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RESEARCH ARTICLE

Administration of Binahong (*Anredera Cordifolia (Ten) Steenis*) Leaves Extract Fixes Pancreatic β-cell Damage through Lowering Blood Glucose and Advanced Glycation End Products (AGEs) Level in Hyperglycemic Wistar Rat

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Abstract

Background: Increasing production of free radicals is one of the factors that cause hyperglycemia. Chronic hyperglycemia can increase the production of reactive oxygen species (ROS), resulting in oxidative stress. The aim of this study is to determine the effectiveness of binahong (Anredera cordifolia (ten) steenis) leaf extract in fixing the pancreas ß-cell damage rate in alloxan induced hyperglycemic Wistar rat. Methods: This study used randomized pre and post-test control group design. Fifty Wistar rats were divided into two control groups and three experimental groups administered with difference doses of binahong leaves extract; 0.5 mg/kg BW (P1), 2.0 mg/kg BW (P2), and 5.0 mg/kg BW (P3). Hyperglycemic state obtained by inducing the rats with alloxan. Results: The result showed that mean blood glucose levels were decreased in the positive control group (glibenclamide) (185.98 mg/dL) and the experimental groups; P1, P2, and P3 (181.36 mg/dL, 184.61 mg/dL, and 163.36 mg/dL, respectively). The mean differences between P1 and P2 and between P1 and P3 were statistically significant ($p < 0.05$). The AGEs level for the positive control group (glibenclamide) is 0.224 mol/L and for the experimental groups are 0.219 mol/L, 0.212 mol/L, and 0.188 mol/L for P1, P2, and P3, respectively. The differences were statistically significant. Conclusions: Administration of binahong leaves extract can improve the damage rate of pancreas ß-cell through lowering the blood glucose and AGEs level in hyperglycemic Wistar rat and that the effect is significant at the dose of 5.00 mg/kg bw per day.

Keywords: *Anredera cordiofolia (ten) steenin, blood glucose and AGEs, hyperglycemia.*

Introduction

In general, a complex chronic disease involving abnormal metabolism of carbohydrates, proteins, and fats as well as the development of its microvascular, macrovascular, and neurological complication is the definition of diabetes mellitus. Diabetes mellitus is a group of heterogeneous disorder characterized by increasing blood glucose level (hyperglycemia).

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Hyperglycemia is a condition in which the fasting blood glucose is above 110 mg/dL and 2 hours post prandial blood glucose level is above 140 mg/d [1]. Hyperglycemia can increase the reactive oxygen species (ROS) either through the enzymatic process and

phosphorylation (ox-phos) or the non-enzymatic process by forming gluco oxidant, and glycation [2]. Hyperglycemia is caused by a disorder in insulin secretion or insulin mechanism of action. A hyperglycemic condition in diabetes can increase the production of free radical and lower the anti-oxidant level, resulting in oxidative stress. Hyperglycemia can increase the free radical through glucose autooxidation, production of advanced glycation end products (AGEs), and increasing polyol (sorbitol) path activity [3]

Inflammation is commonly found in hyperglycemic patients because it is ease for them to get an infection. A prolonged inflammation can increase the production of a pro-inflammatory cytokine such as tumor necrosis factor-α (TNF- α). Besides the increased TNF-α level, the adiponectin level also decreased, resulting in insulin resistance. In prolonged insulin resistance, pancreatic cells can no longer compensate the insulin demands, lead to hyperglycemia. Hyperglycemia and excessive free fatty acid release will be the substance to produce triglyceride in the liver [4].

Glycoside reaction starts with forming of irreversible Schiff base, which will be rearranged to form an Amadori product [5].

This stable amadori product then will undergo a chain of reactions with dicarbonyl, an intermediate substance, to form advanced glycation end products (AGEs). Beside AGEs, glycoside reaction can also produce free radical as byproducts such as hydroxyl radicals, hydroperoxyl radicals, peroxyl radicals, and lipid peroxyl radicals. Free radical is an atom or molecule that loses an electron on its outer orbital, which makes it very reactive. Because of that reactivity, free radical can react with almost all components of biomacromolecules. For proteins, free radical will cause cross-linking in protein, which then changes the structure and function of the protein itself, resulting in damage to the cell [6-7].

