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Original Research Article

Oral acute toxicity of pericarp extract from *Lepisanthes rubiginosa* (Roxh) Leenh in rats

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Abstract

Purpose: *To investigate the acute toxicity of Lepisanthes rubiginosa (Roxh.) Leenh. (Sapindaceae) pericarp extract in rats.*
Methods: *A total of 25 male and female rats were orally administered the extract at different doses (5, 50, 300 and 2,000 mg/kg) while control rats received distilled water. The body weights were recorded weekly for 2 weeks. After administration, the relative organ weights were calculated and the hematological values and biochemical examination including lipid profiles, and liver and kidney function parameters were measured from fasting blood samples collected by cardiac puncture. Liver and kidney tissue of rats were collected by abdomen dissection after 2 weeks of the experiment for histological study.*
Results: *At all doses tested, the extract neither caused death in rats nor were there signs of toxicity within 24 h in the first instance and after 14 days. There was significant increase in the mean weekly weights of rats ($p < 0.05$) but did not affect relative organ weight. The morphology of blood cells showed no abnormalities suggesting that the extract had no acute toxic effect on the circulatory system of the rats that received the extract even at the highest dose of 2,000 mg/kg. The rats did not experience changes in the histological characteristics of the liver. It is thus been proposed that the extract has no liver toxicity. There was no significant change in blood urea nitrogen (BUN) and creatinine ($p < 0.05$) and no changes in the histological features of the kidneys were observed.*
Conclusion: *The extract is safe at all tested doses and is devoid of hepatotoxicity, nephrotoxicity and abnormalities in the circulatory system.*

Keyword: *Thailand, Lepisanthes rubiginosa, Oral acute toxicity, Pericarp*

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INTRODUCTION

Thailand is characterized by a landscape rich in natural resources and high biodiversity, which is an important basic source of livelihood for plants and animals [1]. Recently, consumers now pay more attention to health via emphasis placed on safety in consumption. Herbs and herbal products play significant role in consumption trend. Therefore, there has been extensive

studies on herbs. Efforts have been made to invent new products from herbs to meet the demand for consumption, as well as collection and application of traditional herbal wisdom in both prevention and treatment of various diseases [2].

The genus *Lepisanthes* consists of 26 species. Geographical distribution of this genus ranges from India and Sri Lanka to Malaysia, Philippines, Vietnam and Papua New Guinea. In Southeast Asia including Thailand, *Lepisanthes rubiginosa* (*L. rubiginosa*) (Family Sapindaceae) is a common species [3]. The young leaf of *L. rubiginosa* is traditionally used for the reduction of muscle soreness in Malaysia. The ripe fruits are consumed for relief of fever, flatulence and postpartum blues, and for treatment of diarrhea, dysentery and jaundice [3].

The study on the phytochemicals and pharmacological effect of this plant shows ethyl acetate extract contains phytochemicals including lupeol, diosmetin, heptadecanoic acid, ß-sitosterol, and ß-sitosterol-3-O-ß-D-glucopyranoside [4]. A new farnesol derivative, rubiginoside was in *Lepisanthes rubiginosa* (Roxb.) Leenh. extract [5]. The phytochemical and pharmacological study of *Lepisanthes rubiginosa* (Roxb.) Leenh. ethanol leaf extract exhibited antioxidant, analgesic, antidiabetic and anti-diarrhea effect in animal models [6]. The biological activity of essential oils of *Lepisanthes rubiginosa* (Roxb.) Leenh. flower revealed that the essential oil of *Lepisanthes rubiginosa* flower has an inhibitory effect on lung cancer cells type NCI-H187 and has good antioxidant activity by ABTS method, while both essential oils from *Lepisanthes rubiginosa* flowers and fruit had good antimicrobial activity [7].

Parts of *Lepisanthes rubiginosa* (Roxb.) Leenh. such as the fruit and seeds have been used as food and herbal medicine for a long time. The acute toxicity profile of *Lepisanthes rubiginosa* (Roxb.) Leenh. has not been tested hence this study investigates the quality of *Lepisanthes rubiginosa* (Roxb.) Leenh. extract in terms of toxicity using animal model.

EXPERIMENTAL

Preparation of samples of medicinal plant

Lepisanthes rubiginosa (Roxh.) Leenh. ripe fruits were collected from the plant genetic conservation areas by Her Royal Highness's initiative, Khok Dong Keng Forest, Phra That Subdistrict, Na Doon District, Maha Sarakham Province, Thailand, from April to May 2022. They were authenticated at the Department of Biology, Faculty of Science, Mahasarakham University. Voucher specimens were deposited in the herbarium of the Faculty of Science, Mahasarakham University in Thailand (voucher specimen number is MSUT-7804).

