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Original Article

Acute and subchronic toxicity study of the water extract from *Harrisonia perforata* Merr. in rats.

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Abstract

The water extract from *Harrisonia perforata* Merr. was studied for acute and subchronic toxicities. The extract at a single dose of 5,000 mg/kg was administered orally to female and male rats (5 males, 5 females). After 14 days, signs and behavioral changes, mortality, gross and histopathological changes of internal organs were examined. The body weight of the male treated rats was significantly decreased when compared to the control group. The extract did not produce signs of toxicity. For the subchronic toxicity test, the extract at doses of 300, 600 and 1,200 mg/kg body weight were orally administered to rats daily for 90 days (10 males, 10 females). Observation of signs, behavior and health status showed no abnormality in the test groups as compared with the controls. However, the body weight of all male treated rats was significantly decreased when compared to the control group. At the end of the study, necropsy and histopathology examination were performed in all animals in the control group, the test groups and the satellite group in which the extract was discontinued for another 28 days. Body and organ weights, hematological and blood clinical chemistry were also examined. The results suggest that the water extract of *Harrisonia perforata* Merr. does not cause acute and subchronic toxicities in rats.

Keywords: acute toxicity, subchronic toxicity, *Harrisonia perforata* Merr.

1. Introduction

Harrisonia perforata Merr. (Thai name "Khon Tha") is belonged to Family Simaroubaceae. The leaves, wood and root-bark of *H. perforata* have been used medically (Perry, 1980). The extracts of the leaves and the branches showed *in vitro* antimalarial activity against *Plasmodium falciparum* (Bremner, 1992). *H. perforata* (roots and stem) exhibited a bactericidal effect against *Mycobacterium smegmatis* (Chea *et al*., 2007). Moreover, this plant showed interesting antiplasmodial activity (Nguyen-Pouplin *et al*., 2007) and inhibited adherence of *S. mutans* ATCC 25175 (Limsong *et al*., 2004).

Four chromones, perforamone A, B, C, and D have been isolated together with six known compounds, peucenin-7-methyl ether, O-methylalloptaeroxylin, perforatic acid, eugenin, saikochromone A and greveichromenol, from the branches of *H. perforata* (Tuntiwachwuttikul *et al*., 2006).

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Limonoids were isolated from a sample of *H. perforata* leaves, named haperforins C2, F and G (Khuong-Huu *et al.*, 2001) and haperforine A and haperforine E (Khuong-Huu *et al.*, 2000). Nonetheless, the toxicity of *H. perforata* has never been evaluated. The present study aimed to assess the adverse effects related to different doses in order to find the acceptable safety level of the water extract from *H. perforata* in rats by determining both oral acute and subchronic toxicities.

2. Materials and Methods

2.1 Plant material

Barks of *H. perforata* Merr. were collected from Songkhla, Thailand. The voucher specimen (SBK 0010) was kept and identified by the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

2.2 Preparation of plant extract

Powder of *H. perforata*, 500 grams, was wrapped in a calico bag and put into a stainless boiler. Ten liters of water were added, then boiled for 3-4 hours and collected the filtrate. The residue was extracted again. The combined filtrates were concentrated in a rotary evaporator.

2.3 Laboratory animals

Male and female Sprague-Dawley rats, weighing 130-190 g were obtained from the National Laboratory Animal Center, Nakorn Pathom, Thailand. They were all clinically healthy and maintained in environmentally controlled conditions at 24+1^{o}C under a 12-hour-dark-light cycle, and given a standard diet and water *ad libitum*, throughout the experimental period. All of the experimental protocols in animals were ethically approved by The Animal Ethics Committee of Faculty of Medicine, Thammasat University.

