ORIGINAL ARTICLE

Chronic toxicity study of crude extract of *Derris scandens* Benth

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Abstract

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Chronic toxicity study of crude extract of *Derris scandens* Benth
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Derris scandens Benth, or "Thao-Wan-Priang" in Thai, is one of the most commonly used medicinal plants in Thailand; however, the safety of this herb upon long-term consumption has never been reported. Therefore, a six-month chronic toxicity study of 50% ethanolic extract of *Derris scandens* was performed in four groups of 20 Wistar rats of each sex. Water control group received 10 ml of water/kg BW/day. The three treatment groups were given the extract at the doses of 6, 60 and 600 mg/kg BW/day, which were equivalent to dried stems 0.03, 0.3 and 3 g/kg BW/day or 1, 10 and 100 fold the therapeutic dose, respectively. No difference of initial or final body weights between extract-treated and control groups was detected. It was found that the extract did not produce any significant dose-related changes of hematological parameters or serum chemistry, and no histopathological lesion of any internal organ that could be due to the toxic effect of the extract was observed. The results indicated that 50% ethanolic extract of *D. scandens* at the doses given did not produce any toxicity in the rat.

Key words : *Derris scandens*, toxicity, Thao-Wan-Priang

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การศึกษาพิษเรื้อรังของสารสกัดหยาบของเถาวัลย์เปรียง (*Derris scandens* Benth)
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เถาวัลย์เปรียง (*Derris scandens* Benth) เป็นสมุนไพรไทยที่ใช้กันมากอีกชนิดหนึ่ง โดยที่ไม่มีรายงานความปลอดภัยของสมุนไพรนี้หากใช้เป็นเวลานาน ดังนั้น จึงได้ศึกษาพิษเรื้อรัง (6 เดือน) ของสารสกัดด้วย 50% เอธานอลของเถาวัลย์เปรียงในหนูขาวพันธุ์วิสตาร์ 4 กลุ่ม ๆ ละ 20 ตัวต่อเพศ กลุ่มควบคุมได้รับน้ำ 10 มล/น้ำหนักตัว 1 กก/วัน ขณะที่หนูอีกสามกลุ่มได้รับสารสกัดในขนาด 6, 60 และ 600 มก/น้ำหนักตัว 1 กก/วัน หรือเทียบเท่าผงเถาวัลย์เปรียงแห้ง 0.03, 0.3 และ 3 กรัม/น้ำหนักตัว 1 กก/วัน หรือ 1, 10 และ 100 เท่าของขนาดใช้ในคนต่อวัน ผลการศึกษาพบว่าสารสกัดของเถาวัลย์เปรียงไม่ทำให้เกิดการเปลี่ยนแปลงของค่าทางโลหิตวิทยา ค่าทางชีวเคมีของซีรั่ม หรือจุลพยาธิสภาพของอวัยวะภายในที่มีความสัมพันธ์กับขนาดของสารสกัด และไม่พบความผิดปกติใด ๆ ที่สามารถสรุปได้ว่าเนื่องมาจากความเป็นพิษของสารสกัด

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Derris scandens Benth. is a medicinal plant in the Leguminosae family commonly known in Thai as "Thao-Wan-Priang" (Muanwongyathi and Supatwanich, 1981). It is a woody vine widely distributed throughout Thailand. Its dried stem is used in Thai traditional medicine as expectorant, antitussive, diuretic, antidysentery, and for the treatment of muscle ache and pain, while the root is used as fish poison (Tiangburanatham, 1996, Anonymous, 1967). Phytochemical studies show that the stem contains eturunagarone, warangalone, 8-γ, γ-dimethylallyl-wighteone, 3'-γ,γ-dimethylallyl-wighteone, scandinone, robustic acid, and 4,4'-Di-O-methyl scandenin (Rao, *et al.*, 1994). The root is reported to contain scandenin, nallanin, chadanin (Wang, *et al.*, 1997) osajin, scandenone (warangalone), scandinone (Rao and Seshadri, 1946), chandalone, lonchocarpic acid and lonchocarpenin (Pelter and Stainton, 1966, Falshaw, *et al.*, 1969). It was recently found that warangalone, robustic acid, 8-γ, γ-dimethylallylwighteone, and 3'-γ,γ-dimethylallylwighteone are selective and potent inhibitors of rat liver cyclic AMP-dependent protein kinase catalytic subunit (cAK) with IC_{50} at 3.5, 10, 20, 24 and 33 μM, respectively. It was then suggested that the potent inhibitory action of warangalone and robustic acid on cAK may play a role in *in vivo* biological activity and insecticidal activity of *D. scandens* (Wang, *et al.*, 1997). Recently, our laboratory has reported that 50% ethanolic extract of *D. scandens* showed marked *in vitro* immunomodulating activity in mouse splenic lymphocytes (Chuthaputti and Chavalittumrong, 1998).

