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RESEARCH ARTICLE

Effects of Binahong (*Anredera Cordifolia*) Leaf Ethanol Extracts on Blood Glucose Levels and Pancreas Histopathology in Hyperglycemic Rats

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Abstract

Background: The use of binahong leaf has been carried out for medical purposes for centuries. Several studies have shown that binahong contain alkaloids, saponins, tannins, glycosides, and terpenes, which can be known to have effects to reduce blood sugar levels. Objective: This study to determine the effect of binahong leaf ethanol extract in reducing blood glucose and histopathological change in hyperglycemic rats. Method: This study was an experimental study using Wistar rats that were divided into five groups. The normal group was given with distilled water (P0), the positive control group received glibenclamide (P1), and intervention group was given orally binahong leaf extract of ethanol with a dose of 10mg/kg (P2), 15 mg/kg (P3) and 20 mg/kg (P4). Blood sugar level was evaluated from the initial day of measurement to two weeks. Pancreas tissue was obtained to evaluate the histopathological change. Results: Mean blood sugar level in each group respectively 94.28±6.19 mg/dL, 133.61±47.05 mg/dL, 117.56±26.73 mg/dL, 124.77±37.05 mg/dL, and 136.57±47.55 mg/dL. The result of Kruskal-Wallis test was p=0.011. Multiple comparison test showed significant result between P4 with P0 (p=0.011). Conclusion: Binahong leaf ethanol extract with a dose of 20 mg/kg significantly reduce blood glucose level in the hyperglycemic rat.

Keywords: *Binahong, Anredera cordifolia, Blood glucose, Hyperglycemic rat.*

Introduction

Hyperglycemia lasts for years can cause various complications and death. The condition of hyperglycemia is one of the basic diagnoses of diabetes mellitus [1, 2]. Diabetes mellitus is a disease that attacks many Indonesian people. The most recent data in 2015 by the Endocrinology Association states that the number of diabetics in Indonesia has reached 9.1 million people. Currently, Indonesia is called the fifth most diabetics in the world. Since 2000, people with diabetes mellitus in Indonesia have experienced an increase.

The World Health Organization (WHO) predicts that in 2030, people with diabetes mellitus will reach 21.3 million people [3]. Lately, people use traditional medicine more often. The use of traditional medicines by the community is considered safer than using synthetic drugs. Experience also proves that not all synthetic drugs can overcome various health problems optimally [4, 5]. One of the medicinal plants that can be used as an antidiabetic drug is the binahong (Anredera cordifolia). Binahong leaf has been cultivated for centuries throughout mainland China, Europe, which eventually developed into central and eastern Asia [5, 7]. Plant parts (leaf) have been used in traditional medicine, generally for the treatment of several diseases such as burns [6]. Binahong leaf positively contains alkaloids, terpenes, tannins, saponins, and glycosides.

Alkaloids have been shown to have the ability to regenerate damaged pancreatic ß cells [5, 6, 8].Terpen serves as an antidiabetic because terpenes are the main component of essential oils while saponins function to increase glucose homeostasis by increasing insulin sensitivity [9], [10]. The use of binahong leaf as an antidiabetic drug is still rare.

There has been no scientific study of the effects of giving binahong leaf ethanol extract on decreasing blood glucose levels, toxicity testing, and showing changes in histopathological features [4, 6]. This study will examine the effect of giving binahong ethanol extract on decreasing blood sugar levels, toxicity testing, and changes in picture pancreatic histopathology in hyperglycemic rats.

Materials and Method

The method used in this study was quasi-experimental by comparing the effects of binahong leaf extract on hyperglycemic rats. The study was conducted at the Forensic Laboratory of the Poltabes, Denpasar, Bali. The material used is binahong leaf, which was obtained from Getakan Village, Banjarangkan District, Klungkung Regency, Bali. This research already received the ethical clearance from Ethical Committee Veterinary Faculty of Udayana University with number of the ethical clearance: 291/KE-PH-Lit-4/V/2018.

Binahong Leaf Extraction

At first, 4 kg binahong leaf was thinly sliced in a sterilized place aerated for four days, after dry blended, obtained a constant weight of 1 kg binahong leaf powder extracted by maceration using 96% ethanol until all the powder was submerged in a solvent. Soaking is done for ± 48 hours repeatedly until a clear filtrate is obtained. The clear filtrate is then seen with the thin-layer chromatography (TLC) plate to find out all the compounds that have been extracted.

The ethanol extract was filtered and separated from the solvent by rotary vacuum evaporator until thick extract was produced. Thus, thick ethanol extract fractionated using water, n-hexane, ethanol, and ethyl acetate. The fractionation results were then evaporated, and dosage preparations were made for the initial test of the most active compounds to lower blood glucose in hyperglycemic rats. Blood sugar levels and body weight of rats were studied for 14 days.