2.4 Acute toxicity study

Acute toxicity test was performed according to the World Health Organization (WHO) guideline (WHO, 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (OECD, 2001). The extract was prepared at the concentration of 2,500 mg/ml in distilled water. Five animals per sex were administered a single oral dose of 5,000 mg/kg body weight while distilled water was given to the other group of rats as a control. All rats were observed at the first, second, fourth and sixth hours and thereafter once daily over 14 days for clinical signs of toxicity such as respiratory pattern, color of body surfaces, frequency and nature of movement, marked involuntary contraction or seizures of contraction of voluntary muscle, and loss of reflex etc. After the experimental period, all animals were sacrificed and their internal organs including heart, lungs, livers, kidneys, spleen, adrenals, sex organs and brain were weighed and taken for gross pathological examination.

2.5 Subchronic toxicity study

The method was performed following the protocol described by the WHO guideline (WHO, 2000) and the OECD guideline for testing of chemicals (OECD, 1981). Male and female rats were randomly divided into four groups of ten. The extract was prepared at concentrations of 300, 600 and 1,200 mg/ml in distilled water. The extract was orally administered to treated groups at doses of 300, 600 and 1,200 mg/kg/day, while distilled water was given to the control group. An additional group was devised as the satellite group in order to observe the reverse sign of any toxicity. The satellite group was orally treated with the extract at a daily dose of 1,200 mg/kg/day for 90 days, and no further treatment for the following 28 days before termination of the study.

At the end of the study, all rats were fasted overnight and anesthetized with ether for blood collection. Blood was collected from the common carotid artery into a heparinized tube for hematological studies (complete blood count, red blood cell count, platelet count and red cell indices). The serum was separated for determining the concentrations of glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total bilirubin, direct bilirubin, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP).

After the blood collection, internal organs including heart, lungs, thymus, liver, kidneys, spleen, adrenals, small intestine, stomach and duodenum, muscle with sciatic nerve, thoracic spines, brain, eyes, sex organs, uterus and epididymis were examined for gross pathology. All tissues were fixed in 10% buffered formalin solution. After routine processing, the paraffin sections of each tissue were cut at 5 mm thickness and stained with haematoxylin and eosin for histopathological examination.

2.6 Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. The data obtained from acute toxicity studies were analyzed using Student's paired *t*-test. P values less than 0.05 were considered significant.

3. Results and Discussion

In the acute toxicity study, rats fed with the water extract of *H. perforata* at the dose of 5,000 mg/kg did not show any sign of toxicity over 14-day period of observation. However, in the male groups, rats treated with *H. perforata*

Table 1. Body weights of rats in the acute toxicity study of the water extract of *Harrisonia perforata* Merr.

	Body weight (g)			
	Day 0	Day 7th	Day 14th	Weight gain on day 14th
Female				
Control	137.20±11.74	168.00±8.37	182.80±9.22	45.60±6.30
H. perforata 5,000 mg/kg	127.20±1.20	162.00±4.05	174.80±1.62	47.60±2.79
Male				
Control	142.00±4.43	190.00±7.56	228.00±10.53	86.00±6.60
H. perforata 5,000 mg/kg	138.80±3.88	189.60±4.45	205.20±5.12*	66.40±3.92*

Values are expressed as mean ± S.E.M., n = 5.
* Significantly different from control, $p<0.05$.

showed significantly lower of body weight on the 14th day, and the total body-weight gain over 14 days was also significantly less than those of the control group (Table 1). Nevertheless, there was no significant difference of body weight among female rats when compared to the control (Table 1). The weights of internal organs from both male and female rats were not significantly different from those of the control group (Table 2). Moreover, gross and histological examinations of the internal organs revealed no pathological abnormality. These results suggest that the water extract of *H. perforata* is not toxic to the rats after an acute exposure at the dose of 5,000 mg/kg.