The previous study reported that binahong (*Anredera cordifolia* (*ten*) *Steenis*) leaf could decrease the blood glucose level in male white rat with diabetes Mellitus, by improving insulin production due to pancreatic ß-cells regeneration. This plant is also proved to have benefit in treating heart disease by lowering the lipid, triglyceride, and LDL level, and by increasing the HDL level. This plant also can be used to treat diarrhea and as an insecticide.

The chemical compounds contain in this plant are phytal acetate, neophytadeine, phytol, and some other compounds. The phytol compound in the binahong extract can inhibit the activity of insulin kinase, which often happens in complex network signal intracellular or defects in signal transduction, also known as post-receptor steps, such as glucose transporter translocation and glucose transport in carbohydrate metabolism [8].

Binahong has dense foliage, and it is a fast growing plant. Based on research about the phytochemical compounds, binahong leaves extract has various beneficial effects in treating various conditions. These benefits make people shift into binahong leaves extract as an alternative to the prevention and conventional treatment.

Binahong leaves extract was used as anti-dispersion, weight loss remedy, anti-diabetic, and some other conditions. Binahong leaves extract acts as anti-glycemic because it contains phytol compound, 2-methyl 5H-dibenzib flazepine, neophytadiene, and phytol acetate, which stimulate insulin secretion by pancreatic ß-cells, lowering the blood glucose and AGEs level [9]. Various mechanisms have been proposed regarding the contribution of hyperglycemia disease as a result of oxidative stress, one of them is a possibility of glucose oxidation as the source of ROS [10].

Oxidative stress will cause various disorders in blood vessels. This condition is caused by excessive ROS, exceeding the capability of endogen anti-oxidant. There are two types antioxidant known, enzymatic and non-enzymatic antioxidant. Enzymatic antioxidants are antioxidants in the organism itself, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [11]. While non-enzymatic antioxidants are antioxidants that were obtained from outside the body of

the organism itself, such as vitamin C, vitamin E, beta-carotene, uric acid, bilirubin, and ubiquinol. These exogenous antioxidants can be obtained directly from fruits and vegetables or by separation of natural compounds from certain plants. Previous studies showed that *pare* (*Momordica charantia*), *pasak bumi* (*Eurycoma longifolia*), *brotowali* (*Tinospora cordifolia*), *tapakdara* (*Catharanthus roseus*), *belimbing wuluh* (*Averrhoa bilimbi*), noni (*Morinda citrifolia*), and *daun dewa* (*Gynura divaricata*) can lower the blood glucose level and inhibit glycosylation reaction [12].

The antioxidant effect of binahong leaves extracts good enough, with percent reduction above 50%, which is 82.90%. This result proved that binahong leaves extract has potency in increasing the antioxidant capacity and reduce the oxidative stress.

Active compounds of Binahong have been determined, the process continues to an in vivo hyperglycemic test, using alloxan (streptozocin) induced Wistar rat to investigate the effectiveness in lowering blood glucose and AGEs level. The aim of this study is to investigate the effectiveness of binahong leaves extract in repairing pancreatic ß-cell through lowering the blood glucose and AGEs level in hyperglycemic Wistar rat.

Methods

This study was done in Chemistry Research Laboratory, Faculty of Mathematics and Natutal Sciences, Udayana University, technical implementation unit of the analytical laboratory of Udayana University and join Laboratory Faculty of Mathematics and Natural Sciences, Udayana University.

The instruments used in this study are gas chromatography-mass spectrometry (GC-MS), spatula, blender, glass jar, glass tools, funnel, rotary vacuum evaporator, analytical scale, centrifuge machine, test tube, syringe, EDTA tube, filter paper, aluminum foils, pipette, water bath, sonde, gloves, and mask. Fresh binahong leaves were determined, with maceration process using technical ethanol solvent (72 hours).

