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Original Article

Chronic Toxicity of Leaf Extract from *Sphagneticola trilobata* (L.) Pruski

Areeya Suchantabud[1*], Teeraporn Katisart[2], Chusri Talubmook[2]

ABSTRACT

Context: *Sphagneticola trilobata* (L.) Pruski. is a member of the family Asteraceae and has used traditionally in the prevention and treatment of various diseases. **Aim**: The research was aimed to determine chronic toxicity of 80% ethanolic leaf extract from *S.trilobata* (STLE). **Materials and Methods**: STLE at the doses of 200 or 400 mg/kg b. w. was oral given to the healthy Wistar rats daily for 90 days. **Statistical analysis used:** Statistical analysis was carried out using *F-test* (One-Way ANOVA) followed by Duncan's New Multiple Range Test. **Results**: STLE did not produce any signs or symptoms of chronic toxicity. And also, the mortal rat was not observed during a period of an observation. Furthermore, STLE did not alter the body weight, relative organ (liver, pancreas, kidney and heart) weight, hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), neutrophil, lymphocyte, monocyte, platelet, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, blood cell characteristics, ultrastructure of RBC, and histological features of hepatic, pancreatic and renal tissues in the STLE treated rats comparing to control rats. **Conclusions**: These findings indicate that the leaf extract from *S. trilobata* exerts non chronic toxicity in rats and can be used safely as a traditional medicine or diet complement without any effect on hepatic and renal functions.

Key words: *S. trilobata*, Chronic toxicity, Hematological values, Blood biochemistry, Histological feature.

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INTRODUCTION

Medicinal plants are the major resource of traditional medicines. Traditional and alternative medicines are extensively practiced in the prevention and treatment of various ailments.[1]

Sphagneticola trilobata (L.) Pruski or *Wedelia trilobata* (L.) A.S. Hitch., is a member of the family Asteraceae. Its leaves or aerial parts are used for backache, muscle cramp, rheumatism, sores, swelling and arthritic painful joints.[2] The leaves and stems are used in childbirth and in the treatment of bites and stings, fever and infection,[3] wound healing.[2] *S. trilobata* has antioxidant,[4] antibacterial,[5] analgesic,[6] and antimicrobial activities,[7] and is a potential candidate in the management of diabetes.[8] These pharmacological activities may depend on its phytoconstituents such as tannin, saponins, flavonoids, phenol and terpenoids.[3]

Despite non acute toxicity of the leaf extract from *S. trilobata* has been reported in our previous study.[9] To see whether it is safe for long term administration, the present study was therefore, designed to determine the chronic toxicity of 80% ethanolic leaf extract from *S. trilobata* to Wistar rats for 90 days.

MATERIALS AND METHODS

Preparation of STLE

Fresh and mature leaves of *S. trilobata* were collected from the local gardens in Maha Sarakham and Roi-Et Provinces, Thailand and identified by the Plant Varieties Protection Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. A voucher specimen (Code: MSU-Sci/PA001) is deposited in the Department of Biology, Faculty of Science, Mahasarakham University, Thailand. The plant leaves were washed with tap water, sliced into small pieces, air dried at ambient temperature and ground into powder. The plant powder was extracted by macerating in 80% ethanol for 7 days and the mixture was then filtered. The filtrate was evaporated in a rotary evaporator followed by freeze drying. The obtained powder extract (STLE) was stored at 4°C until be used.

Animals

The animals used in the study were albino Wistar rats weighing 180-200 g purchasing from the National Laboratory Animal Centre, Mahidol University, Thailand. The rats were housed in the clean cages under the conditions at 25 ± 2°C, 50 ± 5% RH with a 12 h D/L cycle in the animal laboratory at the Department of Biology, Faculty of Science, Mahasarakham University. All animals were given a standard laboratory diet and water *ad libitum*. The experimental protocol and performance of the rats were approved by the Institutional Ethical Committee for the Purpose of Use and Control, and Supervi-

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sion on Experiment in Animals, Mahasarakham University (License No. 0004/2013).