In the subchronic toxicity study, results of the body weight and the total body-weight change over 90 days of all tested rats are shown in Table 3. On the day 90th, the body weights of male rats treated with the extract at all three doses (300, 600, and 1,200 mg/kg/day) and of female rats treated with the extract at 300 and 600 mg/kg/day were significantly lower when compared with the control groups. Nevertheless, all animals appeared to be healthy as shown by the normal appearance of respiratory pattern, color of body surfaces, frequency and nature of movement, marked involuntary contraction or seizures of contraction of voluntary muscle, and loss of reflex etc. As shown in Table 4, the weights of internal organs (including heart, kidney and ovary in female rats and heart, spleen and kidney in male rats) of the treated groups were significantly lower than those of the control while the weight of testis of male rats was significantly higher than the control (Table 4). It is noteworthy that these differences of internal-organ weights may be due to the variation in size of internal organs of the animals (Auletta, 1995). There were no signs of behavioral changes in any treated group. Moreover, following necropsy and pathological examination, no abnormal change of the internal organs of any rat was observed (Figure 1-4). To determine hematological effect and bone marrow activity in rats treated with the extract, parameters, including Hb, Hct, RBC counts and indices, and platelets counts, were examined and results are presented in Table 5 (for female rats) and Table 6 (for male rats). In female rat treated with 600 mg/kg and satellite groups, mean corpuscular hemoglobin concentration (MCHC) was significantly lower than the control whereas the Hct in the satellite group was significantly higher (Table 5). In the male group, Hb concentration in rats treated with 300 and 600 mg/kg/day was significantly lower than that of the control while MCV and MCH were significantly higher than the control among rats in the satellite group (Table 6). The differential white blood cell count results are shown in Table 7. In the female treated groups, there were a significant decrease in per cent neutrophils and a significant increase in per cent lymphocytes in the satellite group when compared to the control (Table 7). In the male group, rats treated with 600 mg/kg/day and the satellite group had significantly lower per cent eosinophils than the control while rats treated with

Table 2. Organ weights of rats in the acute toxicity study of the water extract of *Harrisonia perforata* Merr.
Organ weight (g)

	Control	*H. perforata* 5,000 mg/kg
Female		
Lung	1.06±0.06	0.99±0.03
Heart	0.76±0.04	0.76±0.01
Liver	7.63±0.39	6.78±0.17
Spleen	0.50±0.02	0.51±0.01
Adrenal	0.04±0.00	0.03±0.00
Kidney	0.79±0.03	0.81±0.02
Ovary	0.06±0.00	0.06±0.00
Male		
Lung	1.14±0.05	1.02±0.02
Heart	0.99±0.04	0.88±0.03
Liver	8.38±0.88	8.07±0.71
Spleen	0.77±0.06	0.68±0.02
Adrenal	0.04±0.00	0.03±0.00
Kidney	1.03±0.04	0.95±0.03
Testis	1.80±0.07	1.29±0.02

Values are expressed as mean ± S.E.M., n = 5.
There were no significant differences at $p<0.05$.

Table 3. Body weights of rats in the subchronic toxicity study of the water extract of *Harrisonia perforata* Merr.

	Body weight (g)			
	Day 0	Day 90th	Day 118th	Weight gain on day 90th
Female				
Control	150.20±3.96	274.40±4.43	-	124.20±6.91
H. perforata 300 mg/kg	150.80±2.56	260.20±5.10*	-	109.40±6.59
H. perforata 600 mg/kg	142.00±3.18	254.40±4.44*	-	113.40±5.72
H. perforata [a] 1,200 mg/kg	151.20±3.40	263.20±3.07	-	112.00±3.54
H. perforata [b] 1,200 mg/kg	151.20±2.11	270.60±4.88	281.00±7.05	119.40±4.30
Male				
Control	176.80±6.45	433.60±15.43	-	256.80±18.29
H. perforata 300 mg/kg	183.40±6.03	390.30±5.42*	-	206.90±6.90*
H. perforata 600 mg/kg	179.64±4.90	364.360±6.73*	-	184.73±10.05*
H. perforata [a] 1,200 mg/kg	183.40±5.48	383.60±16.24*	-	200.20±15.67*
H. perforata [b] 1,200 mg/kg	182.80±5.96	377.20±9.30*	405.80±9.42	194.40±11.46*

Values are expressed as mean ± S.E.M., n = 10
a: A group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.
* Significantly different from control, $p<0.05$.

Table 4. Organ weights of rats in the subchronic toxicity study of the water extract of *Harrisonia perforata* Merr.

