Songklanakarin J. Sci. Technol.
30 (5), 611-619, Sep. - Oct. 2008

Songklanakarin Journal of Science and Technology
http://www.sjst.psu.ac.th

*Original Article*

# Acute and subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels in rats

Seewaboon Sireeratawong[1,5*], Nirush Lertprasertsuke[2], Umarat Srisawat[3], Amornat Thuppia[4], Anongnad Ngamjariyawat[4], Nadthaganya Suwanlikhid[4] and Kanjana Jaijoy[5]

[1] *Division of Pharmacology, Department of Preclinical Science, Faculty of Medicine,*

[3] *Division of Physiology, Department of Preclinical Science, Faculty of Medicine,*

[4] *Division of Anatomy, Department of Preclinical Science, Faculty of Medicine,*

[5] *Research Unit of Pharmacology and Toxicology of Herbal Medicine, Research Center, Faculty of Medicine, Thammasat University, Rungsit Campus, Klong Luang, Pathum Thani, 12120 Thailand.*

[2] *Department of Pathology, Faculty of Medicine, Chiang Mai University, Muang, Chiang Mai, 50200 Thailand.*

Received 4 June 2008; Accepted 9 August 2008

**Abstract**

The study was carried out to evaluate acute and subchronic toxicities of the water extract from *Tiliacora triandra* (Colebr.) Diels. A single oral administration of the extract at a dose of 5,000 mg/kg body weight (5 males, 5 females) 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 males, 10 females) 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 health monitoring showed no abnormalities in the test groups as compared to the controls. The test and control groups (on the 90$^{th}$ day) and the satellite group (on the 118$^{th}$ 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 *T. triandra* does not cause acute or subchronic toxicities in either male or female rats.

**Keywords:** Acute toxicity, Subchronic toxicity, *Tiliacora triandra* (Colebr.) Diels

## 1. Introduction

*Tiliacora triandra* (Colebr.) Diels (Family: Menispermaceae) had a Thai name Ya Nang. Tilitriandrin is a new bisbenzylisoquinoline alkaloid from *T. triandra* (Pachaly and Khosravian, 1988a). Moreover, alkaloids magnoflorine, nortiliacorine A, and tiliacorinin-2'- N-oxide, two new bisbenzylisoquinoline alkaloids, noryanangine and norisoyanangine were isolated from the aerial parts of *T. triandra* Diels (Pachaly and Khosravian, 1988b). However, the pharmacological activity and toxicity of *T. triandra* has not been intensively studied. The present study aimed to evaluate the safety of the water extract from *T. triandra* in rats by determining both oral acute and oral subchronic toxicities.

*Corresponding author.
Email address: seewaboon@gmail.com

## 2. Materials and Methods

### 2.1 Plant material

The whole plant of *Tiliacora triandra* (Colebr.) Diels was collected from Songkhla, Thailand. The voucher specimen (SBK 0018) was kept and identified at the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

### 2.2 Preparation of plant extract

500 grams of *T. triandra* 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 *T. triandra* for 3-4 hours and filtered when it 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. The weight and percentage yield of the crude extract and the thin layer chromatography (TLC) fingerprints of the extract were recorded.

### 2.3 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 ($24\pm1^{o}$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.

### 2.4 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 necropsy. All rats were weighed and sacrificed on the $15^{th}$ day after administration, and then the vital organs including heart, lungs, livers, kidneys, spleen, adrenals, sex organs and brain were grossly and histopathologically examined.

### 2.5 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 weighed, 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.

