The Thai Journal of Pharmaceutical Sciences

Volume 21
Issue 4 *1997*

Article 3

1-1-1997

Toxicological Study of Crude Extract of Tinospora crispa Mier ex Hook F. & Thoms(การศึกษาความเป็นพิษของสารสกัดของขอระเบ็ด)

Pranee Chavalittumrong

Aimmanas Attawish

Anchalee Chuthaputti

Pranee Chuntapet

Thai J. Pharm. Sci., 1997, Vol. 21, No. 4 pp. 199-210

Toxicological Study of Crude Extract of *Tinospora crispa* Mier ex Hook F. & Thoms

Pranee Chavalittumrong[1], Aimmanas Attawish[1],
Anchalee Chuthaputti[1] and Pranee Chuntapet[2].

[1]Medicinal Plant Research Institute
[2]Division of Clinical Pathology
Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand
Received July 25, 1997; accepted September 22, 1997

ABSTRACT: Acute toxicity study of ethanolic extract of *Tinospora crispa* stem in mice showed that the extract at the highest oral dose of 4.0 g/kg of body weight (g/kg BW), which was equivalent to powdered crude drug 28,95 g/kg BW, did not produce any signs of toxicity. Six-month chronic toxicity study of the extract was performed in five groups of 16 Wistar rats of each sex. Water control group received 10 ml of water/kg BW/day while tragacanth control group received 10 ml of 0.5% tragacanth suspension/kg BW/day. The three treatment groups were given the extract at the doses of 0.02, 0.16 and 1.28 g/kg BW/day which were equivalent to dried stems 0.145, 1.16 and 9.26 g/kg BW/day, respectively. It was found that the body weight of female rats receiving 1.28 g extract/kg BW was significantly lower than that of the tragacanth control group which might be due to lower food intake in this group of animals. Hematological studies showed no significant dose-dependent difference between tragacanth control groups and all extract-treated groups in both sexes. Blood chemistry studies indicated that both male and female rats receiving 1.28 g/kg BW of the extract had significantly higher cholesterol levels but significantly lower glucose levels than those of water control and tragacanth control groups. Animals of both sexes receiving the highest dose of the extract had significantly higher alkaline phosphatase (ALP) levels, alanine aminotransferase (ALT) levels and relative liver weights than those of the water control and tragacanth control groups. Histopathological study indicated that male rats receiving the highest dose of the extract had significantly higher incidence of bile duct preliferation and focal liver cell hyperplasia than the two control groups. These two pathological findings may explain the significant increase of ALP level in this group of male rats. The results suggested that high doses of the extract may cause hepatotoxicity that could alter both function and morphology of the liver. Male rats receiving the extract also had significantly higher creatinine levels than that of the tragacanth control group. In addition, female rats given 0.16 and 1.28 g extract/kg BW also had higher creatinine levels than that of the tragacanth control group. However, histopatological examination of the kidneys showed no significant difference between extract-treated groups and both of the control groups. The results suggested that the extract at the highest dose may also affect kidney function.

Taken together, the results of our chronic toxicity study of ethanolic extract of *Tinospora crispa* suggest that, due to the hepatottoxic and renal toxic potential of the extract observed in rats, prolonged use of high doses of *T. crispa* in humans should be avoided or if signs of liver or renal toxicities occur while using *T. crispa*-containing herbal medicine, the drug should be discontinued immediately.

KEY WORDS: *Tinospora crispa,* Toxicity

INTRODUCTION

Tinospora crispa Mier ex Hook F. & Thoms (Synonyms: *T. rumphii* Boerl, *T. tuberculata* Beaumee, 'Boraphet' in Thai) is a woody and glabous climber in the family Menispermaceae (1). Its young stems are smooth but old stems are prominently tuberculate and contain exceedingly bitter sap (2). In traditional medicine, the decoction of fresh or dried stem is used as an antipyretic in Thailand, and as a stomachic and antipyretic in Indonesia (2,3). The stem contains tinosporine, tinosporidine, picro-rectin, N-trans-feruloyl tyramine, N-cis-feroloyl tyramine, tinotuberide, borapetoside A, borapetol A, ceryl alcohol, β-sitosterol, stigmasterol, phytosterol (2), syringin, borapetoside B (4), borapetol B (5), tinocrisposide (6), while fresh leaves contain tinotufolin C, D, E, F (7).

Scientific studies on this plant indicate that it possesses several pharmacological actions (8-11). *T. crispa* stem was shown to exhibit hypoglycemic activity in experimental animals. When an aqueous extract of *T. crispa* stem was given orally to normal and alloxan-diabetic rats a hypoglycemic effect was observed in moderately diabetic rats with concomitant improvement in insulinemia (8). Acute intravenous treatment with the extract (50 mg/kg) caused an increase in plasma insulin levels in rats (8). In an *in vitro* study, water extract of the stem induced potentiation of basal and glucose-stimulated insulin secretion from both rat and human islets of Langerhans. This insulinotropic action of the extract may explain the hypoglycemic effect of *T. crispa* (9). With regard to antipyretic and anti-inflammatory actions, water extract of the stem at the doses of 100-300 mg/kg could reduce fever in Wistar rats (10), while 50% methanol extract at the dose of 10 mg/kg decreased carrageenan-induced hind paw edema in rats (11).

Currently, this plant is used in Thailand as a bitter tonic and antipyretic; however its toxicological data is still lacking, chronic toxicity study in the rat was therefore undertaken to determine any potential toxic effect that this plant may produce upon prolonged use so that proper warning can be issued to the public.

METHODS

Preparation of the Extract: T. crispa stems were collected and identified by the Botany Section, Division of Medicinal Plant Research and Development, then cut, dried at 50°C, ground and extracted with 95% ethanol in a soxhlet apparatus and the extract was dried under vacuum in a rotary evaporator. Prior to the experiment, the extract was diluted to the desired concentrations with 5% tragacanth suspension.

