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Original Article

Acute and subchronic toxicity study of the water extract from root of *Sida rhombifolia* Linn. in rats

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Abstract

Acute and subchronic toxicities of the water extract from the root of *Sida rhombifolia* Linn. were studied in both male and female rats. Oral administration of the extract at a single dose of 5,000 mg/kg body weight (5 males, 5 females) did not produce signs of toxicity, behavioral changes, mortality or differences on gross appearance of internal organs. The subchronic toxicity was determined by oral feeding the test substance at the doses of 300, 600 and 1,200 mg/kg body weight for 90 days (10 males, 10 females). The examinations of signs, animal behavior and health monitoring showed no signs of abnormalities in the test groups as compared to the controls. The test and control groups (on the 90th day) and the satellite group (on the 118th day) were analyzed by measuring their final body and organ weights, taking necropsy, and examining hematological parameters, blood clinical chemistry and histopathology features. The results suggest that *S. rhombifolia* administered orally did not cause acute or subchronic toxicities to male and female rats.

Keywords: acute toxicity, subchronic toxicity, *S. rhombifolia*

1. Introduction

Sida rhombifolia Linn., family Malvaceae (Thai name Khat Mon) is widely used in Ayurvedic medicine for the treatment of fever as well as a diuretic. Seven ecdysteroids, including the three new compounds 1-3, were isolated from *Sida rhombifolia*. Their structures and configurations were determined by extensive spectroscopic techniques in combination with chemical derivatization. The four known compounds-ecdysone (4), 20-hydroxyecdysone (5), 2-deoxy-20-hydroxyecdysone-3-O-beta-D-glucopyranoside (6), and 20-hydroxyecdysone-3-O-beta-D-glucopyranoside (7) are reported for the first time from this plant (Jadhav *et al*., 2007). The root of *S. rhombifolia* has been found to exert antioxidant activity (Dhalwal *et al*., 2007). The crude extracts from the leaves of *S. rhombifolia* showed cytotoxicity and antibacterial activities (Islam *et al*., 2003). Nonetheless, the toxicity of *S. rhombifolia* has never been evaluated. The present study was aimed to assess the adverse effects related to different doses in order to find the acceptably safe level of the water extract from root of *S. rhombifolia* in rats by determining both oral acute and subchronic toxicities.

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2. Materials and Methods

2.1 Plant material

The roots of *Sida rhombifolia* Linn. were collected from Songkhla, Thailand. The voucher specimen (SBK 0017) 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

Root powder of *S. rhombifolia* 500 grams were wrapped in a calico bag and put into a stainless steal boiler. Ten liters of water were added and boiled for 3-4 hours, then filtered when it had cooled down. The residue from the filtration was boiled and filtered again with the same procedure. The filtrates were collected and evaporated in a rotary evaporator until concentrated.

2.3 Experimental animals

Male and female Sprague-Dawley rats, weighing 130-190 g were obtained from the National Laboratory Animal Center, Nakhon Pathom, Thailand. They were housed under standard environmental conditions of temperature at $24\pm1^{o}C$ under a 12 h dark-light cycle, and allowed free access to drinking water and standard pelleted diet. Rats were deprived of food except water 16-18 hour prior the experiments. All experimental protocols were approved by the Animal Ethics Committee of Faculty of Medicine, Thammasat University.

2.4 Acute toxicity

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, TG420 (OECD, 2001), 10 rats were randomly divided into two groups of 5 animals per sex. The extract at a single dose of 5,000 mg/kg body weight was given orally to the treated group (the extract at concentration 2,500 mg/ml in distilled water), while the control group received water vehicle. Body weight, signs of toxicity and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for 14 days. On the 15^{th} day, all rats were fasted for 16-18 hours, and then sacrificed for necropsy examination. The internal organs were excised and weighed. The gross pathological observations of the tissues were performed by histopathological examination.