D. scandens is said to be one of the most commonly used medicinal plants in Thailand (Pongboonrod, 1950). Rural people usually take infusion of roasted dried stem of *D. scandens* or alcoholic macerate of *D. scandens* stem for the relief of muscle ache after work or as tonic. In addition, Chao Praya Apaipubate General Hospital at Prachin Buri province, which is the forefront user of herbal medicines for the treatment of common diseases, dispensed this dried powdered plant at the dose of 1.5-3 g for the treatment of muscle ache. However, there has been no report of toxicity study of this plant especially upon repeated dosing basis. Since, our laboratory found that 50% ethanolic extract of *D. scandens* possessed immunostimulating activity, chronic toxicity study of this extract was then perfomed in rats in order to evaluate the safety of this extract prior to the evaluation of its therapeutic efficacy in humans.

Materials and Methods

Plant material and preparation of plant extract

Dried stem of *D. scandens* was obtained from Chao Praya Apaipubate Hospital, Prachin Buri province. The plant was identified by Miss Supaporn Pitiporn, the head pharmacist of the hospital. Fifty percent ethanolic extract of *D. scandens* was prepared by reflux method and the extract was dried under vacuum in a rotary evaporator. Prior to the experiment, the extract was diluted to the desired concentrations with water.

Treatment of the animals

Eighty male Wistar rats weighing 160 ± 10 g and 80 female rats weighing 140 ± 10 g from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom 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 ± 1 °C with 60% relative humidity. The animals were allowed to have free access to food and clean water.

Chronic toxicity study

Eighty Wistar rats of each sex were randomly divided into 4 groups of 20 animals per sex. Group 1 (water control) received water 10 ml/kg BW/day and Groups 2-4 were given the extract at the doses of 6, 60 or 600 mg/kg BW/day which were equivalent to 0.03, 0.3 or 3 g of dried powdered plant/kg BW/day or 1, 10 or 100 fold the therapeutic dose (1.5 g/50-kg person/day), 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.

Hematological analysis was performed using an automatic hematological analyser (Cell dyne 3500, Abbott). Hematological parameters measured were white blood cell (WBC), %neutrophil, %lymphocyte, %monocyte, %eosinophil, %basophil, red blood cell (RBC), hemoglobin, hematocrit (Hct), and platelet.

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

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 veterinary pathologist.

Statistical analysis

The data were analyzed by one-way ANOVA followed by Duncan's 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

Effects of the extract 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 extract-treated groups and control groups from the start until the end of the experiment (Figure 1). It was found that food consumption of animals receiving the extract was significantly higher than that of the control groups for several weeks, i.e. in male rats during 2nd-9th weeks and female rats during 21st - 23rd weeks of the study (Figure 2). However, the final body weights of male and female animals receiving the extract were not significantly different from that of the control groups (Table 1). Male rats treated

