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by Ebta Narasukma Anggraeny

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The Effect of *Clinacanthus nutans* (Burm.f.) Lindau Water Fraction Addition on Hypoglycemia

C Retnaningsih, V K Ananingsih, Meiliana .E N Anggraeny, IM Cahyani, B Nugraheni, R Efendi

Abstract. This research aimed to investigate the effect of administering water fraction of *Clinacanthus nutans* [Burm.F.] Lindau (the local name of daun dandang gendang) in different doses on hypoglycemia. The research method was using the test of dandang gendis leaf (*Clinacanthus nutans* (Burm.f.) Lindau) water fraction on wistar rats using pre and post test method randomized control group design. The induction using a high-fat diet consisting of pork oil: duck egg (3:1) and fructose 1.8 g/ kgBW for 30 days was applied. Rats were divided into 6 groups: normal control (aquadest), negative control (carboxyl methyl cellulose/CMC), positive control (metformin), dose I 15,89 mg/ kgBW (water fraction of *Clinacanthus nutans* [Burm.F.] Lindau), dose II 31,78 g / kgBW, and dose III 47,67 mg / kgBW. The result showed that a dose of 15,89 g/ kg BW was an effective dose to reduce blood glucose level. of wistar male rats induced by fructose and high-fat feed.

Keywords: *Clinacanthus nutans* [Burm.F.] Lindau, water fraction, hypoglycemia

1. Introduction

The International Diabetes Federation [4] states that in 2015 Indonesia occupied the world's seventh position for the prevalence of diabetes. The prevalence of people with diabetes in Indonesia showed a tendency to increase from 1.1% (2007) to 2.1% (2013) and 2/3 people with diabetes did not know if they had diabetes (Riskesdas 2013; vi). Increased prevalence of type 2 diabetes due to urbanization or movement from rural to urban areas which then led to changes in one's lifestyle. Among them are eating habits that are not balanced will cause obesity. This condition of obesity will lead to type 2 diabetes mellitus [7].

The high consumption of sugar and fat causes obesity which triggers diabetes mellitus [17]. About 90% of type 2 diabetics are experienced by individuals who are obese. One sign of diabetes is hyperglycemia. This situation accelerates the formation of reactive oxygen compounds (Kostić, 2009). Increased oxidative stress can be inhibited by antioxidants. Antioxidants are free radical stabilizers that work by supplementing the electron deficiency of free radicals and inhibiting the occurrence of chain reactions of free radical formation [3].

Many Indonesian plants are potential as antidiabetic, one of which is widely used is dandang gendis leaves (*Clinacanthus nutans*). Many previous studies have been conducted on the content of dandang gendis leaves such as Nainggolan's research [9] about phytochemical screening of dandang gendis leaves, obtained by alkaloids, triterpenoids / free steroids, tannins, saponins, flavonoids, and essential oils.

The n-hexane fraction found at least 6 triterpenoid / steroid compounds, 3 chloroform fractions of alkaloids and a water fraction found in flavonoids and tannins. This is also supported by research from Nugroheni [11] that the phytochemical screening results of the water fraction of dandang gendis leaves contains phenolic compounds, polyphenols, tannins, flavonoids, alkaloids, saponins, and steroids.

In the study of Abdulah and Kasim [1] stated that the ethanol extract of dandang gendis (EDG) leaves had the most significant inhibitory effect of α -amylase as an antibiotic compared to other extracts in vitro. In Ramlan's research [13] said that the percentage of accumulated decreases in blood glucose levels of mice after administration of EDG at 75 mg and 100 mg / kg BW was not significantly different compared to glibenclamide dose of 1 mg / kg bw. In a study conducted by Sirega et al [14] that administration of high-fat and high-cholesterol foods can cause insulin resistance and worsen metabolic index. In a study conducted by Sirega et al [14] that administration of high-fat and high-cholesterol foods can cause insulin resistance and worsen metabolic index. Giving dandang dendis leaf extract can be used as a prevention of good insulin resistance. In the study of Anggraeny et al (2017), the water fraction of dandang gendis leaves had antioxidant activity which was relatively better compared to other extracts and fractions and had an EC50 value of 532.24 $\mu\text{g} / \text{mL}$.

