ORIGINAL ARTICLE

Subchronic toxicity of *Cissus quadrangularis* Linn.

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Abstract

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***Cissus quadrangularis* Linn (*C. quadrangularis*), or "Phet-Cha-Sung-Khaat" in Thai, is one of the most commonly used medicinal plants in Thailand for the treatment of hemorrhoid; however, the safety of this herb upon long-term consumption has never been reported. Toxicity study was conducted to evaluate the three-month subchronic toxicity of *C. quadrangularis* powder in five groups of 12 Wistar rats of each sex. Water control group received orally 10 ml of water/kg BW/day. The dried-stems powder was given orally to the four treatment groups at the doses of 0.03, 0.3, 3.0 and 3.0 g/kg BW/day, which were equivalent to 1, 10, 100 and 100 fold of the therapeutic dose in human, respectively, the last group was the recovery group. No difference of initial or final body weights between *C. quadrangularis*-treated and control groups was detected. It was found that *C. quadrangularis* did not produce any significant dose-related changes of hematological parameters or serum clinical chemistry, and no histopathological lesion of any internal organ that could be due to the toxic effect of *C. quadrangularis* was observed. The results indicated that *C. quadrangularis* at the doses given did not produce any toxicity in the rats during the administration period of 3 months.**

Key words : *Cissus quadrangularis* Linn., subchronic toxicity

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toxicity testing (30 days) in Wistar rats, it was found that *C. quadrangularis* caused low levels of serum creatinine, albumin and WBC. These occurrences disappeared within 14 days when the rats were allowed to recover (Limpanussorn, *et al.*, 2000). In addition, the Chao-Praya-Apaipubate General Hospital in Prachinburi province, the leading hospital in utilizing herbal medicines for the treatment of common diseases, is dispensing the dried stem powder in the dose of 1.5-3.0 g for the treatment of hemorrhoid. However, no report for long-term toxicity test has been made. Thus in order to evaluate the safety of this plant, the present study, using an OECD guideline (1998), was conducted to investigate the subchronic toxicity of *C. quadrangularis* when administered orally for three months in rats.

Materials and Methods

Plant material and preparation

The plant was collected from the botanical garden of Chao-Praya-Apaipubate Hospital in Prachinburi province, and was verified by Miss Supaporn Pitiporn, the head pharmacist of the hospital. A voucher specimen (BKF 092329) was deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

The plant was washed with tap water, cut, dried in a hot air oven at 50ºC, ground and sieved with sieve No.100, then the powder stored in a brown bottle with cap at room temperature. The powder was suspended to the desired concentrations with distilled water. Phytochemical determination showed the quercetin constituent equivalent to 0.1216 microgram percent.

Treatment of the animals

Sixty male Wistar rats weighing 210±10 g and 60 female rats weighing 170±10 g from the National Laboratory Animal Center, Mahidol University, Nakornpathom province, were used. The animals were housed in the animal facility of the Department of Medical Sciences. The temperature in the animal room was kept at 25±1ºC with 60% relative humidity. The animals were allowed to have free access to food and clean water.

Three months toxicity study

According to the OECD guideline (1998), sixty Wistar rats of each sex were randomly divided into 5 groups of 12 animals per sex. Group 1 (water control) received water 10 milliliter/kilogram of body weight/day (ml/kg BW/day). Groups 2-5 were given the suspensions which were equivalent to 0.03, 0.3, 3.0 and 3.0 grams of dried powdered plant /kilogram of body weight/day (g/kg BW/day), which were equivalent to 1, 10, 100 and 100 folds of therapeutic dose (1.5 g/50-kg person/day), respectively. The last group was the recovery group. Body weight and food-intake were measured weekly and the animals were observed for signs of abnormalities for 3 months. At the end of the treatment period, the 1^{st} - 4^{th} groups of rats 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. The 5^{th} group of rats was withdrawn from of the feeding of the plant for another 14 days before being sacrificed.

Hematological analysis was performed using an automatic hematological analyzer (Cell Dyne 3500, Abbott). The parameters of the blood samples measured were: hematocrit (Hct), hemoglobin (Hb), red blood cell (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC), % neutrophil (%N), % lymphocyte (%L), % monocyte (%M), % eosinophil (%E), % basophil (%B), platelet, mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW) and reticulocyte.

