ORIGINAL ARTICLE

Acute and subchronic toxicity study of the water extract from dried fruits of *Piper nigrum* L. in rats

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Abstract

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Acute and subchronic toxicity study of the water extract from dried fruits of *Piper nigrum* L. in rats

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The study was carried out to evaluate acute and subchronic toxicities of the water extract from the dried fruits of Piper nigrum L. A single oral administration of the extract at a dose of 5,000 mg/kg body weight (5 male, 5 female) did not produce signs of toxicity, behavioral changes, mortality, changes on gross appearance or histopathological changes of internal organs. The subchronic toxicity was determined by oral feeding both male and female rats (10 male, 10 female) daily with the test substance at the doses of 300, 600 and 1,200 mg/kg body weight continuously for 90 days. The examinations of signs, animal behavior and

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health monitoring showed no 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 hematology, blood clinical chemistry and histopathology. The results suggest that the water extract from the dried fruits of P. nigrum does not cause acute or subchronic toxicities in either male or female rats.

Key words : acute toxicity, subchronic toxicity, *Piper nigrum* L.

บทคัดย่อ

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การศึกษาความเป็นพิษเฉียบพลันและกึ่งเรื้อรังของสารสกัดน้ำจากผลแห้งของพริกไทยในหนูขาว

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การศึกษาความเป็นพิษเฉียบพลันและพิษกึ่งเรื้อรังของสารสกัดน้ำจากผลแห้งของพริกไทย (*Piper nigrum* L.) พบว่า การป้อนสารสกัดครั้งเดียวทางปากขนาด 5,000 มก./กก.น้ำหนักตัว (เพศผู้ 5 ตัว เพศเมีย 5 ตัว) ไม่ก่อให้เกิดอาการแสดงความเป็นพิษ การเปลี่ยนแปลงพฤติกรรม การตายและความแตกต่างของลักษณะทางจุลกายวิภาคของอวัยวะภายใน ผลการศึกษาความเป็นพิษกึ่งเรื้อรังโดยการป้อนสารสกัดทางปากแก่หนูขาวทั้งเพศผู้และเพศเมีย (เพศผู้ 10 ตัว เพศเมีย 10 ตัว) ทุกวันในขนาด 300 600 และ 1,200 มก./กก.น้ำหนักตัวเป็นเวลา 90 วัน ไม่พบความผิดปกติทางอาการ พฤติกรรม และสุขภาพของหนูขาวกลุ่มที่ได้รับสารสารสกัดเมื่อเทียบกับกลุ่มควบคุม ผลการชั่งน้ำหนักตัวสุดท้าย การผ่าพิสูจน์ซากสัตว์ทดลอง การชั่งน้ำหนักอวัยวะ การตรวจค่าโลหิตวิทยา ค่าเคมีคลินิกของเลือด และการตรวจจุลกายวิภาคของหนูกลุ่มทดสอบและกลุ่มควบคุมในวันที่ 90 และกลุ่มติดตามผล (satellite) ในวันที่ 118 พบว่าปกติ ดังนั้นการศึกษานี้สามารถสรุปได้ว่าสารสกัดน้ำจากผลแห้งของพริกไทย ไม่ก่อพิษเฉียบพลันและพิษกึ่งเรื้อรังในหนูขาว

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The black pepper, *Piper nigrum* L. (Piperaceae) has traditionally been used as both spice and medicine. It contains small quantities of chemopreventive compounds such as β-carotene, piperine, tannic acid and capsaicin. The antimutagenic activity of black pepper could be related to the large number of potent chemopreventive compounds. Black pepper has been reported to be rich in glutathione peroxidase, glucose-6-phosphate dehydrogenase, and vitamin E (Karthikeyan and Rani, 2003). Both water extract and ethanol extract of black pepper exhibited a strong antioxidant activity (Gulcin, 2005). Besides, Vijayakumar *et al*. (2004) reported that supplementation with black pepper or piperine can reduce high-fat diet-induced oxidative stress to the cells. Piperine, an active alkaloid, is known to possess several pharmacological actions such as antimetastatic (Pradeep and Kuttan, 2002), antimutagenic (El Hamss *et al*., 2003), antioxidant (Mittal and Gupta, 2000; Selvendiran *et al*., 2006). The capability of piperine to enhance cellular antioxidants in lung-cancer-bearing mice has been reported (Selvendiran *et al*., 2005b). Piperine modulates benzo[a]pyrene

metabolism by direct interaction with the cytochrome P4501A1 enzyme (Reen *et al*., 1996) and it counteracts aflatoxin B1 toxicity by suppressing cytochrome P-450-mediated bioactivation of the mycotoxin (Reen *et al*., 1996; Singh *et al*., 1994). The recent studies suggested that piperine might protect human from various types of cancer (Luo *et al*., 2003; Sunila and Kuttan, 2004). It reduces experimentally induced colon, liver, and pulmonary cancers (Pradeep and Kuttan, 2002; Reen *et al*., 1996; Singh *et al*., 1994). Pradeep and Kuttan (2002) demonstrated the antimetastatic activity of piperine in mice. Selvendiran *et al*. (2005a) also reported the protective effect of piperine against B(a)p-induced experimental lung carcinogenesis in Swiss albino mice by altering the tricarboxylic acid (TCA) cycle enzymes, reducing phase I enzymes and by enhancing the activity of glutathione-metabolizing enzymes. In addition, piperine plays an important role against B(a)p-induced lung carcinogenesis in Swiss albino mice by protecting the glycoprotein levels in serum and tissue, as it is one of the indicators of tumorigenesis (Selvendiran *et al*., 2006).