Based on the description, the study was carried out on the water fraction of dandang gendis leaves to determine the effect of decreasing blood sugar levels in Wistar male rats induced by fructose and high-fat feed. .

2. Research Methodology

2.1 Material

The samples used in this study were dandang gendis leaves obtained from temukencono, Semarang, pork oil: duck egg yolk (1), fructose 1.8 g / kg body weight (BW) rat, feed BR II, metformin as a positive control with a dose of 0.126 g / kg BW rat, male white rat wistar strain aged 1-2 months weighing 150-200 g.

2.2 Tools

Mouse cages, oral sonde, and drinking places, ohaus scales, mikrolab 300 (EliTech Selectra), tube racks, glassware from pyrex, micropipets (Skoret, Switzerland), centrifuges (Hettichzentrifugen EBA 20), hematocrit needles (Nesco), digital scales (Shimadzu), ependrof

2.3 Methods

2.3.1 Sample Preparation

Dandang gendis leaves were cleaned from the dirt and unused parts, then washed with running water until clean. After being drained, the leaves were dried using a dryer cup at a temperature of approximately 40-50°C for 1-2 the day until dry evenly. Then, the simplicia was ready to be mashed and sieved with 30 and 40 mesh sieve. Two hundred g of dandang gendis leaves powder was extracted using 96% maceration method of ethanol in 2 liters for 5 days. The aqueous extract was separated from ethanol solvent using a rotary vacuum evaporator at 40°C. The extract was fractionated in stages with the liquid-liquid partition method using a separating funnel. Fractionation was carried out by 10 g of concentrated ethanol extract dissolved with 100 mL of distilled water and stirred until all extracts were dissolved. Then the mixture was put into a separating funnel with a capacity of 250 mL and fractionated using 100 mL of n-hexane solvent to make a shuffle. Two layers would be produced and a layer of water needed to be taken. This process was repeated 3 times. The water residue obtained was then fractionated using 100 mL ethyl acetate solvent to do the same as the treatment of n-hexane solvent. The mixture was shaken and left to form two layers, namely the ethyl acetate fraction and the water fraction. The two layers were separated and then the n-hexane fraction, ethyl acetate fraction, and the water fraction obtained were evaporated using a waterbath at a temperature of 80°C. The fraction obtained was weighed and the rendering value was calculated.

2.3.2 Phytochemical screening of dandang gendis leaves

Preliminary tests were carried out to determine the efficacious substances contained in dandang gendis leaves, the substances tested included identification of flavonoids, alkaloids, saponins, and tannins.

2.3.3. Animal Test Treatment

Experimental animals were 35 rats adapted for \pm 7 days. Furthermore, 10 rats were not induced and 25 rats were induced by fructose 1.8 grams / kgBW of rats and high-fat feeds (lard and duck egg yolks (3: 1)) for 30 days before data were measured as data (days after induction). Test animals are then taken blood through the eye vein.

Group I (negative control): Fructose 1.8 g / kgBW + High Fat Diet from day 1 to 30 (for 30 days), and CMC Na suspension 0.5% orally from 31 to 45 days (for 14 days) per day orally.

Group II (positive control): Fructose 1.8 g / kgBW + High Fat Diet from 1 to 30 days (for 30 days) and metformin at a dose of 0.126 g / kgBW mice from days 31 to 45 (for 14 days) orally.

Group III, IV and V: Fructose 1.8 g / kgBW + High Fat Diet from day 1 to 30 (for 30 days) days and suspension of water fraction of dandang gendis leaves 15.89 mg / KgBW; 31.78 mg / KgBW; 47.67 mg / KgBW of mice from 31 to 45 days (for 14 days) orally.

2.3.4. Blood glucose measurement

Test animals were fasted 8-10 hours after the last treatment. Examination of fasting blood glucose levels is done after a person does not eat and drink calorie drinks 8-10 hours. Patients can still drink water during the period of time (Yanuar, 2015; 34), then the blood of the test animals is taken through the eye vein. Measurements using rat blood serum are made by centrifuging the blood of rats at 3000 rpm for 10 mins until serum is formed. Then the serum was taken 10 μ l and the 1000 μ l Glucose PAP reagent was measured using mikrolab 300. Blood glucose levels will be read in mg / dL units.

3. