2000 N = 15	2000R N = 15
Initial body weight	175.13 ± 8.41	175.31 ± 5.30	173.66 ± 9.83	175.51 ± 7.86	178.39 ±11.80	172.41 ± 9.95
Final body weight	313.39 ±26.00	327.75 ±30.93	315.03 ±24.96	33.85 ±29.48	32.25 ±35.92	311.44 ±34.77
Brain	0.65 ± 0.05	0.63 ± 0.07	0.65 ± 0.06	0.65 ± 0.05	0.63 ± 0.07	0.64 ± 0.06
Heart	0.30 ± 0.03	0.29 ± 0.03	0.29 ± 0.03	0.28 ± 0.02	0.29 ± 0.03	0.28 ± 0.02
Lung	0.40 ± 0.03	0.41 ± 0.06	0.41 ± 0.04	0.40 ± 0.04	0.41 ± 0.04	0.42 ± 0.05
Liver	2.32 ± 0.14	2.28 ± 0.20	2.33 ± 0.17	2.35 ± 0.17	2.49 ± 0.16	2.46 ± 0.19
Stomach	0.53 ± 0.07	0.49 ± 0.07	0.50 ± 0.05	0.51 ± 0.06	0.53 ± 0.07	0.52 ± 0.07
Spleen	0.21 ± 0.02	0.21 ± 0.04	0.21 ± 0.04	0.22 ± 0.02	0.24 ± 0.03	0.21 ± 0.02
Right kidney	0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.27 ± 0.02	0.27 ± 0.03*	0.27 ± 0.02
Left kidney	0.24 ± 0.02	0.23 ± 0.03	0.24 ± 0.02	0.25 ± 0.02	0.27 ± 0.03*	0.25 ± 0.02
Right adrenal	0.012 ± 0.001	0.012 ± 0.003	0.013 ± 0.002	0.013 ± 0.003	0.011 ± 0.002	0.012 ± 0.003
Left adrenal	0.013 ± 0.003	0.013 ± 0.002	0.014 ± 0.003	0.014 ± 0.002	0.013 ± 0.002	0.014 ± 0.003
Bladder	0.031 ± 0.013	0.028 ± 0.007	0.032 ± 0.011	0.030 ± 0.003	0.029 ± 0.006	0.028 ± 0.006
Uterus	0.280 ± 0.127	0.232 ± 0.060	0.256 ± 0.061	0.234 ± 0.056	0.207 ± 0.044	0.237 ± 0.068

Note : The values are expressed as mean ± SD.

***Significantly different from the control group ($p<0.05$).**

Table 3 Hematological values of male rats receiving *Thunbergia laurifolia* extract for 6 months

