ORIGINAL ARTICLE

Acute and subchronic toxicity study of the water extract from root of *Citrus aurantifolia* (Christm. et Panz.) Swingle in rats

Siharat Chunlaratthanaphorn[1,5] Nirush Lertprasertsuke[2] Umarat Srisawat[3] Amornnat Thuppia[4] Anongnad Ngamjariyawat[4] Nadthaganya Suwanlikhid[4] and Kanjana Jaijoy[5]

Abstract

Chunlaratthanaphorn, S., Lertprasertsuke, N., Srisawat, U., Thuppia, A., Ngamjariyawat, A., Suwanlikhid, N., and Jaijoy, K.

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Acute and subchronic toxicities of the water extract from the roots of *Citrus aurantifolia* 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 male, 5 female) 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 male, 10 female). The examinations of signs, animal behavior and health monitoring showed no signs of abnormalities in the test groups as compared to

[1]Ph.D.(Pharmacology), Asst. Prof., [3]M.S.(Physiology), [4]M.S.(Anatomy), Department of Preclinical Science, [5]M.S.(Pharmacology), Researcher, Research Unit of Pharmacology and Toxicology of Herbal Medicine, Research center, Faculty of Medicine, Thammasat University, Rungsit Campus, Klong Loung, Pathum Thani, 12120 Thailand. [2]Ph.D.(Pathology), Assoc. Prof., Department of Pathology, Faculty of Medicine, Chiang Mai University, Muang, Chiang Mai, 50200 Thailand.

Corresponding e-mail: siharat@yahoo.com

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 oral administration of 1,200 mg/kg/day of the extract of *C. aurantifolia* in male and female rats caused a significant increase in the liver enzymes, which remained within the normal range, but did not produce a significant histopathological change in the internal organs. In conclusion, the extract from the roots of *C. aurantifolia* administered orally did not cause acute or subchronic toxicities to male and female rats.

Key words : acute toxicity, subchronic toxicity, *C. aurantifolia*

บทคัดย่อ

สีหรัฐ จุลรัฐธนาภรณ์[1] นิรัชร์ เลิศประเสริฐสุข[2] อัมรัตน์ ศรีสวัสดิ์[1] อมรณัฏฐ์ ทับเปีย[1] อนงค์นาฎ งามจริยาวัตร[1] ณัฏฐกัญญา สุวรรณลิขิต[1] และ กาญจนา ใจจ้อย[2]

การศึกษาความเป็นพิษเฉียบพลันและกึ่งเรื้อรังของสารสกัดน้ำจากรากของต้นมะนาวในหนูขาว

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การศึกษาครั้งนี้เป็นการทดสอบความเป็นพิษเฉียบพลันและพิษกึ่งเรื้อรังของสารสกัดจากรากของต้นมะนาว (*C. aurantifolia*) ในหนูขาวทั้งเพศผู้และเพศเมีย ผลการป้อนสารสกัดครั้งเดียวทางปากในขนาด 5,000 มก./กก. น้ำหนักตัว (เพศผู้ 5 ตัว เพศเมีย 5 ตัว) ไม่พบอาการแสดงของความเป็นพิษ การเปลี่ยนแปลงพฤติกรรม การตาย และความแตกต่างของลักษณะทางจุลกายวิภาคของอวัยวะภายใน ผลการศึกษาความเป็นพิษกึ่งเรื้อรังโดยการป้อนสารสกัดทางปากในขนาด 300 600 และ 1,200 มก./กก.น้ำหนักตัวทุกวันเป็นเวลา 90 วัน (เพศผู้ 10 ตัว เพศเมีย 10 ตัว) พบว่าหนูขาวกลุ่มที่ได้รับสารสารสกัดมีอาการ พฤติกรรม และสุขภาพเป็นปกติเมื่อเปรียบเทียบกับกลุ่มควบคุม ผลการวิเคราะห์น้ำหนักตัวสุดท้าย น้ำหนักอวัยวะภายใน ค่าทางโลหิตวิทยา ค่าเคมีคลินิกของเลือดและการตรวจจุลกายวิภาค ของกลุ่มทดสอบและกลุ่มควบคุมในวันที่ 90 และของกลุ่มติดตามผล (satellite) ในวันที่ 118 พบว่า ระดับเอนไซม์จากตับเพิ่มสูงขึ้นแต่ยังอยู่ในช่วงที่ปกติในหนูขาวเพศผู้และเพศเมียที่ได้รับสารสกัดมะนาวขนาด 1,200 มก./กก.น้ำหนักตัว แต่ไม่พบการเปลี่ยนแปลงทางจุลกายวิภาคของอวัยวะภายในของสัตว์ทดลอง ดังนั้นผลการศึกษานี้สรุปได้ว่าสารสกัดน้ำจากรากต้นมะนาวไม่ก่อพิษเฉียบพลันและพิษกึ่งเรื้อรังในหนูขาวเพศผู้และเพศเมีย

