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Original Research Article

Anti-hyperglycemic activities of *Lepisanthes rubiginosa* (Roxb.) Leenh. leaf and pericarp extracts in streptozotocin-induced diabetic rats

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Abstract

Purpose: *To evaluate the anti-hyperglycemic activities of Lepisanthes rubiginosa (Roxb.) Leenh. Leaf and pericarp extracts in streptozotocin-induced diabetic rats.*

Methods: *Doses of 500 mg/kg of the leaf and pericarp extracts were administered orally once daily to both control and streptozotocin (65 mg/kg)-induced diabetic rats for 28 days. Changes in body weight, blood glucose, triglycerides, liver and kidney function tests as well as histopathological examination of the liver cells post-administration of the extracts were evaluated and compared.*

Results: *Administration of 500 mg/kg of these two extracts to diabetic rats resulted in a significant increase ($p < 0.05$) in body weight, a significant reduction ($p < 0.05$) in fasting blood glucose, and a decrease in triglycerides after 4 weeks of treatment compared to diabetic controls, without affecting other hematological values. Liver function tests showed a decrease ($p < 0.05$) in aspartate aminotransferase levels, while kidney function tests indicated a significant reduction ($p < 0.05$) in blood urea nitrogen levels.*

Conclusion: *Lepisanthes rubiginosa (Roxb.) Leenh. Leaf and pericarp extract effectively lower blood glucose levels in diabetic rats by restoring pancreatic histopathology, resulting in an increase in the size of islets of Langerhans and promoting the cells thereby leading to increased insulin secretion.*

Keywords: *Fasting blood glucose, Triglyceride, Blood urea nitrogen, Aspartate aminotransferase, Pancreatic histopathology*

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INTRODUCTION

Diabetes mellitus is caused by defective insulin secretion by the pancreatic β-cells coupled with reduced efficiency of insulin-sensitive tissues for glucose homeostasis. When glucose is not used, it accumulates in the blood and organs, leading to excessive urination and causing the body to become dehydrated. Diabetics feel thirsty and are unable to metabolize sugar for energy. The replacement of protein energy from the muscles and fat results in lean, fat-free, and atrophied muscles. Weakness, resulting from the presence

of glucose in various organs causes body disorders and leads to further complications [1].

By 2025, the number of global diabetic patients is projected to increase to 300 million [2]. Diabetes impacts the economies of many countries where the medical and public health systems rely on new technologies comprising drugs and medical supplies. The Thai economy is heavily impacted by the cost of diabetes treatment [3]. The Thai Government is now actively promoting the use of herbal medicines as alternative health care because herbs are cheap and have few side effects [4]. Herbal therapy is considered wisdom that has been passed down from Thai ancestors.

Lepisanthes rubiginosa (Roxb.) Leenh. (local name: Ma Huad), a plant in the family Sapindaceae and has long been used as food and eaten as a fruit by people in South and Southeast Asia including Thailand. The root is boiled and drunk to treat fever, headache, skin rashes, itchy skin, tuberculosis and heat poisoning. It is also used to detoxify internal abscesses and for deworming [5]. Studies on the phytochemicals and pharmacological activity of *Lepisanthes rubiginosa* found that the extract contained phytochemicals including lupeol, diosmetin, heptadecanoic acid, ß-sitosterol and ß-sitosterol-3-O-ß-D-glucopyranoside [6]. Phytochemicals of the tree bark extracted with methanol by the steaming method revealed derivatives of farnesol, a new type of rubiginoside [7].

Hasan *et al* [8] studied the phytochemical and pharmacological activities of the steamed fruit leaf ethanol extract of *Lepisanthes rubiginosa*. Results showed that the extract had antioxidant, analgesic, antidiabetic, brain tonic and diarrheal effects in experimental animals. A comparative study on the biological activities of essential oils from the flower and fruit of *Lepisanthes rubiginosa* revealed that the essential oil of the flower had an inhibitory effect on lung cancer cells, NCI-H187 and antioxidant properties were indicated by the ABTS assay [9]. The study also found that the extract showed good inhibitory effects on the growth of certain fungi and bacteria. The leaves and fruits of *Lepisanthes rubiginosa* have long been used as food and medicine. Their phytochemicals and some biological activities have been studied but *in vivo* antidiabetic activities have not been investigated.

Therefore, this study assessed the antidiabetic effects of the leaf and pericarp extracts from *Lepisanthes rubiginosa*.