Biochemical analysis of serum samples was performed using an automatic chemistry analyzer (Hitachi model 912). Biochemical parameters measured were alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), p-amylase, total protein, albumin, bilirubin, creatinine, glucose, uric acid, triglyceride, cholesterol, sodium, potassium and chloride.

The positions, shapes, sizes and colors of internal organs, namely, brain, heart, both kidneys and lungs, trachea, esophagus, stomach, liver, pancreas, intestine, spleen, bladder, salivary gland, adrenal gland 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% phosphate buffered formalin solution. Tissue slides were prepared and stained with hematoxylin and eosin and histopathological examinations were performed by a veterinary 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 set at $p < 0.05$.

Results and Discussion

Effects of the *C. quadrangularis* on body weight, food intake and relative organ weight

In both male and female animals, there was no difference in the average body weights between *C. quadrangularis*-treated groups and control group throughout the experimental period of 3 months (Figure 1). It was found that food consumption of animals receiving the *C. quadrangularis* was significantly different from the control groups for several weeks. Male rats receiving *C. quadrangularis* 3.0 g/kg/day had lower food intake than the male control group during 4^{th} - 12^{th} weeks and female rats receiving *C. quadrangularis* 0.03 g/kg/day had higher food intake than the female control group during 1^{st} - 13^{th} weeks of the study (Figure 2). In male rats, there was no difference in the relative organ weight between *C. quadrangularis*-treated group and control group (Table 1). Female rats treated with *C. quadrangularis* at the dose of 0.03 g/kg/day had lower relative organ weights of the brain and heart than the control group (Table 2).

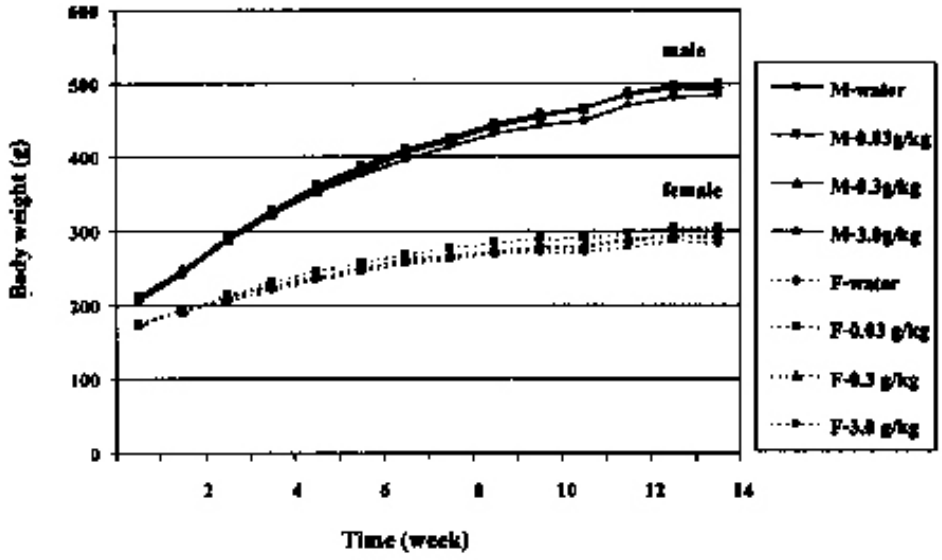

Figure 1. Growth curves of male and female rats receiving *C. quadrangularis* for 3 months

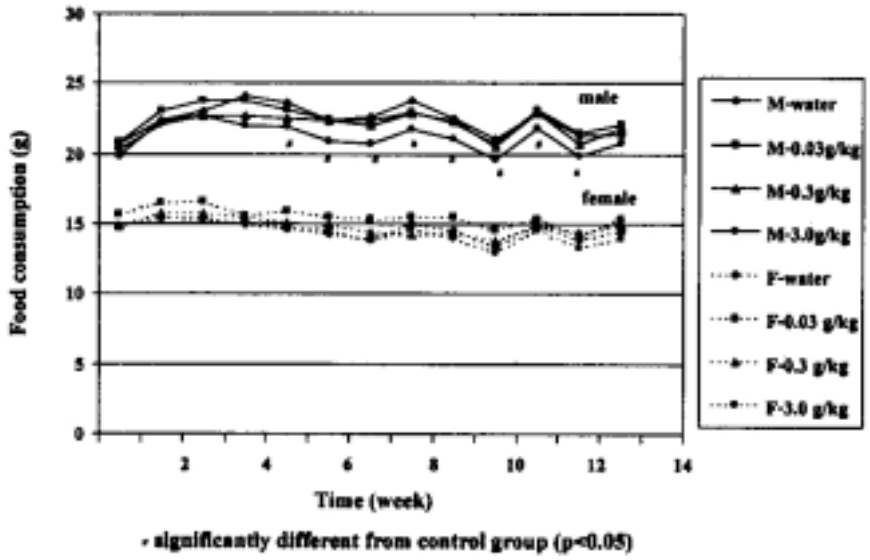

Figure 2. Food consumption of male and female rats receiving *C. quadrangularis* for 3 months

Table 1. Body weight (g) and relative organ weight (g/kg) of male rats receiving *C. quadrangularis* for 3 months

Organs	Dose of *C. quadrangularis* (g/kg BW/day)				
	control	0.03	0.3	3.0	3.0-R
	n = 12	n = 12	n = 12	n = 12	n = 12
Initial body weight	205±12	211±10	208±10	208±9	202±7
Final body weight	493±37	502±47	498±29	485±37	482±34
Brain	4.28±0.32	4.21±0.36	4.27±0.30	4.26±0.18	4.25±0.36
Heart	2.62±0.18	2.55±0.25	2.69±0.20	2.55±0.18	2.60±0.30
Lung	3.57±0.38	3.51±0.32	3.38±0.29	3.52±0.32	3.29±0.28
Liver	25.54±1.19	25.56±1.09	25.55±2.48	25.75±1.78	25.18±1.70
Stomach	3.72±0.32	3.73±0.35	3.77±0.39	3.88±0.37	3.65±0.20
Spleen	1.87±0.20	1.88±0.18	1.88±0.18	1.89±0.14	1.88±0.26
Right Kidney	2.58±0.18	2.55±0.14	2.64±0.17	2.61±0.18	2.59±0.19
Left Kidney	2.51±0.18	2.47±0.16	2.52±0.15	2.50±0.17	2.48±0.16
Right Testis	6.61±0.42	6.52±0.57	6.36±0.41	6.78±0.69	6.45±0.70
Left Testis	6.53±0.41	6.46±0.45	6.26±0.34	6.59±0.86	6.30±0.65
Right Adrenal	0.079±0.012	0.077±0.013	0.080±0.015	0.076±0.014	0.077±0.013
Left Adrenal	0.083±0.014	0.082±0.018	0.084±0.016	0.081±0.013	0.082±0.024
Bladder	0.255±0.042	0.274±0.035	0.240±0.042	0.262±0.037	0.238±0.054

R: Recovery group
The values are expressed as mean ± SD.

Table 2. Body weight (g) and relative organ weight (g/kg) of female rats receiving *C. quadrangularis* for 3 months

Organs	Dose of *C. quadrangularis* (g/kg BW/day)				
	control	0.03	0.3	3.0	3.0-R
	n = 12	n = 12	n = 12	n = 12	n = 12
Initial body weight	176±9	175±10	176±7	171±6	172±9
Final body weight	292±20	306±26	294±18	284±22	291±23
Brain	6.82±0.45	6.34±0.51*	6.71±0.44	6.91±0.54	6.75±0.62
Heart	3.06±0.27	2.81±0.22*	3.08±0.27	2.87±0.25	3.09±0.39
Lung	4.46±0.42	4.54±0.22	4.28±0.30	4.57±0.47	4.23±0.42
Liver	23.84±2.98	24.57±1.82	24.19±1.95	24.57±1.71	24.23±3.32
Stomach	4.98±0.76	4.89±0.60	4.94±0.32	5.14±0.50	5.11±0.59
Spleen	2.37±0.27	2.20±0.22	2.43±0.24	2.41±0.33	2.49±0.31
Right Kidney	2.84±0.24	2.78±0.20	2.79±0.27	2.78±0.25	2.86±0.32
Left Kidney	2.71±0.23	2.59±0.21	2.69±0.27	2.66±0.23	2.66±0.25
Right Adrenal	0.148±0.037	0.146±0.028	0.160±0.032	0.144±0.019	0.138±0.024
Left Adrenal	0.163±0.042	0.148±0.033	0.165±0.027	0.149±0.026	0.153±0.024
Uterus	3.00±0.75	2.80±1.06	3.03±0.56	2.50±0.31	2.96±0.75
Bladder	0.272±0.058	0.295±0.060	0.274±0.064	0.280±0.059	0.246±0.029

*** significantly different from control group ($p<0.05$)**
R: Recovery group
The values are expressed as mean ± SD.

Effect of *C. quadrangularis* on hematological parameters

There was no difference of hematocrit, the number of red blood cells, hemoglobin, MCV, MCH, MCHC, the number of white blood cell, %N, %L, platelet, PCT, PDW, MPV or the number of reticulocyte between *C. quadrangularis*-treated groups and control groups of either male or female rats (Tables 3 and 4). However the groups of male rats receiving *C. quadrangularis* had significantly lower %B than that of the control. Male rats receiving *C. quadrangularis* at the dose of 3.0 g/kg/day had significantly lower %M than that of the control. Male rats receiving *C. quadrangularis* at the dose of 0.3 g/kg/day had significantly lower percent of reticulocyte than that of the control but these values were within normal range (Gad, 1992). Female rats receiving *C. quadrangularis* at the dose of 3.0 g/kg/day had significantly higher %E than that of the control but these values were within normal range (Gad, 1992). In recovery groups, it was found that *C. quadrangularis* caused high %N and low %B in male, but caused high %B and low MCHC in female, however these values were within normal range (Gad, 1992).

Effect of the *C. quadrangularis* on blood chemistry

In male and female rats, no difference in the serum levels of ALP, ALT, AST, BUN, p-amylase, total protein, albumin, bilirubin, creatinine, glucose, uric acid, triglyceride, cholesterol, sodium, potassium and chloride was found between *C. quadrangularis*-treated groups and the control groups. It was found that *C. quadrangularis* caused high level of serum bilirubin, but caused low level of serum uric acid in male rats which were allowed to recover (Tables 5 and 6), however the values were

Table 3. Hematological values of male rats receiving *C. quadrangularis* for 3 months

Parameters	Dose of *C. quadrangularis* (g/kg BW/day)				
	control	0.03	0.3	3.0	3.0-R
	n = 12	n = 12	n = 12	n = 12	n = 12
Hematocrit (%)	45.70±3.04	45.64±2.22	46.30±1.36	44.95±3.33	44.99±2.37
RBC ($x10^6$ cells/mm^3)	9.11±0.71	8.90±0.34	9.11±0.34	8.84±0.39	8.78±0.45
Hemoglobin (g/dl)	15.68±0.55	15.62±0.59	15.62±0.40	15.48±0.48	15.45±0.52
MCV (μm^3/red cell)	50.18±1.81	51.25±1.76	50.85±1.55	50.79±2.40	51.25±1.03
MCH (pg/red cell)	17.30±1.22	17.55±0.54	17.15±0.39	17.57±0.62	17.63±0.69
MCHC (g/dl RBC)	34.52±2.51	34.28±1.13	33.75±0.56	34.72±2.29	34.43±1.53
WBC ($x10^3$ cells/mm^3)	5.62±1.38	5.70±1.28	5.90±1.26	5.86±0.91	5.18±0.79
Neutrophil (%)	11.72±2.89	11.17±3.01	13.58±4.3	13.34±3.83	15.53±6.35*
Eosinophil (%)	1.60±0.73	1.37±0.63	1.78±0.66	2.07±0.64	1.74±0.67
Lymphocyte (%)	81.77±5.55	84.25±4.36	81.07±5.23	82.17±3.65	79.07±6.66
Monocyte (%)	3.09±2.42	1.94±1.49	2.39±1.27	1.33±1.43*	2.44±1.26
Basophil (%)	1.83±1.19	1.27±0.30*	1.17±0.27*	1.10±0.44*	1.21±0.39*
Platelet ($x10^3$ cells/mm^3)	949±140	866±67	909±100	913±93	865±77
PCT (%)	0.86±0.20	0.79±0.16	0.86±0.11	0.76±0.17	0.86±0.11
PDW (%CV)	17.57±2.61	17.51±2.79	18.29±0.41	16.60±3.62	18.56±0.66
MPV (fl/platelet)	9.06±1.47	9.04±1.54	9.42±0.51	8.53±1.94	9.93±1.15
Reticulocyte ($x10^3$ cells/mm^3)	235±43	234±41	205±29	204±33	245±30
Reticulocyte (%)	2.61±0.39	2.62±0.46	2.26±0.33*	2.34±0.36	2.81±0.25

*** significantly different from control group ($p<0.05$)**
R: Recovery group
The values are expressed as mean ± SD.

within normal range (Gad, 1992). The significant change of serum sodium in recovery groups was less than that of the control group, however the lowered serum sodium had no clinical effect. This change did not occur in any of the treated groups. Hence, this change may not be due to the effect of *C. quadrangularis*.

Table 4. Hematological values of female rats receiving *C. quadrangularis* for 3 months

Parameters	Dose of *C. quadrangularis* (g/kg BW/day)				
	control	0.03	0.3	3.0	3.0-R
	n = 12	n = 12	n = 12	n = 12	n = 12
Hematocrit (%)	44.52±2.35	44.12±1.77	45.02±1.65	44.30±1.98	45.54±2.28
RBC ($x10^6$ cells/mm^3)	8.02±0.41	7.88±0.30	8.13±0.30	8.08±0.42	8.13±0.22
Hemoglobin (g/dl)	15.01±0.51	14.78±0.71	15.16±0.51	14.86±0.42	14.98±0.76
MCV (xm^3/red cell)	55.54±1.93	56.03±2.07	55.46±2.96	54.91±1.39	56.02±1.97
MCH (pg/red cell)	18.75±0.73	18.78±0.91	18.70±1.05	18.48±0.63	18.44±0.76
MCHC (g/dl RBC)	33.77±0.89	33.51±0.79	33.70±0.52	33.66±0.82	32.91±0.50*
WBC ($x10^3$ cells/mm^3)	2.66±0.65	2.79±0.53	2.89±0.87	2.71±0.57	2.40±0.47
Neutrophil (%)	10.99±4.00	14.77±5.22	12.20±4.59	14.61±6.02	14.85±3.65
Eosinophil (%)	1.60±0.31	1.53±0.47	1.88±0.85	2.27±1.10*	1.53±0.44
Lymphocyte (%)	84.53±4.12	80.12±6.83	83.27±5.86	79.62±6.79	79.22±5.37
Monocyte (%)	1.93±0.89	2.77±2.07	1.78±1.80	2.58±1.61	3.10±2.16
Basophil (%)	0.96±0.43	0.82±0.31	0.86±0.41	0.94±0.36	1.30±0.48*
Platelet ($x10^3$ cells/mm^3)	868±54	872±88	919±103	873±61	883±75
PCT (%)	0.79±0.05	0.80±0.08	0.85±0.08	0.80±0.07	0.83±0.06
PDW (%CV)	18.01±0.46	18.20±0.22	18.15±0.43	18.07±0.31	18.00±0.21
MPV (fl/platelet)	9.18±0.59	9.20±0.49	9.25±0.60	9.11±0.43	9.43±0.38
Reticulocyte ($x10^3$ cells/mm^3)	261±65	275±59	264±75	240±45	298±70
Reticulocyte (%)	3.29±0.79	3.46±0.72	3.24±0.88	2.98±0.53	3.66±0.83

*** significantly different from control group ($p<0.05$)**
R: Recovery group
The values are expressed as mean ± SD.

Effect of the *C. quadrangularis* on histopathology of internal organs

Upon gross examination of internal organs, no abnormal signs were observed. Histopathological results indicated that there was no lesion of salivary gland, spleen, pancreas or intestine in any group of animals (Tables 7 and 8). The incidence of peribronchiolar lymphoid aggregation in all

Table 5. Biochemical values of male rats receiving *C. quadrangularis* for 3 months

Parameters	Dose of *C. quadrangularis* (g/kg BW/day)				
	control	0.03	0.3	3.0	3.0-R
	n = 12	n = 12	n = 12	n = 12	n = 12
ALP (U/L)	64.00±11.39	64.08±8.94	65.92±8.73	60.17±8.17	66.50±8.02
ALT (U/L)	32.83±8.32	31.67±5.42	34.75±7.42	38.50±6.29	38.67±14.64
AST (U/L)	71.42±11.44	8.33±4.72	65.08±8.34	65.92±10.09	66.25±10.70
p-amylase (U/L)	1989±266	1960±185	2154±288	2070±205	1958±216
Total protein (g/dl)	6.57±0.76	6.50±0.32	6.66±0.31	6.49±0.42	6.72±0.22
Albumin (g/dl)	3.33±0.40	3.35±0.23	3.38±0.23	3.32±0.24	3.23±0.08
Bilirubin (mg/dl)	0.07±0.03	0.07±0.02	0.07±0.02	0.06±0.03	0.11±0.03*
BUN (mg/dl)	18.32±2.55	17.18±2.29	19.49±3.41	18.60±2.97	19.38±3.19
Creatinine (mg/dl)	0.64±0.07	0.64±0.04	0.65±0.05	0.62±0.06	0.62±0.04
Glucose (mg/dl)	170.48±25.77	169.16±18.26	175.09±23.06	170.54±18.18	157.54±11.48
Uric acid (mg/dl)	2.12±1.05	1.64±0.58	1.94±0.62	1.80±0.71	1.42±0.43*
Triglyceride (mg/dl)	127.54±46.73	125.72±38.37	144.11±33.39	145.38±48.91	131.76±12.78
Cholesterol (mg/dl)	72.39±24.92	62.24±8.62	70.04±10.98	67.13±15.03	59.36±12.78
Na^+ (mmol/l)	148±3	144±8	146±5	145±6	140±1*
K^+ (mmol/l	5.72±1.12	5.22±0.53	5.30±0.55	5.32±0.51	5.88±0.62
Cl^- (mmol/l)	108±4	106±5	106±2	107±3	110±2

*** significantly different from control group ($p<0.05$)**
R: Recovery group
The values are expressed as mean ± SD.

treated groups was significantly lower than the control group, and this incidence was increased in the recovery group. Hence, this change should be due to the effect of *C. quadrangularis*. Park and his colleague (2001) reported that β-sitosterol from the ethanol extract of cactus (*Opuntiaficus indica*) showed anti-inflammatory action in adjuvant-induced chronic inflammation model in mice. Because of β-sitosterol the same constituent in *C. quadrangularis*, this plant may have anti-inflammatory action too. In male rats receiving *C. quadrangularis* at the dose of 0.03 g/kg/day, the incidence of hepatocyte degeneration of the liver was not significantly different from that of the control. Cortex fatty degeneration of adrenal gland was noted in half of the male control group. However the incidence of fatty degeneration of the adrenal gland in all groups of male rats receiving *C. quadrangularis* was significantly lower than that of control, and there was no difference of the incidence between recovery and control group. This incidence seemed to be sex-related (Gad, 1992).

Table 6. Biochemical values of female rats receiving *C. quadrangularis* for 3 months

Parameters	Dose of *C. quadrangularis* (g/kg BW/day)				
	control	0.03	0.3	3.0	3.0-R
	n = 12	n = 12	n = 12	n = 12	n = 12
ALP (U/L)	28.42±3.87	28.17±5.37	29.17±5.49	27.25±4.35	27.92±6.57
ALT (U/L)	26.50±5.18	28.75±6.18	26.42±5.68	29.08±5.87	27.83±5.69
AST (U/L)	64.08±8.35	67.00±9.12	63.08±6.96	64.17±10.61	61.58±6.23
p-amylase (U/L)	1150±242	1168±180	1126±200	1081±183	1054±284
Total protein (g/dl)	6.92±0.40	6.91±0.36	6.72±0.42	6.61±0.58	6.98±0.25
Albumin (g/dl)	3.71±0.17	3.69±0.26	3.65±0.26	3.61±0.42	3.54±0.14
Bilirubin (mg/dl)	0.10±0.04	0.10±0.06	0.10±0.04	0.09±0.07	0.09±0.03
BUN (mg/dl)	19.48±1.88	19.74±2.17	19.56±3.04	20.19±3.55	19.43±3.70
Creatinine (mg/dl)	0.71±0.05	0.72±0.04	0.67±0.06	0.70±0.09	0.69±0.04
Glucose (mg/dl)	145.16±14.78	147.90±16.33	128.92±21.28	132.21±28.77	132.82±17.49
Uric acid (mg/dl)	1.62±0.50	1.37±0.50	1.37±0.50	1.48±0.57	1.42±0.61
Triglyceride (mg/dl)	79.05±32.62	73.85±23.84	79.58±38.35	86.26±26.42	84.15±32.48
Cholesterol (mg/dl)	71.71±22.00	73.85±17.98	68.07±12.91	67.99±12.77	67.10±14.15
Na^+ (mmol/l)	150±3	148±4	146±6	146±10	141±1*
K^+ (mmol/l)	5.00±0.66	4.79±0.60	4.88±1.01	4.61±0.90	4.99±0.68
Cl^- (mmol/l)	114±4	112±4	111±2	110±8	113±2

*** significantly different from control group ($p<0.05$)**
R: Recovery group
The values are expressed as mean ± SD.

Therefore, *C. quadrangularis* may reduce fat accumulation in the adrenal cortex. Fraser (1994) reported that β-sitosterol inhibited the absorption of both endogenous and exogenous cholesterol and in moderate doses lowered serum cholesterol. As in female rats receiving *C. quadrangularis*, though there were some lesions detected microscopically in the liver and kidneys in some groups of animals, but this change was not significantly different from control group. We found that only the congestion of adrenal gland in recovery group was significantly higher than that of control; however, this lesion might be caused by the stress from anesthetic process as well (Koplewitz, *et al.*, 1998; Pugachev, 1977; Bassett and Cairncross, 1975).

Conclusion

Three-month subchronic toxicity study of *C. quadrangularis* Linn. in Wistar rats indicated that the dried stem powder at the doses of 0.03, 0.3 and 3.0 g/kg BW/day, which were equivalent to 1, 10

Table 7. Histopathological values of male rats receiving *C. quadrangularis* for 3 months

Organs	Microscopic findings	Dose of *C. quadrangularis* (g/kg BW/day)				
		control	0.03	0.3	3.0	3.0-R
		n = 12	n = 12	n = 12	n = 12	n = 12
Lung	Peribronchiolar lymphoid aggregation	7/12	0/12*	0/12*	2/12*	3/12
Heart	Focal myocarditis	1/12	0/12	0/12	0/12	1/12
Liver	Hepatocyte degeneration	1/12	1/12	0/12	0/12	0/12
Kidney		0/12	0/12	0/12	0/12	0/12
Spleen		0/12	0/12	0/12	0/12	0/12
Pancreas		0/12	0/12	0/12	0/12	0/12
GI tract		0/12	0/12	0/12	0/12	0/12
Testis		0/12	0/12	0/12	0/12	0/12
Adrenal gland	Cortex fatty degeneration	6/12	0/12*	0/12*	1/12*	6/12
Salivary gland		0/12	0/12	0/12	0/12	0/12

*** significantly different from control group ($p<0.05$)**
R: Recovery group
The results are expressed as number of rats with pathological findings/ total number of rats examined.

and 100 fold the therapeutic dose did not produce any significant dose-related changes of hematological parameters, serum biochemistry or histopathology of any internal organs. Therefore, it is concluded that *C. quadrangularis* at the given doses did not produce any significant toxic effect in rats during the period of treatment for 3 months.

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References

Banjob, M., Pecharaply, D., Chaiyaraj, S. and Sawangwong, C. 2000a. Medicinal plants in North Eastern, Part 2. Institute of Medicinal Plant Research, Department of Medical Sciences, Ministry of Public Health. Thanasinjaroen Printing, Bangkok, Thailand. p.55. (in Thai)

Banjob, M., Pecharaply, D., Chaiyaraj, S. andSawangwong, C. 2000b. Medicinal plants in North Eastern, Part 3. Institute of Medicinal Plant Research, Department of Medical Sciences, Ministry of Public Health. S.R. Printing Massproducts Co. Ltd., Bangkok, Thailand. p.68. (in Thai)

Bassett, J.R. and Cairncross, K.D. 1975. Morphological changes induced in rats following prolonged exposure to stress. Pharmacol. Biochem. Behav. 3(3): 411-20.

Table 8. Histopathological values of female rats receiving *C. quadrangularis* for 3 months

Organs	Microscopic findings	Dose of *C. quadrangularis* (g/kg BW/day)				
		control	0.03	0.3	3.0	3.0-R
		n = 12	n = 12	n = 12	n = 12	n = 12
Lung	Peribronchiolar lymphoid proliferation	3/12	0/12*	0/12*	1/12*	2/12
Heart	Focal myocarditis	0/12	0/12	0/12	0/12	0/12
Liver	Lymphoid aggregated periportal area	0/12	1/12	1/12	0/12	1/12 (focal necrosis)
Kidney	Tubular cast cyst	7/12	9/12	4/12	6/12	7/12
	Lymphoid aggregation	0/12	0/12	0/12	0/12	0/12
Spleen		0/12	0/12	0/12	0/12	0/12
Pancreas		0/12	0/12	0/12	0/12	0/12
GI tract		0/12	0/12	0/12	0/12	0/12
Ovary Uterus Cervix		0/12	0/12	0/12	0/12	0/12
Adrenal gland	Congestion	0/12	0/12	0/12	0/12	6/12*
Salivary gland		0/12	0/12	0/12	0/12	0/12

*** significantly different from control group ($p<0.05$)**
R: Recovery group
The results are expressed as number of rats with pathological findings/total number of rats examined.