Piperine demonstrates not only chemopreventive effect (Selvendiran *et al*., 2003), but also genoprotective effects against B(a)-p induced mutagenesis in Swiss albino mice. Oral supplementation with piperine can reduce DNA damage and DNA protein cross-links by enhancing phase II enzymes. It can induce apoptosis in B(a)p-induced lung carcinogenesis in Swiss albino mice (Selvendiran *et al*., 2005b). Furthermore, black pepper supplementation can protect the colon by decreasing the activity of bacterial enzymes β-glucuronidase and mucinase, in the presence of the procarcinogen 1,2-dimethyl hydrazine (DMH) in rats (Nalini *et al*., 1998).

Piperine potentiates pentobarbitone-induced hypnosis in rats in a dose dependent manner (Mujumdar *et al*., 1990). Oral administration of aqueous extract of black pepper seeds is not only useful in controlling the glucose and lipid levels but also helpful in strengthening the antioxidant potential in alloxan-induced diabetic rats (Kaleem *et al*., 2005). Piperine increases permeability of rat intestinal epithelial cells, possibly by interacting with the lipid environment (Johri *et al*., 1992). Furthermore, an aqueous extract (10% w/v) of black pepper increases gastric acid secretion in anesthetized albino rats (Vasudevan *et al*., 2000). However, the toxicity of the water extract from *P. nigrum* has not been intensively studied. The present study aimed to evaluate the safety of the water extract from *P. nigrum* in rats by determining both oral acute and oral subchronic toxicities.

Materials and methods

Plant material

The dried fruits of *P. nigrum* was collected from Songkhla, Thailand. The voucher specimen (SBK 0014) was kept and identified the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Preparation of plant extract

The dried fruits, comprising 500 grams of *P. nigrum* seeds, was wrapped in a calico bag and put into a stainless boiler. Ten liters of water was added, then boiled to extract the substances in *P. nigrum* for 3-4 hours and filtered when it cooled down. 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 and the thin layer chromatography (TLC) fingerprints of the extract were recorded. The percentage yield was 35.40.

Experimental animals

Male and female Sprague-Dawley rats weighing 130-190 g were obtained from the National Laboratory Animal Center, Nakorn Pathom, Thailand. All animals were maintained in a controlled environment condition of temperature (24±1^{o}C) on alternative 12 h light/dark cycles. Before each experiment, the animals were

fasted overnight with free access to water. All experimental protocols were approved by the Animal Ethics Committee of Faculty of Medicine, Thammasat University.

Acute toxicity study

The acute oral toxicity was evaluated following the World Health Organization (WHO) guideline (WHO, 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for chemical testing (OECD, 2001). Briefly, rats were divided into two groups of ten (five males, five females). The treated group was orally given the aqueous extract in a single dose of 5,000 mg/kg body weight, while the control group received only water vehicle. The animals were monitored for apparent signs of toxicity for 14 days. The animals that died within this period were subjected to necropsies. All rats were weighted and sacrificed on the 15th day after administration, and then the vital organs including heart, lungs, livers, kidneys, spleen, adrenals, sex organs and brain were grossly and histopathological examined.

Subchronic toxicity study

The method was performed following the WHO guideline (WHO, 2000) and the OECD guideline (OECD, 1981). Briefly, male and female rats were randomly divided into three groups of ten. The treated group of each sex (ten males, ten females) was orally given the extract orally at the dose of 300, 600 and 1,200 mg/kg body weight daily for 90 days, while the control group received the vehicle at the same volume. The satellite group was treated orally with the extract at the daily dose of 1,200 mg/kg/body weight for 90 days and continually maintained without treatment for 28 days in order to detect a delayed occurrence of toxic effect.

All rats were observed for apparent signs of toxicity or behavioral alterations during the experimental period. At the end of each experiment, the rats were fasted 12 hours, and then anesthetized with ether. Blood was collected from a common carotid artery for hematological study. The serum was separated and the levels of glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) measured.

After the blood collection, the animals were sacrified for tissue examinations. The following tissues and organs were weighted, examined, and then fixed in 10% buffered formaldehyde solution: heart, lungs, thymus, livers, kidneys, spleen, adrenals, small intestine, stomach and duodenum, muscle with sciatic nerve, thoracic spines, brain, eyes, sex organs, uterus and epididymis. The fixed organs from all animals were examined by histopathological method.

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 was analyzed using Student's paired t-test. P values less than 0.05 were considered significant.