EXPERIMENTAL

Preparation of plant samples

Leaf and pericarp samples of *Lepisanthes rubiginosa* were collected during April and May 2022, from the Plant Genetic Conservation Area, Khok Dong Kheng Forest, Phra That Sub-district, Na Doon District, Maha Sarakham Province. The herbal samples were used to prepare dried plant specimens (voucher specimen no. MSUT-7804) and compared with dried plant specimens at the Herbarium of the Faculty of Science, Mahasarakham University in Thailand.

Preparation of plant extracts

The leaves and pericarps of the plant were washed with clean water, then cut into small pieces and dried at 60 °C separately in a hot air oven. Well-dried separated leaf and pericarp pieces were ground into a fine powder then filtered through sieve No. 100 and extracted with distilled water via the hot decoction method. The ratio of leaf or pericarp powder and distilled water used was 1 g: 6 mL. The extracts were refluxed and freeze-dried to obtain the extract in the form of a fine powder and stored in a refrigerator at 4 °C for further experiments.

Preparation of animals

Male Wistar rats weighing between 180 – 200 grams were raised in the Northeast Experimental Animal Center. The animals were maintained at 23 °C and relative humidity of 30 – 60 % with a dark-light cycle of 12 hours (light on at 6:00 a.m., light off at 6:00 p.m.) to ensure that the rats adapted to the laboratory environment.

They were handled in accordance with the Institutional Animal Care and Use Committee of Khon Kaen University [10] and approval was received from the Ethics of Animal Experimentation of the National Research Council of Thailand (approval no. IACUC-KKU-138/66).

Induction of diabetes

Diabetes induction was performed by a single intraperitoneal injection of streptozotocin (STZ) dissolved in 20 mM citrate buffer, pH 4.5 and freshly prepared at 65 mg/kg [11]. After the STZ injection, the rats were given 2 % sucrose for 48 hours to prevent hypoglycemia. Three days later, the rats were fasted for 8 hours and their blood glucose was determined using a glucometer. Rats with blood glucose levels greater than 126 mg/dL were selected for experimental use [12].

Design

Forty rats were divided into 5 groups, each consisting of 8 animals as follows: Group 1 (Normal control) received distilled water (1 ml/kg) while diabetes was induced in other groups. Group 2 (Diabetic control) received only distilled water (1 ml/kg) while Groups 3, 4 and 5 were treated with standard glibenclamide (5 mg/kg), pericarp extract (500 mg/kg) and leaf extract (500 mg/kg), respectively. Extracts of the leaves and pericarp and other treatments were administered orally to each group of rats using a needle feeder at 09:00 a.m. once daily for 4 weeks. At the end of the treatment, all rats were sacrificed under anesthesia using an intravenous injection of thiopental sodium at a dose of 85 mg/mL/kg [13]. Blood samples (2 mL) were collected from a cardiac puncture and kept in a vacuum tube.

Blood glucose determination

Fasting blood glucose (FBG) was determined weekly. All rats were fasted for 8 – 12 hours before blood was drawn from the tip of the tail. Accu-Chek® inform II (Roche, Germany) was used to measure the blood glucose level, with 70 % ethanol used to clean the wounds and prevent infection [14].

Assay of hematological parameters

The serum obtained was analyzed by an Automatic Blood Analyzer (Flow Cytometry) to determine hematological values [15] at the Community Laboratory for analysis (AMS-KKU Excellence Laboratory), Faculty of Associated Medical Sciences, Khon Kaen University.

Biochemical analysis

Blood urea nitrogen (BUN), creatinine (CREA), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels were analyzed in the serum using an Automatic Blood Chemical Analyzer (BT 2000 Plus, Germany) at the Community Laboratory (AMS-KKU Excellence Laboratory), Faculty of Associated Medical Sciences, Khon Kaen University.

Lipid profile determination

Serum lipid profiles comprising total cholesterol, triglyceride and high-density lipoprotein (HDL) were analyzed using an Automatic Blood Chemical Analyzer (BT 2000 Plus, Germany) at the Community Laboratory (AMS-KKU Excellence Laboratory), Faculty of Associated Medical Sciences, Khon Kaen University.

Histopathological study

The rats were dissected and the pancreas tissues were removed to compare histopathological characteristics using the paraffin embedding method. The pancreatic tissues were stained with hematoxylin and eosin to investigate pancreatic histopathology, especially islets of Langerhans. The tissue slides were then examined under a microscope and photographed [16].

Statistical analysis

All data were presented as mean ± standard error of the mean (SEM), with statistical analyses conducted using a one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test. *P*-value < 0.05 was considered statistically significant.