Preparation of extract

The pericarp of *Lepisanthes rubiginosa* (Roxb.) Leenh. were cleaned with tap water, cut into small pieces and dried at 50 ºC until dry. After drying, it was grounded into a fine powder, filtered with a sieve no. 100, then extracted with distilled water by Reflux apparatus with a pericarp powder to solvent ratio of 1:6, then filtered out with a thin white cloth, followed by Whatman filter paper no. 1, and freeze-dried. The extract was in fine powder form and stored in the refrigerator at 4 ºC for future experiments.

Preparation of animals

Both male and female Wistar rats, weighing 180 - 200 g were selected for the study. They were acquired from and kept at Northeastern Laboratory Animal Center, Khon Kaen University, Khon Kaen, Thailand, which is an Animal Biosafety Level 1 (ABSL1) facility with a temperature of 23 ºC, relative humidity is about 30 – 60 %, and a dark-light cycle every 12 h. Rats used were raised in ABSL-1 animal room of Northeastern Laboratory Animal Center, with food and water round the clock.

The animal protocol was approved by the Animal Ethics Committee of Khon Kaen University, Thailand (approval no. IACUC-KKU-6/66). The experiments were conducted according to the Organization for Economic Co-operation and Development (OECD) 420 guideline for acute toxicity study [8].

Design

The acute toxicity of *Lepisanthes rubiginosa* (Roxh.) Leenh. pericarp extract (LRPE) was evaluated according to OECD 420 guideline. The maximum extract dose was 2,000 mg/kg. Administration of the extract is done once. Male and female rats were divided into 5 groups, each group consisting of 10 rats, 5 females and 5 males as follows:

Group 1 control rats were orally administered with distilled water 10 mL/kg.

Group 2 normal rats orally administered with LRPE at a dose of 5 mg/kg.

Group 3 normal rats orally administered with LRPE at a dose of 50 mg/kg.

Group 4 normal rats orally administered with LRPE at a dose of 300 mg/kg.

Group 5 normal rats orally administered with LRPE at a dose of 2,000 mg/kg.

Distilled water was used as a solvent. The rats in groups 2 to 5 were given the extract orally with an orogastric tube while control rats were given distilled water instead of the same amount of extract. After administering the extract, signs of toxicity such as seizures, urination, staggering, lethargy, vomiting, loss of appetite or death were observed within 24 hrs of extract. Symptoms of toxicity and the number of rat deaths were recorded. After that, the rats were further observed for 14 days to see the effects of continued toxicity. The body weight of rats before treatment and on days 7 and 14 of the experiment were recorded and symptoms of toxicity or the number of rat deaths in each group for 14 days was also noted.

The study of acute toxicity was evaluated using the rats' body weight, relative organ weight, blood lipids, hematopoiesis and hematopoietic morphology. On day 1 of the study, the extract/distilled water was first administered and then rats were fed and observed for 14 days. On day 14 of the study, the rats were fasted for 8 hours, then sacrificed ethically by using Thiopental sodium anesthesia injected intraperitoneally at a dose of 85 mg/ml/kg [9]. Then blood was withdrawn from the heart by opening the chest cavity and using a 1-inch syringe No. 23 injected into the left ventricle the blood was withdrawn to fill the 3 ml syringe for hematological analysis including RBC (Red blood cells), WBC (White blood cells) and platelets. Blood chemistry of rats including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and Creatinine, Total cholesterol, Triglyceride, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) were also performed using Automatic blood chemical analyzer (BT 2000 plus, Germany), done in AMS-KKU Excellence Laboratory, Faculty of Allied Medical Sciences, Khon Kaen University. The organs were also harvested, the abdomen of rats was wiped with 95 % ethanol, then opened up to the tissue layer, then muscle layer, and then the targeted organs were harvested which included liver, kidneys, heart, lungs and spleen. 0.85 % sodium chloride solution was used to wash away any blood left in the tissues. The tissues were dried and weighed to calculate their relative weight (g %). Once the organ weight was obtained, the relative organ weight was calculated using a mathematical formula.

Hematological measurement

After 14 days, rats were fasted for 8 h and sacrificed with thiopental sodium injected intraperitoneally at a dose of 85 mg/ml/kg [9]. After 2 - 3 mins of anesthesia, fasting blood was withdrawn via a cardiac puncture and introduced into a 2 mL volume blood collection tube. The sample was centrifuged at 3,000 rpm for 10 min to separate serum and the resulting serum was used for hematological analysis by an Automatic Blood Analyzer which uses sensors to count the number of cells and identify the type of blood cells called the flow cytometry technique [10] to check for complete blood count (CBC) by counting various elements such as size, shape and arrangement of red blood cells. Blood samples were analyzed at AMS-KKU Excellence Laboratory, Faculty of Allied Medical Science, Khon Kaen University.

