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Antidiabetic potential of karamunting leaves ethanolic extract as a natural herb: Blood glucose levels and pancreatic islets histomorphology on diabetic rats model

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ABSTRACT

Background: Karamunting leaf is a wetland herb with antioxidant compounds that bind free radicals, reduce oxidative stress, reduce insulin resistance, and prevent the development of pancreatic cell dysfunction and damage.

Objective: This study aims to prove the antidiabetic potential of the karamunting leaves ethanolic extract in treating diabetes in white male rats.

Methods: This study used a true experimental with a pre-test and post-test control group design. Thirty rats were divided into four groups (control: C, ethanol extract of karamunting dose of 100 mg: P1, 200 mg: P2, and 400 mg: P3). Data on fasting blood glucose levels were taken before (pre-test) and after treatment (post-test). Data on pancreatic islets histomorphological changes (size, number, constituent cells) were analysed quantitatively. Fasting blood glucose levels were analysed using paired t-test, while the histomorphological data of the pancreatic islets were analysed using ANOVA. The data were considered significant if the p-value <0.05 (95% CI).

Results: Karamunting leaf ethanol extract treatment reduced fasting blood glucose levels in diabetic rats. A 200 mg/kgBW was demonstrated as the most effective dose that improves the islets and cells number compiles the pancreatic islets, potentially treating diabetes mellitus (DM).

Conclusion: Karamunting leaf ethanol extract potential as a natural herbal medicine in treating DM.

Latar belakang: *Daun Karamunting merupakan herbal lahan basah yang mempunyai efek antioksidan yang mampu mengikat radikal bebas sehingga dapat mengurangi stres oksidatif. Berkurangnya stres oksidatif dapat mengurangi resistensi insulin dan mencegah perkembangan disfungsi dan kerusakan sel β pankreas.*

Tujuan: *Tujuan penelitian ini adalah membuktikan potensi antidiabetes ekstrak etanol daun Karamunting terhadap tikus putih jantan diabetes.*

Metode: *Rancangan penelitian yang digunakan adalah true experimental design menggunakan* pretest *dan* posttest control group design*. Tiga puluh ekor tikus dibagi menjadi 4 kelompok (control:C, ekstrak etanol karamunting dosis 100 mg: P1, 200 mg: P2 dan 400 mg: P3). Data kadar gula darah puasa diambil sebelum terapi (pretest) dan juga paska terapi (post-test). Data perubahan histomorfologi (ukuran, jumlah, sel penyusun) insula pankreas dianalisis secara kuantitatif. Kadar glukosa darah puasa dianalisis*

menggunakan uji t berpasangan, sedangkan data histomorfologi insula pancreas dengan Anova. Data dinyakatan bermakna jika nilai p<0.05 (CI 95%)
Hasil: *Terapi ekstrak etanol daun Karamunting mampu menurunkan kadar glukosa darah puasa pada tikus DM. Karamunting mengakibatkan peningkatan jumlah insula dan juga jumlah sel penyusun insula pankreas. Dosis ekstrak etanol daun Karamunting yang mempunyai potensi sebagai untuk terapi diabetes mellitus yaitu dosis 200 mg/kgBB.*
Kesimpulan: *Ektrak etanol daun Karamunting mempunyai potensi sebagai obat herbal alami dalam terapi penyakit diabetes melitus.*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterised by hyperglycaemia due to the body's relative and absolute insulin deficiency.[1] Insulin function insufficiency is caused by impaired or deficiency of insulin production by β cells of the pancreas or caused by the lack of body cells to respond to insulin.[2,3] According to International Diabetes Federation (IDF), Indonesia's status has diabetes alert because it ranks seventh out of 10 countries with the highest number of diabetic patients. The prevalence of people with diabetes reaches 6.2%, which means that there will be more than 10.8 million people with diabetes by 2020.[4]

In DM, reactive oxygen species (ROS) are easy to produce. ROS is a free radical compound that is reactive and reacts with other compounds that cause tissue damage. Oxidative stress also damages pancreatic islets function and cause insulin resistance, thus worsening the condition of diabetes.[5]

