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The Effect of Binahong Leaf Extract Emulsion (Anredera cordifolia) on Histopathological Description of Commond Carotid Artery Study on Male Wistar (Rattus novergicus) Rats Induced by Diabetes Mellitus Using Streptozotocin

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ABSTRACT

Background: Diabetes mellitus (DM) is a chronic metabolic disease that can cause microvascular and markrovascular complications.The formation of superoxide (O2-) by dysfunctional mitochondria in diabetes has been recognized as a major driver of diabetic complication. Therefore the usage of antioxidants can be beneficial. Andredera cordifolia contains flavonoids as antioxidants.
Objective: To know the effect of binahong leaf extract on the picture of commod carotid artery atherosclerosis.
Methods: This research used laboratory experimental method with randomized posttest control group design. Research subjects were 15 Wistar rats (Ratus novergicus). In this research the making of extract by maseration process using 96% ethanol (1:3) and emulsification by adding sunflower-oil, CMC, Span80 and twen 20. Anredera cordifolia extract content analysis such as flavonoid and IC50. Intervention will be conducted for 14 days. K- 1 ml aquades/day; K+ 45mg/kgBW metformin; P1 200 mg / kgBW EAC; P2 400 mg/kbBW EAC. The DM induction using single dose 50 mg/kgBW streptozotocin intraperitoneal. Blood glucose measurements was 14th days after treatment induction using GOD-PAP method and histopathological examination of the commond carotid artery using eosin hemactocillin staining at a magnification of 100-400x.
Results: Total flavonoid in binahong leaves is 1525 mg/100 g extract and IC50 contain is 1,9 ppm. ANOVA shows there were a significantly difference among group 0,002($p<0.05$) for fasting blood glucose level. Post hoc test showed that 400 mg/kgBW doses can decrease the glucose level up to 305,75 mg/dl $p=0,001$($p<0,05$). Mann-withney shows there were a significantly difference among group 0,041($p<0.05$) for inhibition of atherosclerosis common carotid artery.
Conclusion: there was a difference in the administration of 400 mg / kgBB binahong leaf emulsion extract with a negative control group on the histopathological picture of atherosclesosis of the commond carotid artery.

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1. Introduction

Diabetes mellitus (DM) is a public health problem in all corners of the world. In 2013, 382 million (8.3% of the total population) were affected by DM. [1] This disease has increased every year from 4.7% in 1980 and 8.5% in 2014. [2] In 2017 it is estimated that 451 million (aged 19-99 years) are affected by DM and cause 5 million deaths in patients estimated to increase to 693 millions in 2045 in the world. [3] Diabetes mellitus is the third largest disease that causes the biggest deaths in Indonesia with 6.7% of the total deaths. [4]

Diabetes mellitus is categorized as a metabolic disease where there is an increase in blood sugar levels due to a lack of insulin and or a decrease in the sensitivity of its receptors.[5] Various complications can be caused by DM including nephropathy, neuropathy, retinopathy, and heart and blood vessel disease. [6]

Atherosclerosis is caused by an inflammatory process in the endothelium due to an imbalance between oxidant and anti-oxidant agents, inflammation and anti-inflammatory, proliferation and anti-proloferation. Oxidative stress and high blood glucose levels are factors that trigger damage to endothelial function. [7] Reactive Oxygen Species (ROS) react with other components in the body such as fat, protein and nucleic acids in cells and degeneration processes occur. ROS induces the production of Advanced Glication End Products (AGE) or activation of Protein Kinase C (PKC)

which is another complication factor. [7] Oxidative stress in patients with diabetes mellitus increases due to glucose auto-oxidation, increased glycolysis, and AGE-RAGE activation. In physiological conditions the formation of superoxide as oxidative stress occurs in the process of phospholiration in the mitochondria during electron transfer, but in patients with diabetes mellitus there is an increase in glycolysis which causes an increase in electron flow in the mitochondria and superoxide production in cells. [7] Therefore the use of antioxidants to neutralize superoxide due to diabetes mellitus which functions to prevent the process of atherosclerosis formation can be used. [8-10]