Parameters	Dose of *Thunbergia laurifolia* extract (mg/kg/day)					
	Control N = 15	20 N = 15	200 N = 15	1000 N = 15	2000 N = 15	2000R N = 15
Hematocrit (%)	47.25 ± 2.10	47.03 ± 1.24	46.91 ± 2.99	47.30 ± 2.58	47.42 ± 3.05	46.11 ± 1.23
RBCs ($\times 10^6$ cells/mm^3)	9.01 ± 0.43	9.02 ± 0.44	8.93 ± 0.70	8.62 ± 0.40	8.35 ± 0.53*	8.25 ± 0.22*
Hemoglobin (g/dl)	15.50 ± 0.63	15.47 ± 0.42	15.34 ± 0.87	15.42 ± 0.76	15.38 ± 0.87	15.09 ± 0.41
MCV ($\times m^3$/red cell)	52.50 ± 2.18	52.23 ± 2.33	52.61 ± 2.17	54.86 ± 1.91*	56.84 ± 1.69*	55.87 ± 1.39*
MCH (pg/red cell)	17.23 ± 0.80	17.20 ± 0.81	17.23 ± 0.73	17.88 ± 0.56	18.43 ± 0.57*	18.28 ± 0.54*
MCHC (g/dl RBC)	32.81 ± 0.43	32.93 ± 0.31	32.73 ± 0.47	32.61 ± 0.31	32.44 ± 0.48	32.73 ± 0.61
WBCs ($\times 10^3$ cells/mm^3)	3.79 ± 0.58	2.80 ± 1.10	2.83 ± 0.87	3.10 ± 0.71	3.54 ± 1.30	3.71 ± 1.08
Neutrophils (%)	16.23 ± 6.94	24.49 ± 9.24	23.87 ± 5.82	21.56 ± 9.02	15.99 ± 5.03	20.42 ± 10.17
Eosinophils (%)	1.87 ± 0.81	1.74 ± 0.48	1.35 ± 0.38	1.58 ± 0.65	1.25 ± 0.35	1.44 ± 0.39
Lymphocytes (%)	71.52 ± 10.16	61.33 ± 11.29	60.71 ± 9.09	64.45 ± 10.56	70.63 ± 9.45	67.76 ± 12.10
Monocytes (%)	7.39 ± 2.11	8.79 ± 2.50	10.59 ± 4.20	9.14 ± 3.94	8.67 ± 4.29	7.47 ± 2.39
Basophils (%)	3.00 ± 1.48	3.65 ± 2.01	3.47 ± 1.59	3.27 ± 1.38	3.47 ± 1.39	2.90 ± 0.73
Platelets ($\times 10^3$cells/mm^3)	968.93 ± 115.41	930.14 ± 180.80	956.20 ± 137.80	981.37 ± 100.96	976.83 ± 159.26	956.67 ± 65.15

Note : The values are expressed as mean ± SD.

***Significantly different from the control group ($p<0.05$).**

tion of male and female rats receiving TLE at any dose were not significantly different from those of their corresponding control groups throughout the six-month period (data not shown). There was no abnormality in physical appearance and behavior in any group of rats receiving TLE as well as in the control group. TLE-treated rats did not show any toxic signs and mortality throughout the experiment. Initial and final body weights of each group are expressed in Tables 1 and 2.

Table 4 Hematological values of female rats receiving *Thunbergia laurifolia* extract for 6 months

Parameters	Dose of *Thunbergia laurifolia* extract (mg/kg/day)					
	control	20	200	1000	2000	2000R
	N = 15	N = 15	N = 15	N = 15	N = 15	N = 15
Hematocrit (%)	45.57 ± 2.48	45.70 ± 1.44	46.31 ± 2.77	46.38 ± 2.08	46.10 ± 2.78	47.06 ± 2.10
RBCs ($\times 10^6$ cells/mm^3)	7.87 ± 0.45	8.02 ± 0.36	7.97 ± 0.50	7.88 ± 0.36	7.78 ± 0.43	8.01 ± 0.49
Hemoglobin (g/dl)	15.19 ± 0.75	15.18 ± 0.42	15.31 ± 0.79	15.26 ± 0.61	15.14 ± 0.92	15.58 ± 0.60
MCV ($\times m^3$/red cell)	57.89 ± 1.03	56.99 ± 1.56	58.11 ± 1.10	58.85 ± 1.55	59.24 ± 2.00	58.84 ± 1.77
MCH (pg/red cell)	19.33 ± 0.75	18.93 ± 0.53	19.23 ± 0.48	19.36 ± 0.39	19.46 ± 0.52	19.48 ± 0.64
MCHC (g/dl RBCs)	33.37 ± 0.99	33.22 ± 0.41	33.09 ± 0.66	32.91 ± 0.41	32.86 ± 0.36	33.12 ± 0.42
WBCs ($\times 10^3$ cells/mm^3)	1.71 ± 0.41	2.07 ± 0.45	2.33 ± 0.69	2.77 ± 0.54*	2.46 ± 0.84*	2.23 ± 0.70
Neutrophils (%)	18.76 ± 6.00	18.58 ± 5.32	16.00 ± 3.49	22.17 ± 8.95	20.57 ± 12.82	20.26 ± 9.52
Eosinophils (%)	1.33 ± 0.69	1.32 ± 0.74	1.26 ± 0.69	1.24 ± 0.70	0.97 ± 0.43	1.28 ± 0.69
Lymphocytes (%)	70.45 ± 8.27	66.93 ± 9.02	74.35 ± 5.83	69.18 ± 10.82	70.23 ± 13.01	68.18 ± 13.08
Monocytes (%)	7.10 ± 3.27	8.27 ± 5.98	6.47 ± 5.08	5.31 ± 2.99	5.95 ± 2.51	8.49 ± 4.37
Basophils (%)	2.36 ± 1.00	2.45 ± 1.34	1.93 ± 0.93	2.11 ± 1.18	2.27 ± 0.87	1.77 ± 0.84
Platelets ($\times 10^3$ cells/mm^3)	921.33 ± 82.02	926.07 ± 73.75	949.51 ± 146.13	916.60 ± 81.77	995.43 ± 69.71	941.07 ± 117.86