2.5 Subchronic toxicity

According to WHO guideline (WHO, 2000) and the OECD TG408 (OECD, 1981), rats were divided into 5 groups of 20 animals (10 male and 10 female). The extract at concentration 300, 600 and 1,200 mg/ml in distilled water was given orally to each groups of rats daily for 90 days, while the control group received water vehicle. In order to assess reversibility effect, the extract at the dose of 1,200 mg/kg was given once daily to the fifth group of rats for 90 days, and kept for another 28 days post treatment. Toxic manifestations such as signs of toxicity, mortality and the body weight changes were monitored daily.

Rats were anesthetized with ether on day 91^{st} and 118^{th} (satellite groups). Heparinized blood samples were taken for determining complete blood count, red blood cell count, platelet count and red cell indices. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis.

All rats were sacrificed after the blood collection. The internal organs and some tissues were weighed to determine relative organs weights and observed for gross lesions. All tissues were preserved in 10% buffered formadehyde solution for histopathological examination.

2.6 Statistical analysis

Results were expressed as mean $\pm$ 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

Changes in general behaviors, body weight and internal organ weight are critical for the objective evaluation of the effect of a compound on test animals, since such changes are often the first signs of toxicity (Auletta, 1995). In acute toxicity study, after the water extract from root of *S. rhombifolia* at a single dose of 5,000 mg/kg was orally given to the rats, neither sign of toxicity nor death of rats was observed during the 14 days of the experimental period. Toxicity evaluation was further carried out by observing both body weight gain and internal organ weight as showed in Tables 1 and 2, respectively. Neither body weight nor internal organ weight of treated rats was significantly changed relative to the control group. Furthermore, gross and histopathological examinations of the internal organs revealed no pathological abnormality as compared with the control. These results suggest that the water extract from root of *S. rhombifolia* is practically not toxic after an acute exposure.

In the subchronic toxicity study, the body weight and body weight gain in treated groups of male and female rats showed a decrease significantly different from those of the control group (Table 3). Neither changes in animal behaviors nor toxic signs were detected in the treated rats. Usually, the major toxic effect involves one or two organs and they represent target organs of toxicity of the particular substance. The degree of the toxic effect is also varied in different

Table 1. Body weights of rats in the acute toxicity testing of the water extract from root of *Sida rhombifolia* Linn.

	Body weight (g)			
	Day 0	Day 7th	Day 14th	Weight gain on day 14th
Female				
Control	130.80±10.67	158.00±6.32	172.00±9.34	41.20±1.62
S. rhombifolia 5,000 mg/kg	119.80±3.01	164.00±4.69	180.40±4.02	60.60±1.78*
Male				
Control	139.60±3.97	184.40±5.71	227.20±3.61	87.60±2.04
*S. rhombifolia*5,000 mg/kg	139.60±3.97	184.40±5.71	227.20±3.61	87.60±2.04

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

Table 2. Organ weights of rats in the acute toxicity testing of the water extract from root of *Sida rhombifolia* Linn.

	Organ weight (g)	
	Control	*S. rhombifolia* 5,000 mg/kg
Female		
Lung	1.09 ± 0.06	1.17 ± 0.03
Heart	0.80 ± 0.02	0.84 ± 0.03
Liver	8.03 ± 0.38	7.66 ± 0.35
Spleen	0.53 ± 0.01	0.61 ± 0.03*
Adrenal	0.04 ± 0.00	0.05 ± 0.00
Kidney	0.85 ± 0.04	0.87 ± 0.01
Ovary	0.06 ± 0.00	0.07 ± 0.00
Male		
Lung	1.15 ± 0.05	1.30 ± 0.03*
Heart	0.98 ± 0.05	1.01 ± 0.05
Liver	8.78 ± 0.91	8.12 ± 0.52
Spleen	0.79 ± 0.04	0.77 ± 0.02
Adrenal	0.04 ± 0.00	0.03 ± 0.00
Kidney	1.02 ± 0.04	1.06 ± 0.06
Testis	1.36 ± 0.02	1.34 ± 0.03