Animal Study Preparation

After the adaptation process for one week, the samples were randomly divided into five groups consisting of 3 rats in each group, two control groups, and three treatment groups.

The normal group only receives distilled water (P0). The positive control of rats was induced by alloxan and given glibenclamide, which was a standard anti-diabetic drug (P1). The three treatment groups each was induced by alloxan and given binahong leaf ethanol extract at a dose of 10.0 mg/kg BW (body weight) (P2), 15.0 mg/kg BW (P3), and 20.0 mg/kg BW (P4).

After fasting, hyperglycemia condition made by administering a single dose of alloxan injection of 125 mg/kg BW for three days .After injection, rats are fed and drunk as usual. Determination of blood glucose levels was measured in venous blood of rats using the glucose kit method in mg/dL units after fasted for 10-12 hours. If there was an increase in blood glucose levels, which was ≥125 mg/dL, the rats could be considered hyperglycemia. The examination

Histopathological Examination

The pancreas sample that has been taken then preserved for 24 hours, hydrated, chopped, infiltrated, cut, and finally colored with HE (hematoxylin-eosin), then observed under a microscope with 100X magnification.

Statistical Analysis

Data from the research results were analyzed statistically using the ANOVA/Kruskal-Wallis test method with the SPSS program (Statistical Product and Services Solution). Post hoc test will be analyzed after the null hypothesis is rejected or statistically significant ($p<0.05$) with a 95% confidence interval.

Results

The results of the preliminary test showed that the ethyl fraction decreased significantly compared to other fractions, seen from the decrease in blood glucose levels in succession to ethyl acetate, ethanol, and n-hexane fractions respectively 55 mg/dL, 38 mg/dL, and 26 mg/dL. From these results, ethyl acetate fraction was chosen to be further tested, because ethyl acetate fraction has the highest potential in reducing blood glucose levels.

Phytochemical screening performed on ethyl acetate fractions includes alkaloid compounds, flavonoids, terpenoids, steroids, saponins, and phenolics. Data from phytochemical screening showed that ethyl

acetate fraction did not contain steroid compounds, but contained alkaloid compounds, flavonoids, terpenoids, saponins, and phenolics (Table 1). The identification results using gas chromatography-mass spectrometry (GC-MS) obtained a chromatogram with six peaks in the fraction (Figure 1). The results of the chromatogram and the compounds contained can be seen in Table 2.

Table 1: Phytochemical analysis

Compound	Reactor	Result
Flavonoid	Willst atter	+
Phenolic	FeCl3	+
Alkaloid	Wagner	+
Terpenoid	Lieberman Burchard	+
Steroid	Lieberman Burchard	-
Saponin	Hot aquades + HCl	+

Note (+) = detected; (-) = not detected

Table 2: Identified compound

Peak	RT	Area	MW	Compound
1	4.41	23.90	100	Vinyl propionate
2	4.52	2.85	73	Butyl formate
3	1.60	2.29	150	2-Methoxy-4-vinylphenol
4	1.90	1.71	194	13-oxadispiro[5.0.5.1]tridecan-1- one
5	1.68	19.68	178	Methyl iso- eugenol 1
6	2.14	20.14	166	Phenol, 3-i sopropoxy-5-methyl-

Note: RT=reaction time (minutes); MW= molecular weight

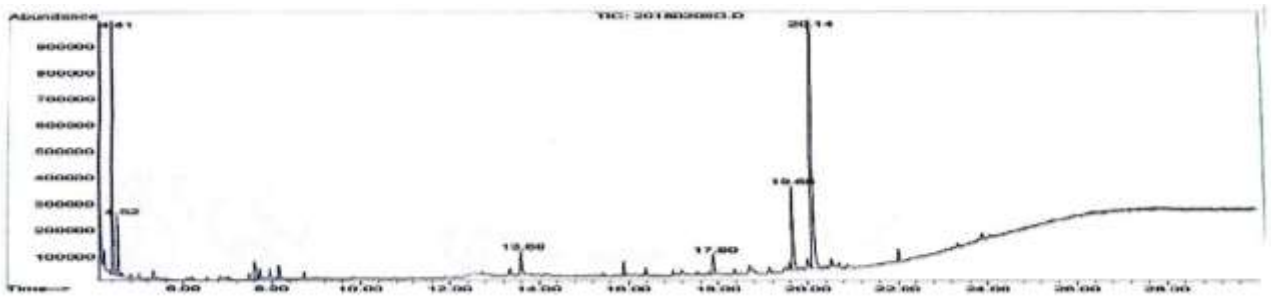

Figure 1: Binahong leaf ethanol extracts chromatogram

Blood glucose levels and body weight from day 0 to day 14 can be seen in Table 3. The results show that the administration of ethanol extract of binahong leaf with various doses can reduce blood glucose levels in hyperglycemic mice. Mean blood glucose level and body weight in each group for 14 days can be seen in Table 4.