Traditional medicine is one form of community participation and is also a potential appropriate technology to support national development.[6–9] One of the uses of herbal ingredients widely used by the public to treat DM is the karamunting plant, usually used by Kalimantan people, Indonesia, to lower blood glucose levels.[9,10] Karamunting leaves contain phenol, such as flavonoids, saponins, tannins, steroids and triterpenoids.[11] Some organic compounds from karamunting leaves have been isolated, including flavon glycosides such as myricetin-3-O-α-L- rhamnoside and ellagitannin groups 2,3-hexahydroxyl D-glucose.[12] Flavonoids are mainly water-soluble compounds that have activities such as antioxidants, antimicrobials, antibacterial, antifungal, antiviral, hepatoprotective, anti-inflammatory and antidiabetic.[12,13]

Flavonoids have antidiabetic activity through their function as antioxidants.[14,15] Antioxidants in karamunting leaves bind to free radicals to reduce oxidative stress. Thus reduce insulin resistance and prevent dysfunction and pancreatic β cells damage.[16,17] This study aims is to find an effective and optimal dose as a reference for the study development on karamunting leaves as a natural herbal medicine in treating DM.

METHODS

Study design

This study used a true experimental with a pre-test and post-test control group design. Thirty white male rats (2–6 months) and weights 200 – 300 g were divided into four groups: a control group (C) and three treatment groups (P). All the animals were streptozotocin (STZ) induced at a 40 mg/kgBW dose intraperitoneally. Rats were considered diabetic when blood glucose levels were more than 180 mg/dl. Treatment with karamunting leave extract were given 100 mg/kgBW (P1), 200 mg/kgBW (P2), and 400 mg/kgBW (P3). This study has received ethical approval from The Ethical Committee of Health Medical Study Medical Faculty, University of Lambung Mangkurat No. 576/KEPK-FK ULM/EC/VI/2021.

Plant determination and extraction

Determination of karamunting plants was conducted in the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat. Samples of herbarium leaf parts from karamunting plants are collected from the Wetland Martapura area, South Kalimantan. The karamunting leaves ethanolic extract was

conducted in the Laboratory of Pharmacology and Treatment, Faculty of Medicine, University of Lambung Mangkurat. Karamunting leaves cleaned, dried, blended and then macerated with ethanol 96 % until obtained thick extract.

Fasting blood glucose and histological tissue

Three days after STZ induction, treatment with karamunting leaves ethanolic extract was performed for 14 days. A fasting blood glucose test was performed on the 15th day of the treatment period, followed by termination. The pancreatic organs were then prepared for histological paraffin blocks. The pancreatic tissue was stained by hematoxylin-eosin.

Histomorphological measurement of the pancreatic islets

The histomorphological changes of the pancreas were observed quantitatively. The parameters analysed included islets size, the number, and the number of cells that compile the islets. Imaging of each islet was performed at 400x magnification. Histomorphologic white islets were examined under a binocular light microscope in five fields of view (top, middle, right middle, left middle, and bottom). The size and number of cells that compile the islets were measured using raster image software. The size measurement of islets was carried out by measuring each islets' shortest and longest diameters.

Data analysis

The fasting blood glucose levels were analysed using a paired t-test, while the histomorphological measurement of the pancreatic islets (size, number, and cells) was analysed with an ANOVA test.

RESULTS

Table 1. Fasting blood glucose levels of diabetic rats before and after treatment with karamunting leaves ethanolic extract

Group (dosage)	Average fasting blood glucose levels		p-value
	Pre-test	Post-test	
Control (C)	355.45	350.06	0.100
100 mg (P1)	360.25	154.88	0.006*
200 mg (P2)	370.13	148.38	0.000*
400 mg (P3)	421.00	92.75	0.000*

Note: * $p < 0.05$

Table 2. Histomorphological measurement of the pancreatic islets

Group	Pancreatic islets mean					
	Size of the islets (μm)	p-value	Number of islets (per field of view)	p-value	Number cells in the islets (per field of view)	p-value
Control (C)	87543.88	0.075	1.75	0.030*	29.00	0.008*
100 mg (P1)	90849.88		1.25		37.75	
200 mg (P2)	105787.88		1.63		59.13a*	
400 mg (P3)	75281.75		1.13a*		37.75	