RESULTS

Effect of leaf and pericarp extracts on body weight

Following 28 days of administering 500 mg/kg of leaf and pericarp extracts to diabetic rats, distinct patterns in body weight changes were observed. Normal control rats and diabetic rats treated with leaf and pericarp extracts as well as diabetic rats treated with glibenclamide showed a statistically significant weekly weight gain ($p < 0.05$; Table 1). In contrast, diabetic control rats exhibited a statistically significant weight loss ($p < 0.05$).

Table 1: Effect of administration of extracts of *L. rubiginosa* on body weight of rats

Treatment	Body weight (g)				
	Initial B.W.	Week 1	Week 2	Week 3	Week 4
Group 1	303.86±4.07^{b}	370.38±4.79^{c}	406.68±7.09^{c}	440.20±5.92^{c}	495.07±13.46^{c}
Group 2	268.91±4.99^{a}	258.52±5.02^{a}	255.47±4.81^{a}	248.93±6.48^{a}	220.05±1.52^{a}
Group 3	249.77±8.80^{a}	317.20±3.49^{b}	311.92±2.58^{b}	327.83±11.76^{b}	359.97±15.59^{b}
Group 4	250.16±9.31^{a}	312.39±4.38^{b}	324.67±8.97^{b}	323.11±9.96^{b}	350.30±13.83^{b}
Group 5	247.29±10.06^{a}	311.33±2.93^{b}	321.06±8.87^{b}	323.35±11.78^{b}	342.38±18.60^{b}

^{a,b,c} indicate significant differences among the treatment groups ($p < 0.05$)

Blood glucose levels

Normal rats maintained stable and consistent blood glucose levels throughout the study, showing no significant changes from week to week. Conversely, diabetic rats exhibited a statistically significant weekly increase in blood glucose levels ($p < 0.05$; Table 2), indicating poor glycemic control under diabetic conditions.

Hematological parameters

Analysis of hematological parameters showed no significant differences ($p > 0.05$) amongst normal control rats, diabetic control rats, diabetic rats treated with *L. rubiginosa* leaf and pericarp extracts and diabetic rats treated with glibenclamide (Table 3). These findings indicate that the leaf and pericarp extracts did not adversely affect the hematological parameters in diabetic conditions.

Lipid profiles

Normal control rats maintained stable and unaltered levels of total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol. There was a statistically significant decrease in total cholesterol and triglyceride levels ($p < 0.05$), alongside a significant increase in HDL cholesterol levels ($p < 0.05$) following treatment with both extracts. In contrast, diabetic rats exhibited significant increases in total cholesterol and triglyceride levels ($p < 0.05$), indicating dyslipidemia under diabetic conditions (Table 4).

Liver function

Table 5 presents the effect of the administration of the extract on the liver function parameters in streptozotocin-induced diabetic rats. Normal rats maintained stable and unaltered liver function levels including aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). In contrast, diabetic rats exhibited a significant increase in liver function markers, highlighting impaired liver function under diabetic conditions (Table 5). On the other hand, administration of the extracts caused a significant decrease in the AST, ALT and ALP levels ($p < 0.05$).

Table 2: Blood glucose levels of rats receiving leaf and pericarp extracts of *L. rubiginosa*

Treatment	Fasting Blood Glucose (mg/dL)				
	Initial FBG	Week 1	Week 2	Week 3	Week 4
Group 1	85.13±1.68^{a}	86.13±1.81^{a}	85.13±1.46^{a}	86.25±0.59^{a}	87.38±0.91^{a}
Group 2	280.75±9.43^{b}	316.63±13.02^{c}	338.88±14.18^{c}	358.50±12.80^{c}	371.25±9.80^{c}
Group 3	277.75±6.05^{b}	207.88±5.18^{b}	173.75±6.24^{b}	162.50±7.08^{b}	129.25±1.69^{b}
Group 4	272.13±5.46^{b}	196.50±4.40^{b}	170.88±5.03^{b}	153.25±3.89^{b}	126.88±1.13^{b}
Group 5	278.25±5.39^{b}	216.63±7.12^{b}	189.50±2.48^{b}	162.88±4.47^{b}	130.38±2.06^{b}

^{a,b,c} indicate significant differences among the treatment groups ($p < 0.05$)

Table 3: Hematological parameters of rats treated with leaf and pericarp extracts of *L. rubiginosa*