[1]สถานวิทยาศาสตร์พรีคลินิก [2]ศูนย์วิจัย คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต อำเภอคลองหลวง จังหวัดปทุมธานี 12120 [2]ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ อำเภอเมือง จังหวัดเชียงใหม่ 50200

Citrus aurantifolia (Christm. et Panz.) Swingle, family Rutaceae (Thai name Manao, Common name: Lime) is commonly known as familiar food and medicine, yet its therapeutic effectiveness in a variety of diseases has been suggested in traditional medicine (Chavallier, 1996; Bunyapratphatsara, 2000). The juice of citrus fruit shows its actions as a cytotoxic P-glycoprotein inhibitor by decreasing transepithelial electrical resistance (TEER) and viability of Caco-2 cells (Xu, 2003). Furthermore, lime extract has an antimicrobial activity against upper respiratory tract bacterial pathogens (Adeleye and Opiah, 2003). Gharagozloo *et al*. (2002) documented the anti-proliferative activity of concentrated lime juice extract on a lymphoblastoid cell line. In addition, they reported the immunomodulatory effects on activated human lymphocytes, likely due to the protein components of the extract (Gharagozloo and Ghaderi, 2001).

The root of *C. aurantifolia* is used in traditional medicine for the treatment of fever. The wide range of bioactive ingredients from the Citrus species has been found to exert anti-infection and

anti-inflammatory activities (Murakami, 2000; Rodrigues, 2000). The flavonoids, limonoids, and ascorbic acid are groups of citrus phytochemicals and micronutrients, which are responsible for the anti-inflammatory and antitumor activities (Guthrie and Carroll, 1998; Hollman, 1996; Kawaii, 1999; Lam and Hasegawa, 1989). Citrus limonoids inhibit the chemically induced colon carcinoma in a dose-dependent manner accompanied by suppression of cell proliferation in a rat model (Tanaka *et al*., 1998). In addition, the potential use of Citrus flavonoids has been suggested in cancer treatment (Rooprai *et al*., 2001), as they shows an inhibitory effect on breast cancer cell lines (Guthrie and Carroll, 1998; So *et al*., 1996).

Nonetheless, the toxicity of *C. aurantifolia* 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 *C. aurantifolia* in rats by determining both oral acute and subchronic toxicities.

Materials and methods

Plant material

The roots of *C. aurantifolia* were collected from Songkhla, Thailand. The voucher specimen (SBK 0012) was kept and identified by the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Preparation of plant extract

Root powder of *C. aurantifolia* 500 grams were wrapped in a calico bag and put into a stainless boiler. Ten liters of water were added and boiled for 3-4 hours, then filtered when cool. The residue from the filtration was boiled and filtered again with the same ratio. The filtrates were collected and evaporated in a rotary evaporator until concentrated. The weight and percentage yield of the crude extract was 29.26.

Experimental animals

Male and female Sprague-Dawley rats, weighing 130-190 g were obtained from the National Laboratory Animal Center, Nakorn Pathom, Thailand. They were housed under standard environmental conditions of temperature at 24±1^{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.

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 15th 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.

Subchronic toxicity

According to WHO guideline (WHO, 2000) and the OECD TG407 (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 91st and 118th (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.

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.

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 (Carol, 1995). In acute toxicity study, after the water extract from root of *C. aurantifolia* 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 *C. aurantifolia* 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 slight increase but were not significantly different from those of the control group, except the male and female rats receiving the extract at the dose of 1,200 mg/kg/day (Table 3). Their body weights on the 90th day showed a significant decrease, yet the average body weight of the satellite groups stayed normal at the end of the experiment. 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

Table 1. Body weights of rats in the acute toxicity testing of the water extract from root of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	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
C. aurantifolia 5,000 mg/kg	128.80±1.20	166.40±3.60	184.00±4.42	55.20±4.58
Male				
Control	136.00±3.69	182.40±6.24	217.60±9.12	81.60±6.05
C. aurantifolia 5,000 mg/kg	138.8 0±3.88	189.60±4.44	205.20±5.12	66.4±3.92

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 *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Organ weight (g)	
	Control	***C. aurantifolia* 5,000 mg/kg**
Female		
Lung	1.09±0.06	1.14±0.04
Heart	0.80±0.02	0.85±0.02
Liver	8.03±0.38	7.18±0.57
Spleen	0.53±0.01	0.58±0.01
Adrenal	0.04±0.00	0.05±0.00
Kidney	0.85±0.04	0.81±0.01
Ovary	0.06±0.00	0.07±0.00
Male		
Lung	1.15±0.05	1.18±0.04
Heart	0.98±0.05	1.04±0.06
Liver	8.78±0.91	9.20±0.46
Spleen	0.79±0.04	0.66±0.01
Adrenal	0.04±0.00	0.03±0.00
Kidney	1.02±0.04	1.02±0.02
Testis	1.36±0.02	1.35±0.01