Lipid profile measurement

As previously described, rats were sacrificed with thiopental sodium injected into the abdomen after 14 days. After 2 - 3 min of anesthesia, blood samples were drawn from the heart and placed in 2 mL vacuum tubes. The collected blood samples were spun with a centrifuge at 3,000 rpm for 10 min to separate serum. The serum was analyzed using an automatic blood chemical analyzer (BT 2000 Plus, Germany) to determine blood urea nitrogen (BUN), creatinine (CREA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglyceride, LDL and HDL. Blood samples were analyzed at AMS-KKU Excellence Laboratory, Faculty of Allied Medical Science, Khon Kaen University.

Histopathological investigation

The organs (liver and kidney) were fixed in 10 % formalin, and histological characteristics were compared in these animals. Paraffin embedding which involves implanting the desired tissue in paraffin wax and then cutting with a microtome was done. After cutting the tissue, it is also used for staining. Hematoxylin and Eosin stains and photographed under a light microscope [11].

Statistical analysis

All values were reported as mean ± standard error of mean (SEM). One-way ANOVA was

used to evaluate mean comparisons by using SPSS software version 23, statistical analysis was performed with the significance level at $p < 0.05$.

RESULTS

Mean body weight gain per week

Average weekly weight gain in both male and female Wistar rats showed statistically significant difference between rats receiving fruit extract over two weeks, with a statistically significant difference ($p < 0.05$) in average weight gain per week compared to control group (Table 1).

Effects of extract on relative organ weight of rats

In both male and female rats who received the extract at different doses, it was found that at two weeks of administration, there was no statistically significant difference in the average relative organ weight of the liver, kidney, heart, spleen, or lungs compared to control group ($p < 0.05$) (Table 2).

Effect of extract on hematological indices

In this study, all groups of rats fed with the extract at different doses over two weeks showed no statistically significant difference in hematological values (Table 3).

Effect on blood lipid profiles

There was no statistically significant difference of total cholesterol, triglyceride, HDL and LDL between male and female rats compared to control (Table 4).

Effect of extract on liver function

The ALT, AST and ALP of all groups of rats fed with the extract at different doses (5, 50, 300 and 2,000), showed no statistically significant difference between male and female rats compared to control group (Table 5).

Table 1: Effect of pericarp extract from *Lepisanthes rubiginosa* on body weight gain in male and female rats (mean ± SEM)

Group	Treatment	Body weight (%) (mean ± SEM)	
		Week 1	***Week 2***
Male rats	Control	28.03±2.83	50.59±5.35*
	L. rubiginosa 5 mg/kg	32.27±1.16	62.68±2.30*
	L. rubiginosa 50 mg/kg	30.27±1.32	56.38±2.43*
	L. rubiginosa 300 mg/kg	29.69±1.53	57.59±2.15*
	L. rubiginosa 2,000 mg/kg	21.25±1.32	48.77±1.56*
Female rats	Control	18.25±1.18	28.62±2.27*
	L. rubiginosa 5 mg/kg	19.97±1.36	32.36±3.18*
	L. rubiginosa 50 mg/kg	22.75±0.91	36.96±1.38*
	L. rubiginosa 300 mg/kg	16.72±4.90	29.20±5.90*
	L. rubiginosa 2,000 mg/kg	26.90±5.38	41.84±6.29*

*$P < 0.05$ vs. week 1

Table 2: Effect of pericarp extract from *Lepisanthes rubiginosa* on relative organ weight in male and female rats (mean ± SEM)

Group	Organ	Relative organ weight (mean ± SEM)				
		Control	***L. rubiginosa 5 mg/kg***	***L. rubiginosa 50 mg/kg***	***L. rubiginosa 300 mg/kg***	***L. rubiginosa 2,000 mg/kg***
Male rats	Liver	3.97±0.12^{b}	3.93±0.10^{ab}	3.85±0.08^{b}	4.06±0.06^{a}	4.00±0.08^{ab}
	Kidney	0.97±0.03^{ab}	1.02±0.04^{a}	0.89±0.03^{b}	0.96±0.05^{a}	0.98±0.03^{ab}
	Heart	0.45±0.02^{a}	0.43±0.02^{a}	0.41±0.00^{a}	0.42±0.01^{a}	0.39±0.02^{a}
	Spleen	0.33±0.01^{b}	0.38±0.01^{a}	0.32±0.01^{b}	0.34±0.01^{ab}	0.35±0.01^{ab}
	Lung	0.61±0.04^{a}	0.52±0.02^{a}	0.54±0.02^{a}	0.52±0.03^{a}	0.52±0.01^{a}
Female rats	Liver	3.97±0.09^{b}	3.62±0.05^{ab}	3.65±0.09^{a}	4.15±0.28^{a}	3.66±0.18^{ab}
	Kidney	1.04±0.03^{a}	0.90±0.03^{a}	0.90±0.03^{a}	0.99±0.05^{a}	0.90±0.05^{a}
	Heart	0.38±0.00^{b}	0.38±0.02^{a}	0.37±0.00^{a}	0.43±0.03^{a}	0.39±0.02^{a}
	Spleen	0.30±0.01^{b}	0.35±0.02^{a}	0.31±0.01^{ab}	0.38±0.03^{a}	0.30±0.01^{b}
	Lung	0.57±0.01^{b}	0.58±0.02^{a}	0.55 ±0.01^{a}	0.64±0.04^{a}	0.56±0.02^{a}

$P < 0.05$ for different letters in the same row

Table 3: Effect of pericarp extract from *Lepisanthes rubiginosa* on hematological values in male and female rats (mean ± SEM)

Group	Hematological values	Treatment				
		Control	*L. rubiginosa* 5 mg/kg	*L. rubiginosa* 50 mg/kg	*L. rubiginosa* 300 mg/kg	*L. rubiginosa* 2,000 mg/kg
Male	WBC (10^3/µL)	5.23±0.88^a	5.63±1.33^a	5.06±0.47^a	4.93±0.43^a	5.91±0.54^a
	RBC(10^3/µL)	6.67±0.30^{ab}	6.38±0.12^b	6.51±0.22^b	6.34±0.23^b	7.17±0.14^a
	HGB (g/dL)	14.04±0.55^{ab}	13.18±.037^b	13.20±0.41^b	13.08±0.15^b	14.60±0.30^b
	HCT (%)	44.60±1.89^{ab}	42.20±1.24^{ab}	42.40±1.33^{ab}	44.60±0.40^b	46.20±1.07^a
	MCV (fL)	66.94±1.00^a	66.06±2.01^a	64.92±1.10^a	64.42±1.49^a	64.64±0.60^a
	MCH (pg)	21.08±0.24^a	20.68±0.61^a	20.28±0.21^a	20.72±0.56^a	20.36±0.17^a
	MCHC (g/dL)	31.48±0.28^a	31.34±0.17^a	31.30±0.20^a	32.12±0.30^a	31.52±0.31^a
	PLT(10^3/µL)	982.00±45.25^a	924.00±201.31^a	1180.00±77.25^a	1032.00±52.08^a	1219.40±46.82^a
	PMN (10^3/µL)	13.60±3.6^a	13.60±3.71^a	14.40±1.21^a	14.80±0.49^a	3.40±3.40^b
	LYMPH (10^3/µL)	78.80±1.93^a	63.20±15.89^a	80.40±1.50^a	80.00±0.89^a	17.20±0.97^a
	MONO (10^3/µL)	3.40±0.68^a	3.40±0.98^a	3.40±0.75^a	3.60±0.51^a	5.60±0.60^a
	EOS(10^3/µL)	1.40±0.24^a	1.00±0.00^a	1.00±0.00^a	1.40±0.24^a	1.40±0.24^a
Female	WBC (10^3/µL)	3.88±0.44^a	4.10±0.91^a	4.16±0.52^a	4.04±0.30^a	4.94±0.78^a
	RBC (10^3/µL)	6.96±0.19^a	6.74±0.12ab^a	6.84±0.21^a	6.84±0.90^a	6.94±0.12^a
	HGB (g/dL)	14.12±0.46^a	13.80±0.25^a	13.88±0.39^a	13.78±0.28^a	14.02±0.15^a
	HCT (%)	43.60±1.75^a	42.20±1.16^a	43.60±1.21^a	43.00±0.63^a	43.40±0.60^a
	MCV (fL)	62.58±0.85^a	62.58±0.46^a	63.52±1.14^a	62.62±0.82^a	62.42±1.36^a
	MCH (pg)	20.28±0.21^a	20.48±0.06^a	20.32±0.34^a	20.16±0.33^a	20.24±0.40^a
	MCHC (g/dL)	32.42±0.31^a	32.72±0.25^{ab}	31.98±0.04^{ab}	32.18±0.27^{ab}	32.40±0.12^b
	PLT (103/µL)	1063.20±35.02^a	1125.60±96.30^a	1154.60±45.31^a	1048.80±68.64^a	1116.60±75.99^a
	PMN (10^3/µL)	9.20±4.31^a	11.40±2.42^a	12.60±1.33^a	12.00±1.64^a	12.00±2.61^a
	LYMPH (10^3/µL)	77.60±3.01^a	81.20±5.07^a	82.40±1.63^a	81.80±1.98^a	83.60±3.17^a
	MONO (10^3/µL)	2.20±0.58^a	3.00±1.05^a	3.00±0.84^a	3.20±0.37^a	2.80±0.58^a
	EOS (10^3/µL)	1.80±0.20^a	3.40±1.66^a	2.00±0.55^a	2.80±1.11^a	1.60±0.24^a