Treatment of the Animals: Twenty ICR mice (Nation Institute of Health Thailand) of each sex weighing 25±2 g were used in acute toxicity study. For chronic toxicity study, 80 male rats weighing 230±20 g and 80 female rats weighing 200±20 g (The National Laboratory Animal Center, Mahidol University, Salaya, Thailand) were used. The animals were housed in the animal facility of the NIH. The temperature in the animal room was kept at 25 ± 1°C with 60% relative humidity. The animals were allowed to have free access to food and clean water.

Acute Toxicity Study: For oral route of administration, 4 groups of 5 male and 5 female mice were used. For extract-treated groups, the extract was administered p.o. at the doses of 1, 2 and 4 g/kg BW which were equivalent to crude drug 7.24, 14.47 and 28.94 g/kg BW, respectively, while control group received 0.5% tragacanth suspension 10 ml/kg BW. The animals were observed for mortality or any signs of abnormalities periodically during the first 24 hours and twice daily for 7 days thereafter.

Chronic Toxicity Study: Eighty Wistar rats of each sex were randomly divided into 5 groups of 16 animals per sex. Group 1 (water control) received water 10 ml/kg BW/day and Group 2 (tragacanth control) received 0.5% tragacant

suspension 10 ml/kg BW/day. Groups 3-5 were given the extract at the doses of 0.02, 0.16 or 1.28 g/kg BW/day which were equivalent to dried stem 0.14, 1.16 and 9.26 g/kg BW/day or 1, 8 and 64 folds of the therapeutic dose, respectively. Body weight and food intake were measured weekly and the animals were observed for signs of abnormalities throughout the study. At the end of 180-day treatment period, the animals were fasted for 18 hours, then anesthetized with ether and sacrificed by drawing blood samples from the inferior vena cava for hematological and biochemical examinations. Hematocrits were determined while white blood cell and platelet counts were performed using counting chambers. Biochemical studies of serum samples and the assay procedures used were alkaline phosphatase or ALP (12), aspartate aminotransferase or AST and alanine aminotransferase or ALT (13), creatinine (Jaffe's reaction), blood urea nitrogen or BUN (Diacetylmonoxime method), cholesterol (enzymatic reaction), total protein (biuret method), albumin (dye binding with bromcresol green), and globulin was determined by subtracting albumin level from total protein level.

The positions, shapes, sizes and colors of internal organs, namely, brain, heart, both kidneys and lungs, trachea, esophagus, stomach, liver, pancreas, intestine, spleen, bladder, and testis in male rats or ovary and uterus in female rats were visually observed for any signs of gross lesions. These organs were then collected, weighed to determine relative organ weights, and preserved in 10% buffered formalin solution. Tissue slides were prepared and stained with hematoxylin and eosin and histopathological examinations were performed by a medical pathologist.

Statistical Analysis: The data were analyzed by one-way ANOVA followed by Duncan multiple range test, using SPSS/PC program, to determine significant differences between groups at $p<0.05$. Histopathological data were evaluated by the Fisher Exact test and the significance level was also set at $p<0.05$.

RESULTS

Acute Toxicity Study: When ethanolic extract of *Tinospora crispa* stem was given orally in mice at the doses of 1, 2 or 4 g/kg BW, it was found that the extract even at the highest dose of 4 g/kg BW, which was equivalent to powdered crude drug 28.95 g/kg BW did not produce any signs of toxicity.

Chronic Toxicity Study

Effect of the Extract on Body Weight, Food Intake, and Relative Organ Weight: At the start of the experiment, there was no difference in average body weights between groups of animals. However, after 4 weeks of treatment until the end of the experiment, the group of female animals receiving the highest dose of the extract had significantly lower body weight than those in the tragacanth and water control groups. Similarly, the body weights of the groups of male rats receiving the highest dose and the medium dose of the extract started to become significantly lower than that of the water control group, but not tragacanth control group, after 6 and 4 weeks of treatment, respectively (Figure 1). The reduction of body weights in these groups of animals may, in part, be due to significantly lower food intake as compared with water and tragacanth control groups in several weeks. In male rats, the difference in food consumption had been observed since the second week of the experiment until the end of the study, while in female rats the significant difference in food intake was also detected in the twelfth week and many weeks thereafter; however, the difference was not observed every week as seen in male rats (Figure 2). At the end of the experiment, body weights and weight gain of female animals receiving the extract at the dose of 1.28 g/kg BW/day were significantly lower than that of the tragacanth control groups (Table 1). Male rats treated with this dose of the extract had significantly higher relative weights of the liver and spleen, while in the female rats, relative weights of the brain, bladder, liver and stomach were significantly higher than those of the tragacanth control groups.