Treatment	WBC (10^3/µL)	RBC (10^6/µL)	Hb (g/dL)	MCH (pg)	MCHC (g/dL)	RDW (%)
Group 1	7.38±1.08	7.84±0.16	14.56±0.30	18.56±0.17	33.35±0.24	16.73±0.46
Group 2	8.14±0.88	8.42±0.15	15.18±0.29	18.11±0.21	33.75±0.24	18.10±0.61
Group 3	7.69±0.77	8.34±0.11	15.16±0.14	18.17±0.22	34.48±0.22	16.42±1.26
Group 4	8.57±0.99	8.15±0.18	15.06±0.20	18.50±0.21	34.57±0.24	17.26±0.64
Group 5	9.38±0.79	8.26±0.24	15.16±0.40	18.37±0.21	34.12±0.24	17.37±0.54

^{a,b,c} indicate significant differences among the treatment groups ($p < 0.05$)

Table 4: Lipid profiles of rats treated with leaf and pericarp extracts of *L. rubiginosa*

Treatment	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)
Group 1	58.00±3.90^{a}	137.62±5.40^{a}	41.12±2.17^{c}
Group 2	88.50±3.68^{c}	235.00±9.35^{c}	30.12±1.61^{a}
Group 3	61.62±4.41^{ab}	201.75±10.53^{b}	35.87±1.30^{b}
Group 4	67.87±4.19^{ab}	215.50±6.91^{bc}	34.75±0.97^{ab}
Group 5	71.25±3.91^{b}	228.00±11.23^{bc}	33.50±1.48^{ab}

TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein. ^{a,b,c} indicate significant differences among the treatment groups ($p < 0.05$)

Table 5: Liver function values of rats treated with leaf and pericarp extracts of *L. rubiginosa*

Treatment	AST (IU/L)	ALT (IU/L)	ALP (mg/dL)
Group 1	61.25±5.52^{a}	41.38±1.66^{a}	105.25±2.40^{a}
Group 2	106.13±1.27^{d}	84.88±1.75^{c}	185.88±2.07^{d}
Group 3	71.25±0.88^{b}	42.13±1.38^{a}	111.88±2.29^{ab}
Group 4	79.00±1.15^{bc}	58.63±1.60^{b}	126.13±3.21^{c}
Group 5	81.50±2.09^{c}	56.88±1.38^{b}	118.25±2.44^{b}

^{a,b,c} indicate significant differences among the treatment groups ($p < 0.05$)

Kidney function

Normal control rats maintained stable kidney function parameters, with no significant changes observed in blood urea nitrogen (BUN) and creatinine levels. However, diabetic rats exhibited a statistically significant increase in kidney function markers ($p < 0.05$), indicating impaired renal function under diabetic conditions (Table 6). The extracts reduced the kidney function markers including BUN and creatinine in diabetic rats, suggesting the improvement of renal function such as glomerular filtration.

Table 6: Kidney function values of rats administered leaf and pericarp extracts of *L. rubiginosa*

Treatment	BUN (mg/dL)	Creatinine (mg/dL)
Group 1	16.88±0.35^{a}	0.28±0.02^{a}
Group 2	36.75±0.49^{d}	1.28±0.04^{c}
Group 3	19.00±0.33^{b}	0.33±0.02^{ab}
Group 4	20.13±0.35^{c}	0.38±0.02^{b}
Group 5	20.00±0.33^{bc}	0.36±0.02^{b}

BUN = blood urea nitrogen. ^{a,b,c} indicate significant differences among the treatment groups ($p < 0.05$)

Histopathological characteristics of the pancreas

The Islets of Langerhans, containing beta cells responsible for insulin production, were markedly smaller in the control diabetic group compared to normal rats, indicating impaired pancreatic function. However, in diabetic rats treated with the leaf and pericarp extracts, the Islets of Langerhans were larger than those in untreated diabetic rats but still smaller than in normal control rats. In contrast, normal control rats showed large and intact Islets of Langerhans in pancreatic tissue. The findings suggested that the leaf and pericarp extracts supported the preservation and modest enhancement of pancreatic islet size in diabetic rats (Figure 1).

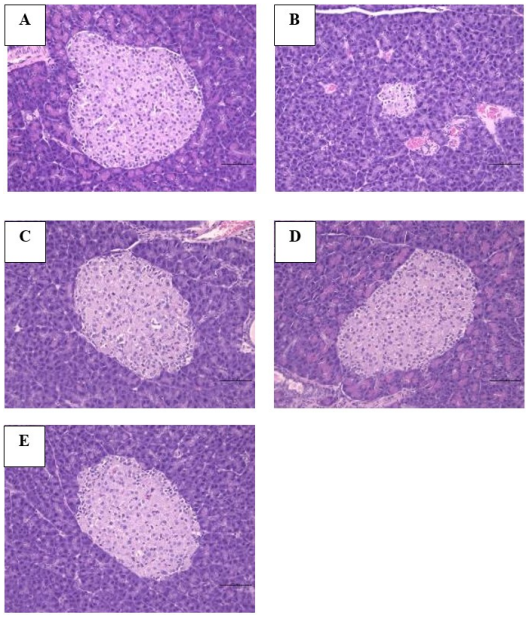

Figure 1: Characteristics of pancreatic histopathology: Normal control rats (A), Control diabetic rats (B) Diabetic rats receiving standard glibenclamide (C) Diabetic rats receiving leaf (D), and pericarp (E) extracts of *L. rubiginosa* (scale bar = 20 µm)