Chronic toxicity test

The experimental protocols were maintained in accordance with the OECD452 Guidelines. The rats were randomly assigned into 3 groups with 8 rats in each. Group I: normal control; rats received 0.5% Tween 80, Group II-III: rats received 200 or 400 mg/kg b. w. STLE respectively.

Prior to an administration, STLE was suspended in 0.5% Tween 80 (Polysorbate 80 or Polyoxyethylene (20)* Sorbitanmonooleate (emulsifier). Volume of 10 ml/kg b. w. of STLE or 0.5% Tween 80 was administered orally and daily to the rats for 90 days. Observation for the gross behavioral, neurological and autonomic effects, and also signs of toxicity such as skin and fur, eyes, salivation, lethargy, sleep, diarrhea or coma, and mortality of the rats were conducted twice daily over a period lasting 90 days. Body weight of the rats was recorded weekly. At the end of the experiments, the rats were fasted overnight and sacrificed by using a cervical dislocation technique. Blood samples were then collected from the rat hearts and put into the heparinized and non-heparinized tubes for the determinations of blood biochemistry, hematological values, blood cell characteristics and ultrastructure of red blood cells. Visceral organs including liver, pancreas and kidneys were removed and weighed for a calculation of relative organ weight, and used for a histological examination.

Determination of Blood Biochemistry

The blood samples from non-heparinized tubes were centrifuged at 3500 rpm for 20 min to separate blood serum. The serum was then used for an investigation of blood biochemistry including total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN), creatinine, total protein (TP), albumin (Alb), and alkaline phosphatase (ALP) by using an automatic blood chemical analyzer (BT 2000 plus, Germany).

Determination of Hematological Values

Heparinized blood was used for a determination of hematological values including white blood cell (WBC), red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) by using an automatic blood chemical analyzer (BT 2000 plus, Germany).

Histological Examination

The tissues from liver, pancreas and kidney of all rats were fixed in 10% formaldehyde and embedded in paraffin. The specimens in paraffin blocks were cut using a microtome. Sections at 5 µm thickness were stained with hematoxylin and eosin. The histological changes in the stained sections were examined under a light microscope (LM).

Investigation of blood cell characteristics

Blood from non-heparinized tubes was also smeared, fixed in 95% methanol for 5 min and stained with Wright-Giemsa. Structures of blood cells were investigated and photographed under a light microscope.

Ultrastructure of red blood cells examination

Blood was removed and dropped into 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 at 4°C overnight. The specimens were then washed in the same buffer. They were post-fixed with 1% osmium tetroxide for 2 h, rinsed with distilled water, dehydrated in 20%, 40%, 60%, 80%, 100% and 100% acetone respectively, and then embedded in Epon resin. The specimens were sectioned, put on copper grid and stained with lead citrate and uranyl acetate. The ultrathin sections were examined using Transmission Electron Microscope (TEM; JEM 1230).

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using *F-test* (One-Way ANOVA) followed by Duncan's New Multiple Range Test. The criterion for statistical significance was at a *p*-value less than 0.05.

RESULTS AND DISCUSSION

Body weight and Relative organ weight

STLE at the doses of 200 and 400 mg/kg did not produce any signs of toxicity (diarrhoea, sleep, lethargy, salivation, eyes, skin and fur) and mortality of the rats observed twice daily during a period lasting 90 days.

Body weight of each rat was recorded weekly. The results revealed that the initial (day 0) body weight of controls and STLE treated rats was not different. The same result also occurred at the final (day 90) body weight (Table 1). Relative organ weight, the relationship between each organ weight and body weight, between controls and STLE treated rats was not different (Table 1).

Blood biochemistry and Hematological values

Glucose, BUN, creatinine, Uric, TP, Alb, Glob, TB, AST, ALT, and ALP from the rats treated with different doses of STLE were not different, and were not different from controls (Table 2). Hematological values including Hb, Hct, RBC, MCV, MCH, MCHC, WBC, neutrophils, lymphocytes, monocytes and platelet from the rats treated with different doses of STLE and from controls were not different (Table 2).