Note: *P* < 0.05 for different letters in the same row

Table 4: Effect of pericarp extract from *Lepisanthes rubiginosa* on lipid profile in male and female rats (mean ± SEM)

Group	Treatment	Lipid profiles			
		cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Male	Control	65.00±3.48^a	95.20±14.52^b	41.00±1.58^a	4.80±0.92^a
	L. rubiginosa 5 mg/kg	64.20±3.97^a	68.20±9.57^a	40.80±3.34^a	9.60±1.78^a
	L. rubiginosa 50 mg/kg	66.20±3.65^a	90.00±24.08^b	42.00±2.85^a	8.00±2.35^a
	L. rubiginosa 300 mg/kg	69.60±2.64^a	104.60±16.83^b	43.00±1.38^a	7.60±2.62^a
	L. rubiginosa 2,000 mg/kg	70.60±4.99^a	69.60±8.98^a	45.40±2.77^a	11.40±3.25^b
Female	Control	77.60±4.61^a	49.60±2.89^{ab}	54.20±3.22^a	13.40±1.78^{ab}
	L. rubiginosa 5 mg/kg	74.20±3.22^a	51.60±9.35^{ab}	51.80±2.37^{ab}	11.80±2.18^b
	L. rubiginosa 50 mg/kg	80.20±1.50^a	62.60±4.17^a	55.20±1.59^a	12.60±1.30^{ab}
	L. rubiginosa 300 mg/kg	86.20±6.15^a	44.00±2.61^b	60.20±4.49^a	17.80±1.77^a
	L. rubiginosa 2,000 mg/kg	79.00±3.42^a	53.00±4.70^{ab}	54.40±2.16^a	13.80±1.16^{ab}

Note: *P* < 0.05 for different letters in the same row

The result of this study on liver function and histological characteristics of the liver in rats that received the extract at doses of 5, 50, 300 and 2,000 mg/kg body weight showed that male rats in all groups had similar levels of ALP. Statistical analysis showed no significant differences and was not different from those in control group, but in the male rats, the levels of ALT and AST were different from control group, with lower levels of ALT in male rats than control group but the values remained within the normal range. However, female rats who received *Lepisanthes rubiginosa* extract had different levels of ALT and AST than control group, with a lower ALT level in female rats than in control and AST in female rats were lower than normal in rats that received *Lepisanthes rubiginosa* extract at a dose of 50 mg/kg body weight compared to control.

Effect on kidney function

BUN and Creatinine of all rats fed with the extract at different doses (5, 50, 300, and 2,000, showed no statistically significant difference between male and female rats compared to control group (Table 6).

Table 5: Effect of pericarp extract from *Lepisanthes rubiginosa* on liver function values in male and female rats (mean ± SEM)

Group	Liver function values	Treatments				
		Control	*L. rubiginosa* 5 mg/kg	*L. rubiginosa* 50 mg/kg	*L. rubiginosa* 300 mg/kg	*L. rubiginosa* 2,000 mg/kg
Male	ALT (U/L)	47.20±4.57^{a}	40.20±3.01^{abc}	38.75±0.43^{bc}	40.60±2.27^{ab}	35.60±1.96^{bcd}
	AST (U/L)	132.20±13.06^{abc}	112.60±8.57^{c}	119.20±8.58^{bc}	133.20±7.36^{abc}	114.60±12.88^{c}
	ALP (U/L)	252.20±29.26^{a}	230.80±9.64^{a}	227.60±10.22^{a}	234.80±14.36^{a}	220.20±10.10^{a}
Female	ALT (U/L)	37.20±2.62^{bcd}	32.00±1.92^{cd}	37.00±2.85^{bcd}	33.80±2.75^{bcd}	29.80±1.46^{d}
	AST (U/L)	139.60±8.23^{abc}	135.00±11.84^{abc}	155.40±7.24^{a}	147.80±8.37^{ab}	141.40±6.36^{abc}
	ALP (U/L)	143.40±5.99^{b}	148.20±7.29^{b}	144.40±13.13^{b}	130.40±10.85^{b}	142.80±12.85^{b}