Note : The values are expressed as mean ± SD.

***Significantly different from the control group ($p<0.05$).**

Table 5 Clinical chemistry values of male rats receiving *Thunbergia laurifolia* extract for 6 months

Parameters	Dose of *Thunbergia laurifolia* extract (mg/kg/day)					
	Control	20	200	1000	2000	2000R
	N = 15	N = 15	N = 15	N = 15	N = 15	N = 15
ALP (U/L)	58.13 ± 8.64	57.40 ± 8.86	54.00 ± 12.76	56.47 ± 10.22	56.27 ± 7.27	53.20 ± 4.68
ALT (U/L)	33.20 ± 6.70	41.53 ± 24.65	35.27 ± 15.41	26.93 ± 9.25	24.60 ± 3.60	30.53 ± 7.94
AST (U/L)	79.73 ± 9.11	94.93 ± 34.90	85.87 ± 21.32	74.87 ± 11.78	8.60 ± 8.17	82.20 ± 10.50
Total protein (g/dl)	6.71 ± 0.29	6.85 ± 0.25	6.91 ± 0.46	6.80 ± 0.34	6.90 ± 0.36	6.89 ± 0.21
Albumin (g/dl)	4.39 ± 0.19	4.49 ± 0.12	4.45 ± 0.11	4.60 ± 0.13*	4.62 ± 0.17*	4.43 ± 0.12
Bilirubin (mg/dl)	0.06 ± 0.03	0.09 ± 0.03	0.07 ± 0.03	0.08 ± 0.02	0.10 ± 0.04*	0.7 ± 0.03
BUN (mg/dl)	18.09 ± 2.23	18.10 ± 2.65	19.59 ± 2.53	19.25 ± 1.95	19.54 ± 2.05	18.31 ± 2.20
Creatinine (mg/dl)	0.75 ± 0.08	0.73 ± 0.07	0.78 ± 0.07	0.74 ± 0.07	0.75 ± 0.06	0.73 ± 0.05
Glucose (mg/dl)	172.24 ± 29.35	165.33 ± 23.77	181.00 ± 37.13	189.43 ± 48.37	185.62 ± 37.08	172.43 ± 18.75
Uric acid (mg/dl)	1.99 ± 1.55	1.91 ± 1.19	2.37 ± 1.53	2.75 ± 1.85	3.12 ± 1.81	1.87 ± 0.89
Triglyceride (mg/dl)	131.90 ± 44.29	108.04 ± 24.87	112.43 ± 38.70	103.17 ± 22.27	99.15 ± 27.92	135.33 ± 38.04
Cholesterol (mg/dl)	71.63 ± 14.26	64.83 ± 12.52	65.06 ± 9.25	64.86 ± 10.94	71.08 ± 20.56	76.59 ± 13.98
Sodium (mmol/l)	145.87 ± 1.18	147.73 ± 1.98	147.33 ± 1.88	147.24 ± 1.83	147.07 ± 1.39	147.73 ± 1.39
Potassium (mmol/l)	5.23 ± 0.97	4.85 ± 1.05	4.95 ± 1.07	5.72 ± 1.06	6.35 ± 1.22	5.25 ± 0.80
Chloride (mmol/l)	107.93 ± 1.71	109.47 ± 1.85	109.13 ± 1.25	109.60 ± 1.96	109.27 ± 1.67	109.87 ± 1.68*

Note : The values are expressed as mean ± SD.

***Significantly different from the control group ($p<0.05$).**

Effects on relative weight of organs

Male rats receiving TLE at the dose of 2,000 mg/kg/day showed significant increases in relative spleen and kidney weights when compared with the control group (Table 1). Female rats receiving TLE at the highest dose had a significant increase in relative kidney weight compared with the controls (Table 2).

Table 6 Clinical chemistry values of female rats receiving *Thunbergia laurifolia* extract for 6 months

Parameters	Dose of *Thunbergia laurifolia* extract (mg/kg/day)					
	Control	20	200	1000	2000	2000R
	N = 15	N = 15	N = 15	N = 15	N = 15	N = 15
ALP (U/L)	23.80± 7.01	22.33 ± 6.50	25.27 ± 6.74	21.00± 5.45	20.73 ± 5.70	26.07± 5.92
ALT (U/L)	29.47± 9.74	28.40 ±12.60	25.20 ± 8.43	25.53±11.67	19.20 ± 3.40*	23.00± 6.33
AST (U/L)	84.20±24.89	89.33 ±35.70	81.27 ± 15.27	74.20±15.13	70.07 ±11.16	78.20±11.03
Total protein (g/dl)	7.17± 0.26	7.15 ± 0.30	7.07 ± 0.35	7.00± 0.37	7.14 ± 0.43	7.13± 0.28
Albumin (g/dl)	5.16± 0.26	5.20 ± 0.20	5.13 ± 0.25	5.11± 0.16	5.23 ± 0.23	5.05± 0.21
Bilirubin (mg/dl)	0.10± 0.04	0.11 ± 0.04	0.11 ± 0.03	0.13± 0.04	0.15 ± 0.05*	0.12± 0.03
BUN (mg/dl)	24.49± 4.55	24.01 ± 4.12	23.92 ± 3.58	25.49± 4.39	25.69 ± 6.08	24.59± 4.72
Creatinine (mg/dl)	0.86± 0.08	0.89 ± 0.11	0.89 ± 0.11	0.82± 0.09	0.85 ± 0.16	0.89± 0.14
Glucose (mg/dl)	129.75±11.73	123.56 ±14.68	133.35 ± 28.73	124.26±11.51	130.51 ±20.46	131.00±18.95
Uric acid (mg/dl)	1.11± 0.89	1.37 ± 1.18	1.46 ± 1.23	1.09± 1.05	1.29 ± 1.37	1.90± 0.91*
Triglyceride (mg/dl)	82.54±32.47	88.95 ±31.84	74.32 ± 28.39	57.45±20.92	59.95 ±23.10	73.20±26.31
Cholesterol (mg/dl)	70.85±12.93	66.01 ±11.33	63.95 ± 14.15	64.17±16.69	63.97 ± 9.56	67.13±19.08
Sodium (mmol/l)	146.40± 1.12	147.13 ± 1.60	147.47 ± 1.46	147.47± 1.12	147.02 ± 1.03	148.53± 1.36*
Potassium (mmol/l)	4.26± 0.61	4.34 ± 1.25	4.17 ± 1.37	4.05± 0.93	4.13 ± 0.49	4.78± 1.18
Chloride (mmol/l)	110.47± 1.51	111.27 ± 0.96	111.53 ± 1.36	111.60± 1.64	110.73 ± 2.19	112.47± 1.25*

Note : The values are expressed as mean ± SD.

***Significantly different from the control group ($p<0.05$).**

Table 7 Histopathological results of male rats receiving *Thunbergia laurifolia* extract for 6 months

Organs	Microscopic findings	Dose of *Thunbergia laurifolia* extract (mg/kg/day)					
		Control	20	200	1000	2000	2000R
		N = 15	N = 15	N = 15	N = 15	N = 15	N = 15
Lung	Lymphoid proliferated peribronchioles	7/15	10/15	3/15	4/15	6/15	7/15
Heart	Focal myocardiosis	3/15	1/15	3/15	0/15	0/15	1/15
Liver	Fatty degeneration	6/15	8/15	6/15	7/15	2/15	5/1
Small intestine	Gut-associated lymphoid tissue hyperplasia	5/15	1/15	1/15	2/15	4/15	5/15
Large intestine	Gut-associated lymphoid tissue hyperplasia	1/15	4/15	1/15	2/15	1/15	2/15
Adrenal gland	Cortical fatty degeneration (mild)	0/15	2/15	8/15*	1/15	0/15	0/15

Note : The results are expressed as number of rats with pathological findings / total number of rats examined.

***Significantly different from the control group ($p<0.05$)**

Table 8 Histopathological results of female rats receiving *Thunbergia laurifolia* extract for 6 months

Organs	Microscopic findings	Dose of *Thunbergia laurifolia* extract (mg/kg/day)					
		Control	20	200	1000	2000	2000R
		N = 15	N = 15	N = 15	N = 15	N = 15	N = 15
Lung	Lymphoid proliferated peribronchioles	1/15	2/15	2/15	5/15	3/15	3/15
Heart	Focal myocardiosis	0/15	0/15	1/15	0/15	0/15	0/15
Small Intestine	Gut-associated lymphoid tissue hyperplasia	1/15	2/15	0/15	2/15	2/15	1/15
Large intestine	Gut-associated lymphoid tissue hyperplasia	0/15	0/15	0/15	0/15	1/15	0/15

Note: The results are expressed as number of rats with pathological findings/total number of rats examined.

Effects on hematological parameters

The number of RBCs in the male rats receiving TLE at the dose of 2,000 mg/kg/day and in the 2000R group was significantly decreased when compared with that of their control group. Male rats receiving TLE at doses of 1,000 and 2,000 mg/kg/day and the 2000R group had significantly increased MCV values. MCH values for male rats treated with 2,000 mg/kg/day of TLE and those of the 2000R group were significantly higher than those of their control group (Table 3). Female rats receiving TLE at doses of 1,000 and 2,000 mg/kg/day showed significant increases in WBC numbers (Table 4).

Effects on clinical chemistry parameters

Male rats receiving TLE at doses of 1,000 and 2,000 mg/kg/day had significantly higher albumin levels than their control group. The bilirubin level of male rats receiving TLE at the dose of 2,000 mg/kg/day was significantly higher than that of the control group (Table 5). The ALT level of the female rats receiving the highest dose of TLE was significantly lower than that of their control group, whereas the bilirubin level was significantly increased (Table 6).

Effects on histopathological alterations of visceral organs

Necropsy revealed no remarkable gross lesions in any organs of both male and female rats receiving each dose of TLE, including their corresponding control groups. The incidence and degree of histopathological alterations in some organs of the TLE-treated male and female rats did not show any dose-dependency (Tables 7 and 8). There were no remarkable histopathological lesions in other organs of the TLE-treated groups and their controls.

Discussion

There was no significant difference in body weight and food consumption of rats treated with TLE, suggesting that TLE does not adversely affect the general health status of such animals. Although the relative weights of spleens in male rats and those of kidneys in both sexes of rats receiving 2,000 mg/kg/day of TLE were significantly increased; there were no gross pathological and histological alterations of these organs. The increase of kidney weights in this study was consistent with the study of Wisitpongpan et al., that rats receiving *rang chuet* leaf extract at 500 mg/kg for 28 days had significant increases in the weight of both kidneys.[9] Despite the statistically significant decrease in RBCs in male rats receiving TLE at 2,000 mg/kg/day and in the 2000R group, these alterations were still within the normal range.[10,11] Although the MCV values of male rats receiving TLE at doses of 1,000 and 2,000 mg/kg/day were significantly higher than that of the controls, and showed a tendency toward a dose-dependent response and the MCH value of the male rats receiving TLE at 2,000 mg/kg/day was significantly higher than that of the control group, these values were within the normal range.[10,11] The increases in WBCs in female rats receiving TLE at doses of 1,000 and 2,000 mg/kg/day were consistent with the reports of Wisipongpan et al. and Techakitiroj,[9,13] that is, they revealed a significant increase in WBCs in rats treated with *rang chuet* extract. Therefore, this phenomenon may be attributed to TLE. The increase in albumin levels in male rats receiving TLE at doses of 1,000 and 2,000 mg/kg/day and the decrease in the ALT level in female rats treated with the highest dose of TLE were within the rats' normal range.[10,12] The significant increases in total bilirubin levels in both sexes of rats receiving TLE at the highest dose were consistent with the study of Techakitiroj.[13] In addition, Wisitpongpun et al. reported an increase in conjugated bilirubin in rats receiving *rang chuet* extract for 28 days.[9] The mechanism of this change definitely remains unknown; however, these alterations were within the normal range.[10] The incidence and severity of the histopathological findings in some organs of the rats receiving TLE (Tables 7 and 8) had no correlation with the doses of TLE given; therefore, these changes may not be due to TLE.

In conclusion, the chronic toxicity study of TLE extract at doses ranging from 20 to 2,000 mg/kg/day did not affect the body weight, food consumption, behavior and general health of Wistar rats. TLE did

not cause any significant gross and histological lesions in the visceral organs of Wistar the rats; The extract at doses of 1,000 and 2,000 mg/kg/day produced slight alterations of some hematological and clinical chemistry parameters of the rats; however, most were within the normal range. Therefore, this study suggests that prolonged use of high doses of *T. laurifolia* aqueous extract may affect the hematopoietic system.

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References

1. Backer CA, Bakhuizen van den Brink RC. Acanthaceae. Flora of Java 1965;2:544-53.
2. Hutchinson J. The family of flowering plants. Oxford: The Clarendon Press; 1973. p. 609-10.
3. The Forest Herbarium Royal Forest Department. Thai Plant Names (Tem Smitinand Revised Edition 2001). Bangkok: Phachachon Press; 2001. p. 526.
4. Perry LM, Metzger J. Medicinal plants of East and Southeast Asia. Cambridge: MIT Press; 1980. p. 6.
5. Association of Thai Traditional Medicine School. Pramuan Supakun Yathai Part 3. Bangkok: Ampolpittaya Printing; 1973. p. 110. (in Thai)
6. Tejasen P, Thongtharb C. Experimental using Thunbergia laurifolia as antidote for insecticide poisoning. Chiang Mai Medical Bulletin 1980;19:105-14. (in Thai)
7. Dunkhoom, P. Effectiveness of Thunbergiaceae on reducing insecticide residue level in blood circulation of agriculturists. FDA Journal 2001;8:42-9. (in Thai)
8. Pramyothin P, Chirdchupunsare H, Rungsipipat A, Chaichantipyuth C. Hepatoprotective activity of *Thunbergia Laurifolia* Linn extract in rats treated with ethanol: in vitro and in vivo studies. J Ethnopharmacol 2005;102:408-11.
9. Visitpongpant W , Ruamgyuthikarn V, Rujjanavej C, Pantong A , Vinijkhetkamnuan, Lertprasertsuk N. Testing of toxicity of *Thunbergia laurifolia* Linn. abstract in mice. J Med Hearb 2003;10:23-36.
10. Gad SC. The rat: Pathology. In : Gad SC, Chengellis CP, editors. Animal Models in Toxicology. New York: Marcel Dekker; 1982. p.78-81.
11. Campbell TW. Mammalian hematology: Laboratory animals and miscellaneous species. In: Troy DB, editor. Veterinary hematology and clinical chemistry. Baltimore: Lippincott, Williams and Wilkins; 2004. p. 215.
12. Pimainog Y, Yothinarak A, Jornrakate P. Reference ranges for hematological and clinical chemistry values in Wistar rats. Bulletin of the Department of Medical Sciences 2003;45:27-36. (in Thai)
13. Techakitiroj V. The effect of *rang chute* (*Thunbergia laurifora* Linn.) extract on rat splenocytes by Fourier Tranform Infrared Spectroscopy. Thesis for Master's Degree (Pharmacology). Chulalongkorn University. 2004.