Histological examination

Hepatic tissues: Light micrograph of the differential of histological feature on Wistar rat hepatic tissues showed that the hepatocytes are organized into plates separated by vascular channels (sinusoids), an arrangement supported by a reticulin network. Hepatocytes in the controls and the rats received 200 or 400 mg/kg b. w. STLE were not different (Figure 1).

Pancreatic tissues: Light microscopic observation revealed that the islet of Langerhans, which are the regions of the pancreas that contain its endocrine cells, of the controls and the rats received 200 or 400 mg/kg b. w. STLE were not different (Figure 1).

Renal tissues: Histological section of renal tissues showed the glomerulus which is the round blind beginning of the nephron. It is invaginated by a tuft of capillaries at the vascular pole of the glomerulus. The renal tissues of the controls and the rats received 200 or 400 mg/kg b.w. STLE were also not different (Figure 1).

Table 1: Body weight and relative organ weight of control and STLE treated rats

Parameters	Treatments		
	Control	200 mg/kg STLE	400 mg/kg STLE
Body weight (g)			
Initial	275.00 ± 6.95	280.00 ± 6.17	272.66 ± 4.48
Final	391.00 ± 6.91	376.85 ± 6.54	388.33 ± 3.98
Relative organ weight (%)			
Liver	3.23 ± 0.13	3.16 ± 0.25	3.45 ± 0.25
Pancreas	0.19 ± 0.02	0.18 ± 0.02	0.20 ± 0.03
Kidneys	0.67 ± 0.04	0.70 ± 0.51	0.72 ± 0.06
Heart	0.34 ± 0.02	0.31 ± 0.01	0.32 ± 0.01

The values represent the mean+SEM within the same row followed by the different superscript letters are significantly different at the p<0.05

Table 2: Blood biochemistry and hematological values of control and STLE treated rats

Parameters	Treatments		
	Control	200 mg/kg STLE	400 mg/kg STLE
Blood biochemistry			
Glucose (mg/dL)	101.75 ± 2.32	112.25 ± 2.49	106.25 ± 2.78
BUN (mg/dL)	22.02 ± 0.54	21.90 ± 0.60	22.30 ± 0.81
Creatinine (mg/dL)	1.00 ± 0.04	1.05 ± 0.06	0.95 ± 0.05
Uric (mg/dL)	3.17 ± 0.53	3.13 ± 0.61	2.92 ± 0.25
TP (mg/dL)	6.25 ± 0.16	6.00 ± 0.09	5.97 ± 0.13
Alb (g/dL)	3.47 ± 0.11	3.37 ± 0.13	3.35 ± 0.11
Glob (g/dL)	2.77 ± 0.07	2.62 ± 0.04	2.62 ± 0.02
TB (mg/dL)	0.37 ± 0.04	0.27 ± 0.02	0.22 ± 0.02
AST (U/L)	163.00 ± 8.55	162.25 ± 5.39	167.50 ± 7.45
ALT (U/L)	49.50 ± 1.19	54.00 ± 2.67	50.25 ± 1.75
ALP (U/L)	78.50 ± 7.27	75.25 ± 2.78	76.00 ± 5.00
Hematological values			
Hb (g/dL)	15.35 ± 0.42	15.25 ± 0.33	15.25 ± 0.60
Hct (%)	46.00 ± 1.29	45.75 ± 1.18	46.00 ± 1.29
RBC (x10^6 cells/mm^3)	8.27 ± 0.43	8.12 ± 0.11	8.07 ± 0.14
MCV (fL)	55.70 ± 1.57	55.00 ± 1.15	56.25 ± 2.21
MCH (pg)	18.62 ± 0.52	18.57 ± 0.41	18.90 ± 0.75
MCHC (g/dL)	33.35 ± 0.17	33.92 ± 0.17	33.65 ± 0.13
WBC (x10^3 cells/mm^3)	3.15 ± 2.06	3.42 ± 2.17	3.30 ± 2.38
Neutrophils (%)	11.75 ± 0.85	13.25± 0.62	11.50 ± 0.86
Lymphocytes (%)	18.25 ± 1.65	15.00 ± 0.52	15.00 ± 0.82
Monocytes (%)	5. 25 ± 0.47	4.75 ± 0.25	5.25 ± 0.25
Platelet (x10^6 cells/mm^3)	8.43 ± 4.79	7.72 ± 4.11	7.76 ± 9.00

The values represent the mean+SEM within the same row followed by the different superscript letters are significantly different at the p<0.05.

Structure of white blood cells

Light micrographs showed the differential of white blood cells (WBCs) including lymphocytes, monocytes, neutrophils and eosinophil's. All types of WBCs from all experimental rats and controls were not different (Figure1).

Ultrastructure of red blood cells

Figure 2 showed transmission electron micrographs of RBC from normal controls (A), normal rats received 200 mg/kg b. w. STLE (B), and normal rats received 400 mg/kg b. w. STLE (C). The ultrastructure of red blood cells from all experimental rats and those from controls were not different.

DISCUSSION

Phyto-therapeutic using medicinal plants or plant products have become universally popular in primary healthcare, particularly in developing countries. The medicinal plants and plant products are presumed to be safe. Nevertheless, there is a lack of proven scientific studies on the toxicity and adverse and/ or undesirable effect of these remedies. Therefore, oral chronic toxicity study of the leaf extract from *S. trilobata* was investigated.

STLE at the doses of 200 and 400 mg/kg b. w. exerted non chronic toxicity on albino Wistar rats as they did not exhibit any signs of toxicity and mortality of the rats. Furthermore, the body weight, relative organ weight, blood biochemistry, hematological values, histological feature of hepatic, renal and pancreatic tissues, and the structure of red blood cells in the rats treated with such the extracts were not different from those in the untreated rats.

Non toxicity of STLE from this study is in line with the non-toxicity of the other plant extracts such as the shoot tip and leaf ethanolic extracts from *S. trilobata*,[10] leaves of *Ocimum tenuiflorum* (Linn.),[11] flower extract from *Nelumbo nucifera,*[12] and seed powder of *Garcinia kola.*[13] Nevertheless, a controversial result of toxicity of *S. trilobata* (*Wedelia trilobata*) has been reportedly shown the larvicidal and trypanocidal activities.[4] The crude extract from *W. trilobata* exhibited toxicity on *Oreochromis niloticus*, *Pomacea canaliculata* and *Chironomus sp*. With LC_{50} of 22.32, 135.07 and 143.14 mg/L respectively.[14]

Assay of biochemical parameters was performed in order to give insight into pathological changes and nature of the hepatic and renal functions. In this study, assay of the liver profile parameters (TP, AST, ALT and ALP) and renal profile parameters (Glucose, BUN, creatinine, Uric, Alb and Glob) revealed normal functioning of the liver and kidney after 90 days of the administration of STLE as these profile parameters of experimental animals and the controls were not different. This result is concomitant with the studies on the shoot tip and leaf ethanolic extracts of *S. trilobata*,[10] the aqueous leaf extract of *Ocimum tenuiflorum* (Linn.)[11] and ethanol leaf extracts of *Carica papaya* Linn.[15]

Analysis of hematological parameters is relevant to evaluate the risk of alterations of the process of blood cell formation. In this study, admini-