Bhutani, K.K., Kapoor, R. and Atal, C.K. 1984. Two unsymmetric tetracyclic triterpenoids from *Cissus quadrangularis*. Phytochemistry. 23(2): 407-10.

Chopra, S.S., Patel, M.R. and Awadhiya, R.P. 1976. Studies on *Cissus quadrangularis* in experimental fracture repair: a histopathological study. Indian J. Med. Res. 64:1365-75.

Chopra, S.S., Patel, M.R., Gupta, L.P. and Datta, I.C. 1975. Studies on *Cissus quadrangularis* in experimental fracture repair: effect on chemical parameters in blood. Indian J. Med. Res. 63:824-8.

Fraser, GE. 1994. Diet and coronary heart disease: beyond dietary fats and low-density-lipoprotein cholesterol. Amer. J. Clin. Nutri. 59: 1117-23.

Gad, S.C. 1992. The Rat: Pathology. In: Gad, S.C., Chengellis, C.P. (Eds.), Animal Model in Toxicology, Marcel Dekker, New York, USA. pp. 81, 111-3.

Koplewitz, B.Z., Daneman, A., Cutz, E. and Hellmann, J. 1998. Neonatal adrenal congestion: a sonographic-pathologic correlation. Pediatr. Radiol. 28(12): 958-62.

Lhieochaiphunt, S. and Sangdee, P. 1985. An acute toxicity testing of *Cissus quadrangularis*;

Vitaceae. Research Report. Faculty of Pharmacy, Chieng Mai University. pp.15. (in Thai)

Limpanussorn, J., Poongchompu, S., Thisayakorn, K. *et al.* 2000. Toxicity study of *Cissus quadrangularis* L. In: Guideline for Medicinal Plant Development of Thailand. Thailand Institute of Scientific and Technological Research. p.47. (in Thai)

Madan, M.G. and Ram, K.V. 1990. Unsymmetric tetracyclic triterpenoid from *Cissus quadrangularis*. Phytochemistry. 29(1): 336-7.

OECD. 1998. OECD Principles of Good Laboratory Practice, Section 2. OECD, Paris, France.

Park, E.,Kahng, J., Lee, S.H., Shin, K. 2001. An anti-inflammatory principle from cactus. Fitoterapia. 72(3): 288-90.

Pongboonrod, S. 1950. Mai Thed Muang Thai. Kaseambunnakit Printing, Bangkok, Thailand. pp.428-9. (in Thai)

Pugachev, M.K. 1977. Problem of thickening of the adrenal cortex in acute thermal stress. Arkh. Anat. Gistol. Embriol. 72(3): 73-7.

Quisumbing, E. 1951. Medicinal plants of the Philippines. Tech. Bull. 16: 1-10.

Saburi, A.A., Rene, N., Marie-Therese, M., *et al*. 1999. Stilbene derivatives from *Cissus quadrangularis*. J. Nat Prod. 62: 1694-5.

Segsunviriya, C. and Choomprabutra, S. 1989. A clinical study of *Cissus quadrangularis* Linn. in hemorrhoid patients. Seminar of Research and Development of Medicinal Plant. Division of Medical Research, Department of Medical Sciences, Ministry of Public Health. pp. 54-5. (in Thai)

Sen, S.P. 1964. Active constituents (oxo steroids) of *Cissus quadrangularis*. Indian J. Pharmacy. 26(9): 247-8.

Tiangburanatham, W. 1996. Dictionary of Thai Medicinal Plants. Prachumtong Printing, Bangkok, Thailand. pp. 572-3. (in Thai)

Pluemjai, T. and Saifah, E. 1986. Constituents of *Cissus quadrangularis* Linn. Th. J. Pharm.Sci. 11(4): 205-11.

Udupa, K.N. and Prasad, G. 1964. Biomechanical and 45-CA studies on the effect of *Cissus quadrangularis* in fracture repair. Indian J. Med. Res. 52(5): 480-7.

Udupa, K.N., Prasad, G and Sen, S.P. 1965. The effect of phytogenic anabolic steroid in the acceleration of fracture repair. Life Sci. 4(3): 317-27.

Wuthithammawej, W. 1994. Thai Traditional Medicine. O.S. Printing House, Bangkok, Thailand. p. 337. (in Thai)