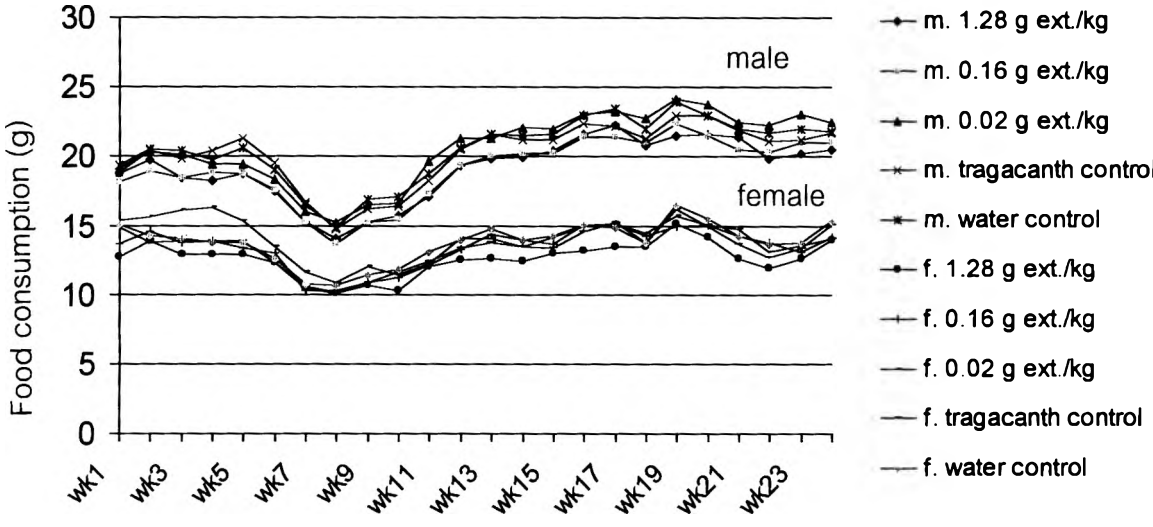

Figure 1 Food consumption of male and female rats. Water control and tragacanth control groups received 10 ml/kg BW/day of water or 0.5% tragacanth suspension, while treatment groups (m=male, f=female rats) were given 0.02, 0.16 or 1.28 g extract/kg BW/day for 180 days. Each point represents average body weight (g) at the end of each week.

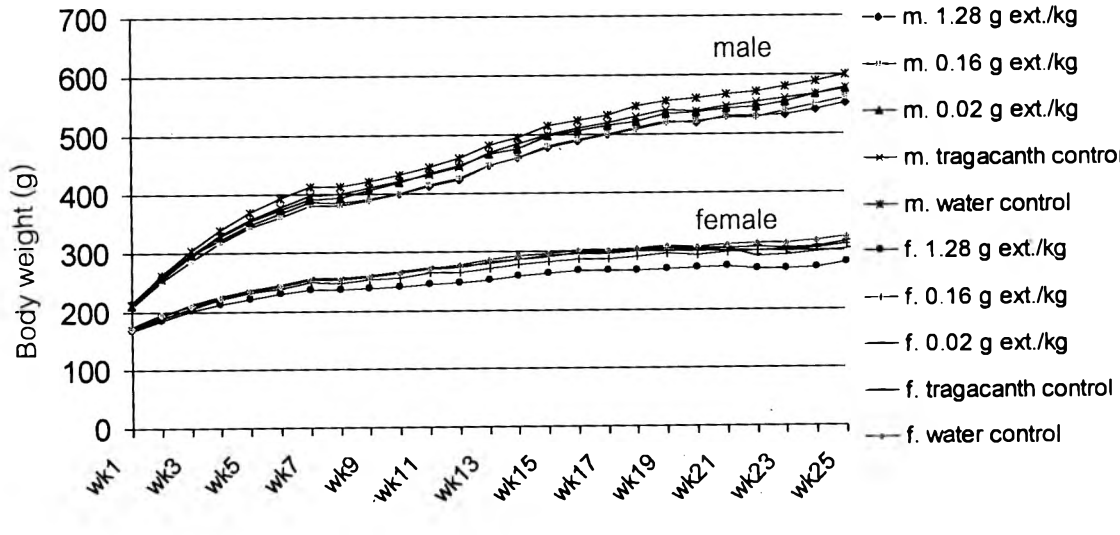

Figure 2 Growth curve of male and female rats. Water control and tragacanth control groups received 10 ml/kg BW/day of water or 0.5% tragacanth suspension, while treatment groups (m=male, f=female rats) were given 0.02, 0.16 or 1.28 g extract/kg BW/day for 180 days. Food consumption of animals housed in each cage (4 rats/cage) was measured once a week. Average daily food intake/animal in each week was calculated and shown in this figure

Table 1 % Relative organ weight (g/100 g BW) and body weight of male rats.

	Groups of animals				
	Water control	**Tragacanth control**	**0.02 g ext./kg/day**	**0.16 g ext./kg/day**	**1.28 g ext./kg/day**
	n = 16	**n = 16**	**n = 16**	**n = 15**	**n = 15**
Initial weight (g)	149.19 ± 10.34	147.06 ± 8.77	146.25 ± 8.82	145.47 ± 11.30	150.80 ± 12.84
Final weight (g)	590.50 ± 53.09	571.44 ± 57.93	563.31 ± 41.58	548.00 ± 61.13*	538.33 ± 39.02*
Different wt. (g)	441.31 ± 52.22	424.38 ± 56.62	417.06 ± 41.41	402.53 ± 57.68	387.53 ± 41.91*
Brain	0.378 ± 0.038	0.386 ± 0.050	0.394 ± 0.032	0.395 ± 0.045	0.406 ± 0.020
Heart	0.255 ± 0.032	0.262 ± 0.034	0.263 ± 0.030	0.286 ± 0.045*	0.277 ± 0.019
Right kidney	0.259 ± 0.023	0.256 ± 0.031	0.264 ± 0.016	0.240 ± 0.021*	0.261 ± 0.021
Left kidney	0.245 ± 0.023	0.244 ± 0.026	0.249 ± 0.014	0.232 ± 0.021	0.247 ± 0.017
Urinary bladder	0.030 ± 0.006	0.036 ± 0.017	0.034 ± 0.012	0.033 ± 0.008	0.033 ± 0.011
Liver	2.556 ± 0.212	2.605 ± 0.259	2.617 ± 0.128	2.695 ± 0.250	3.579 ± 0.205*,**
Spleen	0.183 ± 0.017	0.177 ± 0.018	0.181 ± 0.028	0.181 ± 0.016	0.214 ± 0.016*,**
Stomatch	0.390 ± 0.044	0.410 ± 0.031	0.392 ± 0.034	0.411 ± 0.038	0.403 ± 0.043
Lung	0.362 ± 0.029	0.380 ± 0.045	0.359 ± 0.045	0.372 ± 0.043	0.383 ± 0.034
Right testis	0.595 ± 0.053	0.563 ± 0.059	0.602 ± 0.060	0.601 ± 0.078	0.631 ± 0.078**
Left testis	0.603 ± 0.057	0.569 ± 0.068	0.604 ± 0.059	0.608 ± 0.099	0.624 ± 0.097

Water control and tragacanth control groups received 10 ml/kg BW/day of water or 0.5% tragacanth suspension while extract-treated groups were given 0.02, 0.16 or 1.28 g extract/kg BW/day for 180 days. At the end of the study, each organ was weighed and calculated as % relative organ weight (g/100 g BW). Each value represents mean ± SD.

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

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

Table 2 % Relative organ weight (g/100 g BW) and body weight of female rats.

	Groups of animals				
	Water control	**Tragacanth control**	**0.02 g ext./kg/day**	**0.16 g ext./kg/day**	**1.28 g ext./kg/day**
	n = 16	**n = 16**	**n = 16**	**n = 15**	**n = 15**
Initial weight (g)	142.56 ± 7.41	144.31 ± 13.05	143.60 ± 11.81	139.67 ± 9.47	139.07 ± 8.75
Final weight (g)	315.69 ± 21.50	304.25 ± 35.42	310.20 ± 29.20	298.33 ± 28.70	270.80 ± 18.79*,**
Different wt. (g)	173.12 ± 21.73	159.94 ± 29.82	160.60 ± 28.46	158.67 ± 27.73	131.73±18.42*,**
Brain	0.636 ± 0.043	0.663 ± 0.063	0.657 ± 0.063	0.675 ± 0.056	0.758 ± 0.060*,**
Heart	0.314 ± 0.032	0.320 ± 0.035	0.304 ± 0.032	0.331 ± 0.051	0.325 ± 0.032
Right kidney	0.282 ± 0.025	0.307 ± 0.042	0.284 ± 0.020**	0.281 ± 0.029**	0.319 ± 0.022*
Left kidney	0.266 ± 0.023	0.282 ± 0.027	0.262 ± 0.015**	0.263 ± 0.023**	0.305 ± 0.018*,**
Urinary bladder	0.031 ± 0.006	0.031 ± 0.004	0.028 ± 0.004	0.030 ± 0.004	0.035 ± 0.008*,**
Liver	2.604 ± 0.378	2.804 ± 0.435	2.677 ± 0.226	2.913 ± 0.367	4.401 ± 0.257*,**
Spleen	0.227 ± 0.032	0.251 ± 0.035	0.232 ± 0.030	0.234 ± 0.042	0.270 ± 0.033*
Stomatch	0.528 ± 0.064	0.544 ± 0.067	0.511 ± 0.060	0.546 ± 0.058	0.590 ± 0.038*,**
Lung	0.461 ± 0.044	0.498 ± 0.049	0.476 ± 0.048	0.492 ± 0.057	0.513 ± 0.041*

Water control and tragacanth control groups received 10 ml/kg BW/day of water or 0.5% tragacanth suspension while extract-treated groups were given 0.02, 0.16 or 1.28 g extract/kg BW/day for 180 days. At the end of the study, each organ was weighed and calculated as % relative organ weight (g/100 g BW). Each value represents mean ± SD.

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

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

Effect of the Extract on Hematological Parameters: In male rats, there was no difference in hematological parameters between control and extract-treated groups (Table 3). In female rats, the numbers of monocytes of animals receiving the extract at the doses of 0.16 and 1.28 g/kg BW/day were not different from that of the water control group but were significantly lower than that of the tragacanth control group (Table 4). However, the number of monocytes of the tragacanth control was significantly higher than that of the water control. The changes of hematocrits or eosinophil numbers found in some groups of female rats as compared to control groups were not dose-related.

Table 3 Results of hematological examinations of male rats

	Groups of animals				
	Water control	**Tragacanth control**	**0.02 g ext./kg/day**	**0.16 g ext./kg/day**	**1.28 g ext./kg/day**
	n = 16	**n = 15**	**n = 16**	**n = 14**	**n = 15**
Hematocrit (%)	46.38 ± 1.82	45.93 ± 2.37	46.31 ± 1.30	47.14 ± 1.70	45.53 ± 1.96
White blood cells X 10^2 cells/mm^3	6.16 ± 1.11	6.16 ± 1.32	6.02 ± 1.12	5.95 ± 1.35	5.80 ± 1.27
Platelet X 10^3 cells/mm^3	666.38 ± 73.19	653.27 ± 62.17	660.06 ± 64.55	660.93 ± 61.02	650.73 ± 64.54
Neutrophil (%)	64.00 ± 9.24	59.53 ± 6.55	58.94 ± 8.10	60.50 ± 9.11	61.33 ± 7.76
Eosinophil (%)	0.50 ± 0.63	1.27 ± 2.05	1.56 ± 1.97	0.93 ± 1.14	1.00 ± 1.13
Lymphocyte (%)	32.38 ± 10.11	35.13 ± 5.63	36.06 ± 9.86	34.50 ± 8.84	34.53 ± 8.11
Monocyte (%)	3.12 ± 2.22	4.07 ± 1.98	3.44 ± 2.56	4.07 ± 2.09	3.13 ± 1.68

Water control group received 10 ml/kg BW/day while tragacanth control group received the same dose of 0.5% tragacanth suspension. The three extract-treated groups were given the extract at the doses of 0.02, 0.16 or 1.28 g/kg/day which were equivalent to dried stem 0.14, 1.16 and 9.26 g/kg BW/day, respectively. Blood samples were collected for hematological examination at the end of 180-day treatment period. Each value represents mean ± SD.