Results and discussion

After the rats were orally given a single dose of the water extract from the dried fruits of *P. nigrum* at 5,000 mg/kg, neither signs of toxicity nor death of rats were observed during 14 days of the acute toxicity experimental period. The alterations of body weight gain and organ weights from the control would reflect the toxicity of the substance (Carol, 1995), significant difference in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailey *et al*., 2004). Both body weight gain and internal organs weight were recorded as shown in Table 1 and 2, respectively. Neither body weight nor internal organ weight of treated rats was significantly changed relative to that of the control group. Gross and histopatho-

Table 1. Body weights of rats in the acute toxicity study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Body weight (g)			
	Day 0	**Day 7th**	**Day 14th**	**Weight gain on day 14th**
Female				
Control	144.80±12.82	167.20±8.87	181.60±10.13	36.80±338
P. nigrum 5,000 mg/kg	123.60±2.48	158.80±1.85	172.80±2.42	49.20±3.26
Male				
Control	138.80±5.46	186.80±8.40	224.40±11.75	85.60±6.73
P. nigrum 5,000 mg/kg	132.80±5.00	178.40±6.46	216.40±6.94	83.60±3.12

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 study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Organ weight (g)	
	Control	***P. nigrum* 5,000 mg/kg**
Female		
Lung	1.11±0.06	1.10±0.04
Heart	0.75±0.04	0.81±0.03
Liver	7.92±0.44	7.96±0.44
Spleen	0.51±0.02	0.53±0.02
Adrenal	0.04±0.00	0.04±0.00
Kidney	0.84±0.04	0.79±0.01
Ovary	0.07±0.00	0.08±0.00
Male		
Lung	1.16±0.04	1.13±0.06
Heart	0.99±0.04	0.95±0.06
Liver	8.90±0.86	9.00±0.52
Spleen	0.76±0.06	0.72±0.03
Adrenal	0.04±0.00	0.03±0.00
Kidney	1.03±0.04	0.95±0.02
Testis	1.25±0.06	1.27±0.03

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

logical examinations further confirmed that the substance did not cause any tissue damage. The internal organs revealed no pathological abnormality relative to the control (data not shown). Therefore, the results suggest that the water extract from the dried fruits of *P. nigrum* is not toxic after an acute exposure.

In the subchronic toxicity study, the body weights and body weight gains of both male and female groups treated with various doses showed

Table 3. Body weights of rats in the subchronic toxicity study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Body weight (g)			
	Day 0	**Day 90th**	**Day 118th**	**Weight gain on day 90th**
Female				
Control	151.11±4.21	272.00±3.69	-	120.89±6.88
P. nigrum 300 mg/kg	148.00±1.93	258.20±4.44	-	110.20±4.64
P. nigrum 600 mg/kg	149.60±2.76	256.40±4.66	-	106.80±4.10
P. nigrum 1,200 mg/kg [a]	146.20±2.75	261.60±5.32	-	115.40±3.21
P. nigrum 1,200 mg/kg [b]	143.20±2.96	274.00±8.47	282.80±8.01	130.80±8.46
Male				
Control	186.00±3.86	407.20±16.50	-	221.20±18.70
P. nigrum 300 mg/kg	183.00±5.48	375.20±10.46	-	192.20±8.94
P. nigrum 600 mg/kg	183.60±7.89	373.00±9.57	-	189.40±12.57
P. nigrum 1,200 mg/kg [a]	182.20±4.20	353.40±12.02	-	171.20±9.28
P. nigrum 1,200 mg/kg [b]	184.00±7.09	375.80±8.20	399.00±7.23	191.80±11.56

Values are expressed as mean ± S.E.M., n = 10
a: A group was treated with the water extract from the dried fruits of *P. nigrum* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from the dried fruits of P. *nigrum* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.
There were no significant differences at $p<0.05$.

no significant changes when compared with the control group (Table 3). Furthermore, neither changes in animal behaviors nor toxic signs were detected in the treated rats. Following necropsy, no macroscopic change was observed in the internal organs of all treated rats. As shown in Table 4, the female groups treated with *P. nigrum* at the doses of 300, 600, and 1,200 mg/kg/day showed significant lower weights of heart and kidney than those of the control groups. In the male groups treated with all three doses of the extract, the weights of lung were significantly lower than the control. A significant decrease in the weight of heart was also detected in the male treated at the dose 600 and 1,200 mg/kg/day. The weights of liver and kidney were significantly lower only in the group treated at the dose 600 mg/kg/day. The weights of liver and ovary in the female satellite group were significantly different from those of the control. However, slight changes were found in the weights of other internal organs that may due to the variation in size of internal organs in each animal (Carol, 1995). Next, the histopathological examination was performed to substantiate the results.