Note: * $p < 0.05$, a compared with control group

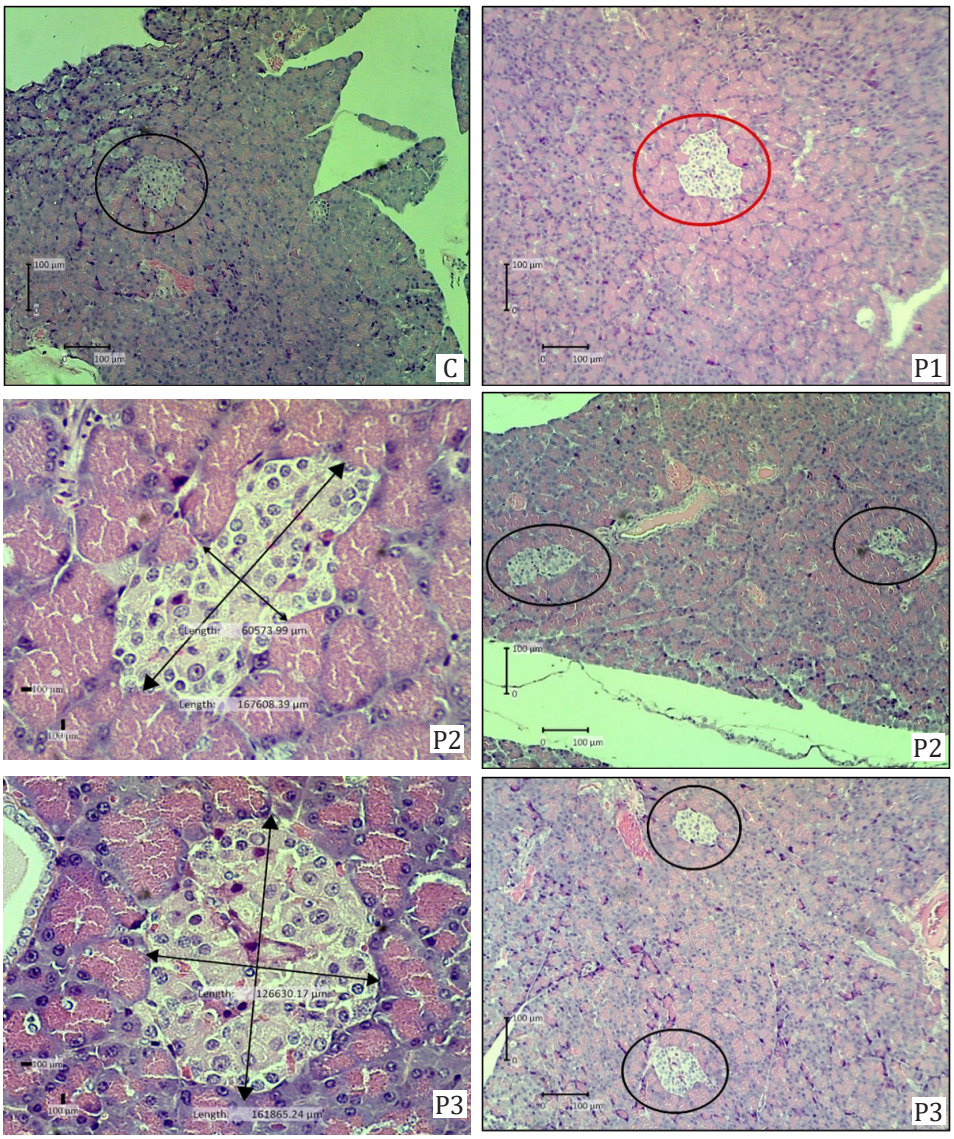

Figure 1. Histopathological morphology of the pancreatic islets on karamunting treatment. karamunting treatment increases the number of constituent cells and also the number of islets. Note: control group: C, karamunting leaves ethanolic extract 100 mg: P1, 200 mg: P2 and 400 mg: P3. HE stains; scale 100 um; magnification 100 x on right side and C group; magnification 400 x on the left side (P2 and P3).