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

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

Table 4 Results of hematological examinations of female rats

	Groups of animals				
	Water control	**Tragacanth control**	**0.02 g ext./kg/day**	**0.16 g ext./kg/day**	**1.28 g ext./kg/day**
	n = 16	**n = 16**	**n = 14**	**n = 12**	**n = 14**
Hematocrit (%)	43.81 ± 2.79	44.31 ± 2.36	45.79 ± 2.22*	45.83 ± 2.08*	43.43 ± 1.55
White blood cells X 10^2 cells/mm^3	6.49 ± 0.74	5.89 ± 1.04	6.39 ± 1.10	5.94 ± 1.36	5.84 ± 0.69
Platelet X 10^3 cells/mm^3	652.12 ± 75.38	668.38 ± 68.99	639.00 ± 73.63	629.75 ± 41.68	627.71 ± 59.63
Neutrophil (%)	61.31 ± 7.50	62.00 ± 7.21	63.14 ± 7.97	61.42 ± 9.59	59.21 ± 6.52
Eosinophil (%)	0.50 ± 0.82	1.56 ± 1.26	1.21 ± 1.63	0.58 ± 1.16**	0.86 ± 0.95
Lymphocyte (%)	35.00 ± 7.71	30.25 ± 6.77	31.64 ± 9.30	35.75 ± 10.13	37.14 ± 8.06
Monocyte (%)	3.19 ± 1.80	5.56 ± 2.34*	4.00 ± 2.48	2.25 ± 1.71**	2.79 ± 2.15**

Water control group received 10 ml/kg BW/day while tragacanth control group received the same dose of 0.5% tragacanth suspension. The three extract-treated groups were given the extract at the doses of 0.02, 0.16 or 1.28 g/kg/day which were equivalent to dried stem 0.14, 1.16 and 9.26 g/kg BW/day, respectively. Blood samples were collected for hematological examination at the end of 180-day treatment period. Each value represents mean ± SD.

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

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

Effect of the Extract on Blood Chemistry: In both male and female rats, no difference in AST levels was found between all extract-treated groups and tragacanth control group (Table 5,6). Male rats receiving the extract 0.16, and 1.28 g/kg BW/day and female rats receiving the extract 1.28 g/kg BW/day had significantly lower ALT levels than their tragacanth controls. In addition, both male and female rats receiving 12.8 g extract/kg BW/day had significantly higher ALP levels than their tragacanth controls.

Creatinine levels of all extract-treated male rats and female rats receiving extract at the doses of 0.16 and 1.28 g/kg BW/day were significantly higher than those of tragacanth controls. However, BUN levels were not different between extract-treated groups and tragacanth control groups in both female and male rats.

Both male and female rats receiving extract 1.28 g/kg BW/day had significantly higher cholesterol levels but lower glucose levels than their tragacanth control groups. In male rats, total protein, albumin and globulin levels were not different among control and extract-treated groups while total protein level of female rats receiving extract 0.16 g/kg BW/day was significantly higher than that of tragacanth control. Sodium and potassium levels of male rats were not different between extract-treated and control groups while potassium level of female rats receiving extract at the dose of 1.28 g/kg BW/day was significantly higher than that of tragacanth control.

Table 5 Blood chemistry of male rats

	Groups of animals				
	Water control	**Tragacanth control**	**0.02 g ext./kg/day**	**0.16 g ext./kg/day**	**1.28 g ext./kg/day**
	n = 16	**n = 16**	**n = 15**	**n = 15**	**n = 15**
AST (U/l)	54.44 ± 9.97	60.31 ± 11.32	61.00 ± 10.80	54.87 ± 7.85	59.47 ± 11.31
ALT (U/l)	17.24 ± 5.89	22.16 ± 9.01*	20.22 ± 4.71	14.56 ± 2.69**	12.07 ± 2.52*,**
ALP (U/l)	58.69 ± 9.57	59.75 ± 16.20	53.13 ± 10.06	64.07 ± 13.37	75.73±8.07*,**
Bilirubin (mg/dL)	0.40 ± 0.27	0.59 ± 0.39	0.35 ± 0.18	0.49 ± 0.34	0.37 ± 0.28
Creatinine (mg %)	0.79 ± 0.08	0.78 ± 0.09	0.88 ± 0.11*,**	0.87 ± 0.14**	1.04 ± 0.09*,**
BUN (mg %)	24.14 ± 3.70	27.15 ± 2.89*	25.06 ± 2.94	25.23 ± 3.90	26.91 ± 2.62*
Cholesterol (mg %)	58.07 ± 16.50	61.05 ± 10.87	57.19 ± 9.43	82.61 ± 27.18*,**	103.63 ± 19.85*,**
Glucose (mg/dL)	110.88 ± 9.28	112.62 ± 15.13	112.40 ± 16.63	108.67 ± 14.36	98.87 ± 7.70*,**
Total protein (g %)	6.53 ± 0.57	6.62 ± 0.36	6.54 ± 0.42	6.63 ± 0.78	6.72 ± 0.61
Albumin (g %)	4.02 ± 0.43	3.98 ± 0.50	4.20 ± 0.26	3.73 ± 0.32	3.83 ± 0.29
Globulin (g %)	2.53 ± 0.57	2.55 ± 0.39	2.33 ± 0.28	2.90 ± 0.83	2.89 ± 0.59
Sodium (mmol/l)	147.26 ± 1.03	146.68 ± 1.71	147.81 ± 1.76	147.64 ± 1.45	146.94 ± 1.10
Potassium (mmol/l)	4.82 ± 0.88	5.37 ± 1.28	4.78 ± 0.89	5.11 ± 0.94	5.20 ± 0.94

Water control group received water 10 ml/kg BW/day while tragacanth control group received the same dose of 0.5% tragacanth suspension. The three extract-treated groups were given the extract at the doses of 0.02, 0.16 or 1.28 g/kg/day which were equivalent to dried stem 0.14, 1.16 and 9.26 g/kg BW/day, respectively. Blood samples were collected for determinations of blood chemistry at the end of 180-day treatment period. Each value represents mean ± SD.

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

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

Table 6 Blood chemistry of female rats

	Groups of animals				
	Water control	**Tragacanth control**	**0.02 g ext./kg/day**	**0.16 g ext./kg/day**	**1.28 g ext./kg/day**
	n = 16	**n = 15**	**n = 15**	**n = 14**	**n = 15**
AST (U/l)	49.06 ± 6.32	54.75 ± 7.02	56.07 ± 8.96*	57.53 ± 10.76*	59.47 ± 9.17*
ALT (U/l)	12.55 ± 5.08	14.19 ± 3.80	15.93 ± 2.83*	13.31 ± 3.42	10.95 ± 2.38**
ALP (U/l)	26.50 ± 7.28	29.75 ± 9.43	26.40 ± 8.88	21.80 ± 4.31**	42.07 ± 6.31*,**
Bilirubin (mg/dL)	0.35 ± 0.15	0.44 ± 0.21	0.62 ± 0.45**	0.47 ± 0.41	0.67 ± 0.42**
Creatinine (mg %)	0.86 ± 0.14	0.90 ± 0.18	0.86 ± 0.10	1.02 ± 0.17*,**	1.06 ± 0.10*,**
BUN (mg %)	25.45 ± 3.89	26.65 ± 4.76	25.23 ± 4.55	23.60 ± 3.78	26.89 ± 3.71
Cholesterol (mg %)	59.26 ± 20.77	79.26 ± 17.63*	64.53 ± 23.96	68.63 ± 34.32	96.89 ± 16.25*,**
Glucose (mg/dL)	101.38 ± 12.86	95.19 ± 8.62	98.73 ± 13.28	95.60 ± 11.84	80.80 ± 6.97*,**
Total protein (g %)	6.67 ± 0.33	6.81 ± 0.57	7.07 ± 0.57	7.52 ± 0.75*,**	7.24 ± 0.90*
Albumin (g %)	4.19 ± 0.45	4.25 ± 0.43	4.48 ± 0.34	4.52 ± 0.42	4.39 ± 0.43
Globulin (g %)	2.42 ± 0.49	2.54 ± 0.40	2.61 ± 0.48	3.02 ± 1.06*	2.85 ± 0.60
Sodium (mmol/L)	145.68 ± 1.62	146.91 ± 1.74	147.73 ± 2.30*	147.16 ± 1.34*	146.56 ± 1.52
Potassium (mmol/L)	4.36 ± 0.75	4.27 ± 0.75	4.38 ± 0.66	4.36 ± 0.71	4.98 ± 0.79*,**

Water control group received water 10 ml/kg BW/day while tragacanth control group received the same dose of 0.5% tragacanth suspension. The three extract-treated groups were given the extract at the doses of 0.02, 0.16 or 1.28 g/kg/day which were equivalent to dried stem 0.14, 1.16 and 9.26 g/kg BW/day, respectively. Blood samples were collected for determinations of blood chemistry at the end of 180-day treatment period. Each value represents mean ± SD.

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

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

Effect of the Extract on Histopathology of Internal Organs: Upon gross examinations of internal organs, no abnormal signs were observed except the livers where spotted lesions were found in all male rats receiving the highest dose of the extract. These lesions became more obvious when the organs were preserved in 10% buffered formalin solution. Histopathological examinations of internal organs were performed on brain, lung, thyroid gland, parathyroid gland, trachea, esophagus, liver, heart, spleen, pancreas, kidney, stomach, intestine, bladder, including testis and prostate gland in male rats or vagina, cervix, uterus and ovary in female rats. As shown in Table 8, in female rats, there was no histopathological difference of internal organs between extract-treated groups and the two control groups. In contrast, the numbers of male rats receiving 1.28 g extract/kg BW that had bile duct proliferation and focal liver cell hyperplasia were significantly higher than those in both groups of control animals (Table 7). In addition, the number of male rats detected with fatty change of the liver was significantly higher in those receiving 0.16 g extract/kg BW than in tragacanth control group.

Table 7 Histopathological evaluations of male rats

Organ	Lesion	Groups of animals				
		Water control	Tragacanth control	0.02 g ext./kg/day	0.16 g ext./kg/day	1.28 g ext./kg/day
Lung	Organizing pneumonia	0/16	0/16	0/16	0/15	0/15
	Foreign body reaction	0/16	0/16	0/16	1/15	1/15
Liver	Bile duct proliferation	0/16	0/16	0/16	0/15	9/15*,**
	Focal liver cell hyperplasia	0/16	0/16	0/16	0/15	9/15*,**
	Fatty change	3/16	2/16	1/16	7/15**	0/15
Heart	Focal myocarditis	2/16	1/16	3/16	1/15	0/15
Thyroid	Nodular goiter	0/16	0/16	0/16	1/15	0/15
Kidney	Nephrocalcinosis	0/16	0/16	0/16	0/15	1/15
	Hydrocalyx	4/16	2/16	3/16	0/15	1/15
Testis	Atrophy	0/16	0/16	0/16	0/15	1/15
	Granuloma	0/16	1/12	0/16	0/15	0/15

Water control and tragacanth control groups received 10 ml/kg BW/day of water or 0.5% tragacanth suspension while extract-treated groups were given 0.02, 0.16 or 1.28 g extract/kg BW/day for 180 days. At the end of the study, internal organs were collected in 10% buffered formalin and prepared for histopathological examinations. Each value represents number of rats with pathological abnormalities/total number of rats examined.

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

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

Table 8 Histopathological evaluations of female rats

Organ	Lesion	Groups of animals				
		Water control	Tragacanth control	0.02 g ext./kg/day	0.16 g ext./kg/day	1.28 g ext./kg/day
Lung	Organizing pneumonia	0/16	0/16	0/15	0/15	0/15
	Foreign body reaction	0/16	0/16	0/15	0/15	0/15
Liver	Bile duct proliferation	0/16	0/16	0/15	0/15	2/15
	Focal liver cell hyperplasia	0/16	0/16	0/15	0/15	1/15
	Fatty change	0/16	0/16	2/15	1/15	1/15
Heart	Focal myocarditis	0/16	0/16	0/15	0/15	0/15
	Nodular goiter	0/16	0/16	0/15	0/15	0/15
Kidney	Nephrocalcinosis	15/16	11/16	11/15	11/15	14/15
	Hydrocalyx	0/16	1/16	0/15	0/15	0/15

Water control and tragacanth control groups received 10 ml/kg BW/day of water or 0.5% tragacanth suspension while extract-treated groups were given 0.02, 0.16 or 1.28 g extract/kg BW/day for 180 days. At the end of the study, internal organs were collected in 10% buffered formalin and prepared for histopathological examinations. Each value represents number of rats with pathological abnormalities/total number of rats examined.

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

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

DISCUSSION

Acute toxicity study of ethanolic extract of *T. crispa* stem indicated that the extract at the doses up to 4 g/kg BW did not produce any signs of toxicity in mice. However, chronic toxicity study of the extract in rats showed that the extract at the highest dose of 1.28 g/kg BW/day, which is equivalent to 64 folds of the therapeutic dose, could significantly decrease the growth of the animals. This decrease in final body weight may explain, at least in part, the significant increases of relative organ weights of several internal organs in both male and female animals receiving the highest dose of the extract.

Hematological study indicated that the extract did not affect the hematocrit or the numbers of white blood cells or platelets of male rats. In female rats, even though there were some statistically significant changes of some hematological parameters in certain groups of extract-treated animals, these changes were not dose-dependent, suggesting that they may not be the result of the extract. Hence, the extract at the doses given did not appear to affect hematological parameters in rats.

Both male and female animals treated with the highest dose of the extract had significantly higher relative liver weights than their two control groups. Upon gross examination of internal organs, spotted lesions were found in the livers of all male rats receiving the highest dose of the extract. These lesions could be seen more easily when the organs were preserved in 10% buffered formalin solution. Histopathological examination of the livers showed a significantly higher incidence of bile duct proliferation and focal liver cell hyperplasia in this group of male animals than in the two control groups. These two pathological findings may partly explain a significant increase of ALP level in this group of male rats (14). This increase of ALP level was also observed in female rats receiving the same dose of the extract. The results suggested that high doses of the extract may induce hepatotoxicity that could alter both function and morphology of the liver.

Both male and female rats receiving the highest dose of the extract had significantly lower glucose levels than their water and tragacanth controls. This change might be the result of the hypoglycemic effect of *T. crispa* (8, 9). In addition, these groups of animals also had significantly higher cholesterol levels than their controls. This increase of serum cholesterol was not likely to be derived from dietary source since these groups of animals had lower food intake than their tragacanth controls. It might be possible that the highest dose of *T. crispa* could increase hapatic synthesis of cholesterol or alter the metabolism of cholesterol, by a yet unknown mechanism, causing serum cholesterol levels in these groups of animals to rise above that of the controls.

In comparison to the corresponding tragacanth control groups, all groups of male rats receiving the extract and groups of female rats receiving 0.16 and 1.28 g extract/kg BW/day had higher creatinine levels. In addition, all groups of extract-treated female rats had significantly higher relative kidney weights than tragacanth control group. Histopathological examinations of the idneys showed high incidence of nephrocalcinosis in female rats and hydrocalyx in male rats; however, the incidence of the two pathological changes of extract-treated groups was not different from that of the control groups. Taken together the results suggested that the extract at high doses may also affect the kidney function.

CONCLUSION

Six-month chronic toxicity study of 95% ethanolic extract of *Tinospora crispa* stem in rats indicated that the extract at the doses of 0.02, 0.16 and 1.28 g/kg BW, which were equivalent to 1, 8 and 64 folds of the therapeutic dose, did not produce any significant dose-related hematological changes. However, in both male and female animals, the extract at the highest dose caused a significant decrease of body weights and alterations of liver and kidney functions. This dose of the extract also caused morphological changes of the liver, i.e. bile duct proliferation and focal liver cel hyperplasia observed in male rats. Hence, due to the

hepatotoxic and renal toxic potential of the extract, prolonged use of high doses of *Tinospora crispa* in humans should be avoided or if signs of liver or renal toxicities occur while using *T. crispa*-containing herbal medicine, the drug should be immediately discontinued.

ACKNOWLEDGEMENTS

The authors would like to thank the World Health Organization for the grant giving to this research project. We are grateful to Mrs. Jaree Bansiddhi, an expert botanist, for the supply and identification of medicinal plants, and Dr. Somnuek Jesdapatarakul for histopathological examinations of tissue samples. Thanks are also due to Mr. Daroon Pecharaply, the director of the Division of Medicinal Plant Research and Development, for his advice on this project.

REFERENCES

1. T. Smitinand. *Thai Plant Names, Botanical Names-Vernacular Names*, Funny Publishing, Bangkok, 1980, pp. 331-332.
2. ASEAN Countries. *Standard of Asean Herbal Medicine Vol. 1,* Jarkata: Aksara Buana Printing, Jarkata, 1993, pp. 421, 433.
3. Department of Medical Sciences, Ministry of Public Health, Thailand. *Manual for Cultivation Production and Utilization of Herbal Medicines in Primary Health Care,* The War Veterans Organization Press, Bangkok, 1990, p. 121.
4. I. Murakoshi, T. Sekine, H. Suzuki, C. Ninomiya, K. Saito, S. Okonogi and F. Ikegami. Monolignan and diterpene glycosides from the stems of *Tinospora rumphii* Boeri. *Thai J. Pharm. Sci.* 17: 33-37 (1993).
5. N. Fukuda, M. Yonemitsu and T. Kumura. Studies on the constituents of the stems of *Tinospora tuberculata* Beumee. III New diterpenoids, borapetoside B and borapetl B. *Chem. Pharm. Bull.* 34: 2868-72 (1985).
6. P. Pachaly and A.Z. Adnan. Tinosporoside a new bitter furanoditerpene glycoside from *Tinospora crispa* Miers. *Archiv Der Pharmazie.* 325: 705-708 (1992).
7. N. Fukuda, M. Yonemitsu, T. Kimura, R. Isobe and T. Komori. Studies on the constituents of the leaves of *Tinospora tuberculata.* 2. Isolation and structure elucidation of 4 new furanoid diterpenes, tinofolin-C-F. *Liebig's Annalen der Chemie.* 7: 755-757 (1994).
8. H. Noor and S.J. Ashcroft. Antidiabetic effects of *Tinoospoora crispa* in rats. *J. Ethnopharmacol.* 27: 149-61 (1989).
9. H. Noor, P. Hammonds, R. Sutton and S.J. Ashcroft. The hypoglycemic and insulinotropic activity of *Tinospora crispa:* studies with human and rat islets and HIT-T 15 B cells. *Diabetologia.* 32: 354-9 (1989).
10. B. Kongsaktrakoon, R. Temsiririrkkul, W. Suvitayavat, S. Nakornchai and Y. Wongkrajang. The antipyretic effects of *Tinospora crispa* Mier ex Hook F. & Thoms. *Mahidol J. Pharm. Sci.* 21: 1-6 (1994).
11. H. Higashino, A. Suzuki, Y. Tanaka and K. Pootakham. Inhibitory effects of Siamese *Tinospora crispa* extracts on the carrageenin-induced foot pad edema in rats. *Nippon Yakurigaku Zasshi.* 100: 339-44 (1992).
12. G.N. Bowers Jr. and R.B. McComb. Measurement of Total Alkaline Phosphatase Activity in Human Serum. *Clin. Chem.* 21: 1988-1995 (1975).
13. R.J. Henry, N. Chaimori O.J. Golub and S. Berkman. Revised spectroophotometric methods for the determination of Glutamic Oxaloacetic Transaminase Glutamic Pyruvic Transaminase and Lactic Acid Dehydrogenase. *Am. J. Clin. Pathol.* 34: 381-398 (1960).
14. G.L. Plaa and M. Charbonneau. Detection and evaluation of chemically induced liver injury. In A.W. Hayes (ed.) *Principles and Methods of Toxicology. 3rd ed.,* Raven Press, New York, 1994, p. 844.

การศึกษาความเป็นพิษของสารสกัดของบอระเพ็ด

ปราณี ชวลิตธำรง[1], เอมมนัส อัตตวิชญ์[1], อัญชลี จูฑะพุทธิ[1], ปราณี จันทเพ็ชร[2]

[1]สถาบันวิจัยสมุนไพร, [2]สถาบันวิจัยวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ นนทบุรี 11000

ได้รับ 25 กรกฎาคม 2540 ตอบรับ 22 กันยายน 2540

บทคัดย่อ : จากการศึกษาพิษเฉียบพลันของสารสกัดด้วยเอธานอลของเถาบอระเพ็ด (*Tinospora crispa* Mier ex Hook F. & Thoms) โดยให้ทางปากแก่หนูถีบจักร พบว่าเมื่อให้สารสกัดในขนาด 4 กรัมต่อน้ำหนักหนู 1 กิโลกรัม (ก./กก.) หรือเทียบเท่ากับลำต้นแห้ง 28.95 ก./กก. ไม่ทำให้เกิดอาการพิษใด ๆ และจากการศึกษาพิษเรื้อรังของสารสกัดในหนูขาวพันธุ์วิสตาร์โดยการป้อนสารสกัดในขนาด 0.02, 0.16 และ 1.28 ก./กก./วัน หรือเทียบเท่าลำต้นแห้ง 0.145, 1.16 และ 9.26 ก./กก./วัน และกลุ่มควบคุม 2 กลุ่มด้วย 0.5% tragacanth และด้วยน้ำในขนาด 10 มล./กก./วัน ตามลำดับ เป็นเวลา 6 เดือน พบว่าน้ำหนักของหนูขาวเพศเมียที่ได้รับสารสกัดขนาด 1.28 ก./กก. ต่ำกว่ากลุ่มควบคุมด้วย tragacanth อย่างมีนัยสำคัญ ($p<0.05$) ทั้งนี้อาจเกิดจากหนูกลุ่มดังกล่าวกินอาหารน้อยกว่ากลุ่มควบคุม การตรวจสอบทางโลหิตวิทยาไม่พบความแตกต่างที่แปรผันตามขนาดของสารสกัดที่ได้รับระหว่างหนูขาวที่ได้รับสารสกัดกับกลุ่มควบคุมในหนูทั้งสองเพศ และจากการตรวจสอบทางชีวเคมีของซีรั่มและการศึกษาทางจุลพยาธิวิทยา พบว่าหนูขาวทั้งสองเพศที่ได้รับสารสกัดในขนาด 1.28 ก./กก. มีค่าโคเลสเตอรอล, alkaline phosphatase (ALP), alanine aminotransferase (ALT), น้ำหนักสัมพัทธ์ของตับและอัตราการเกิด bile duct proliferation และ focal liver cell hyperplasia มากกว่ากลุ่มควบคุมที่ได้รับน้ำและ tragacanth อย่างมีนัยสำคัญ แต่มีระดับน้ำตาลในเลือดต่ำกว่ากลุ่มควบคุมทั้งสองอย่างมีนัยสำคัญ ($p<0.05$) พยาธิสภาพของตับที่ตรวจพบนี้อาจเป็นสาเหตุของการเพิ่มขึ้นของระดับ ALP ในหนูกลุ่มดังกล่าว นอกจากนี้หนูเพศผู้ที่ได้รับสารสกัด 1.28 ก./กก. และเพศเมียที่ได้รับสารสกัด 0.16 และ 1.28 ก./กก. มีค่าครีอาตินินสูงกว่ากลุ่มควบคุมด้วย tragacanth อย่างมีนัยสำคัญ ($p<0.05$) แต่จากการศึกษาทางจุลพยาธิวิทยาไม่พบความแตกต่างของไตจากกลุ่มควบคุม

จากผลการศึกษาพิษเรื้อรังของสารสกัดด้วยเอธานอลของเถาบอระเพ็ดในหนูขาว พบว่าเมื่อให้สารสกัดในขนาดสูงและเป็นระยะเวลานาน อาจทำให้เกิดอาการผิดปกติของการทำงานของตับและไตได้ ดังนั้นหากมีการนำบอระเพ็ดมาใช้เป็นยาในขนาดสูงเป็นเวลานานและเกิดอาการผิดปกติของการทำงานของตับและไต ควรหยุดการใช้สมุนไพรนี้

กุญแจคำ: บอระเพ็ด, ความเป็นพิษ