To determine the intravascular effect and bone marrow activity in rats treated with the extract, hematological parameters of female and male rates were examined as presented in Tables 5 and 6, respectively. The concentration of hemoglobin in the females treated with 600 mg/kg/day of the extract was slightly lower than that of the control group. In all of the male groups, the red blood cell count, hemoglobin and hematocrit contents were significantly decreased. The male satellite group showed increases in the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Although the hemoglobin, hematocrit and these red blood cell indices were helpful in the differential diagnosis of anemia (Gregg and Voigt, 2000), gross examinations of the skin, eye and mucous membrane did not show any clinical defect. In addition, all of

Table 4. Organ weights of rats in the subchronic toxicity study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Control	*P. nigrum*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
Lung	1.29±0.03	1.32±0.04	1.29±0.08	1.27±0.04	1.41±0.05
Heart	1.06±0.03	0.97±0.03*	0.97±0.02*	0.94±0.03*	0.99±0.03
Liver	5.67±0.15	6.16±0.25	6.06±0.18	6.12±0.22	6.54±0.46*
Spleen	0.67±0.02	0.64±0.02	0.67±0.02	0.69±0.02	0.71±0.03
Adrenal	0.05±0.00	0.05±0.00	0.06±0.00	0.04±0.00	0.05±0.00
Kidney	0.97±0.04	0.85±0.02*	0.86±0.02*	0.85±0.01*	0.92±0.04
Ovary	0.10±0.00	0.09±0.00	0.10±0.00	0.09±0.00	0.08±0.00*
Male					
Lung	1.66±0.11	1.47±0.04*	1.40±0.03*	1.38±0.05*	1.54±0.04
Heart	1.45±0.06	1.31±0.03	1.23±0.04*	1.19±0.03*	1.43±0.06
Liver	10.29±0.36	9.33±0.50	9.00±0.47*	10.46±0.39	9.67±0.32
Spleen	0.91±0.04	0.80±0.04	0.77±0.02	0.80±0.03	0.99±0.09
Adrenal	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.05±0.00
Kidney	1.28±0.03	1.18±0.08	1.14±0.02*	1.17±0.02	1.26±0.02
Testis	1.94±0.02	1.87±0.06	1.92±0.03	1.88±0.03	1.83±0.04

Values are expressed as mean ± S.E.M., n = 10
a: A group was treated with the water extract from the dried fruits of *P. nigrum* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from the dried fruits of *P. nigrum* 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 study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Control	*P. nigrum*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells (x10^6/µl)	6.80±0.07	6.67±0.06	6.61±0.10	6.66±0.08	6.61±0.10
Hemoglobin (g/dl)	14.72±0.14	14.58±0.09	14.03±0.16*	14.54±0.13	14.41±0.36
Hematocrit (%)	40.78±0.49	40.50±0.50	40.10±0.40	40.40±0.54	40.30±0.96
Mean corpuscular volume (fl)	59.86±0.39	60.64±0.59	60.64±0.58	60.55±0.38	60.93±0.83
Mean corpuscular hemoglobin (pg)	21.65±0.32	21.86±0.16	21.25±0.37	21.82±0.15	21.81±0.57
Mean corpuscular hemoglobin concentration (g/dl)	36.17±0.47	36.10±0.39	35.07±0.56	36.05±0.26	35.86±0.97
Platelet (x10^5/µl)	7.48±0.12	7.84±0.08	7.72±0.20	7.81±0.12	7.80±0.13

Values are expressed as mean ± S.E.M., n = 10.
a: A group was treated with the water extract from the dried fruits of *P. nigrum* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from the dried fruits of *P. nigrum* 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 from the dried fruits of *Piper nigrum* Linn.

	Control	*P. nigrum*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Red blood cells (x10^6/µl)	8.04±0.16	7.61±0.05*	7.49±0.10*	7.39±0.11*	7.67±0.11*
Hemoglobin (g/dl)	15.66±0.27	14.69±0.13*	14.57±0.20*	14.54±0.23*	15.86±0.25
Hematocrit (%)	46.80±1.03	44.50±0.34*	43.30±0.57*	43.00±0.77*	46.20±0.75
Mean corpuscular volume (fl)	57.50±0.45	58.42±0.24	57.79±0.22	58.20±0.34	60.26±0.59*
Mean corpuscular hemoglobin (pg)	19.06±0.42	19.30±0.22	19.59±0.25	19.68±0.19	20.67±0.16*
Mean corpuscular hemoglobin concentration (g/dl)	33.15±0.62	33.03±0.26	33.90±0.40	33.80±0.21	34.30±0.17*
Platelet (x10^5/µl)	8.04±0.07	8.05±0.06	8.07±0.16	7.93±0.09	8.22±0.21

Values are expressed as mean ± S.E.M., n = 10.
a: A group was treated with the water extract from the dried fruits of *P. nigrum* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from the dried fruits of *P. nigrum* 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 study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Control	*P. nigrum*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Female					
White blood cells (x10^3/µl)	2.89±0.32	3.49±0.16	3.27±0.33	2.92±0.18	3.58±0.47
Neutrophil (%)	20.00±1.65	16.80±0.82	17.80±1.43	15.90±1.48	16.80±1.99
Lymphocyte (%)	73.11±1.82	77.90±1.15	75.80±1.87	77.90±2.23	75.10±1.74
Monocyte (%)	6.00±0.60	4.60±0.73	5.40±0.47	5.30±0.65	6.40±0.63
Eosinophil (%)	0.89±0.35	0.70±0.26	1.00±0.29	0.90±0.23	1.70±0.44
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)	4.05±0.23	3.10±0.26*	3.34±0.26*	3.24±0.26*	4.14±0.18
Neutrophil (%)	14.30±1.46	16.60±2.82	17.40±1.91	11.70±1.18	15.80±1.18
Lymphocyte (%)	78.10±1.29	77.10±2.79	77.60±2.01	81.70±1.66	78.60±1.11
Monocyte (%)	5.40±0.84	5.00±0.65	3.70±0.49	5.40±0.81	5.00±0.53
Eosinophil (%)	2.40±0.47	1.30±0.42*	1.30±0.42*	1.00±0.25*	0.60±0.22*
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 the dried fruits of *P. nigrum* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from the dried fruits of *P. nigrum* at 1,200 mg/kg/day for 90 days followed by no treatment for 28 days.
*** Significantly different from control, p<0.05.**

the changes were still within the normal limits (Feldman *et al*., 2000; Inala *et al*., 2002). The differential white blood cell count values of the female and male treated groups are shown in Table 7. As compared with the control values, no significant changes in any values were observed

Table 8. Clinical blood chemistry values of female rats in the subchronic toxicity study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Control	*P. nigrum*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	103.78±2.59	100.10±3.61	88.90±4.34*	100.30±3.20	126.20±5.12*
BUN (mg/dl)	24.22±1.07	21.50±0.67	19.30±0.94*	23.30±1.02	22.30±1.55
Creatinine (mg/dl)	0.43±0.02	0.38±0.01	0.43±0.03	0.41±0.01	0.42±0.03
Total protein (g/dl)	5.07±0.08	5.26±0.08	5.19±0.11	5.15±0.06	5.58±0.06*
Albumin (g/dl)	3.58±0.06	3.60±0.04	3.63±0.07	3.51±0.05	3.79±0.08*
Total bilirubin (mg/dl)	0.16±0.01	0.13±0.01	0.17±0.01	0.13±0.01	0.15±0.01
Direct bilirubin (mg/dl)	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.01	0.01±0.01
SGOT (U/l)	105.22±2.44	101.00±8.17	98.50±7.76	99.40±6.71	160.70±16.64*
SGPT (U/l)	31.33±1.71	33.30±4.19	29.70±4.43	27.60±1.88	34.80±2.80
ALP (U/l)	41.22±2.49	48.90±3.68	41.00±2.36	43.00±2.44	36.60±2.34

Values are expressed as mean ± S.E.M., n = 10.
a: A group was treated with the water extract from the dried fruits of *P. nigrum* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from the dried fruits of *P. nigrum* 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 study of the water extract from the dried fruits of *Piper nigrum* Linn.

	Control	*P. nigrum*			
		300 mg/kg	600 mg/kg	1,200 mg/kg [a]	1,200 mg/kg [b]
Glucose (mg/dl)	126.00±4.42	110.20±3.88	119.20±9.70	134.90±4.57	120.10±4.29
BUN (mg/dl)	21.00±0.91	20.50±0.82	22.00±0.65	20.70±0.51	19.40±0.74
Creatinine (mg/dl)	0.30±0.02	0.27±0.01	0.31±0.02	0.30±0.02	0.37±0.03*
Total protein (g/dl)	5.53±0.12	5.88±0.12	5.27±0.21	5.63±0.06	5.15±0.10
Albumin (g/dl)	3.42±0.07	3.44±0.04	3.32±0.06	3.38±0.02	3.39±0.04
Total bilirubin (mg/dl)	0.10±0.01	0.11±0.01	0.13±0.01	0.10±0.00	0.10±0.00
Direct bilirubin (mg/dl)	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00
SGOT (U/l)	112.20±4.90	103.90±6.05	78.60±4.06*	105.70±6.35	103.80±5.02
SGPT (U/l)	39.80±4.21	38.00±1.28	36.50±2.65	37.70±1.04	37.40±1.97
ALP (U/l)	68.20±6.09	74.70±3.91	85.70±12.95	96.60±9.08*	51.70±2.86

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

in the female groups at all three given doses including the satellite group. In the male treated groups at all three doses, white blood cells especially eosinophil were significantly decreased relative to the control. However, the alterations were minor and remained within the normal ranges (Feldman *et al*., 2000; Inala *et al*., 2002). Besides, the normal blood smear was detected. These