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 testing of the water extract from of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Body weight (g)			
	Day 0	**Day 90**	**Day 118**	**Weight gain on day 90**
Female				
Control	150.67±4.12	274.22±4.44	-	123.56±7.06
C. aurantifolia 300 mg/kg	148.90±2.84	264.40±7.33	-	115.50±6.26
C. aurantifolia 600 mg/kg	144.80±3.11	261.20±7.59	-	116.40±5.47
C. aurantifolia 1,200 mg/kg [a]	143.00±2.65	254.80±4.46*	-	112.40±4.29
C. aurantifolia 1,200 mg/kg [b]	142.00±3.790	261.60±3.38	288.00±9.71	119.60±3.75
Male				
Control	179.80±6.60	406.00±16.24	-	226.20±20.47
C. aurantifolia 300 mg/kg	172.00±4.92	403.20±9.24	-	231.20±11.77
C. aurantifolia 600 mg/kg	181.20±3.85	400.70±10.06	-	218.60±9.08
C. aurantifolia 1,200 mg/kg [a]	179.60±2.90	365.40±12.23*	-	185.80±14.07*
C. aurantifolia 1,200 mg/kg [b]	183.20±8.16	379.60±11.64	413.10±12.88	194.20±11.02

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

different organs. As shown in Table 4, the female group treated with the extract at the dose of 300 mg/kg/day, had the liver weight significantly higher than the control. In contrast, the weights of heart and kidney were significantly lower than those of their control values in female rats treated with 600 and 1,200 mg/kg/day. Besides, the satellite female group showed a significant decline in the kidney weight when compared with the control. In the male group, the weights of lung and heart were significantly decreased in the group treated with 300 mg/kg/day, while those of heart and kidney 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 the internal organs was not significantly changed in the male satellite group. Nonetheless, all of the increase and decrease 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 (Bailey *et al*., 2004; Carol,1995). Necropsy and histopathology examinations were performed 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 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 anaemia (Gregg and Voigt, 2000). To determine intravascular effect and bone marrow activity in

Table 4. Organ weights of rats in the subchronic toxicity testing of the water extract from root of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Control	*C. aurantifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
Lung	1.31±0.02	1.35±0.05	1.19±0.05	1.27±0.03	1.37±0.05
Heart	1.05±0.03	0.99±0.01	0.94±0.03*	0.92±0.02*	1.01±0.02
Liver	5.72±0.16	6.77±0.41*	5.71±0.15	5.99±0.24	5.88±0.14
Spleen	0.67±0.02	0.74±0.04	0.65±0.01	0.63±0.01	0.71±0.03
Adrenal	0.05±0.00	0.05±0.00	0.05±0.00	0.04±0.00	0.05±0.00
Kidney	0.98±0.04	0.98±0.03	0.85±0.02*	0.85±0.01*	0.84±0.02*
Ovary	0.10±0.00	0.10±0.00	0.10±0.00	0.14±0.04	0.08±0.00
Male					
Lung	1.69±0.11	1.44±0.06*	1.54±0.03	1.36±0.02	1.64±0.07
Heart	1.48±0.06	1.32±0.04*	1.30±0.03*	1.19±0.03*	1.38±0.03
Liver	10.66±0.41	10.32±0.28	10.08±0.40	9.18±0.42*	9.84±0.35
Spleen	0.92±0.04	0.83±0.03	0.87±0.03	0.73±0.01*	0.90±0.03
Adrenal	0.04±0.00	0.04±0.00	0.05±0.00	0.04±0.00	0.04±0.00
Kidney	1.28±0.03	1.21±0.02	1.19±0.18*	1.14±0.03*	1.24±0.02
Testis	1.94±0.02	1.92±0.03	1.90±0.01	1.86±0.03	1.86±0.01

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

Table 5. Hematological values of female rats in the subchronic toxicity testing of the water extract form root of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Control	*C. aurantifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells (x10^6/µl)	6.81±0.06	6.45±0.15*	6.70±0.08	6.75±0.13	6.88±0.07
Hemoglobin (g/dl)	14.63±0.12	13.99±0.28*	14.33±0.18	14.43±0.26	14.62±0.12
Hematocrit (%)	40.78±0.49	39.00±0.83	40.30±0.36	40.00±0.81	41.40±0.45
Mean corpuscular volume (fl)	59.75±0.37	60.56±0.91	60.25±0.54	59.13±0.50	54.67±5.41
Mean corpuscular hemoglobin (pg)	21.47±0.16	21.70±0.27	21.46±0.22	21.35±0.18	21.23±0.11
Mean corpuscular hemoglobin concentration (g/dl)	35.95±0.30	35.84±0.30	35.58±0.29	36.23±0.25	35.31±0.19
Platelet (x10^5/µl)	7.40±0.15	7.54±0.83	7.85±0.35	8.14±0.23	8.34±0.13