Note: $P < 0.05$ for different letters in the same row

Table 6: Effect of pericarp extract from *Lepisanthes rubiginosa* on kidney function values in male and female rats (mean ± SEM)

Group	Kidney function values	Treatment				
		Control	*L. rubiginosa 5 mg/kg*	*L. rubiginosa 50 mg/kg*	*L. rubiginosa 300 mg/kg*	*L. rubiginosa 2,000 mg/kg*
Male	BUN (mg/dL)	13.40±0.51^{a}	12.00±0.84^{a}	12.80±0.80^{a}	13.00±0.32^{a}	12.40±0.68^{a}
	Creatinine (mg/dL)	0.22±0.02^{a}	0.20±0.00^{a}	0.20±0.00^{a}	0.20±0.00^{a}	0.20±0.00^{a}
Female	BUN (mg/dL)	12.60±1.03^{a}	15.80±1.20^{a}	15.60±0.51^{a}	14.60±1.40^{a}	15.60±0.93^{a}
	Creatinine (mg/dL)	0.20±0.00^{a}	0.22±0.02^{a}	0.20±0.00^{a}	0.20±0.00^{a}	0.20±0.00^{a}

Note: $P < 0.05$ for different letters in the same row

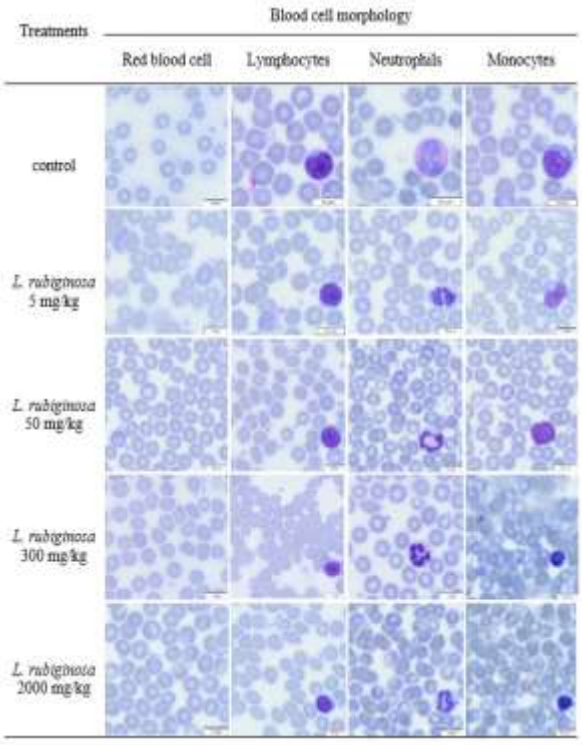

Figure 1: Effect of pericarp extract from *Lepisanthes rubiginosa* on blood cell morphology in male rats

Morphology of blood cells

All groups of rats administered the extract at different doses for 2 weeks compared to control group had similar blood cell morphology. The value of hemoglobin in male and female rats is at normal levels and white blood cells are divided into two groups: It is clear from the results that the white blood cells of all groups are intact (Figure 1 and Figure 2).

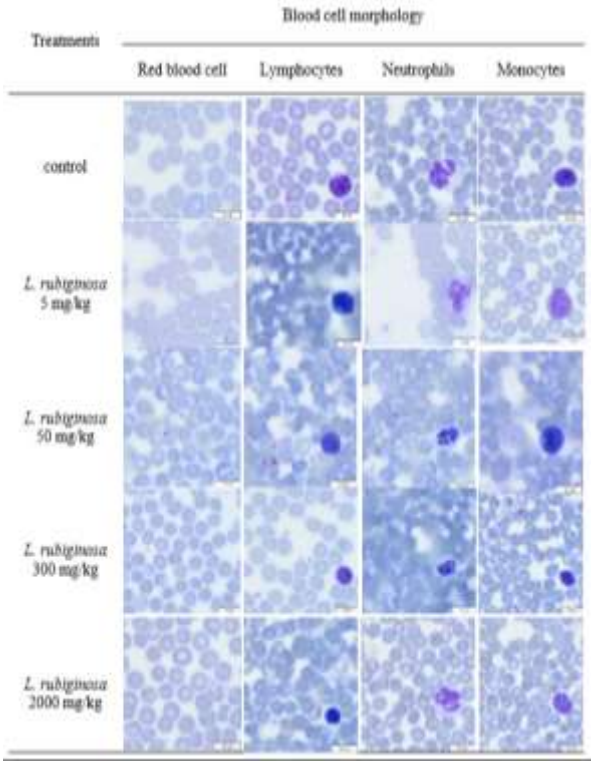

Figure 2: Effect of pericarp extract from *Lepisanthes rubiginosa* on blood cell morphology in female rats