A thick green extract of ethanol was obtained from this process. This is a process of extraction that uses a polar ethanol solvent. In extracting the contents of the cell, the ethanol solvent can loosen up the cell's cellulose structure and dissolve the active components. The ethanol solvent can impair the cell wall, dissolve the bioactive compounds, and preserve the reactivity of a compound. The thick ethanol extract of binahong leaves gives redeem as much as 33.55%,

Table 1:Compounds detected in binahong leaves ethanol extract

Peak number	Retention time (minute)	Area (%)	Molecular formula	Compounds name
1	11.03		$C_{20}H_{40}O$	Phytol
2	15.75		$C_6H_{40}O$	2-Hexadecene, 3,7,15-tetramethy
3	15.50		$C_{20}H_{38}$	(z)-1,3-phytadiene
4	15.25		C_5H_{10}	Cylopentane, 1-ethynl
5	17.65		$C_{20}H_{40}O$	Cis-1,3-Dideuterio-1,3-cyclohexana
6	17.61		$C_{20}H_{40}O$	phytol, acetat
7	18.53		C_2H_4O2	1,3-dimethyl-4-azaphenanthrene
8	19.34		$C_{20}H_{40}O$	1-methyl-2 phenylindole-2- Ethylacridine

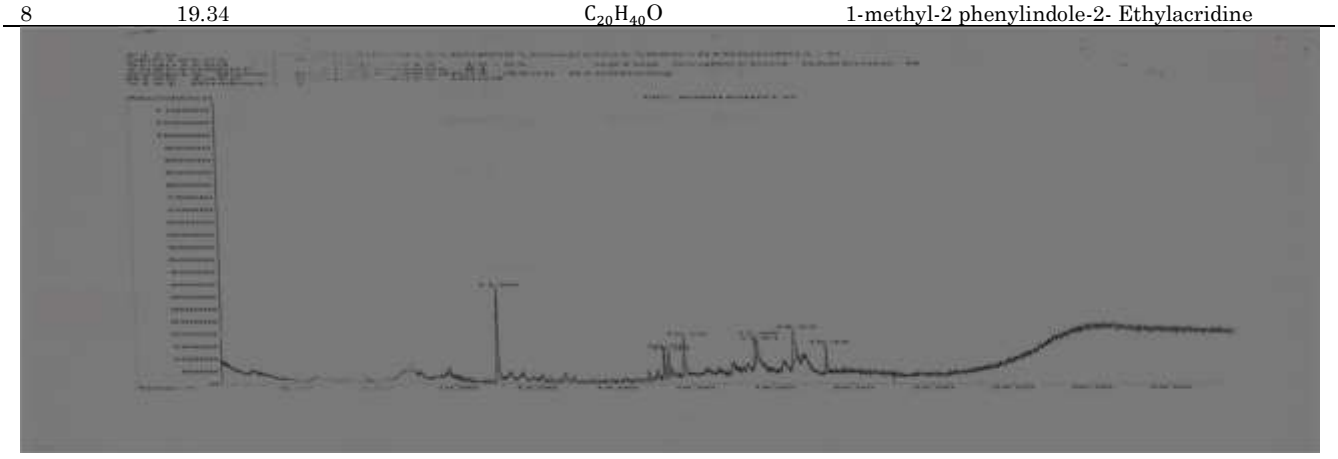

Figure 1: Chromatogram of the active compounds in binahong leaves extract

and has a deep green color. The result of antioxidant capacity towards DPPH (1,1-difenil-2-pikrilhidrazin) showed that binahong leaves extract has percent reduction as much as 60.05% in 5 minutes and 80.90% in 60 minutes. This result showed that binahong leaves extract is a strong inhibitor of DPPH activity, a powerful oxidizer, which associated with the extract's ability as a free radical scavenger. This test has a positive correlation with increased antioxidant capacity as a result of oxidative stress or ROS. A compound is classified as an active antioxidant if the percent reduction is greater than or equal to 50%.[13]