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

organs. As shown in Table 4, the female group treated with the extract at the dose of 300 and 1,200 mg/kg/day, had the kidney weight significantly lower than the control. Besides, the satellite female group showed a significant decline in the kidney and ovary weight when compared with the control. In the male group, the weights of heart, liver, kidney and testis were significantly decreased in the group treated with 300 mg/kg/day, while those of heart and liver decreased in the animals treated with 600 mg/kg/day. At the dose of 1,200 mg/kg/day, a significant weight decrease was found not only in heart and liver, but also spleen and kidney as compared with those of the controls. The weight of heart and liver was significantly changed in the male satellite group. Nonetheless, all of the decreases were minor changes and the differences may have been due to the variation in size of internal organs and/or body weight of the animals (Levine, 1995; Bailey *et al*., 2004). Necropsy and histopathology examinations were carried out to further confirm whether or not the organs or tissue had been damaged. The results showed no macroscopic or microscopic changes in the internal organs of any of the treated rats.

Bone marrow is one of the target sites for the adverse

Table 3. Body weights of rats in the subchronic toxicity testing of the water extract from of *Sida rhombifolia* Linn.

	Body weight (g)			
	Day 0	Day 90	Day 118	Weight gain on day 90
Female				
Control	151.60±3.80	274.20±3.97	-	122.60±6.39
S. rhombifolia 300 mg/kg	149.20±2.60	255.40±4.63*	-	106.20±5.00*
S. rhombifolia 600 mg/kg	147.50±2.07	259.40±4.80*	-	111.90±4.34
S. rhombifolia 1,200 mg/kg [a]	152.00±5.12	259.00±5.10*	-	107.00±4.72*
S. rhombifolia 1,200 mg/kg [b]	145.40±2.38	262.40±3.11	267.20±5.45	117.00±3.50
Male				
Control	177.20±6.09	428.80±18.29	-	251.60±20.53
S. rhombifolia 300 mg/kg	169.50±8.17	372.20±9.40*	-	202.70±10.93*
S. rhombifolia 600 mg/kg	185.40±2.92	391.80±8.87	-	206.40±7.75*
S. rhombifolia 1,200 mg/kg [a]	179.00±3.63	365.20±15.83*	-	186.20±15.43*
S. rhombifolia 1,200 mg/kg [b]	181.60±7.90	379.00±10.00*	398.10±8.59	197.40±14.43*

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

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

b: A satellite group was treated with the water extract from root of *S. rhombifolia* 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 testing of the water extract from root of *Sida rhombifolia* Linn.

	Control	*S. rhombifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
Lung	1.29±0.03	1.21±0.03	1.27±0.01	1.21±0.06	1.25±0.02
Heart	1.06±0.03	0.99±0.02	0.99±0.02	1.05±0.07	0.97±0.02
Liver	5.76±0.16	5.75±0.14	5.98±0.28	6.05±0.30	5.88±0.14
Spleen	0.69±0.01	0.65±0.01	0.69±0.02	0.68±0.03	0.67±0.02
Adrenal	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.00
Kidney	0.92±0.02	0.83±0.01*	0.88±0.02	0.86±0.01*	0.83±0.01*
Ovary	0.10±0.00	0.09±0.00	0.10±0.00	0.10±0.04	0.07±0.00*
Male					
Lung	1.62±0.09	1.45±0.02	1.51±0.04	1.52±0.04	1.64±0.04
Heart	1.47±0.06	1.27±0.03*	1.25±0.03*	1.25±0.03*	1.29±0.04*
Liver	10.33±0.40	9.11±0.22*	9.08±0.25*	9.28±0.34*	9.93±0.23
Spleen	0.90±0.04	0.83±0.02	0.84±0.04	0.76±0.02*	0.83±0.02
Adrenal	0.04±0.00	0.03±0.00	0.05±0.00	0.04±0.00	0.04±0.00
Kidney	1.27±0.03	1.16±0.01*	1.23±0.02	1.16±0.03*	1.18±0.02*
Testis	1.95±0.02	1.86±0.02*	1.94±0.02	1.95±0.02	1.86±0.02*