Table 3: Descriptive data of blood sugar level and body weight

Day	Blood sugar level					Body weight				
	N	Mean	SD	Min.	Max.	N	Mean	SD	Min.	Max.
0	15	89.63	7.17	83.60	102.50	15	231.35	39.01	200.0	300.0
3	15	169.40	44.80	92.90	211.29	15	234.59	38.34	202.5	300.8
7	15	123.07	13.61	97.50	133.20	15	240.30	37.05	208.0	300.5
14	15	103.33	4.30	97.85	111.29	15	267.59	29.38	215.6	304.2

Table 4: Descriptive data of blood sugar level and body weight between groups

Group	Blood sugar level					Body weight				
	N	Mean	SD	Min.	Max.	N	Mean	SD	Min.	Max.
P0	12	94.28	6.19	84.85	100.70	12	297.62	5.23	290.0	403.2
P1	12	133.61	47.05	97.85	210.15	12	222.70	31.88	200.2	276.0
P2	12	117.56	26.73	85.60	155.17	12	222.63	30.58	200.2	275.8
P3	12	124.77	37.05	83.60	178.39	12	215.40	5.10	205.3	222.6
P4	12	136.57	47.55	91.67	211.29	12	258.94	22.36	220.3	289.1

Data distribution analyzed using a normality test, and the result was not normally distributed ($p<0.05$) (Table 5). The parametric test was used to analyze the relationship between blood sugar levels among groups. The result of the Kruskal-Wallis test was significant ($p<0.05$), so the null hypothesis was rejected (Table 6).

Multiple comparisons showed significant result between P0-P1 ($p=0.045$) and P0-P4 ($p=0.011$) (Table 7). The ability to decrease blood sugar level in P1 was similar to P4 compare to the normal group. Histopathological change revealed a similar condition in P0 and P4. Thrombocyte aggregation appeared in P1, P2, and P3 (Figure 2).

Table 5: Normality test of blood sugar level

Group		Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
Blood sugar level	P0	0.198	12	0.200	0.832	12	0.022
	P1	0.323	12	0.001	0.706	12	0.001
	P2	0.199	12	0.200	0.876	12	0.079*
	P3	0.209	12	0.157	0.853	12	0.040
	P4	0.278	12	0.011	0.786	12	0.007

*Normally distributed if p>0.05

Table 6: Nonparametric test of blood sugar level across the group

Null hypothesis	Test	Sig.	Decision
The distribution of blood sugar level is the same across categories of group	Independent-samples Kruskal-Wallis Test	0.011*	Reject the null hypothesis

*Significant if p<0.05

Table 7: Multiple comparisons of blood sugar level between groups

Group-Group	Test statistic	Standard error	Standard test statistic	Sig.	Adjusted sig.
P0-P2	-17.250	7.129	-2.420	0.016	0.155
P0-P3	-18.000	7.129	-2.525	0.012	0.116
P0-P1	-20.250	7.129	-2.840	0.005	0.045*
P0-P4	-23.250	7.129	-3.261	0.001	0.011*
P2-P3	-0.750	7.129	-0.105	0.916	1.000
P2-P1	3.000	7.129	0.421	0.674	1.000
P2-P4	-6.000	7.129	-0.842	0.400	1.000
P3-P1	2.250	7.129	0.316	0.752	1.000
P3-P4	-5.250	7.129	-0.736	0.461	1.000
P1-P4	-3.000	7.129	-0.421	0.674	1.000