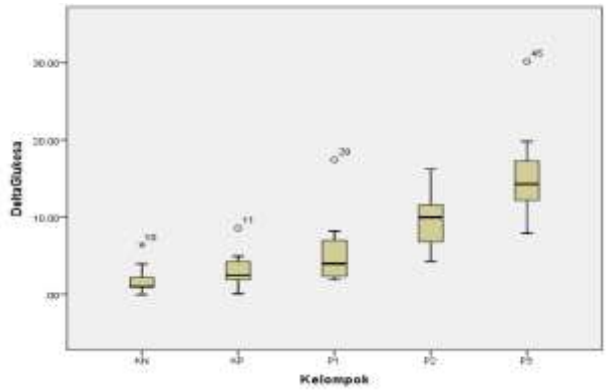

Figure 2: Statistical analysis result of the Wistar rats' blood glucose level

Besides, there is a possibility that this ability is obtained through the synergistic effect of the compounds in reducing the free radicals. The antioxidant capacity in Wistar rat's blood was measured using its ability in catching electron (hydrogen) based on the ability to catch DPPH. The mechanism of action of this reaction is that the electron owned by antioxidant was given away through a redox reaction or electron transfer to DPPH, a strong oxidator. This reaction is marked by color changing, from violet to reddish purple. This color changing reflects an antioxidant activity which can be calculated based on UV-vis spectrofotometer analysis at a wavelength of 340 nm.

Results and Discussion

The separation of the active compounds was done by using GC-MS spectroscopy analysis to indentify the profile of the compounds separated by gas chromatography. The result of GC-MS analysis of binahong leaves extract showed eight peaks of compounds with a different retention time (t_R), peak area (%), and molecule weights.

The compounds detected in ethanol extract of binahong leaves extract is showed in Table 1 and Fig. 1.

Table 2: Mean blood glucose level, before and after the experiment

Group	Blood glucose level (mg/dL)		
	Pre-test		Post-test
Negative-positive control	181.36 ± 3.06		185.98 ± 2.28
Binahong leaves extract 0,5 mg (P1)	177.65 ± 3.19		184.61 ± 3.54
Binahong leaves extract 2,0 mg (P2)	176.26 ± 2.07		183.16 ± 1.71
Binahong leaves extract 5,0 mg (P3)	168.70 ± 2.77		163.65 ± 1.88

Note: All rats were induced with alloxan at the dosage of 125 mg/kg BW. The positive control group was treated with glibenclamide at the dosage of 5 mg/kg BW.

Table 3: Mean blood AGEs level before and after the experiment

Group	Blood AGEs Level (mg/dL)		
	Pre-test		Post-test
Negative-positive control	0.219 ± 3.06		0.222 ± 2.28
Binahong leaves extract 0,5 mg (P1)	0.212 ± 3.19		0.223 ± 3.54
Binahong leaves extract 2,0 mg (P2)	0.207 ± 2.07		0.227 ± 1.71
Binahong leaves extract 5,0 mg (P3)	0.253 ± 2.77		0.188 ± 1.88

Note: All rats were induced with alloxan at the dosage of 125 mg/kg BW. The positive control group was treated with glibenclamide at the dosage of 5 mg/kg BW.

In the chromatogram analysis picture, phytol compounds can be found at peak 1 with retention time 11.05 as the precursor to synthesis vitamin E_1 and K_1. In the picture, can be seen eight peaks, with the highest being phytol compound. The peaks of the other compounds do not have vitamin E_1 and K_1 compounds because there is no separation of chlorophyll. Phytol compound in the extract has a function to modulate the transcription factor peroxisome proliferator-activated receptor-alpha (PPAR-α) and retinoid receptor X (RX). Table 1 shows eights compounds contained in the binahong leaves extract. Phytol compounds have the highest peak, can lower the blood glucose level, and also AGEs.

Pre and post-test data of rat's blood glucose level are shown in Table 2. While the profile of blood glucose level before and after the experiment is shown in Fig. 2. The effect of binahong leaves extract in lowering the

AGEs level is because of to the phytol compound. This compound is a derivate of phenolate which can act as proton donor by releasing a hydrogen atom that bond to the free radicals formed by non enzymatic glycosilation reaction, through an end product of carbohydrate aldehyde group and protein amino group.[14]

Conclusion

Based on the experiment, it can be concluded that administration of binahong leaves extract at the dosage of 5.0 mg/kg BW per day can lower the blood glucose and AGEs level in hyperglycemic Wistar rat induced with alloxan. Further researches need to be conducted on the effect of binahong leaves extract towards another chemical biomarker (i.e. TNF-α and interleukin-6). Despite these findings, a clinical trial in hyperglycemic patients is substantially needed in order to understand the effects of binahong leaves extract in human.

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