บทคัดย่อ

พิษเรื้อรังของสารสกัดรางจืด

ทรงพล ชีวะพัฒน์*, ปราณี ชวลิตธำรง*, เอมมนัส อัตตวิชญ์*, จารีย์ บันสิทธิ์*, ทรงพล ผดุงพัฒน์*

**สถาบันวิจัยสมุนไพร กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข นนทบุรี ๑๑๐๐๐*

รางจืดเป็นพืชสมุนไพรที่ถูกนำมาใช้รักษาอาการพิษอันเกิดจากสารพิษต่าง ๆ. อย่างไรก็ตาม ข้อมูลด้านพิษวิทยาของสมุนไพรชนิดนี้ยังไม่สมบูรณ์ จึงได้ศึกษาพิษเรื้อรังของสารสกัดรางจืดในหนูแรทพันธุ์วิสตาร์ ๑๘๐ ตัว แบ่งออกเป็น ๖ กลุ่ม: กลุ่มที่ ๑ เป็นกลุ่มควบคุมที่ได้รับน้ำกลั่นทางปาก. กลุ่มที่ ๒ ถึง ๖ เป็นกลุ่มทดลองที่ได้รับสารสกัดรางจืดทางปากในขนาด ๒๐, ๒๐๐, ๑,๐๐๐, ๒,๐๐๐ และ ๒,๐๐๐ มก./กก./วัน หรือคิดเป็น ๑, ๑๐, ๕๐ และ ๑๐๐ เท่าของขนาดที่ใช้ในคน เป็นเวลา ๖ เดือน โดยกลุ่มที่ ๖ (2000R) เป็นกลุ่มศึกษาการฟื้นตัวหลังหยุดให้สารสกัดเป็นเวลา ๒ สัปดาห์. จากการศึกษาพบว่าสารสกัดรางจืดไม่มีผลต่อน้ำหนักตัว การกินอาหาร พฤติกรรม และสุขภาพทั่วไปของหนู. สารสกัดรางจืดไม่ทำให้เกิดอาการพิษสะสมและไม่ทำให้หนูตาย. หนูเพศผู้ที่ได้รับสารสกัดรางจืดขนาด ๒,๐๐๐ มก./กก./วัน และกลุ่ม 2000R มีเม็ดเลือดแดงน้อยกว่ากลุ่มควบคุม อีกทั้งมีค่าดัชนีเม็ดเลือดแดงแตกต่างอย่างมีนัยสำคัญ. แต่การเปลี่ยนแปลงเหล่านี้อยู่ในช่วงค่าปรกติ ขณะที่หนูเพศเมียที่ได้รับสารสกัดรางจืดขนาด ๑,๐๐๐ และ ๒,๐๐๐ มก./กก./วัน มีเม็ดเลือดขาวเพิ่มขึ้นอย่างมีนัยสำคัญ. หนูทั้งสองเพศที่ได้รับสารสกัดรางจืดในขนาด ๒,๐๐๐ มก./กก./วันมีระดับบิลลิรูบินเพิ่มขึ้นอย่างมีนัยสำคัญแต่คงอยู่ในช่วงค่าปรกติ. สารสกัดรางจืดไม่ทำให้เกิดการเปลี่ยนแปลงของอวัยวะภายในทั้งระดับมหพยาธิวิทยาและจุลพยาธิวิทยาแต่อย่างใด. อย่างไรก็ตาม การบริโภครางจืดในขนาดสูงเป็นระยะเวลานานต่อเนื่องควรมีการตรวจเลือดเพื่อติดตามดูการเปลี่ยนแปลงของค่าทางโลหิตวิทยาและค่าชีวเคมีที่อาจเกิดขึ้นร่วมด้วย.

คำสำคัญ: **รางจืด, พิษเรื้อรัง**