	Control	*H. perforata*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
Lung	1.29±0.03	1.25±0.03	1.22±0.06	1.19±0.02	1.31±0.04
Heart	1.05±0.03	0.95±0.02*	0.95±0.02*	0.98±0.02	1.04±0.02
Liver	5.75±0.16	6.15±0.20	6.02±0.24	5.68±0.14	6.04±0.20
Spleen	0.68±0.02	0.66±0.02	0.66±0.02	0.63±0.02	0.64±0.01
Adrenal	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.00
Kidney	0.96±0.04	0.89±0.02	0.86±0.04*	0.87±0.02	0.87±0.02
Ovary	0.10±0.00	0.09±0.00	0.08±0.00*	0.10±0.00	0.08±0.00*
Male					
Lung	1.54±0.06	1.52±0.05	1.45±0.03	1.58±0.04	1.62±0.05
Heart	1.43±0.06	1.27±0.02*	1.22±0.02*	1.31±0.05*	1.29±0.02*
Liver	9.98±0.30	9.68±0.40	9.45±0.27	10.33±0.36	9.41±0.25
Spleen	0.89±0.04	0.80±0.02*	0.78±0.02*	0.85±0.02	0.87±0.02
Adrenal	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.05±0.00
Kidney	1.23±0.02	1.20±0.01	1.16±0.02*	1.24±0.02	1.20±0.02
Testis	1.93±0.02	1.91±0.03	1.86±0.02	2.01±0.02*	1.88±0.02

Values are expressed as mean ± S.E.M., n = 10
a: A group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.
* Significantly different from control, $p<0.05$.

1,200 mg/kg/day shower significant higher per cent monocytes (Table 7). However, such changes were minor and might have any important clinical consequence. Most importantly, the white blood cell numbers were not different among treated groups compared to the control and the values remained within the normal range (Feldman *et al*., 2000; Inala *et al.*, 2002).

Clinical blood chemistry examination was performed in order to evaluate any toxic effect on liver and kidney. The results are summarized in Tables 8 and 9. In female groups, there was a significant decrease in the level of total bilirubin among rats treated with 300 and 1,200 mg/kg/day when compared to the control (Table 8). The BUN level was also significantly lower among rats treated with 1,200 mg/kg/day and the satellite group when compared to the control (Table 8). Female rats in the satellite group also had a significantly lower level of ALP than the control (Table 8). In male rats, the BUN level was significantly lower in rats from the satellite group than the control (Table 9). The ALP level was also significantly higher among rats treated with 300 and 600 mg/kg/day (Table 9). Male rats treated with 300 and 1,200 mg/kg/day also showed a significant increase in the total protein level when compared to the control (Table 9). It is worth noting that the levels of these clinical blood chemical parameters, though changed, were still within the normal range (Angkhasirisap *et al*., 2002; Levine, 1995; Caisey and King, 1980; Sacher and McPherson, 1991a, 1991b). Overall, the results suggest that the extract does not induce toxicity to the kidneys and liver. These observations were further confirmed by the histological assessment of the organs shown in Figures 1-4. There were no significant