DISCUSSION

The rats were induced with diabetes using streptozotocin (STZ), which destroys β-cells of the Islets of Langerhans in the pancreas, responsible for the production of insulin, thereby resulting in insulin deficiency [17].

Insulin plays an important role in the absorption of glucose in various processes of the body. Hence, with insulin deficiency, glucose accumulates in the bloodstream and is excreted in urine. Diabetic rats become lethargic, eat more, drink more water than normal rats and urinate frequently because the body cannot use energy from glucose. Therefore, the diabetic rats switch to burning fat to be used as energy, leading to ketosis [18,19]. Results of this study showed that the leaf and pericarp extracts of *L. rubiginosa* lowered blood glucose levels. The body weight of diabetic rats administered extracts increased because blood glucose was used for energy, with no need to break down protein from muscles and fat into energy.

Streptozotocin (STZ) destroys the β-cells of the pancreas, causing the pancreas to produce less or no insulin. When the body lacks insulin, glucose is introduced into the blood as the cells are unable to use the sugar, resulting in its accumulation in the blood, which leads to

hyperglycemia. A decrease in blood glucose levels of the diabetic rats following treatment with the leaf and pericarp extracts resulted from several factors such as the presence of phytochemicals, important substances found in the fruit of *L. rubiginosa*, including high amounts of cyanidin-3-O-glucoside together with hydroxybenzoic acid, chlorogenic acid, vanillic acid, quercetin, rutin, myricetin, lycopene, and cyanidin-3-o-glucoside [20]. This study's results agree with a previous study on the anti-diabetic and immunodeficient effects of red and black jasmine rice from Thailand in rats with diabetes induced using streptozotocin. Black rice, containing anthocyanins such as cyanidin-3-O-glucoside, showed hypoglycemic effect on diabetic rats by increasing insulin sensitivity and contributing to the expression and movement of GLUT-1 and GLUT-4 in fat cells and muscle cells. As a result, the absorption of glucose for energy increased [21]. Results from this study are also consistent with a previous report of the phytochemicals and pharmacological activity of *Lepisanthes rubiginosa*, which examined the glucose tolerance of the ethanol extract from the leaves of this plant. Oral glucose solution was fed to induce hyperglycemia in rats and the leaf extract was then administered at doses of 250 and 500 mg/kg. After receiving the extract, blood glucose levels in the rats reduced after 90 to 150 minutes [8].

Diabetic rats that received the leaf and pericarp extracts showed reduced aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels compared to the control diabetic rats, indicating that the extract possessed anti-inflammatory activity in the liver. However, the extract did not affect the histopathological characteristics of the liver and hematological values in diabetic rats. Cyanidin-3-O-glucoside, an important component with purple-blue pigments in rice [22] and the pericarp extract of this plant, may be responsible for the effect seen on the activity of liver enzymes and the lowered blood glucose levels in diabetic rats. The histopathological characteristics of the pancreas of diabetic rats showed rejuvenation following treatment with extracts of the leaves and pericarp, comparable to the control group of rats with similar histopathological characteristics and those of diabetic rats that received glibenclamide.

CONCLUSION

The leaf and pericarp extracts from *Lepisanthes rubiginosa* demonstrate significant antidiabetic potential. These extracts effectively lower the fasting blood glucose levels in diabetic rats when administered at a dose of 500 mg/kg over 28 days. The treatment leads to an increase in body weight, and decreases in triglycerides, AST levels and BUN levels, without adversely affecting other hematological parameters. The primary mechanism by which the leaf and pericarp extracts exerted antidiabetic effects appears to be through the restoration of pancreatic histopathology and the promotion of the growth of Islets of Langerhans, resulting in increased insulin secretion. The main bioactive ingredient responsible for these effects may be cyanidin-3-O-glucoside. The leaf and pericarp extracts of this plant show promising potential as an alternative therapeutic agent for managing diabetes.

DECLARATIONS

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Ethical approval

Ethical clearance was obtained from the Ethics of Animal Experimentation of the National Research Council of Thailand (no. IACUC-KKU-138/66).

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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