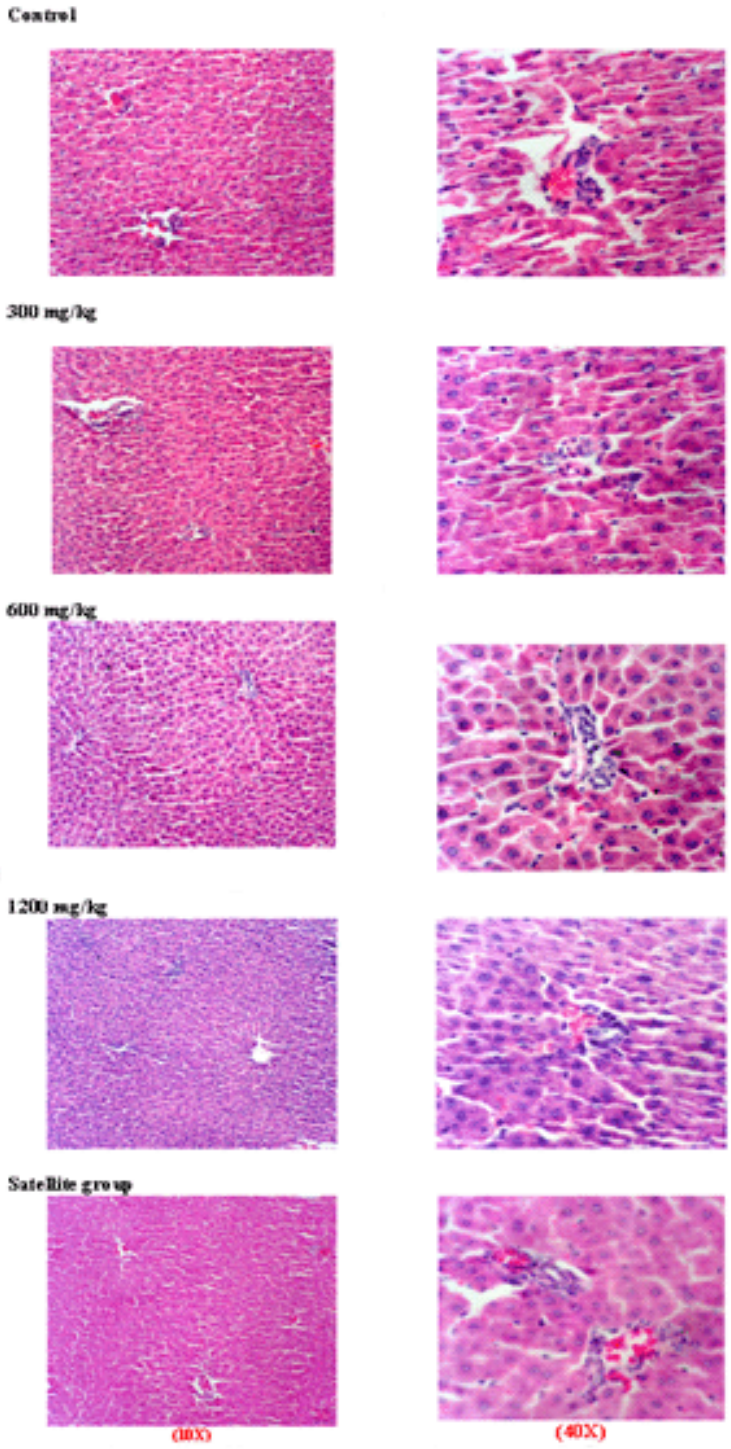

Figure 1. The histology of male liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.
[Color figure can be viewed in the electronic version]