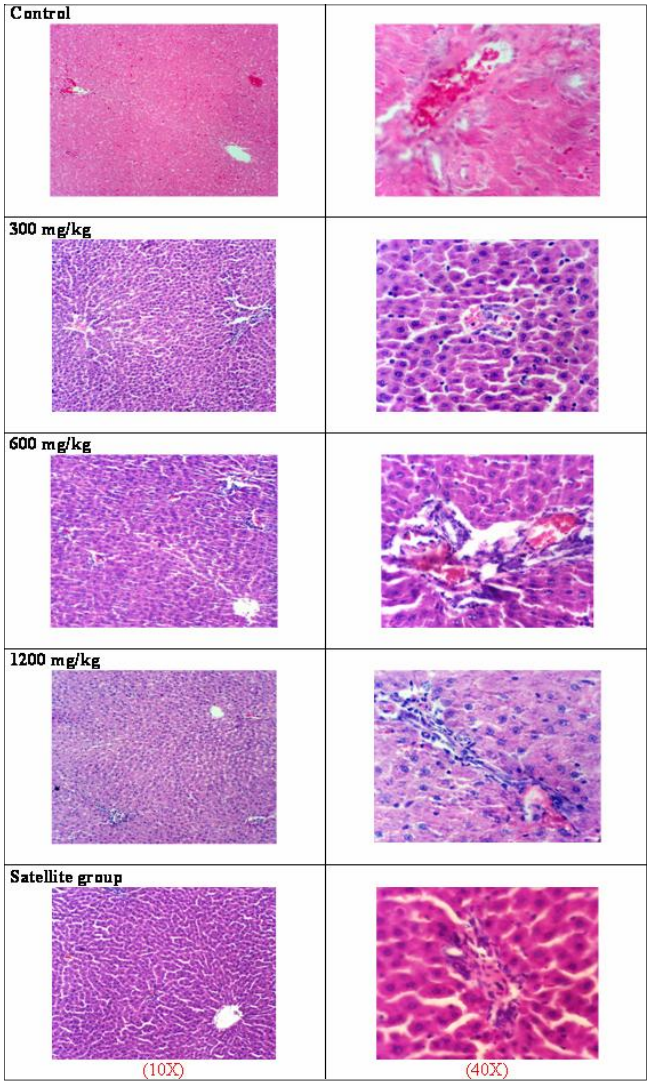

Figure 1. The histology of male liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

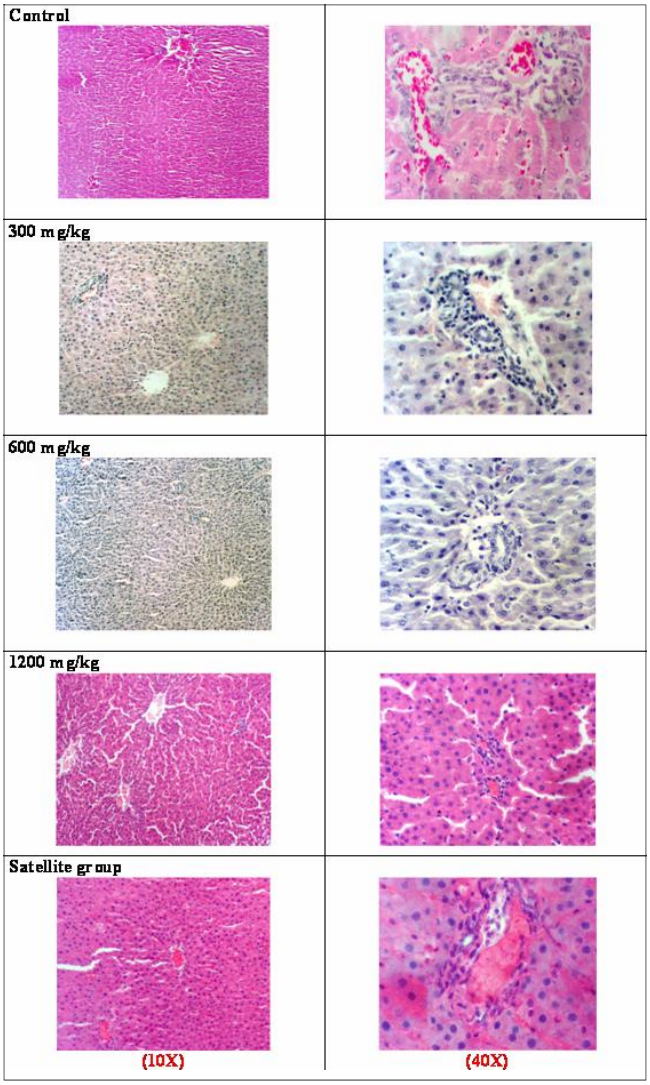

Figure 2. The histology of female liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