2. Methods

This research is an experimental study on rats. This research cunducted at Animal Laboratory, Faculty of Medicine, Diponegoro University. The research was carried out from February 2019 to July 2019. This research is randomized actual experimental type with post-test only with control group design. The treatmen given was aquadest, metfromin, emulsion of binahong, while the outcome was histopathological description of commond carotic artery. The experimental animals, namely male wistar (Rattus novergicus) rats aged approximately three monts weighing 120-200 grams, were selected by inclusion criteria. The study began with acclimatization rats for a week, induced by diabetes mellitus using streptozotocin 50 mg/kg for 2 weeks and rats randomly divided into 4 groups, group K- treatment 1 ml aquades/day; K+ treatment 45mg/kgBW metformin; P1 treatment 200 mg / kgBW EAC; P2 treatment 400 mg/kbBW EAC each consisting of 5 rats. The treatment was given together and there was 5 dead of rats during experimetal after induction diabetes melitus using streptozotocin.

Making binahong leaves extract

In this study, making binahong leaf extract using maceration method. The dried binahong leaves are then macerated with 96% ethanol [ratio 1: 3 (W / V)] in a closed vessel for 3x24 hours, then filtered with a Buchner funnel. The filter results obtained from the liquid extract were evaporated to free from ethanol solvent by using a water bath at 40ºC to produce a thick extract.[11]

Making of Binahong Leaf Extract Emulsion

Thick binahong leaf extract is dissolved using sunflower oil: water: CMC: Span 80 : twen 20 by comparison (10: 90: 900: 1: 1) homogenated with 3000 rpm homogenizer for 15 minutes.[10]

Phytochemistry test

Examination of flavonoids

15 mg of binahong leaf extract is dissolved in 10 ml ethanol. The solution was taken 1 ml and added with 1 ml of $AlCl_3$ and 1 ml of potassium acetate. Then incubated at room temperature for 1 hour. Then read using uv-vis spectrophotometer at 435 nm wavelength triplo, comparing with the standard reading curve in quercetin. [12]

Examination of IC50

Dissolve 10 mg of binahong leaf extract with 10 ml of methanol to produce a solution with a level of 100 ppm, a binahong leaf extract solution diluted to a level of 60 ppm, 40 ppm, 20 ppm, binahong leaf extract solution for each concentration taken 2 ml and a 2 ml 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution then Incubation for 30 minutes then read the absorbance using a uv-vis spectrophotometer using a DPPH solution blank at a wavelength of 517 nm from which data can be calculated IC50 levels of binahong leaf extract.[13]

Blood glucose measurement

Blood sugar levels were measured on the 14th day after streptozotocin (STZ) induction and 14th day after treatment. Blood glucose levels are determined enzymatically using the glucose oxidase-peroxidase (GOD-PAP) method. A blood sample of 1.0 mL was taken from retroorbita. Next, blood samples were centrifuged at 3,000 rpm for 15 minutes. The serum was taken as much as 10.0 µL from the sample then reacted enzymatically with 1.0 mL of GOD-PAP reagent. The next step was incubation at 37 ° C for 15 minutes and absorption was read at λ maximum of 505nm.[14]

Histopathology measurement

All rats each group were anestheized with 5% chlorofoam and conducted servical disslocation. After perfusion, the commond carotid artery ware removed. That was fixed using phospate buffer formaldehyde 10% then dehydrated with serial alcohol. Then, the tissue embedded in OCT parafin. Serial coronal section with thickness 5-6 micrometer using microtome mounted in glass slides and stained with the routine hematoxylin and eosin technique. Tissue examination under light microscope with 100-400 magnification.[17]

3. Result

Extract of Binahong leaves

Extraction using maceration method using 96% ethanol and. Every 1000 grams of dried binahong leaves added 3000 grams of ethanol which was incubated at room temperature for 3x24 hours and evaporated to produce 17.9 grams of thick extract.