*Significant if p<0.05

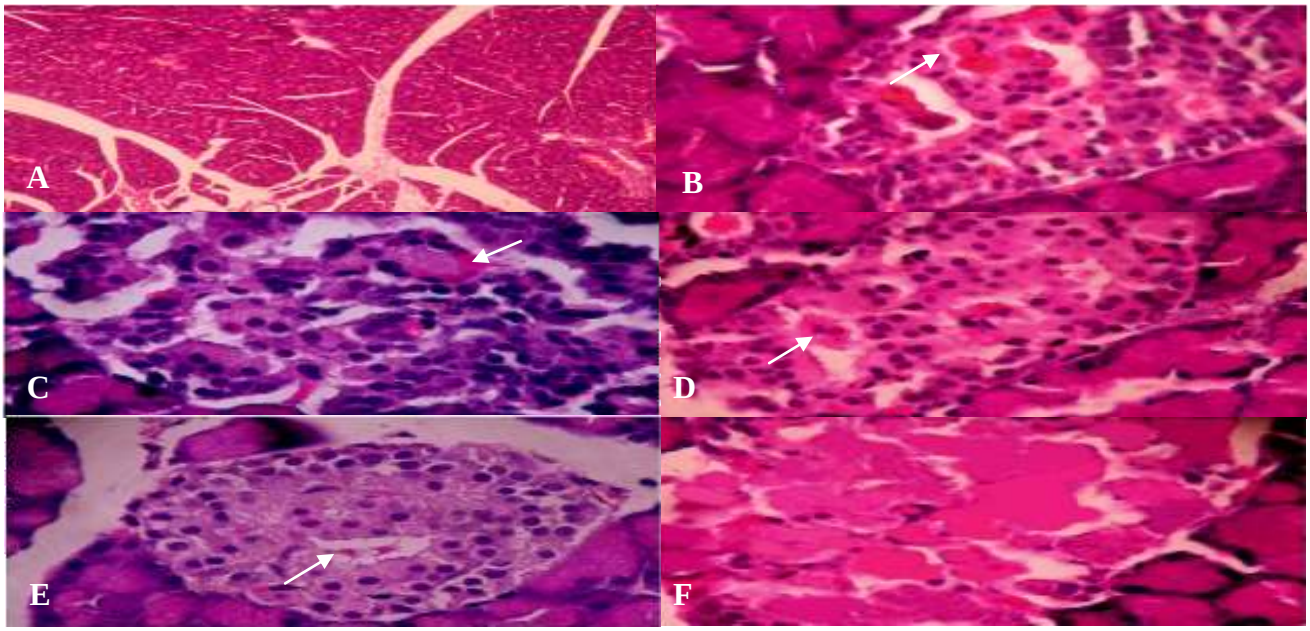

Figure 2.A: Normal pancreas without the presence of leucocyte cell infiltration. B) Change in the pancreas structure, indication of mechanical injury in hyperglycemic condition. C) Pancreas in P2 revealed thrombocyte aggregation on the artery and thickened tunica media. D) Pancreas in P1 revealed small thrombocyte aggregation. E) Pancreas in P3 also revealed thrombocytes aggregation. F) Normal pancreas without thrombocytes aggregation in P4

Discussion

Hyperglycemia is a condition where the glucose level in the blood plasma exceeds the normal limit. Blood glucose levels that occur in carbohydrate metabolism [1, 2].Various studies in India have shown that herbal treatments can reduce blood glucose and toxicity. Herbal remedies are non-toxic (non-toxic) drugs, and free of synthetic drug effects [3, 4]. Hyperglycemia caused by an abnormality in insulin secretion or any disorder of insulin.

This condition can increase the reactive oxygen species (ROS) compounds through enzymatic processes namely oxidation and phosphorylation reactions and ADPH-Oxidase and non-enzymatic processes by forming oxidation of glucose and glycation [1]. Hyperglycemia and the release of excess fatty acids will form triglyceride in the liver. The process of autoxidation of hyperglycemia and the glycation reaction results in the release of electrons. This release of electrons triggers the formation of free radicals.

Increased free radical production results in oxidative stress [11]. Oxidative stress is an event in which free radicals in the form of reactive molecules appear through a biochemical reaction from normal cells that damage cell membranes and cause various bodily dysfunctions. Oxidative stress is one component in the mechanism of tissue damage in humans. Therefore, the condition of hyperglycemia will increase oxidative stress, and oxidative stress will worsen the health condition of the patient so that it is necessary to have antihyperglycemic material [11, 12].In this study, we found that binahong with dose 20 mg/kg BW gives antihyperglycemic effect compared to the normal group.

The group with glibenclamide administration has a similar effect with 20mg/kg BW dose of binahong leaf ethanol extract. This result similar to another study despite the different dose of binahong leaf ethanol extract .Dosage 100 mg/kg BW decrease blood glucose concentration within 14 days of administration compared to the normal and acarbose group ($p<0.05$) [4]. Corrugated binahong leaf contains biologically active compounds such as flavonoids including isorhamnetin, kaempferol and quercetin glycosides, phenylpropanoid derivatives, indole alkaloids, and glucoside sterols [4], [5]. Histopathological examination was similar between the normal group and the group that received dosage 20 mg/kg BW of binahong leaf extract. Thrombocyte aggregation appeared in the group with a dosage 10 mg/kg BW, 15 mg/kg BW, and glibenclamide administration. The work of phenol as an antioxidant is to inhibit the formation of free radicals and protect cells from being oxidized. Phenol has a cardioprotective effect, which is a very powerful antioxidant [11]. The 2-methoxy-4-vinyl phenol, methyl isoeugenol 1 and 3-isopropoxy-5-methyl-phenol compounds are thought to be active compounds that contribute to antioxidant activity and antihyperglycemic in the ethyl acetate fraction of binahong leaf extract [4, 5, 11].

Conclusion

Extract of binahong leaf with a dose of 20 mg/kg body weight significantly reduces blood sugar level similar to the control group in hyperglycemic rats. Histopathological feature in the pancreas was similar between the group with 20 mg/kg binahong extract and normal group.

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