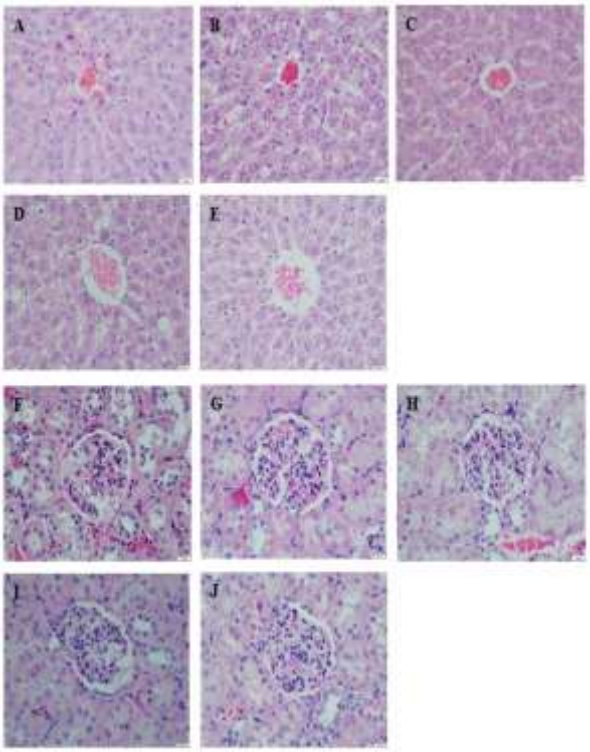

Figure 3: Effect of pericarp extract from *Lepisanthes rubiginosa* on histological characteristic of liver (A-E) and kidney (F-J) in male rats; A, F: control; B, G: 5 mg/kg; C, H: 50 mg/kg; D, I: 300 mg/kg; E, J: 2,000 mg/kg

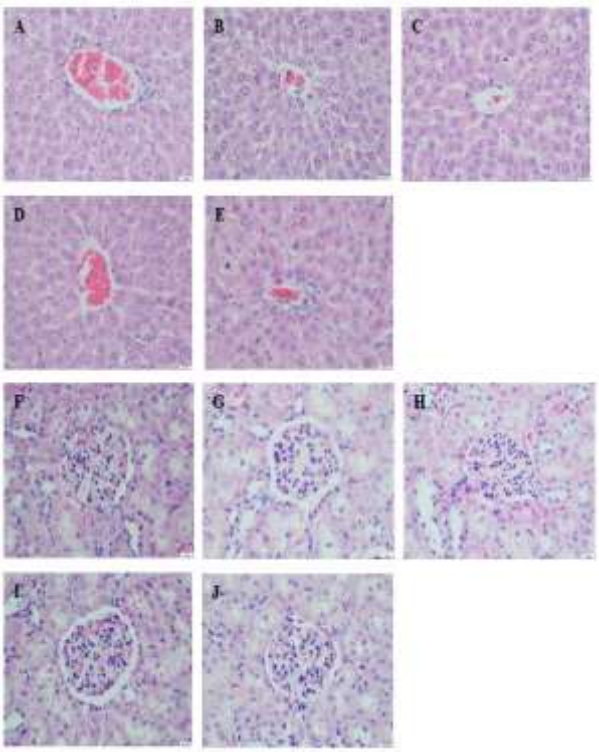

Figure 4: Effect of pericarp extract from *Lepisanthes rubiginosa* on histological characteristic of liver (A-E) and kidney (F-J) in female rats; A, F: control; B, G: 5 mg/kg; C, H: 50 mg/kg; D, I: 300 mg/kg; E, J: 2,000 mg/kg

Histological characteristics of the liver and kidney

The results revealed that hepatocytes have the appearance of hepatocyte, polygonal shape, arranged in sheets, with a single layer of cell thickness. The result shows that at all doses of the extract tested (5, 50, 300 and 2,000 mg/kg), no toxicity to the liver was observed and the liver cell of rats was not inflamed (Figure 3 and Figure 4). The histological characteristics of the kidneys were normal. No swelling or inflammation was observed, or tubular cast in the nephron and interstitium of normal glomerulus and renal tubule size. No hypertrophy or proliferation of mesangial cells and podocytes for arteriolar pole Bowman space and Bowman capsule. No pathological changes were observed. In renal tubules, no tubular cast of all types was found. Tubular cells of both the proximal convoluted tubule and distal convoluted tubule have normal characteristics [12]. Extract at 5, 50, 300 and 2,000 mg/kg had no negative effect on the histological characteristics of rat kidneys. No changes in renal histopathology were observed when compared with control group (Figure 3 and Figure 4).