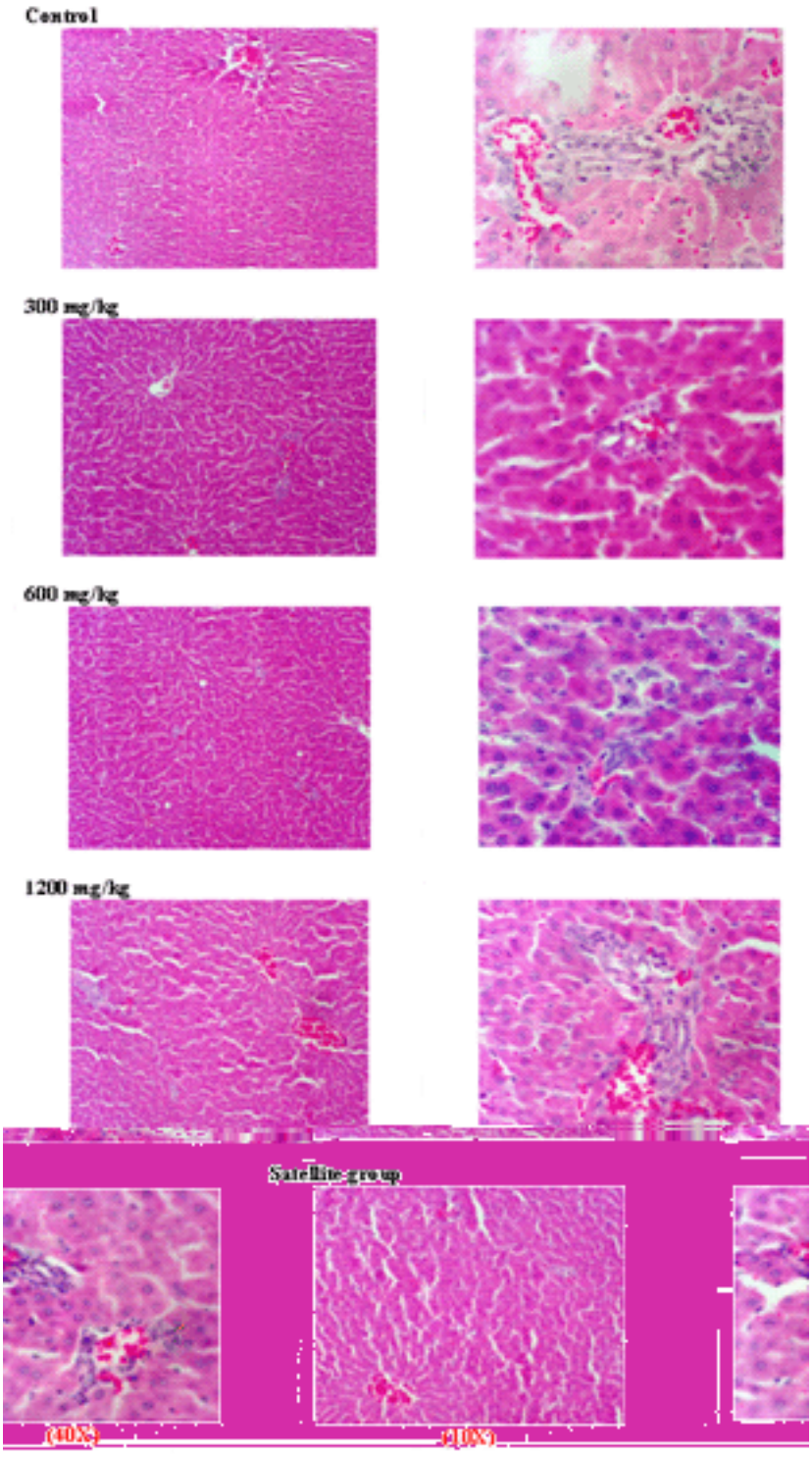

Figure 2. The histology of female liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

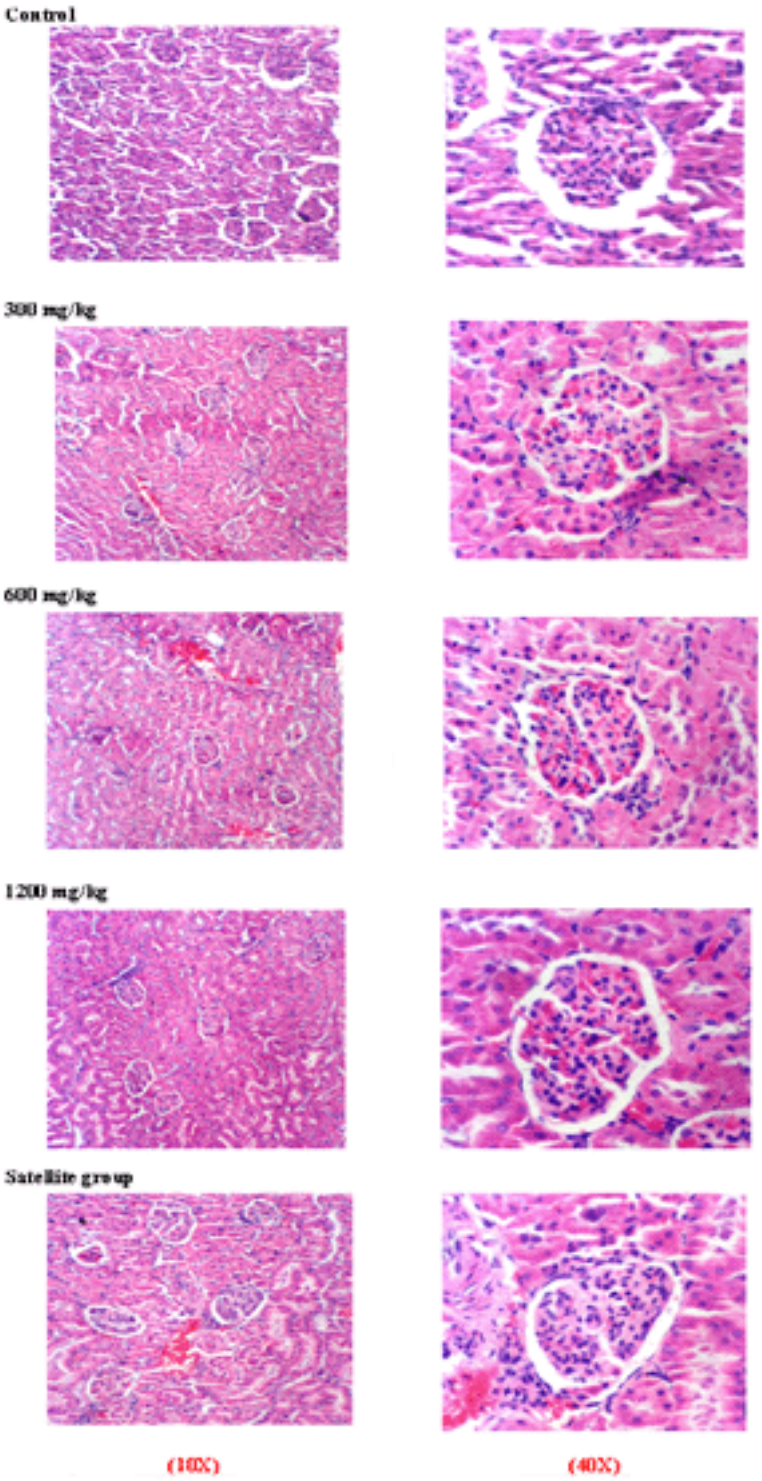

Figure 3. The histology of male kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