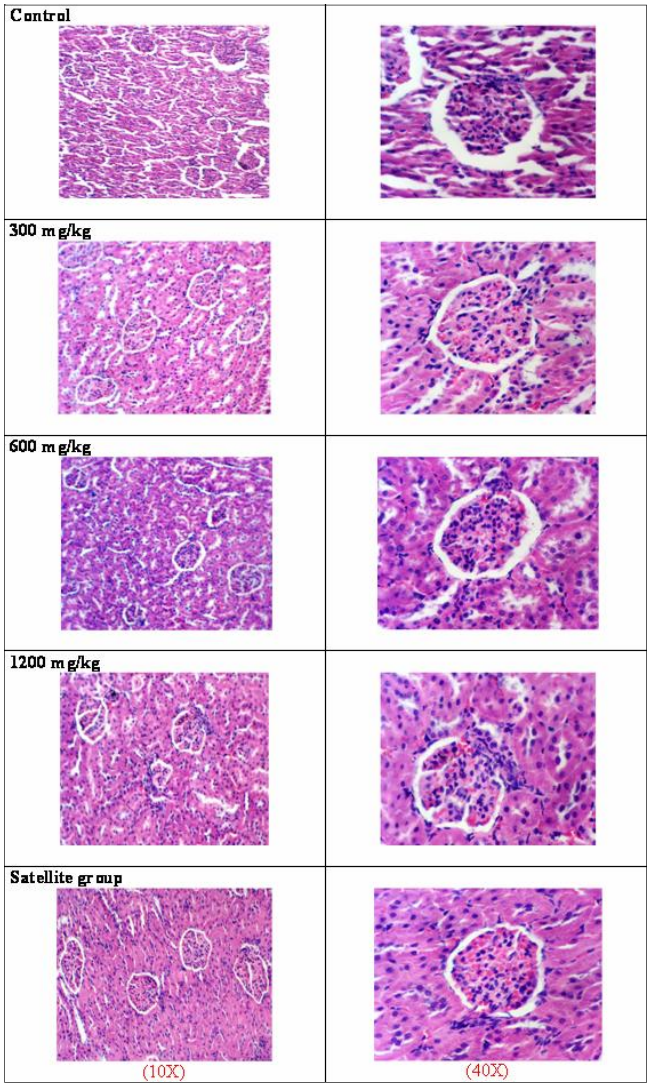

Figure 3. The histology of male kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