DISCUSSION

Acute toxicity evaluation of *Lepisanthes rubiginosa* pericarp extract in rats showed that the highest dose of extract (2,000 mg/kg) did not cause any toxicological symptoms within 24 h. In addition, no rat mortality was observed, and after 14 days of observation, no signs of poisoning and no rat death occurred. Lethal dose (LD_{50}) was greater than 2,000 mg/kg body weight, which was analyzed according to the fixed-dose toxicity test criteria [8]. Extract did not affect the body weight of male and female rats. A new farnesol derivative, rubiginoside, has a pleasant aroma and low toxicity [5]. The methanol bark extract and flesh of the fruit contains flavonoids that help against free radicals, and when used in the right amount, it helps to prevent diseases such as heart disease, stroke and cancer.

After 14 days of treatment, organs such as liver, kidneys, heart, lungs and spleen showed no statistically significant difference in average relative organ weight, suggesting that there is no sign of inflammation found in the internal organ of rats. These findings correspond with the results of previous study which showed that *Lepisanthes rubiginosa* leaf extract exhibited anti-inflammatory activity [6]. Rats that received

the extract had cholesterol, HDL and triglyceride levels that were not significantly different from control. This is consistent with *rubiginosa* and the ethyl acetate extract which has been reported to contain five phytochemicals: lupeol, diosmetin, heptadecanoic acid, ß-sitosterol, and ß-sitosterol-3-O-ß-D-glucopyranoside [4].

Kidney function revealed that male rats that received all doses of extract had lower levels of BUN and creatinine but at similar values that were not significantly different from control group. In female rats, all groups that received extract had higher levels of BUN and creatinine but at similar values as males, so it may be inferred that the extract did not affect biochemical values. It is not likely to cause hepatotoxicity and did not show toxicity to histological characteristics of the liver in laboratory animals. However, long-term studies of the effects of the extract and further toxicological studies on rats are needed.

In the liver tissues, there is a hepatic cord, spreading radially around the central vein, with a gap lined with simple squamous epithelium called sinusoid inserted between the cell plates. In the kidney tissues, the parietal epithelium of the Bowman capsule has the shape of a rectangular, cubic and cellular at the site. The parietal epithelium of the Bowman capsule in female rats is generally round and flattened. The histological features of the kidneys in rats treated with extract showed no morphological changes, no inflammatory cells were found in the kidney tissue, and no histological toxicity of the kidneys was observed in all groups of rats. All groups of rats had normal growth, and no abnormalities were found in the external appearance of rats since the chemical composition of *Lepisanthes rubiginosa* fruit does not contain toxic substances. But on the other hand, it inhibits the growth of NCI-H 187 lung cancer cells. Essential oil of *Lepisanthes rubiginosa* flowers has good antioxidant activity, while essential oils of leaves, flowers and gooseberries have good inhibitory effect on the growth of some fungi and bacteria on the comparative study of the biological activity of essential oils from flowers and fruits of *Lepisanthes rubiginosa*. The essential oil of *Lepisanthes rubiginosa* flowers inhibits lung cancer cells type NCI-H187 and also has antioxidant activity [7]. The effect of the extract on histological characteristics of the liver in rats revealed no changes. This is not different from control group that received 10 mL of distilled water per kilogram of body weight. The liver of female rats showed characteristics that conform to the liver of mammals [12]. The study of chronic toxicity is needed to ensure the safe use of the extract.

The antioxidant property of *Lepisanthes rubiginosa* (Roxb.) Leenh was determined on the 3 stages of fruit ripening. It was found that the ripe fruit of *Lepisanthes rubiginosa* contains high total phenolic and flavonoids [13]. In addition, there were high amounts of lycopene and cyanidin-3-O-glucoside in *Lepisanthes rubiginosa* fruit extracts [14]. These studies confirm that there is no toxic substance in pericarp extracts of *Lepisanthes rubiginosa* suggesting the safe uses of this plant as food or medicine.

CONCLUSION

Lepisanthes rubiginosa extract at all doses has been shown not to cause any negative change in renal and liver histopathology of rats irrespective of sex. Findings from this study may be used to support the use of *Lepisanthes rubiginosa* as food and to develop it further as a medicine that is safe for use.

DECLARATIONS

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Ethical approval

The animal protocol was approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethic of Animal Experimentation of National Research Council of Thailand (Approval number IACUC-KKU-6/66).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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