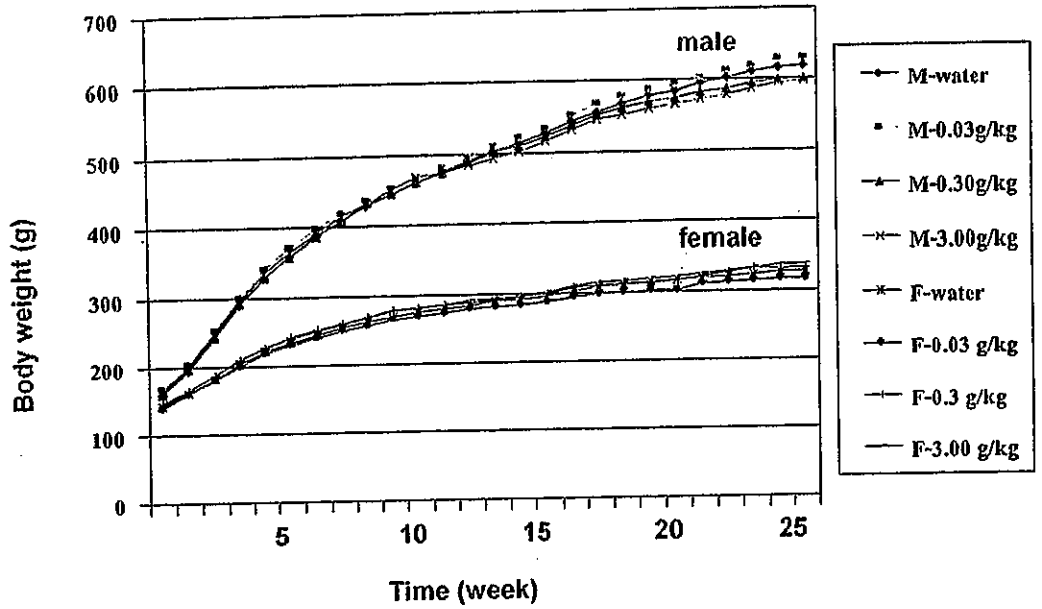

Figure 1 Growth curves of rats treated with *Derris scandens* extract for 6 months

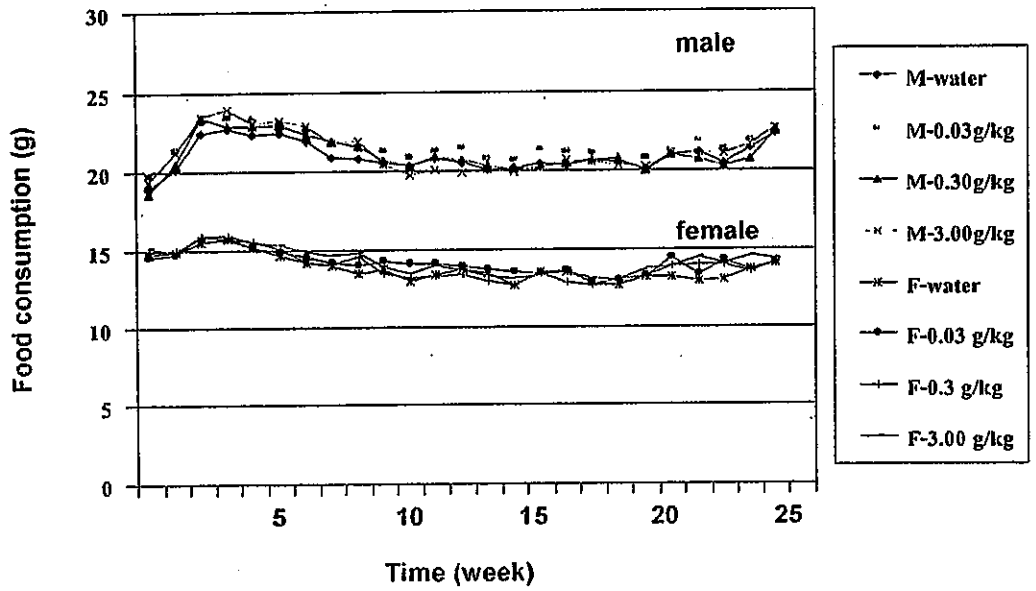

Figure 2 Food consumption of rats treated with *Derris scandens* extract for 6 months.

with the extract at the dose of 600 mg/kg had higher relative weights of the stomach, the lung and the right and left testis than its control group, while male rats receiving the extract 60 mg/kg had higher relative weights of the liver and the right testis than the control group. In contrast, there was no difference of relative organ weights in female rats (Table 1).

Effect of the extract on hematological parameters:

There was no difference of the number of white blood cells, %neutrophil, %lymphocyte, %monocyte, hemoglobin or hematocrit between

Table 1. Relative organ weight[#] (g/kg BW) and body weight of rats treated with *Derris scandens* extract for 6 months.