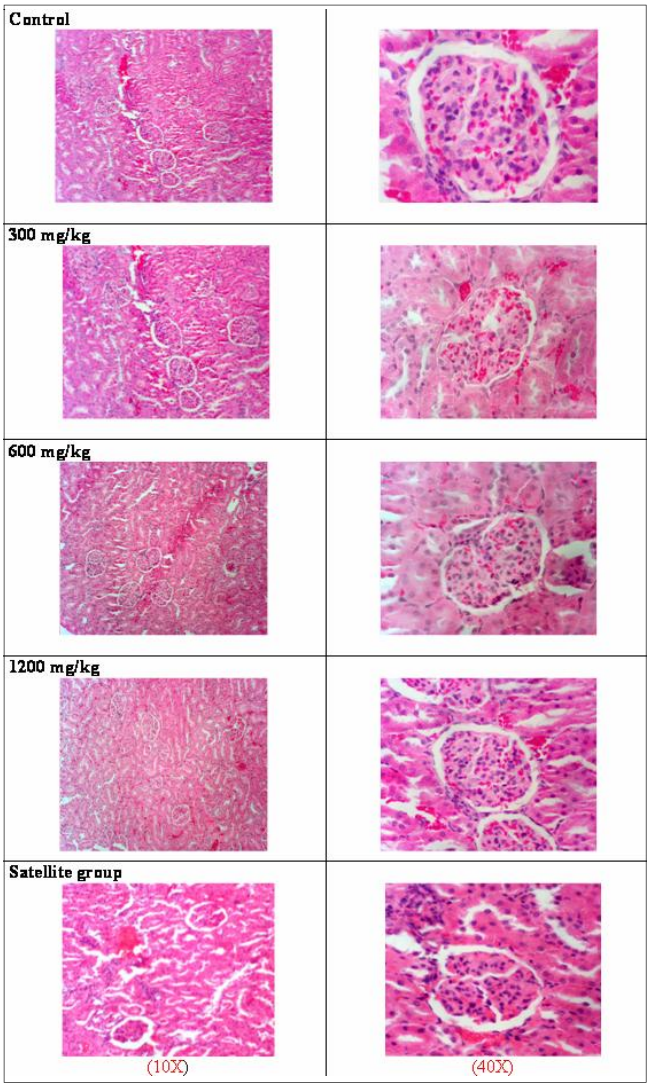

Figure 4. The histology of female kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

damages within liver or kidney. In conclusion, the extract of *H. perforata* at the oral doses treated did not cause either acute or subchronic toxicities in rats.

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Table 5. Hematological values of female rats in the subchronic toxicity study of the water extract of *Harrisonia perforata* Merr.

	Control	*H. perforata*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells ($x10^6$/μl)	6.77±0.08	6.70±0.17	6.84±0.08	6.80±0.06	6.88±0.09
Hemoglobin (g/dl)	14.73±0.14	14.40±0.36	14.53±0.11	14.74±0.16	14.74±0.24
Hematocrit (%)	40.56±0.53	40.40±1.02	41.40±0.47	41.10±0.37	42.90±0.58*
Mean corpuscular volume (fl)	59.80±0.38	60.15±0.39	60.32±0.47	61.37±0.99	62.17±0.47*
Mean corpuscular hemoglobin (pg)	21.75±0.33	21.48±0.12	21.18±033	21.56±0.22	21.52±0.11
Mean corpuscular hemoglobin concentration (g/dl)	36.38±0.49	35.73±0.20	35.07±0.41*	35.86±0.28	34.52±0.40*
Platelet ($x10^5$/μl)	7.42±0.15	7.51±0.42	7.86±0.27	8.15±0.24	7.91±0.09

Values are expressed as mean ± S.E.M., n = 10

a: A group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days.

b: A satellite group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.

* Significantly different from control, $p<0.05$.

Table 6. Hematological values of male rats in the subchronic toxicity study of the water extract of *Harrisonia perforata* Merr.

	Control	*H. perforata*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells ($x10^6$/μl)	7.66±0.37	7.40±0.16	7.54±0.09	7.58±0.11	7.30±0.06
Hemoglobin (g/dl)	15.14±0.53	14.17±0.22*	14.25±0.0*	14.719±0.19	15.05±0.12
Hematocrit (%)	44.60±2.27	43.20±0.94	43.40±0.56	43.60±0.65	43.80±0.62
Mean corpuscular volume (fl)	57.35±0.39	58.27±0.16	57.38±0.15	57.33±0.43	59.93±0.55*
Mean corpuscular hemoglobin (pg)	19.53±0.61	19.07±0.15	18.88±0.09	19.40±0.15	20.61±0.09*
Mean corpuscular hemoglobin concentration (g/dl)	34.08±1.11	32.75±0.25	32.91±0.12	34.80±0.12	34.42±0.33
Platelet ($x10^5$/μl)	7.86±0.12	7.86±0.09	8.01±0.09	7.87±0.08	8.09±0.25

Values are expressed as mean ± S.E.M., n = 10

a: A group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days.

b: A satellite group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.

* Significantly different from control, $p<0.05$.

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Table 7. Differential white blood cell count values of rats in the subchronic toxicity study of the water extract of *Harrisonia perforata* Merr.