DISCUSSION

Hyperglycaemia is associated with the formation of ROS. The excess ROS increases oxidative stress and damages pancreatic β cells. Oxidative stress contributes to damage to the function of pancreatic islets and insulin resistance, thus exacerbating the condition of diabetes.[5] In the current study, the hyperglycaemic condition was shown to change pancreatic islets microscopically. In the control group, there was a significant decrease in the number of pancreatic islets and cells compared to the treatment group. This finding indicates that the hyperglycaemia state in DM causes oxidative stress, thus causing damage to pancreatic islets.

Karamunting leave is one of the traditional medicines believed to treat DM based on local belief in South Kalimantan. Karamunting plants (Rhodomyrtus tomentosa (Aiton) Hassk) thrive in wetland areas. Many studies report that the content of karamunting mainly leaves potentially becoming DM treatment, proven in this study.[14–19]

In the current study, karamunting plants were taken in the Martapura-Banjar district, and then leaves were processed into ethanol extract preparations. Karamunting leaves ethanol extract with various measurable doses was administered in the diabetic rats model for 15 days. Based on the study results, there is antidiabetic potential in karamunting leaves to reduce fasting blood glucose levels, improve the loss of the number of islets and cells in the pancreas due to previous DM conditions. A possible mechanism is that the antioxidant compounds in karamunting leaves bind to free radicals to reduce oxidative stress, thereby reducing insulin resistance and preventing the development of pancreatic β cell dysfunction and damage.[16]

Several studies demonstrated the hypoglycaemic effects of flavonoids using different experimental models. The results showed that plants containing flavonoids had a beneficial effect on DM, either by decreasing glucose absorption or increasing glucose tolerance.[19] Most flavonoids show a mechanism of action as antidiabetic by increasing the function of pancreatic cells and stimulating insulin secretion.[14–16,18–20] Another mechanism is to reduce glucose absorption and regulate the activity of enzymes involved in carbohydrate metabolism.[14–16,18,19]

Tannin compounds contained in karamunting leaves play a role in stimulating glucose and fat metabolism, avoiding accumulating these substances in the blood. Tannins have hypoglycaemic activity and shrink the epithelial membrane in the small intestine, implicating that the absorption of nutrition and glucose intake is inhibited. This condition keeps blood glucose from rising too high. Polyphenols also play a role in lowering blood glucose levels. The possible mechanism is that polyphenols prevent superoxide conversion into hydrogen superoxide and prevent excessive oxidation, thereby protecting pancreatic cells during chronic hyperglycaemia conditions.[16]

This study demonstrates decreased fasting blood glucose levels, increased pancreatic islets number, and increased the number of cells that compile the islets in the treatment group (100 mg/kgBW, 200 mg/kgBW and 400 mg/kgBW) compared to the control group, but the most effective dose is at 200 mg/kgBW. This result is in line with Geri et al. that demonstrate the dose of 280 mg/kgBW was the most effective in reducing fasting blood glucose levels and improving insulin secretion, although there are differences in the dose of karamunting leaves (70 mg/kgBW, 140 mg/kgBW and 280 mg/kgBW) with the current study.[19] The rationale is because the dose of 200 mg/kgBW of karamunting leaves ethanol extract has reached the maximum point to cause improvement to the pancreas organ, which is damaged due to hyperglycaemic conditions.[19]

The limitation of this study was that no further examination was carried out to determine the type of pancreatic islets in the treatment group, which experienced an increase in number compared to the control group. Consequently, this study has not proven whether administration of karamunting leaves ethanolic extract to affect insulin receptor action associated with the pathophysiology of type 2 DM or affects the improvement of an absolute deficiency of cells in the pancreatic islets in type 1 DM.

CONCLUSION

Karamunting leaves ethanolic extract has potential as herbal medicine in treating DM with the most effective dose at 200 mg/kgBW.

CONFLICT OF INTEREST

Authors do not have any conflict of interest with any subject.

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