Phytochemistry examination

Table 1. Phytochemistry examination

Sample	Total Flavonoid	IC50
Binahong leaf extract	1525,7mg / 100g	1,9 ppm

Effect of STZ in blood glucose in rats

Table 2. Blood glucose after STZ induction

Group	N	Blood Glucose (mg/dl)	Kruskal - Walis
		Mean ± SD	
K-	4	214,25 ± 100,35	P=0,794
K+	4	215,25 ± 21,82	
P1	3	193,33 ± 25,48	
P2	4	192,25 ± 13,72	

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In the data above shows that each experimental group had diabetes mellitus.

Effect of Binahong emullsion (EAC) in blood glucose

Table 3. Blood glucose after EAC induction

Group	N	Blood glucose (mg/dl) Mean ± SD	F	ANOVA
K-	4	447 ± 184,03	10,149	P=0,002*
K+	4	118 ± 12,28		
P1	3	156 ± 10,69		
P2	4	141 ± 12,84		

The administration of binahong emultion at a dose of 200 mg/kgBW (group P1) showed an insignificant result when compared to group K+ with a significance value of 0.616 (p> 0.05) and P2 with a significance value of 0.842 (p> 0.05). Whereas, compared to group and group K-, it showed significant results of blood glucose level differences with significance values of 0,002 consecutively (p<0,05).

The administration of binahong emulltion at a dose of 400 mg/kgBW (group P2) showed an insignificant result when compared to group K+ with a significance value of 0.743 (p> 0.05). Whereas, compared to group and group K- it showed significant results of blood glucose level differences with significance values of 0,001 consecutively (p<0,05).

Based on the table above, it was found that the administration of binahong emulltion at a dose of 400 mg/kgBW (group P2) showed the largest decrease in blood glucose to group K- with an average value of blood glucose levels of 305,75 mg/dL

Histopathology of common carotic artery

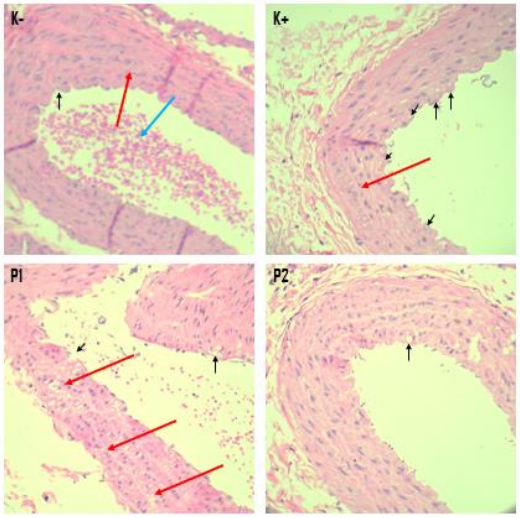

Figure 1. Photograph of HE common carotic artery preparations with 400x magnification (K-,K+, P1, P2). Red arrow : lipid deposits in tunica media. Black arrow: foam cells. Blue arrow : Thrombosis.

In the K - group picture, it is found foam cells and intracellular lipid nuclei in the tunica media and hematomas in the lumen of blood vessels. In the K + and P1 groups, foam cells were sensed and intracellular lipid nuclei were found in the tunica media. In P2 group foam cells were found.

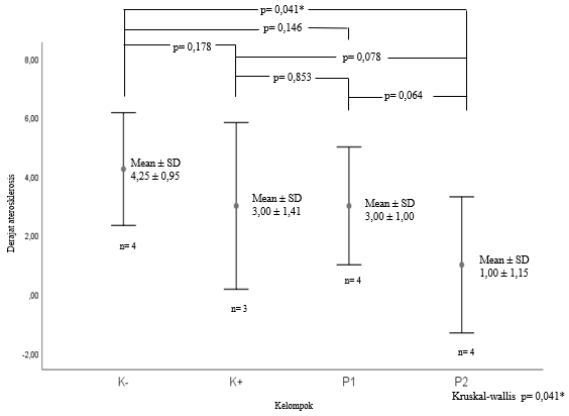

Figure 2. Mann-withney test of atherosclerosis in common carotic artery

The administration of binahong emulsions at a dose of 400 mg / kgBB (P2 group) showed significant results when compared with the K-, K + groups with a significance value of 0.019 (p <0.05). Whereas, compared with groups and groups P1 showed insignificant results with a significance value of 0.078 (p> 0.05)