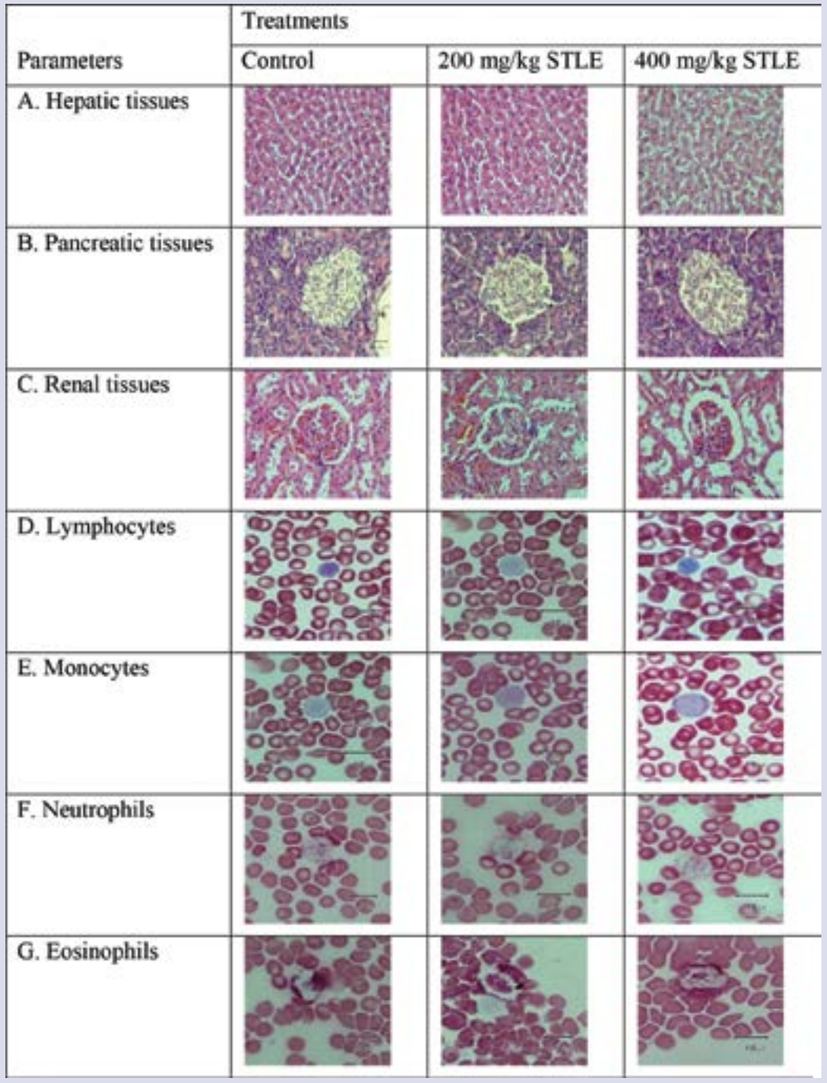

Figure 1 : Light micrographs showed hepatic tissues (A), pancreatic tissues (B), renal tissues (C), lymphocytes (D), monocytes (E), neutrophils (F) and eosinophils (G).

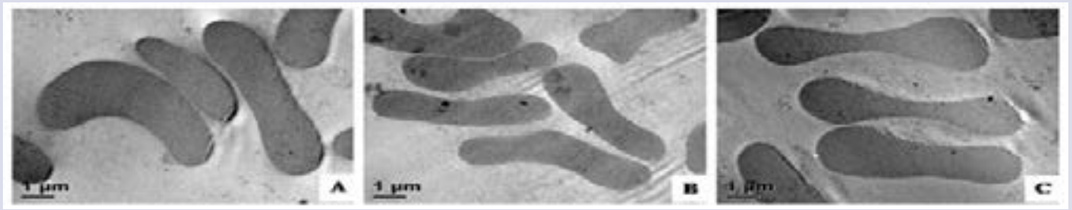

Figure 2: Transmission Electron Micrographs showed lead citrate and uranyl acetate staining of the red blood cell ultrastructure in control (A), rats received 200 mg/kg b.w. STLE (B) and rats received 400 mg/kg b. w. STLE (C).