	Group of male animals				Group of female animals			
	water N= 20	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=18	water N=20	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=19
Initial body weight (g)	158.6±23.8	168.2±20.7	159.3±13.9	162.3±18.3	142.4±12.3	139.6±9.9	146.8±11.8	145.1±9.6
Final body weight (g)	618.4±68.1	628.8±57.4	600.4±44.3	595.4±37.8	327.2±38.9	316.6±31.9	332.7±31.3	336.±41.3
Brain	3.52±0.36	3.35±0.30	3.53±0.27	3.60±0.22	6.09±0.64	6.24±0.54	5.94±0.52	5.88±0.65
Heart	2.42±0.36	2.43±0.22	2.43±0.19	2.52±0.16	2.96±0.29	2.98±0.34	2.84±0.27	2.82±0.28
Right kidney	2.37±0.25	2.37±0.22	2.38±0.20	2.49±0.18	2.68±0.19	2.74±0.28	2.61±0.22	2.60±0.23
Left kidney	2.24±0.23	2.26±0.21	2.36±0.18	2.36±0.16	2.53±0.17	2.61±0.29	2.50±0.20	2.54±0.23
Urinary bladder	0.247±0.08	0.217±0.08	0.225±.05	0.246±.08	0.25±0.05	0.27±0.06	0.25±0.05	0.27±0.06
Liver	23.78±2.44	24.77±1.89	25.31±2.14*	25.13±1.52	24.02±3.11	24.31±2.95	23.05±2.70	25.57±3.82
Spleen	1.84±0.33	1.72±0.16	1.84±0.23	1.75±0.16	2.10±0.20	2.19±0.21	2.04±0.20	2.14±0.34
Stomach	3.41±0.35	3.29±0.83	3.63±0.45	3.78±0.31*	4.92±0.67	5.05±0.74	4.85±0.68	5.35±0.82
Lung	3.00±0.30	3.02±0.34	3.25±0.43	3.32±0.40*	4.29±0.48	4.42±0.66	4.19±0.64	4.17±0.37
Right testis	4.98±0.92	5.24±0.60	5.51±0.52*	5.51±0.54*				
Left testis	5.00±0.87	5.15±0.59	5.36±0.46	5.59±0.49*				

[#] **Relative organ weight = organ weight(g)/body weight(kg), Each value represents mean ± SD.**
*** Significantly different from water control group ($p < 0.05$)**

Table 2. Results of hematological examinations of rats treated with *Derris scandens* extract for 6 months.

	Group of male animals				Group of female animals			
	water N=20	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=18	water N=20	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=17
White blood cells (K/uL)	5.43±1.15	4.79±1.10	5.15±1.03	4.72±0.94	3.05±0.76	3.28±0.75	3.11±0.55	3.25±0.84
Neutrophil (%)	16.65±6.50	17.01±3.48	15.66±3.43	16.21±3.68	14.07±6.52	14.92±5.80	12.71±3.82	16.83±7.11
Lymphocyte (%)	72.36±7.28	71.30±4.86	73.12±4.57	74.24±4.73	74.28±8.67	75.33±7.16	77.35±6.12	73.36±8.77
Monocyte (%)	6.52±2.31	7.50±2.40	7.22±2.23	6.30±2.10	7.54±3.06	6.34±2.12	6.98±3.15	6.94±3.10
Eosinophil(%)	1.23±0.40	1.37±0.49	1.24±0.42	1.29±0.41	1.65±0.44	1.32±0.31*	1.35±0.38*	1.18±0.26*
Basophil (%)	3.23±1.46	2.84±1.37	2.77±1.54	1.98±0.98*	2.47±0.84	2.08±0.71	1.60±0.84*	1.69±0.85*
Erythrocytes (M/uL)	9.13±0.30	9.41±0.41*	9.34±0.39	9.21±0.48	8.63±0.40	8.60±030	8.58±0.39	8.46±0.49
Hemoglobin (g/dL)	15.85±0.56	15.92±0.44	15.84±0.50	15.91±0.27	15.98±0.56	15.83±0.57	15.76±0.51	15.82±0.39
Hematocrit (%)	47.26±1.48	48.10±1.88	47.87±1.65	48.22±1.27	47.30±1.66	47.30±1.96	46.90±1.77	46.88±3.10
Platelet (K/uL)	934±119	1055±136*	1015±121*	1053±114*	899±96	933±91	960±92	914±86

Each value represents mean ± SD.
*** Significantly different from water control group ($p < 0.05$)**

extract-treated groups and control groups of both male and female rats (Table 2). The groups of male and female rats receiving the extract at the dose of 600 mg/kg had significantly lower %basophils than their controls. In male rats, the numbers of platelets in all extract-treated groups were significantly higher than that of the control; however, this change was not dose-dependent and did not occur in female rats. Meanwhile, %eosinophil of all groups of female, but not male, rats treated with the extract were significantly lower than that of the control.