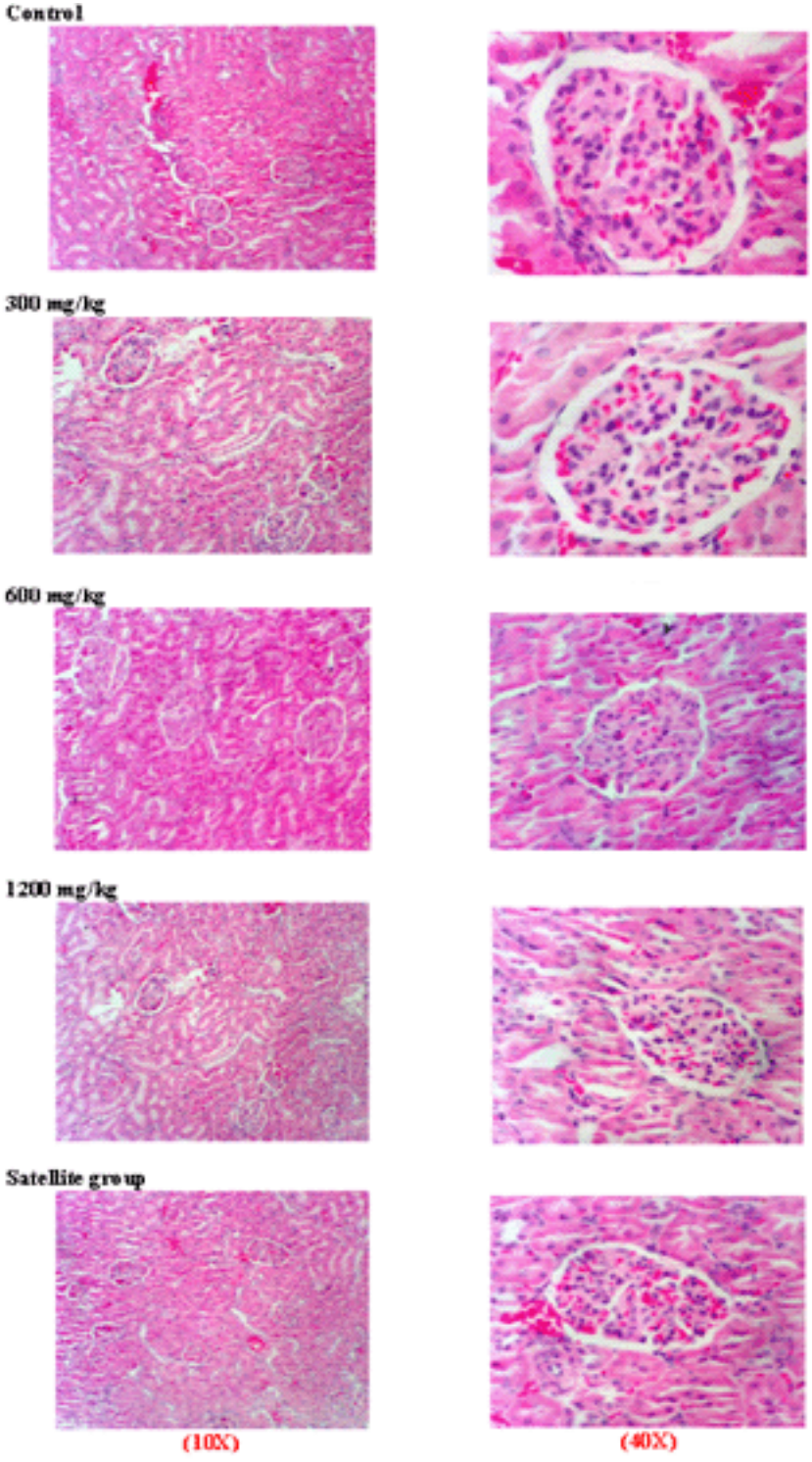

Figure 4. The histology of female kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

results suggest the water extract from the dried fruits of of *P. nigrum* does not cause leucopenia or conditions that affect the bone marrow.

The clinical blood chemistry examination was performed in the female and male rats and the results are shown in Tables 8 and 9, respectively. The data indicate significant decreases in the levels of glucose and BUN in the female rats treated with the extract at the dose of 600 mg/kg/day. The concentrations of glucose, total protein, SGOT and albumin were significantly increased within the normal range in the female satellite group as compared to those of the control group. In the groups of male rats, the SGOT was reduced significantly in the group treated with 600 mg/kg/day as compared to the controls. In addition, the creatinine level was significantly increased in the male satellite group. However, the levels of these clinical blood chemical parameters were still within the normal range (Angkhasirisap *et al*., 2002; Barry, 1995; Caisey and King, 1980; Sacher and McPherson, 1991a, 1991b).

Clearly, highly significant increase in SGOT level of the female satellite group and ALP of the male rats receiving the extract at 1,200 mg/kg/day were detected (Tables 8, 9). These observations were further investigated by the histopathological assessment of the organs. The results showed that the water extract of *P. nigrum* did not produce a significant damage in the internal organs, such as liver and kidney (Figures 1, 2, 3 and 4). In conclusion, the water extract from the dried fruits of *P. nigrum* dose not produce acute or subchronic toxicities in female and male rats. In addition, a chronic toxicity study should be further carried out to assess a long-term safety of the extract.

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