Values are expressed as mean ± S.E.M., n = 10
a: A group was treated with the water extract from root of *C. aurantifolia* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from root of *C. aurantifolia* 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 testing of the water extract from root of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Control	*C. aurantifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells (x10^6/µl)	7.98±0.17	7.62±0.13	7.63±0.14	7.80±0.14	7.25±0.09*
Hemoglobin (g/dl)	15.58±0.27	14.80±0.31*	14.80±0.21*	15.46±0.29	15.08±0.15
Hematocrit (%)	46.30±0.97	43.90±1.02	44.60±0.80	45.60±0.80	43.80±0.77
Mean corpuscular volume (fl)	57.77±0.45	57.71±0.38	58.38±0.14	58.49±0.28	60.49±0.52*
Mean corpuscular hemoglobin (pg)	19.51±0.22	19.41±0.14	19.40±0.09	19.84±0.30	20.79±0.10*
Mean corpuscular hemoglobin concentration (g/dl)	33.78±0.26	33.60±0.18	33.34±0.15	33.89±0.41	34.39±0.28
Platelet (x10^5/µl)	8.42±0.43	8.31±0.25	8.35±0.08	8.61±0.88*	8.83±0.14*

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

rats treated with the extract, hematological parameters of female and male rats were examined as presented in Tables 5 and 6, respectively. The concentrations of red blood cells and hemoglobin in females treated with 300 mg/kg/day of the extract were slightly lower than those of the control values. In the male groups treated with 300 and 600 mg/kg/day, hemoglobin concentration was significantly lower 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 (MCV) and mean corpuscular hemoglobin (MCH) 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

Table 7. Differential white blood cell count values of rats in the subchronic toxicity testing of the water extract from root of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Control	*C. aurantifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
White blood cells (x10^3/µl)	3.08±0.29	2.80±0.34	2.50±0.29	2.62±0.23	2.99±0.21
Neutrophil (%)	19.22±1.87	17.10±1.45	17.30±0.84	18.60±2.06	9.50±1.74*
Lymphocyte (%)	74.22±2.13	76.70±1.85	75.00±1.05	76.00±2.09	82.70±1.59*
Monocyte (%)	5.89±0.61	5.20±0.49	6.40±0.42	5.10±0.56	6.10±0.77
Eosinophil (%)	0.67±0.33	1.00±0.21	1.30±0.21	1.20±0.13	2.10±0.45*
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)	4.02±0.24	3.96±0.51	3.79±0.27	3.77±0.40	3.17±0.30
Neutrophil (%)	14.20±0.1.42	22.00±4.26*	17.50±2.04	15.70±2.03	16.30±1.89
Lymphocyte (%)	78.20±1.29	70.50±4.18*	74.70±2.50	80.30±2.39	77.20±1.89
Monocyte (%)	5.40±0.84	5.90±0.64	5.50±0.61	5.40±1.18	5.70±0.24
Eosinophil (%)	2.40±0.47	1.60±0.42	2.30±0.44	2.60±1.00	1.00±0.18
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 *C. aurantifolia* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from root of *C. aurantifolia* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.
*** Significantly different from control, p<0.05.**

the control values, no significant changes in any values were detected at any of the three given doses in the female treated groups. Furthermore, a significant decrease in neutrophil and significant increase in lymphocyte and eosinophil were observed in the female satellite group. In the male rats treated with 300 mg/kg/day, neutrophil and lymphocyte were slightly but significantly changed from the control values. Nonetheless, all of these changes may have resulted from normal variation among 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 indicates a significant decrease in blood urea nitrogen (BUN) in the female rats treated with the extract at the doses of 600 and 1,200 mg/kg/day. In addition, the concentrations of direct bilirubin and alkaline phosphatase (ALP) in the female rats treated with 1,200 mg/kg/day were significantly increased as compared with those of the controls. In the satellite female group, only BUN, creatinine and ALP were significantly less than their control values. The male rats treated with 1,200 mg/kg/day showed significant increases in BUN, ALP and serum glutamic-pyruvic transaminase (SGPT) concentrations as compared with the control values. Furthermore, creatinine was significantly increased in the male satellite 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