### 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

After the rats were orally given a single dose of the water extract from *T. triandra* 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 (Auletta, 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 Tables 1 and 2, respectively. Body weight gain on day 14 of the female treated group was significantly increased, whereas the male treated group had significantly decreased. Internal organ weight of treated rats was not significantly changed relative to that of the control group except for the testis weight which was significantly increased. Gross and histopathological examinations further confirmed that the substance did not cause any tissue damage. The internal organs revealed no pathological abnormality relative to the

Table 1. Body weight of rats in the acute toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels

| | 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±3.38 |
| *T. triandra* 5,000 mg/kg | 137.60±13.12 | 173.20±4.45* | 181.20±8.11 | 43.60±7.57* |
| Male | | | | |
| Control | 142.00±4.43 | 190.00±7.56 | 228.00±10.53 | 86.00±6.60 |
| *T. triandra* 5,000 mg/kg | 153.60±1.60 | 218.40± 3.25* | 230.00±2.76 | 76.40±1.94* |

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

Table 2. Organ weights of rats in the acute toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels

| | Organ weight (g) | |
|---|---|---|
| | Control | *T. triandra* 5,000 mg/kg |
| Female | | |
| Lung | 1.11±0.06 | 1.06±0.06 |
| Heart | 0.75±0.04 | 0.84±0.03 |
| Liver | 7.92±0.44 | 7.89±0.56 |
| Spleen | 0.51±0.02 | 0.56±0.03 |
| Adrenal | 0.04±0.00 | 0.04±0.00 |
| Kidney | 0.84±0.04 | 0.87±0.03 |
| Ovary | 0.07±0.00 | 0.07±0.00 |
| Male | | |
| Lung | 1.16±0.04 | 1.19±0.04 |
| Heart | 0.99±0.04 | 0.97±0.05 |
| Liver | 8.90±0.86 | 9.67±0.70 |
| Spleen | 0.76±0.06 | 0.71±0.02 |
| Adrenal | 0.04±0.00 | 0.03±0.00 |
| Kidney | 1.03±0.04 | 1.05±0.01 |
| Testis | 1.25±0.06 | 1.44±0.02* |

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

control (data not shown). Therefore, the results suggest that the water extract from *T. triandra* 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 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 *T. triandra* at the doses of 300 mg/kg/day showed significantly lower weights of heart and kidney than those of the control groups. Moreover, kidney weight was significantly decreased in females treated with 1,200 mg/kg/day, whereas the ovary weight significantly decreased. A significant decrease in the weight of heart was also detected in males treated at the dose 300, 600 and 1,200 mg/kg/day. The weight of liver was significantly lower only in the group treated at the dose 300, 1,200 mg/kg/day and satellite group. The weights of spleen in the male treated with 600, 1200 mg/kg/day and satellite group were significantly different from those of the control. In addition, kidney weight was significantly decreased in males treated with 300 and 1200 mg/kg/day. The males treated with 600 mg/kg/day had a significantly decreased testis weight. 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 (Auletta, 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 male satellite group showed increases in the mean corpuscular volume (MCV). Although the hemoglobin, hematocrit and other 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 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 in the female and male groups at any of the three given doses except the satellite group. In the female satellite groups, white blood cells especially neutrophil was significantly decreased and monocyte and eosinophil were significantly increased relative to the control. The male satellite group was significantly decreased in eosinophils. However, the alterations were minor and remained within the normal ranges (Feldman *et al*., 2000; Inala *et al.,* 2002). Further-

Table 3. Body weight of rats in the subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels

| | Body weight (g) | | | |
|---|---|---|---|---|
| | Day 0 | Day 90th | Day 118th | Weight gain on day 90th |
| Female | | | | |
| Control | 147.10±3.59 | 276.00±3.95 | - | 128.90±5.00 |
| *T. triandra* 300 mg/kg | 141.60±2.87 | 257.20±4.45* | - | 115.60±5.32* |
| *T. triandra* 600 mg/kg | 142.60±1.95 | 256.00±4.19* | - | 114.20±4.38* |
| *T. triandra* 1,200 mg/kg [a] | 142.00±3.04 | 261.20±4.48* | - | 119.20±3.30 |
| *T. triandra* 1,200 mg/kg [b] | 142.40±3.13 | 264.00±3.32* | 277.20±3.83 | 121.60±4.26 |
| Male | | | | |
| Control | 181.60±6.67 | 407.20±17.02 | - | 225.60±20.16 |
| *T. triandra* 300 mg/kg | 175.60±3.91 | 415.60±12.06 | - | 240.00±10.38 |
| *T. triandra* 600 mg/kg | 175.60±4.70 | 376.40±9.03 | - | 200.80±8.80 |
| *T. triandra* 1,200 mg/kg [a] | 173.80±6.14 | 367.80±7.96* | - | 194.00±12.85 |
| *T. triandra* 1,200 mg/kg [b] | 180.60±8.92 | 381.00±9.73 | 404.30±12.27 | 200.40±13.19 |

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

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

b: A satellite group was treated with the water extract from *T. triandra* 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 from *Tiliacora triandra* (Colebr.) Diels

| | Control | *T. triandra* | | | |
|---|---|---|---|---|---|
| | | 300 mg/kg | 600 mg/kg | 1,200 mg/kg [a] | 1,200 mg/kg [b] |
| Female | | | | | |
| Lung | 1.28±0.02 | 1.20±0.04 | 1.19±0.02 | 1.22±0.04 | 1.29±0.03 |
| Heart | 1.04±0.02 | 0.96±0.02* | 0.99±0.02 | 0.95±0.03* | 1.02±0.02 |
| Liver | 5.83±0.11 | 5.87±0.18 | 6.00±0.38 | 5.91±0.15 | 6.14±0.11 |
| Spleen | 0.67±0.02 | 0.64±0.01 | 0.65±0.02 | 0.62±0.03 | 0.65±0.02 |
| Adrenal | 0.05±0.00 | 0.05±0.00 | 0.06±0.00 | 0.04±0.00 | 0.05±0.00 |
| Kidney | 0.98±0.04 | 0.82±0.01* | 0.87±0.02* | 0.85±0.01* | 0.88±0.01 |
| Ovary | 0.10±0.00 | 0.10±0.00 | 0.10±0.00 | 0.10±0.00 | 0.08±0.00* |
| Male | | | | | |
| Lung | 1.63±0.10 | 1.49±0.05 | 1.51±0.03 | 1.48±0.05 | 1.50±0.04 |
| Heart | 1.47±0.06 | 1.29±0.05* | 1.30±0.04* | 1.24±0.02* | 1.34±0.02 |
| Liver | 10.59±0.42 | 9.52±0.27* | 9.59±0.46 | 9.24±0.38* | 9.18±0.22* |
| Spleen | 0.91±0.05 | 0.84±0.03 | 0.76±0.02* | 0.80±0.02* | 0.81±0.02* |
| Adrenal | 0.04±0.00 | 0.04±0.00 | 0.04±0.00 | 0.03±0.00 | 0.05±0.00 |
| Kidney | 1.26±0.03 | 1.18±0.01* | 1.21±0.02 | 1.17±0.02* | 1.22±0.01 |
| Testis | 1.92±0.02 | 1.93±0.02 | 1.81±0.04* | 1.87±0.02 | 1.86±0.32 |

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

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

b: A satellite group was treated with the water extract from *T. triandra* 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 *Tiliacora triandra* (Colebr.) Diels

| | Control | *T. triandra* | | | |
|---|---|---|---|---|---|
| | | 300 mg/kg | 600 mg/kg | 1,200 mg/kg [a] | 1,200 mg/kg [b] |
| Red blood cells (x$10^6$/µl) | 6.77±0.08 | 6.65±0.06 | 6.75±0.09 | 6.62±0.14 | 6.72±0.07 |
| Hemoglobin (g/dl) | 14.77±0.12 | 14.51±0.11 | 14.52±0.14 | 14.33±0.22 | 14.48±0.21 |
| Hematocrit (%) | 40.78±0.49 | 40.40±0.52 | 41.10±0.45 | 40.30±0.81 | 40.70±0.65 |
| Mean corpuscular volume (fl) | 60.04±0.35 | 60.50±0.40 | 60.60±0.70 | 60.90±0.50 | 60.75±0.51 |
| Mean corpuscular hemoglobin (pg) | 21.82±0.32 | 21.90±0.13 | 21.54±0.31 | 21.68±0.44 | 21.54±0.24 |
| Mean corpuscular hemoglobin concentration (g/dl) | 36.34±0.49 | 36.09±0.26 | 35.53±0.36 | 35.58±0.63 | 35.42±0.18 |
| Platelet (x$10^5$/µl) | 7.71±0.13 | 7.70±0.16 | 7.78±0.17 | 8.05±0.15 | 7.95±0.09 |

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

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

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

There were no significant differences at $p<0.05$.

Table 6. Hematological values of male rats in the subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels

| | Control | *T. triandra* | | | |
|---|---|---|---|---|---|
| | | 300 mg/kg | 600 mg/kg | 1,200 mg/kg [a] | 1,200 mg/kg [b] |
| Red blood cells (x$10^6$/µl) | 7.76±0.37 | 7.59±0.17 | 7.72±0.16 | 7.63±0.11 | 7.74±0.08 |
| Hemoglobin (g/dl) | 15.27±0.55 | 14.78±0.36 | 15.19±0.24 | 15.43±0.35 | 15.89±0.15 |
| Hematocrit (%) | 45.10±2.30 | 43.50±1.11 | 45.00±0.95 | 44.90±0.72 | 47.40±0.52 |
| Mean corpuscular volume (fl) | 57.40±0.51 | 57.34±0.36 | 58.03±0.25 | 58.79±0.64 | 60.96±0.59* |
| Mean corpuscular hemoglobin (pg) | 19.45±0.61 | 19.42±0.09 | 19.71±0.32 | 20.17±0.31 | 20.52±0.10 |
| Mean corpuscular hemoglobin concentration (g/dl) | 33.92±1.11 | 33.90±0.09 | 33.93±0.50 | 34.290±0.39 | 33.68±0.40 |
| Platelet (x$10^5$/µl) | 8.04±0.12 | 8.05±0.09 | 7.98±0.08 | 7.91±0.11 | 8.16±0.13 |

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

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

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

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

more, the normal blood smear was detected. These results suggest the water extract from *T. triandra* 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. In the female-rat group (Table 8), there was a significant decrease of the BUN levels in the rats treated with the extract at the dose of 300 mg/kg/day and the satellite group. The albumin level was also significantly decreased in the female rats treated with the extract at the dose of 1,200 mg/kg. The alkaline phosphatase (ALP) level was significantly increased in the group treated with 1,200 mg/kg of the extract whereas this level was significantly decreased in the satellite group. The total protein was also increased in the satellite group. Moreover, the total bilirubin level was significantly decreased within the normal range in the females treated with 300, 600 and 1,200 mg/kg/day as compared to those of the control group (Table 8). There was no significant change in the levels of creatinine, direct bilirubin, SGOT or SGPT in these female-rat subjects (Table 8). In the groups of male rats (Table 9), there was a significant increase in the BUN level in the group treated with 1,200 mg/kg/day when compared to the control. The creatinine level was also found to be significantly increased in the male satellite group. In addition, the total protein level was significantly increased in males treated with 300 mg/kg/day (Table 9). There were no significant differences among other parameters tested including albumin, total bilirubin, direct bilirubin, SGOT, SGPT and ALP (Table 9). It is noteworthy that the levels of these clinical blood chemical parameters were

Table 7. Differential white blood cell count values of rats in the subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels

| | Control | *T. triandra* | | | |
|---|---|---|---|---|---|
| | | 300 mg/kg | 600 mg/kg | 1,200 mg/kg [a] | 1,200 mg/kg [b] |
| Female | | | | | |
| White blood cells ($x10^3$/µl) | 2.95±0.32 | 2.96±0.12 | 2.74±0.35 | 2.72±0.26 | 2.76±0.20 |
| Neutrophil (%) | 18.33±1.60 | 16.60±1.18 | 17.70±1.04 | 15.70±1.07 | 9.90±1.05* |
| Lymphocyte (%) | 75.22±1.73 | 77.50±1.57 | 74.20±1.34 | 78.20±1.38 | 78.00±1.44 |
| Monocyte (%) | 5.89±0.61 | 4.60±0.37 | 6.80±0.44 | 5.20±0.57 | 7.90±075* |
| Eosinophil (%) | 0.56±0.24 | 1.30±0.33 | 1.30±0.21 | 0.90±0.27 | 4.20±0.49* |
| 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) | 3.83±0.33 | 3.70±0.34 | 3.91±0.42 | 3.32±0.23 | 3.49±0.27 |
| Neutrophil (%) | 16.40±1.66 | 11.70±1.25 | 20.30±1.26 | 20.10±2.81 | 16.00±0.77 |
| Lymphocyte (%) | 75.80±1.74 | 81.10±1.79 | 73.30±1.55 | 75.10±3.42 | 77.30±0.59 |
| Monocyte (%) | 5.10±0.79 | 5.60±0.54 | 4.90±0.58 | 3.40±0.56 | 5.80±0.68 |
| Eosinophil (%) | 2.70±0.85 | 1.60±0.22 | 1.50±0.50 | 1.40±0.54 | 0.90±0.23* |
| 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 *T. triandra* at 1,200 mg/kg/day for 90 days.
b: A satellite group was treated with the water extract from *T. triandra* 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 from *Tiliacora triandra* (Colebr.) Diels

| | Control | *T. triandra* | | | |
|---|---|---|---|---|---|
| | | 300 mg/kg | 600 mg/kg | 1,200 mg/kg [a] | 1,200 mg/kg [b] |
| Glucose (mg/dl) | 102.60±2.26 | 103.70±3.89 | 99.40±3.13 | 100.60±3.26 | 111.40 ± 4.70 |
| BUN (mg/dl) | 23.33±1.41 | 20.50±1.24* | 21.10±0.72 | 21.70±0.63 | 19.80 ± 0.35* |
| Creatinine (mg/dl) | 0.42±0.02 | 0.40±0.00 | 0.37±0.01 | 0.42±0.02 | 0.39 ± 0.02 |
| Total protein (g/dl) | 5.13±0.07 | 5.07±0.07 | 5.04±0.08 | 5.20±0.08 | 5.52 ± 0.06* |
| Albumin (g/dl) | 3.63±0.05 | 3.60±0.06 | 3.51±0.07 | 3.42±0.07* | 3.72 ± 0.05 |
| Total bilirubin (mg/dl) | 0.17±0.01 | 0.12±0.01* | 0.13±0.01* | 0.13±0.01* | 0.17 ± 0.01 |
| Direct bilirubin (mg/dl) | 0.00±0.00 | 0.01±0.01 | 0.00±0.00 | 0.00±0.00 | 0.00 ± 0.00 |
| SGOT (U/l) | 103.80±2.55 | 105.50±11.01 | 96.00±5.55 | 92.80±4.77 | 101.40 ± 3.97 |
| SGPT (U/l) | 31.40±1.69 | 26.20±1.28 | 24.90±0.90 | 25.90±1.65 | 45.30 ± 15.02 |
| ALP (U/l) | 39.80±1.55 | 43.00±2.05 | 41.80±2.75 | 46.90±2.71* | 28.90 ± 1.72* |

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

still within the normal range (Angkhasirisap *et al*., 2002; Caisey and King, 1980; Sacher and McPherson, 1991a, 1991b).

These observations were further investigated by the histopathological assessment of the organs. The results showed that the water extract of *T. triandra* 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 *T. triandra* dose not produce acute or subchronic toxicities in female and male rats. In addition, a chronic

Table 9. Clinical blood chemistry values of male rats in the subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels

| | Control | *T. triandra* | | | |
|---|---|---|---|---|---|
| | | 300 mg/kg | 600 mg/kg | 1,200 mg/kg [a] | 1,200 mg/kg [b] |
| Glucose (mg/dl) | 119.80±5.86 | 116.40±2.81 | 111.00±8.12 | 115.60±3.13 | 123.20±3.68 |
| BUN (mg/dl) | 21.00±0.86 | 19.20±0.57 | 20.50±0.82 | 23.70±0.55* | 22.90±1.53 |
| Creatinine (mg/dl) | 0.29±0.01 | 0.26±0.01 | 0.28±0.02 | 0.30±0.02 | 0.38±0.02* |
| Total protein (g/dl) | 5.48±0.09 | 5.81±0.12* | 5.73±0.12 | 5.22±0.07 | 5.33±0.06 |
| Albumin (g/dl) | 3.37±0.07 | 3.51±0.08 | 3.41±0.05 | 3.46±0.05 | 3.44±0.04 |
| Total bilirubin (mg/dl) | 0.10±0.01 | 0.10±0.01 | 0.10±0.00 | 0.13±0.010 | 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) | 112.60±4.70 | 118.80±7.57 | 98.80±2.81 | 98.80±3.75 | 108.60±6.17 |
| SGPT (U/l) | 40.20±4.13 | 41.10±2.84 | 37.10±3.96 | 36.70±2.53 | 33.20±3.33 |
| ALP (U/l) | 67.10±6.34 | 71.80±4.79 | 68.40±6.50 | 70.50±4.94 | 53.40±1.28 |

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

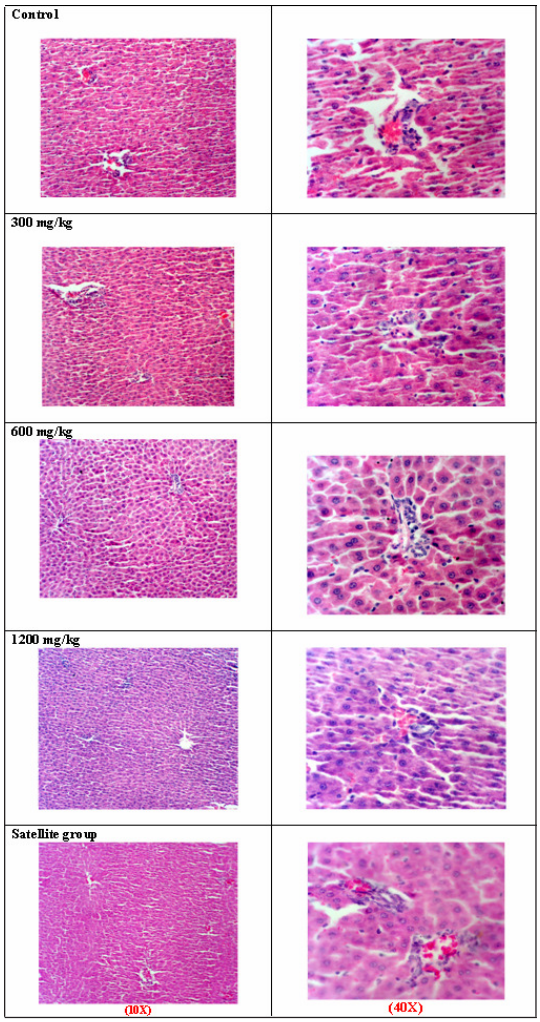


Figure 1. The histology of male liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

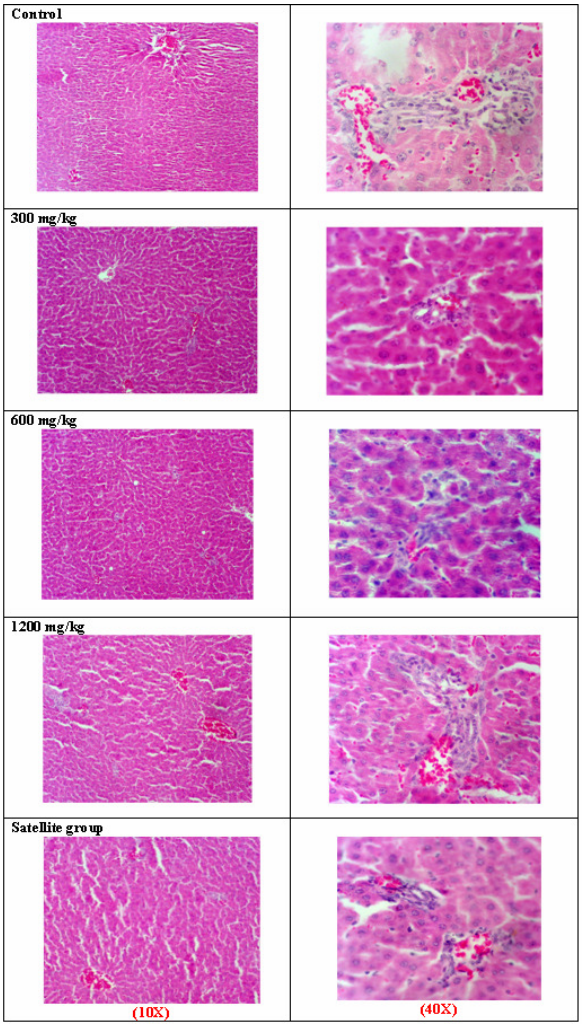


Figure 2. The histology of female liver from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

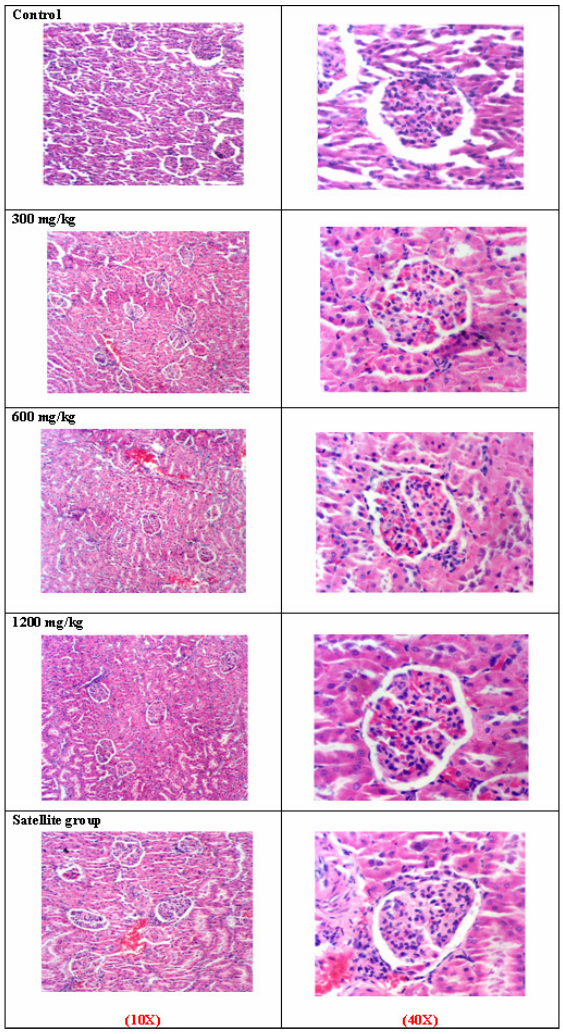


Figure 3. The histology of male kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