stration of STLE at the doses of 200 or 400 mg/kg b. w. for 90 days revealed that STLE had no effect on the hematological values as these parameters in the STLE treated rats and controls were not different. This result is in line with the extracts from *S. trilobata* shoot tip and leaves[10], *S. trilobata* leaves[9], *Nelumbo nucifera* flowers,[12] *Tinospora crispa* stems,[16] *Musa sapientum* flowers,[17] *Cyperus rotundus* rhizomes[18] and *Phyllanthus acidus* L. leaves.[19]

In general, *in vivo* toxicity study is the toxicological analysis of many medicinal plants and its potency to evaluate qualitatively and quantitatively by histopathology and oral chronic toxicity studies. Oral chronic toxicity testing in rats could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose.[20] Histological examination of the liver, pancreas and kidney did not reveal any morphological changes after administration of STLE. The result from this study is in accordance with the other plant extracts such as leaf extract of *Carica papaya* Linn,[15] rhizomes extract from *Ocimum tenuiflorum* (Linn.)[11] and rhizomes extract from *Cyperus rotundus.*[18]

Light micrographs demonstrated that the structure of white blood cells from the rats treated with STLE was similar to controls. The result from this study provided the same results with the extract of *Phillinus ignarius,*[21] *Tinospora crispa* stems extract[16] and flower extract from *Nelumbo nucifera.*[12]

TEM micrographs revealed that the ultrastructure of red blood cells STLE was not different from that in the controls. This study showed the same result as the extracts from *Nelumbo nucifera* flowers,[12] *Piper sarmentosum* stems, and *Tinospora crispa* stems.[16]

CONCLUSION

In conclusions, chronic administration of ethanol leaf extract of *S. trilobata* shows no significant adverse effects on the experimental parameters such as hematological, blood biochemistry and also body weight. And also, the level of the marker enzymes in the liver and kidney of the leaf extract of *S. trilobata* treated rats showed that the extract at the doses of 200 and 400 mg/kg b. w. cannot induce any toxic effects on hepatic and renal functions. It can strongly conclude that the ethanol leaf extract of *S. trilobata* can be adopted for clinical used safely.

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CONFLICT OF INTEREST

None

ABBREVIATIONS USED

STLE: Sphagneticola trilobata leaf extract; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; Hb: hemoglobin; Hct: hematocrit; RBC: red blood cell ; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; WBC: white blood cell; MCHC: mean corpuscular hemoglobin concentration.

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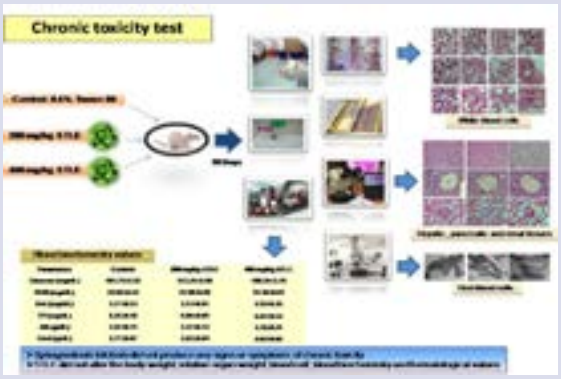
GRAPHICAL ABSTRACT



SUMMARY

- This paper is the first report about chronic toxicity of 80% ethanolic leaf extract from *Sphagneticola trilobata* (L.) Pruski (STLE) in healthy Wistar rats.
- STLE did not produce any signs or symptoms of chronic toxicity.
- STLE did not alter the body weight, relative organ weight, blood biochemistry, hematological values, blood cell characteristics, and histological features of hepatic, pancreatic and renal tissues in the STLE treated rats comparing to control rats.
- The ultrastructure of red blood cells from all experimental rats and those from controls were not different.
- The leaf extract from *S. trilobata* exerts non chronic toxicity in rats and can be used safely as a traditional medicine or diet complement without any effect on hepatic and renal functions.

ABOUT AUTHOR

Areeya Suchantabud, is a Ph.D. Candidate at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand, where she graduated in Master Degree of Biology. Her doctoral research focused on the acute, sub-acute, sub-chronic and chronic toxicities of Thai medicinal plants.

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