Table 8. Clinical blood chemistry values of female rats in the subchronic toxicity testing of the water extract from root of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Control	*C. aurantifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	103.78±2.59	101.40±6.41	102.00±4.05	93.90±3.31	107.40±3.86
BUN (mg/dl)	24.22±1.07	22.10±0.96	21.20±0.71*	21.50±1.15*	19.60±0.71*
Creatinine (mg/dl)	0.43±0.02	0.43±0.01	0.39±0.01	0.40±0.02	0.36±0.02*
Total protein (g/dl)	5.07±0.08	5.14±0.11	4.92±0.08	5.32±0.12	5.35±0.13
Albumin (g/dl)	3.58±0.06	3.49±0.12	3.54±0.10	3.56±0.12	3.57±0.06
Total bilirubin (mg/dl)	0.16±0.01	0.13±0.01	0.13±0.01	0.14±0.01	0.15±0.01
Direct bilirubin (mg/dl)	0.00±0.00	0.00±0.00	0.00±0.00	0.20±0.01*	0.00±0.00
SGOT (U/l)	105.22±2.44	98.40±8.88	99.80±5.46	98.00±5.27	95.20±4.14
SGPT (U/l)	31.33±1.71	26.40±1.13	26.80±1.58	28.00±2.03	29.90±2.39
ALP (U/l)	41.22±2.49	46.70±5.75	42.10±2.53	54.40±6.01*	29.00±1.18*

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

Table 9. Clinical blood chemistry values of male rats in the subchronic toxicity testing of the water extract from root of *Citrus aurantifolia* (Christm. et Panz.) Swingle

	Control	*C. aurantifolia*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	121.40±2.60	124.40±4.51	119.00±3.60	120.70±6.09	131.80±4.82
BUN (mg/dl)	20.20±0.71	19.20±0.81	20.80±0.90	23.10±0.69*	21.50±0.91
Creatinine (mg/dl)	0.30±0.02	0.28±0.01	0.26±0.01	0.31±0.01	0.36±0.01*
Total protein (g/dl)	5.60±0.11	5.67±0.08	5.75±0.11	5.55±0.10	5.28±0.16
Albumin (g/dl)	3.40±0.06	3.44±0.07	3.33±0.07	3.60±0.04	3.48±0.10
Total bilirubin (mg/dl)	0.10±0.01	0.13±0.01	0.12±0.01	0.13±0.01	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)	113.30±4.76	113.10±4.70	104.50±4.30	105.90±10.01	108.10±4.04
SGPT (U/l)	38.10±3.71	39.80±2.09	34.10±1.12	54.60±10.43*	33.70±1.83
ALP (U/l)	61.90±4.69	74.80±4.17	72.80±8.28	84.00±6.09*	66.20±4.71

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

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 (Angkhasirisap *et al*., 2002; Barry, 1995, Caisey and King, 1980; Sacher and McPherson, 1991a, 1991b). The results of the histopathological assessment also showed no significant histopatho-