	Control	*H. perforata*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
White blood cells ($x10^3$/μl)	3.00±0.32	3.44±0.33	2.80±0.26	3.25±0.22	2.87±0.13
Neutrophil (%)	19.00±1.86	17.60±0.96	18.40±1.09	17.80±1.29	12.50±1.39*
Lymphocyte (%)	74.44±2.10	76.10±1.21	75.00±1.06	77.30±1.45	79.00±1.71*
Monocyte (%)	5.67±0.55	5.30±0.63	5.10±0.31	4.30±0.26	6.50±0.91
Eosinophil (%)	0.89±0.35	1.00±0.39	1.80±0.38	0.60±0.16	2.00±0.83
Basophil (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Male					
White blood cells ($x10^3$/μl)	3.48±0.24	3.18±0.22	3.33±0.27	3.07±0.24	3.25±0.15
Neutrophil (%)	16.60±1.86	22.70±3.95	19.60±2.50	15.80±1.38	17.80±0.84
Lymphocyte (%)	76.40±1.85	72.60±3.58	74.10±2.51	73.50±2.32	76.50±0.89
Monocyte (%)	4.40±0.71	3.20±0.51	2.60±0.47	6.90±0.82*	4.80±0.57
Eosinophil (%)	2.80±0.84	1.50±0.30	0.80±0.32*	3.80±0.77	0.90±0.31*
Basophil (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values are expressed as mean ± S.E.M., n = 10

a: A group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days.

b: A satellite group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.

* Significantly different from control, $p<0.05$.

Table 8. Clinical blood chemistry values of female rats in the subchronic toxicity study of the water extract of *Harrisonia perforata* Merr.

	Control	*H. perforata*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	104.80±2.28	96.50±3.78	97.80±4.24	105.40±1.97	108.80±3.61
BUN (mg/dl)	23.33±1.41	21.40±0.77	21.40±0.67	20.50±0.74*	19.20±0.38*
Creatinine (mg/dl)	0.41±0.02	0.44±0.01	0.39±0.02	0.39±0.01	0.38±0.02
Total protein (g/dl)	5.10±0.08	5.25±0.12	5.14±0.08	5.06±0.07	5.25±0.06
Albumin (g/dl)	3.60±0.06	3.58±0.07	3.65±0.06	3.50±0.06	3.77±0.06
Total bilirubin (mg/dl)	0.17±0.01	0.13±0.01*	0.16±0.01	0.10±0.00*	0. 14±0.01
Direct bilirubin (mg/dl)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
SGOT (U/l)	103.10±2.18	115.60±10.97	91.40±2.74	92.80±3.72	103.10±4.45
SGPT (U/l)	31.40±1.69	39.40±9.63	25.20±1.40	24.90±0.82	29.30±1.31
ALP (U/l)	42.30±2.44	43.30±1.66	45.00±2.32	40.00±1.56	30.70±1.12*

Values are expressed as mean ± S.E.M., n = 10

a: A group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days.

b: A satellite group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.

* Significantly different from control, $p<0.05$.

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Table 9. Clinical blood chemistry values of male rats in the subchronic toxicity study of the water extract of *Harrisonia perforata* Merr.

	Control	*H. perforata*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	125.10±4.64	122.10±6.67	132.40±6.45	135.09±6.65	121.30±5.55
BUN (mg/dl)	21.20±0.86	22.30±1.08	21.40±0.67	21.30±0.58	18.30±0.47*
Creatinine (mg/dl)	0.31±0.01	0.32±0.02	0.28±0.02	0.28±0.02	0.35±0.02
Total protein (g/dl)	5.52±0.12	5.87±0.12*	5.62±0.09	5.98±0.11*	5.25±0.10
Albumin (g/dl)	3.41±0.07	3.50±0.05	3.43±0.05	3.34±0.05	3.50±0.06
Total bilirubin (mg/dl)	0.0±0.01	0.12±0.01	0.10±0.00	0.10±0.00	0. 10±0.00
Direct bilirubin (mg/dl)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
SGOT (U/l)	113.40±4.90	110.30±5.16	100.00±6.25	110.10±4.90	101.60±7.37
SGPT (U/l)	40.10±4.15	47.10±4.00	36.70±1.72	41.55±2.49	36.30±2.01
ALP (U/l)	66.60±6.41	96.70±9.23*	91.40±4.85*	78.00±5.43	57.40±1.58

Values are expressed as mean ± S.E.M., n = 10

a: A group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days.

b: A satellite group was treated with the water extract of *H. perforata* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.

* Significantly different from control, $p<0.05$.

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