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

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

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

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

effects of test substances. Blood cells are mainly produced in bone marrow. Any test substance that affects the bone marrow could inhibit certain enzyme activities involved in the production of hemoglobin in red blood cells, and then reduce the ability of the blood to distribute oxygen through out the body, a condition known as anemia (Gregg and Voigt, 2000). To determine intravascular effect and bone marrow activity in rats treated with the extract, hematological parameters of female and male rats were examined as presented in Tables 5 and 6, respectively. In the male groups treated with 1,200 mg/kg/day, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) was significantly higher in the control values. In the satellite male group, a slight but significant decrease in the concentration of red blood cells was observed. Conversely, significant increase of mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (MCHC) was shown. However, the alteration of these values was minor and remained within the normal ranges. The differential white blood cell count values of female and male treated groups are shown in Table 7. As compared with the control values, no significant changes in any values were detected at 300 and

Table 5. Hematological values of female rats in the subchronic toxicity testing of the water extract form root of *Sida rhombifolia* Linn.

	Control	*S. rhombifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells ($x10^6$/µl)	6.71±0.05	6.55±0.14	6.34±0.41	6.63±0.13	6.76±0.10
Hemoglobin (g/dl)	14.63±0.12	14.19±0.17	13.79±0.87	14.05±0.18	14.30±0.09
Hematocrit (%)	40.33±0.44	39.30±0.61	38.70±2.56	39.10±0.67	40.10±0.40
Mean corpuscular volume (fl)	60.01±0.36	60.10±0.56	60.56±0.57	59.08±1.02	59.38±0.42
Mean corpuscular hemoglobin (pg)	21.78±0.33	21.73±0.30	21.78±0.16	21.23±0.39	21.17±0.26
Mean corpuscular hemoglobin concentration (g/dl)	36.32±0.49	36.12±0.30	35.98±0.28	35.94±0.47	35.66±0.24
Platelet ($x10^5$/µl)	7.41±0.15	6.97±0.70	8.22±0.34	8.10±0.19	7.81±0.09

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

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

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

Table 6. Hematological values of male rats in the subchronic toxicity testing of the water extract from root of *Sida rhombifolia* Linn.

	Control	*S. rhombifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells ($x10^6$/µl)	7.97±0.17	7.76±0.13	7.99±0.15	7.63±0.12	7.52±0.09*
Hemoglobin (g/dl)	15.58±0.27	15.31±0.31	15.44±0.29	15.54±0.24	15.54±0.15
Hematocrit (%)	46.30±0.97	45.70±0.92	46.00±0.89	44.90±0.73	45.90±0.87
Mean corpuscular volume (fl)	57.84±0.45	58.58±0.51	57.50±0.24	58.50±0.34	60.88±0.86*
Mean corpuscular hemoglobin (pg)	19.53±0.22	19.71±0.15	19.31±0.14	20.37±0.29*	20.67±0.15*
Mean corpuscular hemoglobin concentration (g/dl)	33.78±0.26	33.63±0.14	33.62±0.16	34.84±0.34*	34.00±0.55
Platelet ($x10^5$/µl)	7.92±0.11	8.00±0.07	7.83±0.14	7.91±0.19	8.30±0.13

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

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

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

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

Table 7. Differential white blood cell count values of rats in the subchronic toxicity testing of the water extract from root of *Sida rhombifolia* Linn.