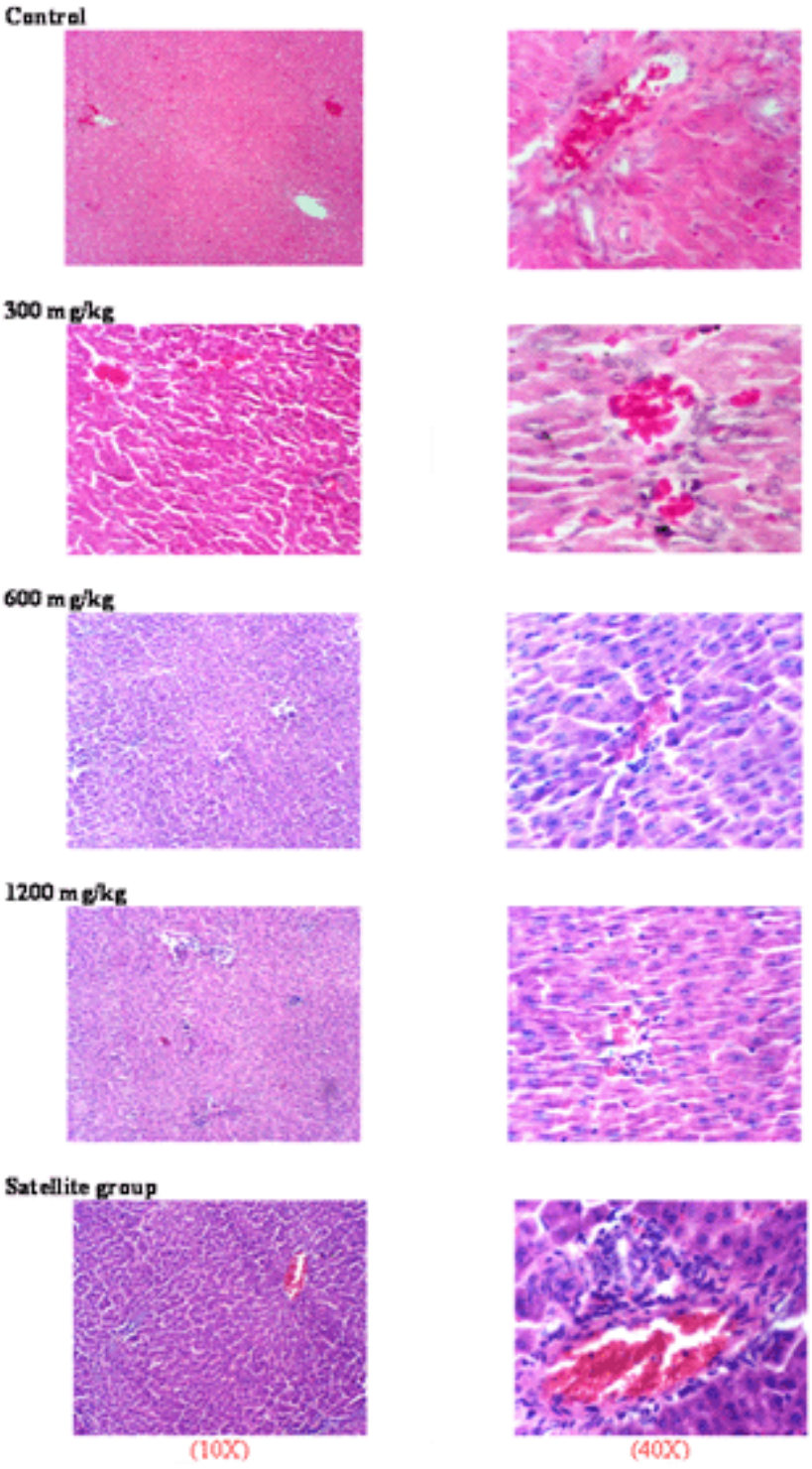

Figure 1. The histology of male liver from the control and treated groups (the 10x and 40 x magnifications). No significant damage was detected in any treatment group.
[Color figure can be viewed in the electronic version]