4. Discussion

In the examination of flavonoid levels in this study obtained levels of 1525.83 mg / 100 grams of extract or 1,525% high levels. Based on research conducted by Fidriyyani, et al 2013 obtained total flavonoid levels of 0,70%. (18) The extraction process is carried out for a longer period of time because of that reason more flavonoid levels are obtained due to the achievement of saturation levels of solvents and solutes and the maximum remaceration process. [19]

Administration of 50 mg/ kg body weight of streptozotocin intraperitoneally significantly increases blood glucose levels on the 14th day after treatment and reaches diabetes criteria with a blood glucose level> 126 mg/ dl. The mechanism of streptozotocin in increasing blood glucose levels begins with the reaction between streptozotocin and pancreatic β-langerhans cells by means of DNA alkalization and as a source of nitric oxide which results in increasing xantine oxidase levels and decreasing ATP and NADP + so that it affects glucose oxidation and decreases biosynthesis and insulin secretion. [20]

In the positive control group (K + group), metformin was given 45 mg / kg body weight and showed a significant effect compared to the K-group. This is consistent with the benefits of metformin which is used as a therapy to reduce blood glucose levels by increasing peripheral glucose absorption by increasing cell sensitivity to insulin, suppressing glucose production by the liver, reducing fatty acids and increasing glucose use in the intestine through

non-oxidative processes [(21)]. P2 group also showed a decrease in blood glucose levels. In vitro studies of binahong leaf extract were carried out on the enzymes α-glucosidase, α-amylase and dipeptidyl peptidase IV (DPP IV). Inhibition of α-glucosidase and α-amylase will reduce hyperglycemic conditions after eating by delaying the process of glucose absorption because these two enzymes play a role in the process of hydrolysis of carbohydrates. Dipeptidyl peptidase (DPP IV) plays a role in the degradation process of incretin, especially GLP-1 (Glucagon Like Peptide-1) which stimulates insulin production. [22-23]

Oxidative stress in diabetes mellitus will cause endothelial dysfunction and excessive production of Reactive Oxygen Species (ROS) will oxidize extracellular LDL. [24] This oxidation of LDL will produce proinflammatory particles and proaterosclerosis which is the development of atherosclerosis. LDL oxidation is captured by macrophage-derived monocytes through the scavenger receptor. Oxidation of LDL which has died due to phagocytosis by macrophages, accumulates to form foam cells. These foam cells will form aggregates and eventually form fat streaks. So that fat strokes can be the accumulation of smooth muscle intracellular lipids and accumulation of extracellular smooth muscle lipids as well as the occurrence of further atherosclerosis with the formation of surface defects, hematomas and even thrombus. [25]

The mechanism of antioxidant effects of binahong leaf flavonids can include: (1) suppress the formation of reactive oxygen species either through inhibition of enzymes or chelating agent elements involved in the production of free radicals; (2) reducing ROS; and (3) enhance or protect antioxidant defenses. Furthermore, they can also meet most antioxidant criteria. It has been hypothesized that their antioxidant properties might protect tissues against oxygen free radicals and lipid peroxidation. Most flavonoids are effective ROS reducing agents. [24]

5. Conclusion

There was a difference in the administration of 400 mg / kgBB binahong leaf emulsion extract with a negative control group on the histopathological picture of atherosclesosis of the commond carotid artery.

Ethical Approval

The research was approved by Health Research Ethics Committee, Faculty of Medicine, Diponegoro University - Dr. Kariadi and carried out by the principles of Declaration of Helsinski.

Conflicts of Interest

The authors verify that they have no competing financial interest or personal relationship that could influence the work reported this paper.

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Author Contributions

Conceptualization, MIF; methodology, MIF, DP DAI; software MIF; formal analysis, MIF, DP, DAI, HI; investigation, HI; resources, MIF, DP; data curation, MIF, HI; writing-original draft preparation, MIF; writing-review and editing,DP, DAI, APW; visualization, MIF; supervision, DP, DAI, APW; project administration, MIF; funding acquisition, MIF".

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