Effect of the extract on blood chemistry

In male and female rats, no difference in the serum levels of ALT, ALP, bilirubin, BUN, triglyceride, total protein, albumin, uric acid or sodium was found between all extract-treated groups and the control groups (Table 3). In male rats, all extract-treated groups had significantly lower AST level than their control. Male rats receiving the extract at the doses of 60 and 600 mg/kg BW had significantly higher potassium levels while those treated with 60 mg/kg extract had significantly higher cholesterol level than the control. Meanwhile, female rats receiving 600 mg extract/kg BW/day had significantly lower creatinine level but higher glucose level than its control.

Effect of the extract on histopathology of internal organs

Upon gross examinations of internal organs, no abnormal signs were observed. Histopathological results indicated that there was no lesion of thyroid gland, spleen, pancreas, and ovary in all groups of animals (Table 4). In female rats receiving the extract at the dose of 6 mg/kg, the incidence of

Table 3. Blood chemistry of rats treated with *Derris scandens* extract for 6 months.

	Group of male animals				Group of female animals			
	water N=19	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=18	water N=20	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=19
AST (U/L)	98.53±18.96	84.65±9.11*	87.00±23.24	77.44±12.56*	100.95±32.74	81.05±6.60	77.30±13.97	87.37±61.98
ALT (U/L)	37.74±6.12	38.95±6.64	37.10±7.16	36.78±7.13	36.25±10.96	30.55±6.71	32.55±5.26	32.95±11.70
ALP (U/L)	73.63±9.16	73.50±13.84	73.60±14.61	68.61±9.03	28.25±6.08	29.05±7.88	27.90±7.10	28.95±8.70
Bilirubin (mg/dL)	0.09±0.02	0.10±0.03	0.10±0.02	0.10±0.03	0.11±0.05	0.11±0.03	0.10±0.03	0.10±0.04
Creatinine (mg/dL)	0.69±0.03	0.68±0.04	0.68±0.06	0.68±0.06	0.74±0.07	0.74±0.05	0.72±0.04	0.70±0.06*
BUN (mg/dL)	18.63±2.56	17.4±2.30	19.30±2.34	17.94±1.66	21.70±4.16	22.55±3.20	20.35±3.23	20.63±3.55
Cholesterol (mg/dL)	71.95±18.03	82.35±16.60	88.65±22.58*	78.39±12.13	77.60±22.24	72.35±17.07	76.45±19.07	77.63±29.36
Triglyceride (mg/dL)	173.32±47.06	192.45±62.99	192.65±59.24	172.56±49.68	129.15±68.97	131.15±65.52	149.35±78.58	139.42±71.79
Total protein (g/dL)	7.14±0.33	7.25±0.25	7.36±0.43	7.23±0.23	7.43±0.49	7.24±0.33	7.37±0.33	7.46±0.45
Albumin (g/dL)	3.45±0.15	3.46±0.13	3.52±0.18	3.52±0.10	3.92±0.27	3.84±0.17	3.91±0.20	3.90±0.25
Uric acid (mg/dL)	1.94±0.59	2.23±0.90	2.41±0.96	2.03±0.77	1.44±0.43	1.48±0.45	1.48±0.37	1.43±0.46
Glucose (mg/dL)	134.38±21.45	142.13±23.50	146.51±23.32	142.89±13.93	104.98±15.21	101.96±14.74	111.85±19.75	120.39±19.61*
Sodium (mmol/L)	148.00±2.38	148.55±1.73	148.15±2.03	148.56±2.26	146.65±4.78	147.10±5.08	147.15±4.37	147.58±4.26
Potassium (mmol/L)	4.58±0.58	4.53±0.57	5.54±1.11*	5.47±1.32*	4.23±0.68	4.64±1.09	4.67±0.67	4.47±0.85

Each value represents mean ± SD.
* Significantly different from water control group ($p < 0.05$)

Table 4. Histopathological evaluations of rats treated with *Derris scandens* extract for 6 months.