	Control	*S. rhombifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
White blood cells (x10^3/µl)	2.80±0.28	2.45±0.27	3.00±0.41	2.82±0.41	3.13±0.20
Neutrophil (%)	18.11±1.42	20.30±1.05	18.60±0.71	13.80±1.26*	11.60±2.03*
Lymphocyte (%)	75.00±1.88	72.70±1.37	73.60±0.70	81.70±1.61*	81.40±1.86*
Monocyte (%)	6.00±0.60	5.70±0.39	6.10±0.34	4.00±0.36*	5.10±0.40
Eosinophil (%)	0.89±0.35	1.30±0.42	1.70±0.26	0.70±0.30	1.90±0.52
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)	3.98±0.24	4.18±0.41	3.60±0.24	3.45±0.25	3.67±0.31
Neutrophil (%)	16.20±1.80	15.80±1.63	17.80±2.97	18.00±3.30	16.70±1.03
Lymphocyte (%)	76.50±1.77	76.20±1.72	74.30±3.43	76.50±2.88	77.50±1.09
Monocyte (%)	5.10±0.79	5.50±0.60	6.40±0.81	4.10±0.65	4.90±0.54
Eosinophil (%)	2.40±0.47	2.50±0.56	1.50±0.26	1.40±0.45	0.90±0.27*
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 from root of *S. rhombifolia* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from root of *S. rhombifolia* 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 testing of the water extract from root of *Sida rhombifolia* Linn.

	Control	*S. rhombifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	103.80±2.31	103.10±3.82	102.10±3.27	104 .00±4.18	110.30±2.23
BUN (mg/dl)	23.40±1.26	18.00±0.51*	22.10±0.92	22.30±2.53	19.30±0.42*
Creatinine (mg/dl)	0.42±0.02	0.39±0.02	0.35±0.02	0.46±0.05	0.37±0.03
Total protein (g/dl)	5.09±0.07	5.10±0.10	4.99±0.08	5.16±0.06	5.36±0.79*
Albumin (g/dl)	3.60±0.05	3.67±0.05	3.46±0.08	3.55±0.08	3.58±0.04
Total bilirubin (mg/dl)	0.17±0.01	0.12±0.01*	0.11±0.01*	0.16±0.01	0.15±0.01
Direct bilirubin (mg/dl)	0.00±0.00	0.00±0.00	0.01±0.01	0.02±0.01	0.01±0.01
SGOT (U/l)	104.40±2.33	110.90±9.72	107.60±8.20	125.30±29.35	107.40±9.81
SGPT (U/l)	31.10±1.55	33.50±7.44	25.90±1.37	51.30±23.69	36.80±7.45
ALP (U/l)	41.70±2.28	38.60±1.80	42.70±2.25	41.50±2.78	30.80±1.57*

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

600 mg/kg/day in the female treated groups. Furthermore, a significant decrease in neutrophil and a significant increase in lymphocyte were observed in the female treated with 1,200 mg/kg/day. In the female satellite group, the neutrophil and lymphocyte were significantly changed from the control group. In the male satellite rats, eosinophil was significantly decreased from the control values. Nonetheless, all of these changes may have resulted from normal variation among

Table 9. Clinical blood chemistry values of male rats in the subchronic toxicity testing of the water extract from root of *Sida rhombifolia* Linn.

	Control	*S. rhombifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	117.90±3.93	118.60±3.12	107.70±5.14	129.00±6.08	130.70±4.69
BUN (mg/dl)	19.50±0.50	20.40±0.52	19.60±0.79	21.30±0.49	20.60±0.83
Creatinine (mg/dl)	0.28±0.02	0.33±0.01	0.27±0.02	0.30±0.02	0.34±0.02*
Total protein (g/dl)	5.68±0.10	5.56±0.11	5.64±0.10	5.27±0.12*	5.33±0.13*
Albumin (g/dl)	3.36±0.05	3.34±0.04	3.27±0.07	3.42±0.05	3.45±0.06
Total bilirubin (mg/dl)	0.10±0.01	0.10±0.01	0.11±0.01	0.13±0.01	0.11±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)	115.30±4.45	102.50±5.01	104.60±6.28	107.30±12.66	119.00±13.78
SGPT (U/l)	37.20±3.74	37.20±1.48	45.10±7.57	57.80±15.43	56.60±13.01
ALP (U/l)	58.50±3.05	68.40±3.79	70.50±9.35	76.70±5.87*	57.70±2.41