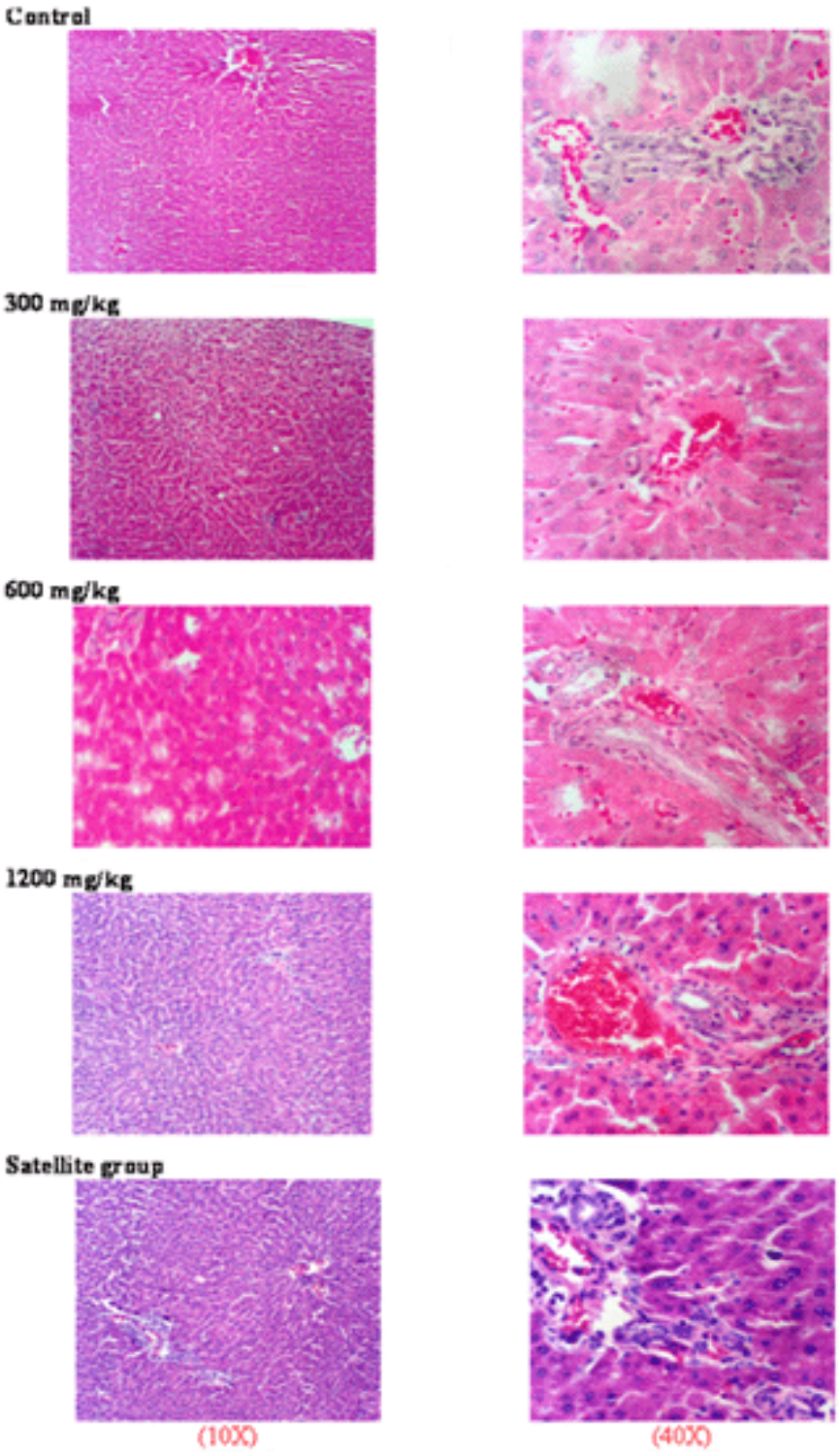

Figure 2. The histology of female liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

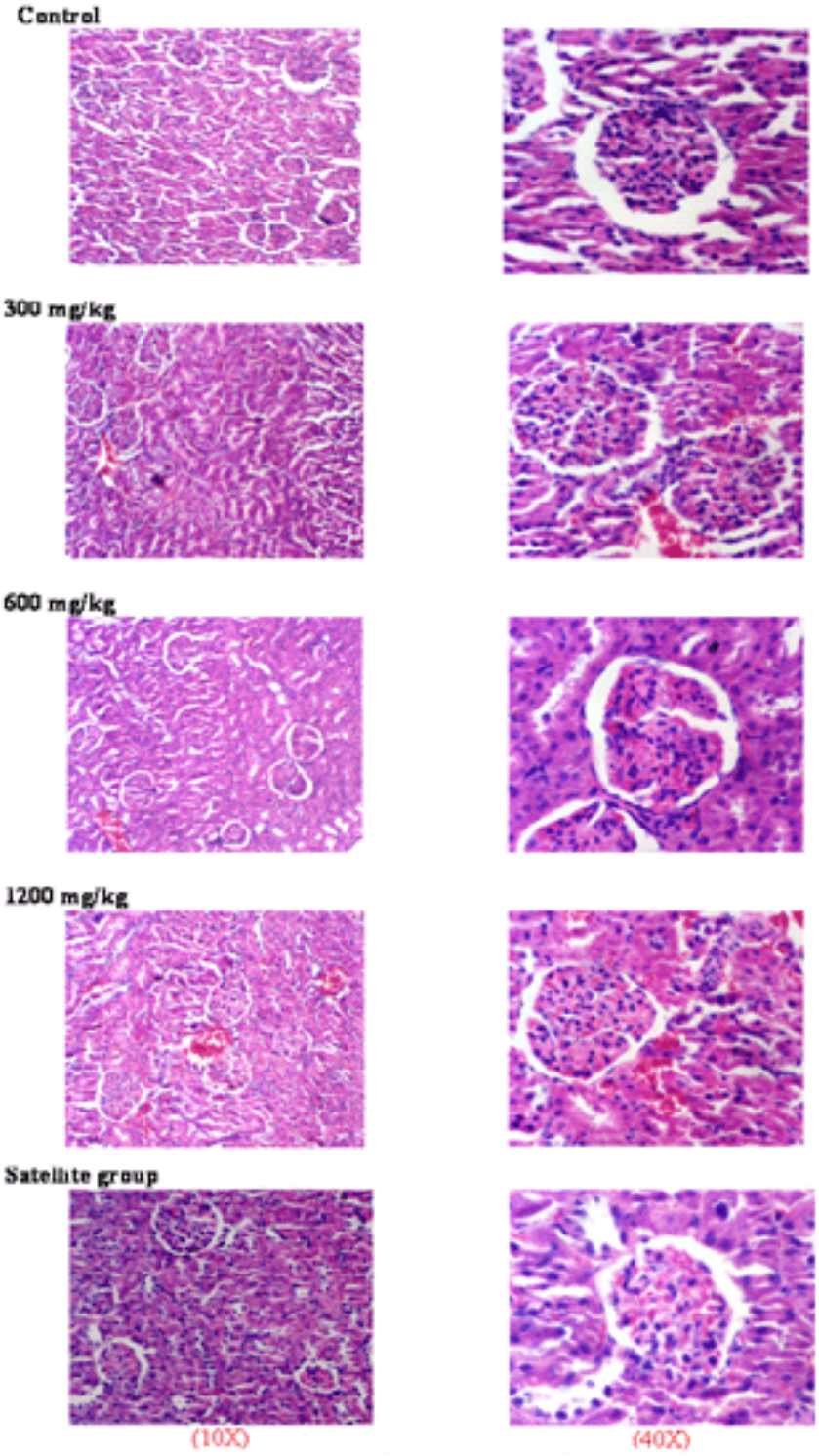

Figure 3. The histology of male kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