Organ	Lesion	Group of male animals				Group of female animals			
		water N=19	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=18	water N=20	6 mg/kg N=20	60 mg/kg N=20	600 mg/kg N=19
Brain	Mild focal non-suppurative meningitis	0/20	1/20	0/20	0/18	1/20	0/20	0/20	0/19
Lung	Increased numbers of alveolar macrophages	0/20	0/20	0/20	0/18	1/20	0/20	0/20	0/19
Thyroid		0/20	0/20	0/20	0/18	0/20	0/20	0/20	0/19
Heart	Mild focal non-suppurative myocarditis	3/20	1/20	1/20	0/18	0/20	0/20	0/20	0/19
Liver	Mild fatty infiltration	4/20	0/20	3/20	1/18	0/20	0/20	2/20	0/19
Kidney	Proteinaceous casts were found in the tubules	3/20	6/20	0/20	1/18	0/20	6/20*	2/20	1/19
Spleen		0/20	0/20	0/20	0/18	0/20	0/20	0/20	0/19
Pancreas		0/20	0/20	0/20	0/18	0/20	0/20	0/20	0/19
GI tract	Increased numbers of eosinophils	4/20	0/20	0/20	0/18	1/20	0/20	0/20	0/19
Testis	Testicular hypoplasia	4/20	0/20	0/20	0/18				
Ovary						0/20	0/20	0/20	0/19

Each value represents number of rats with pathological abnormalities/total number of rats examined.
*** Significantly different from water control group ($p < 0.05$)**

proteinaceous casts in the tubules of the kidney was significantly higher than in the control. However, this change was not dose-dependent. Similarly, there were some lesions detected microscopically in the brain, lung, heart, liver, GI tract and testis on some groups of animals; however, none of these histopathological findings was dose-dependent.

Discussion

In this chronic toxicity study, 50% ethanolic extract of *D. scandens* was given to Wistar rats at the doses of 6, 60 and 600 mg/kg for 6 months. This type of extract was chosen because it should contain most groups of chemicals present in the stem of *D. scandens* that people consume. The doses selected were about 1, 10 and 100 fold the therapeutic dose of *D. scandens*, respectively.

The extract at the doses given did not affect the body weight gain of the animals throughout the study, nor decrease food consumption of the animals even at the highest dose of 600 mg/kg. In contrast, both male and female animals treated with the extract ate more than their controls did during certain periods of the study.

Hematological study indicated that the extract did not affect the numbers of white blood cells, %neutrophil, %lymphocyte, %monocyte,

hemoglobin or hematocrit of either male or female animals. In addition, histopathological study did not show any lesion of the spleens in any group of the animals. However, %eosinophil of all extract-treated female rats, and %basophil of animals receiving 600 mg/kg extract and female rats receiving 60 mg/kg extract were significantly lower than those of the control. Since these two types of polymorphonuclear leukocytes are supposed to be undetectable or present only a very small percentage in blood (Gad, 1992), the lower numbers of these cells in extract-treated groups as compared to their controls should not be harmful to the animals. In all extract-treated male rats, the numbers of platelets were significantly higher than in the control but these changes were within normal range (Smith, 1995).

With regard to the effects of the extract on blood chemistry, it was found that all groups of male rats treated with the extract had significantly lower AST levels than their control; however, this change occurred only in male rats and not in female rats. Furthermore, no histopathological change indicative of hepatotoxic effect of the extract was observed.

The significantly higher levels of chloesterol and potassium in certain groups of male rats were not dose-dependent changes and occurred only in male rats. Hence, these changes should not be due to the effect of the extract. A significantly lower creatinine level and a higher glucose level were found only in female rats treated 600 mg/kg extract; these changes were however still within normal limits (Gad, 1992).

Histopathological findings of some lesions in the brain, lung, heart, liver, kidney, GI tract and testis in certain groups of animals as shown in Table 4 were not dose-dependent; hence, these lesions were not suggestive of toxicity of the extract to any particular organs.

Conclusion

Six-month chronic toxicity study of 50% ethanolic extract of *Derris scandens* in Wistar rats indicated that the extract at the doses of 6, 60 and 600 mg/kg BW/day, which were equivalent to the dried stem 0.03, 0.3 and 3.00 g/kg BW, or 1, 10 and 100 fold the therapeutic dose, did not produce any significant dose-related changes of hematological parameters, serum chemistry, or histopathology of any internal organs. Therefore, it is concluded that 50% ethanolic extract of *D. scandens* at the doses given did not produce any toxic effect in rats.

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