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

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

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

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

animals (Feldman *et al*., 2000; Inala *et al*., 2002). Besides, the physical examination during the experimental period indicated that all animals were healthy. Therefore, these results suggest that the extract did not cause hematological or immunological defects

The liver and kidney are one of the major internal organs in the body and have several important functions. Symptoms of disorder in those organs appear only in serious diseases. To test whether the substance destroys and impairs liver and kidney functions, clinical blood chemistry examination was performed in the female and male rats and the results are summarized in Table 8 and 9, respectively. The data indicate a significant decrease in blood urea nitrogen (BUN) in the female rats treated with the extract at the doses of 300 mg/kg/day. In addition, the concentrations of total bilirubin in the female rats treated with 300 and 600 mg/kg/day were significantly decreased as compared with those of the controls. In the satellite female group, only BUN and ALP were significantly less than their control values, wherever significant increases in total protein as compared with the control values were found. Furthermore, total protein was significantly decreased in the male treated with 1,200 mg/kg/day and satellite group, but ALP was significantly increased in 1,200 mg/kg/day as compared with the control group. Lower-than-normal levels of BUN may indicate impaired kidney function. In addition, increased creatinine level in the blood is a sign of abnormal kidney function due to decreased excretion of creatinine in the urine. Increased SGPT and ALP imply liver damage resulting in the release of these enzymes into the blood circulation. Although statistically significantly different, all of the values were within normal limits i.e. the extract should not cause liver damage or liver failure, as was been confirmed by histopathological study (Caisey and King, 1980; Sacher and McPherson, 1991a, 1991b; Levine, 1995; Angkhasirisap *et al.*, 2002). The results of the histopathological assessment also showed no significant histopathological change in the internal, especially vital, organs (Figures 1, 2, 3 and 4). In summary, the water extract from the root of *S. rhombifolia* administered orally did not cause acute or subchronic toxicities in male or female rats. A chronic toxicity study should be further carried out to assess the long-term safety of the extract.

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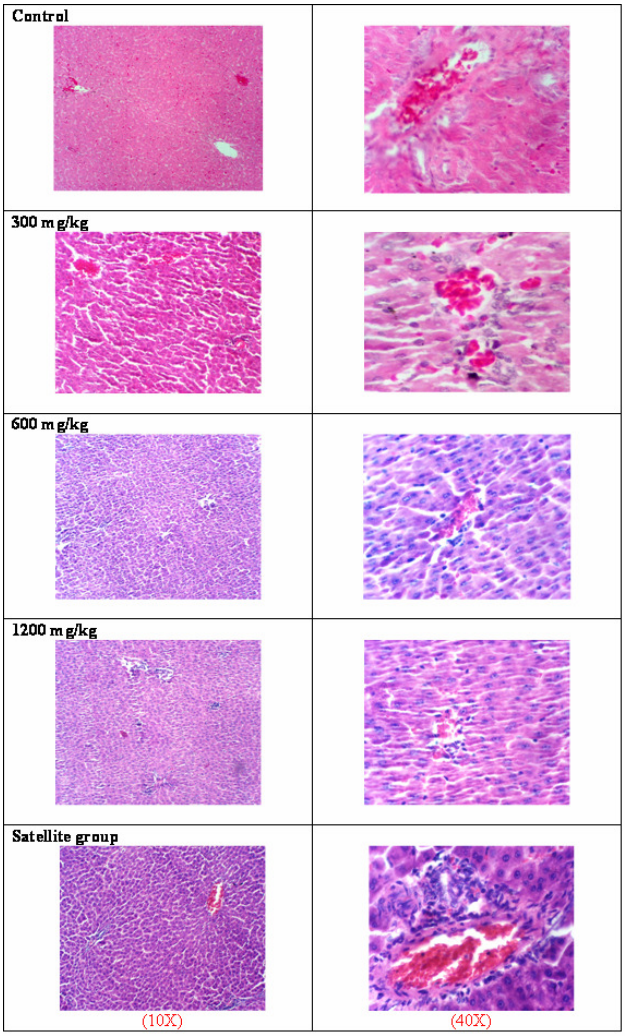