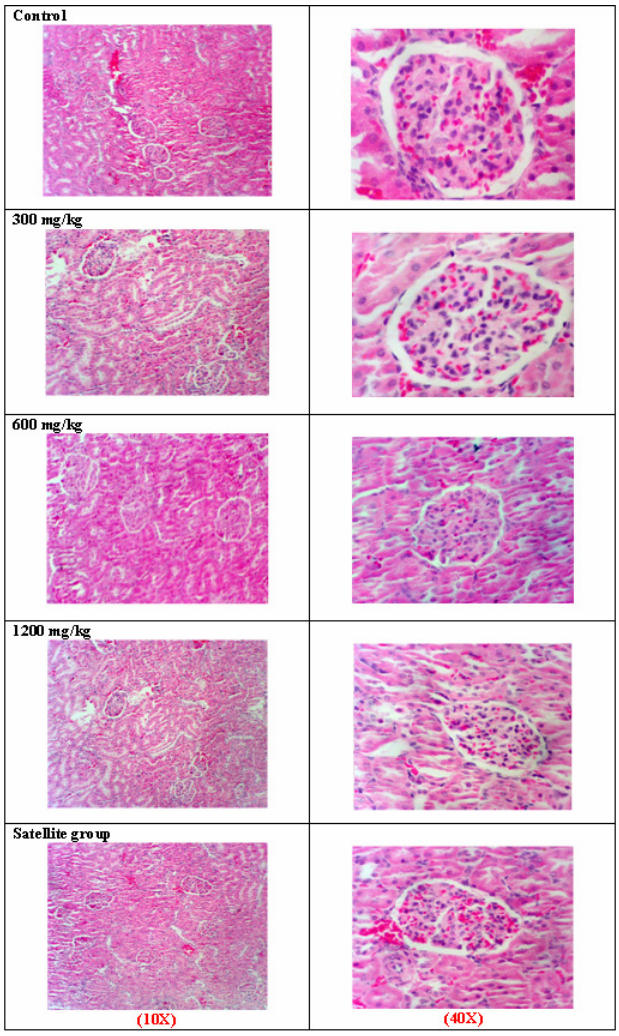


Figure 4. The histology of female kidney from the control and treated groups (the 10x and 40x magnifications). No significant damage was detected in any treatment group.

toxicity study should be further carried out to assess a long-term safety of the extract.

## Acknowledgements

The authors are thankful to the Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health, Bangkok, Thailand, and Dr Somboon Kietinun for financial support. Deep appreciation is especially extended to the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand, for the plant material identification.

## References

Angkhasirisap, W., Inala, P., Sirimontaporn, A., Inpunkaew, R., Rungrojejinda, K., Kengkoom, K., Ratanasak, W. and Buripadi Lawson, D. 2002. Blood chemistry profiles of outbred Sprague-Dawley rat in The Facility of National Laboratory Animal Centre. 28th Congress on Science and Technology of Thailand.

Auletta, C.S. 1995. Acute, Subchronic and Chronic Toxicology. *In* CRC Handbook of Toxicology. M.J. Derelanko, M.A. Hollinger, editors. CRC Press, Inc., U.S.A., pp 51-104.

Bailey, S.A., Zidell, R.H. and Perry, R.W. 2004. Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicologic Pathology. 32(4), 448-466.

Caisey, J.D. and King, D.J. 1980. Clinical chemical values for some common laboratory animals. Clinical Chemistry. 26, 1877-1879.

Feldman, B.F., Zinkl, J.G., Jain, N.C. and Moor, D.M. 2000. Schalm's Veterinary Hematology. 5th ed. Philadelphia, Lippincott Williams & Wilkins.

Gregg, L. and Voigt, D.V.M. 2000. Anemias and Polychythenias. *In* Hematology Techniques and Concepts

for Veterinary Technicians. Iowa State University Press, U.S.A., pp 95-101.

Inala, P., Sirimontaporn, A., Inpunkaew, R., Rungrojejinda, K., Kengkoom, K., Ratanasak, W. and Buripakdi Lawson, D. 2002 Hematological analysis of outbred Sprague-Dawley rat in The Facility of National Laboratory Animal Centre. 28th Congress on Science and Technology of Thailand.

Levine, B.S. 1995. Animal Clinical Pathology. In CRC Handbook of Toxicology, M.J. Derelanko, M.A. Hollinger, editors. CRC Press, Inc., U.S.A., pp 517-537.

Pachaly, P. and Khosravian, H. 1988a. Tilitriandrin: a new bisbenzylisoquinoline alkaloid from *Tiliacora triandra*. Planta Medica. 54(6), 516-519.

Pachaly, P. and Khosravian, H. 1988b. New Bisbenzylisoquinoline Alkaloids from *Tiliacora triandra* 1. Planta Medica. 54(5), 433-437.

Sacher, R.A. and McPherson, R.A. 1991a. General chemistry. *In* Widmann's Clinical Interpretation of Laboratory Test. 10th ed. F.A. Davis Company, U.S.A., pp 318-365.

Sacher, R.A. and McPherson, R.A. 1991b. Test of liver function. *In* Widmann's Clinical Interpretation of Laboratory Test. 10th ed. F.A. Davis Company, U.S.A., pp 416-443.

The Organization of Economic Co-operation and Development (OECD). 1981. The OECD guideline for testing of chemical: 408 Subchronic Oral Toxicity-Rodent: 90-day Study. France.

The Organization of Economic Co-operation and Development (OECD). 2001. The OECD guideline for testing of chemical: 420 Acute Oral Toxicity. France.

World Health Organization (WHO). 2000. General guidelines for methodologies on research and evaluation of traditional medicine. Switzerland.