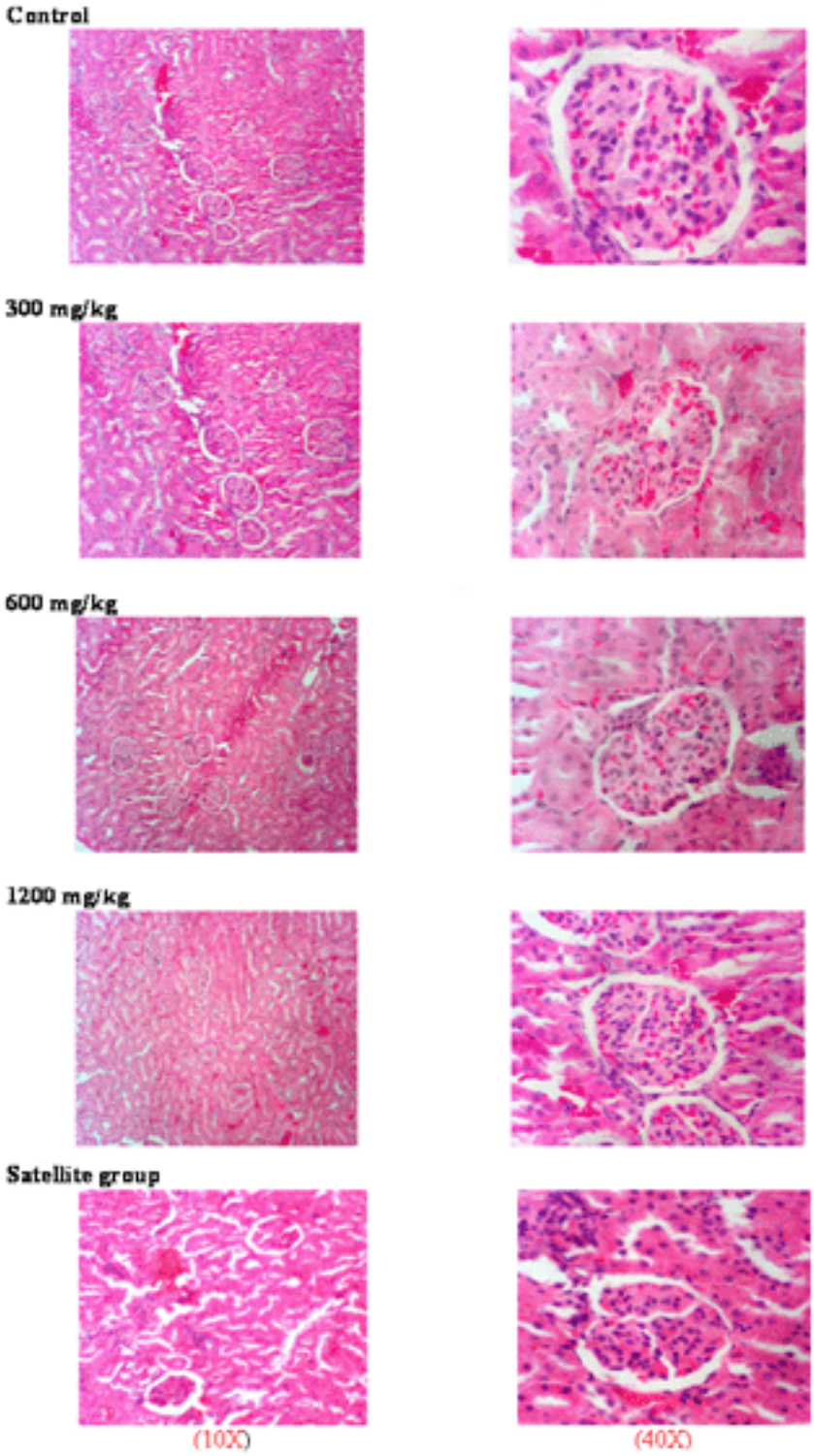

Figure 4. The histology of female kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

logical change in the internal, especially vital, organs (Figures 1, 2, 3 and 4). In summary, the water extract from the root of *C. aurantifolia* 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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