Figure 1. The histology of male liver from the control and treated groups (the 10x and 40 x magnifications). No significant damage was detected in any treatment group.

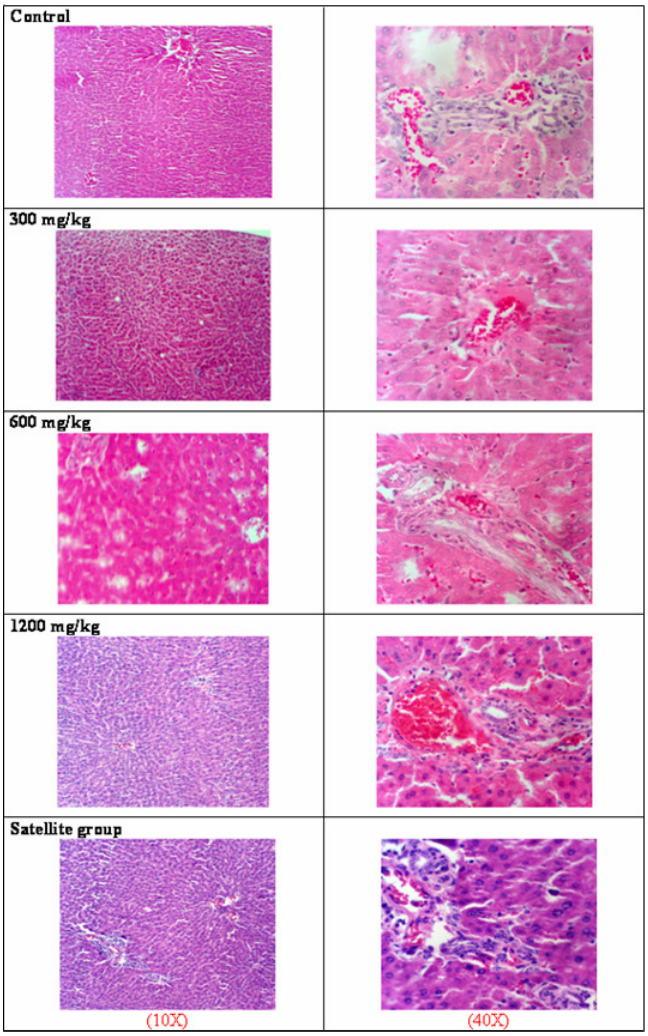

Figure 2. The histology of female liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

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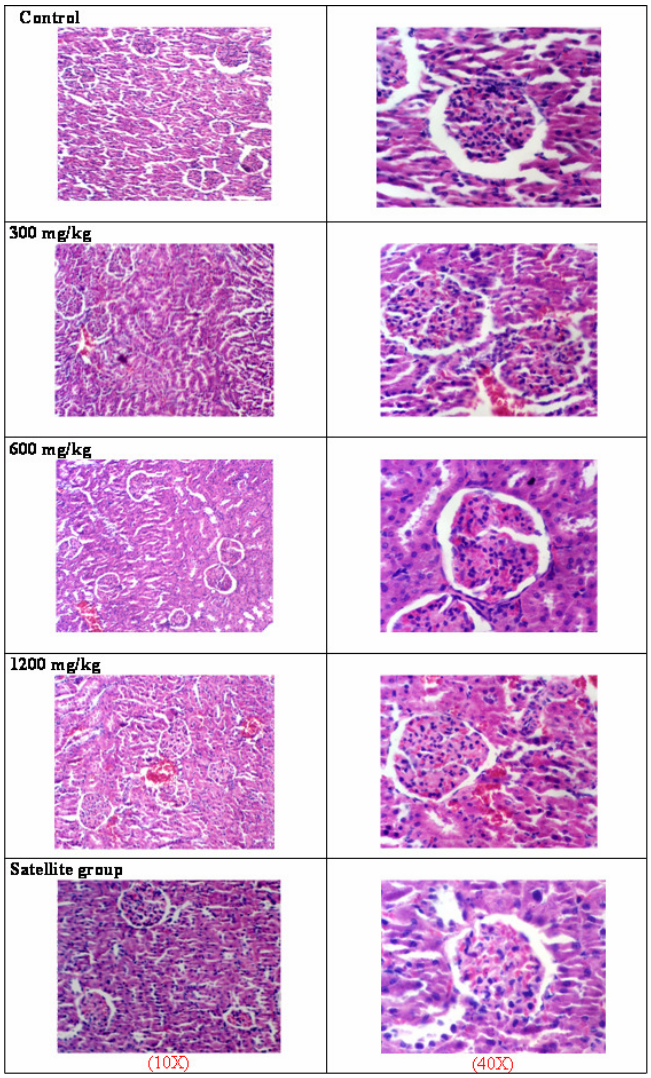

Figure 3. The histology of male kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

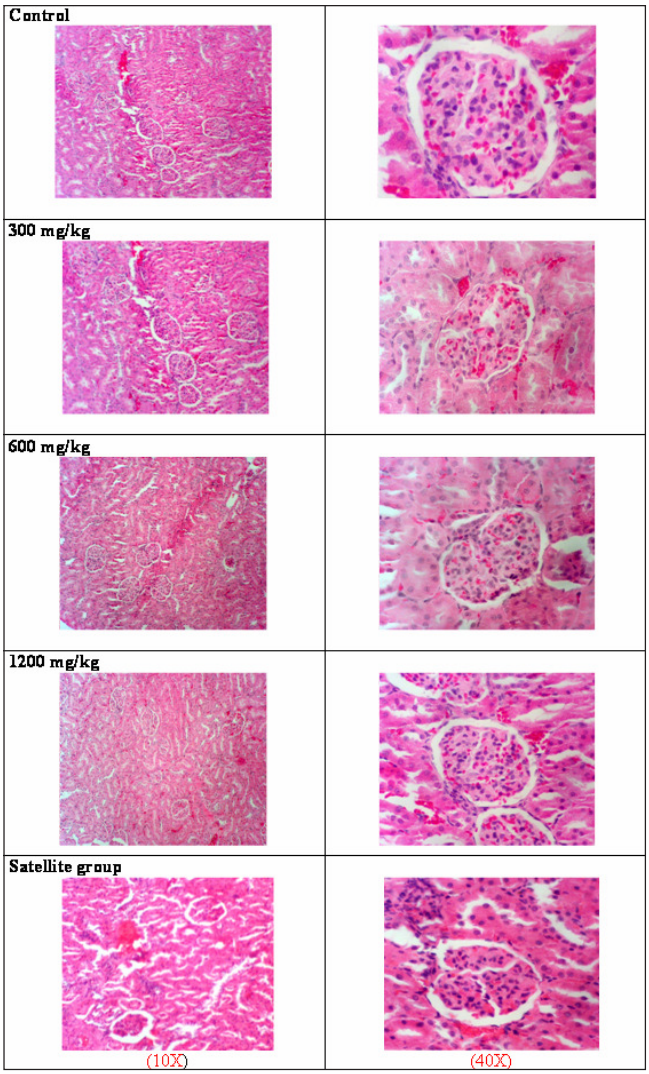

Figure 4. The histology of female kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

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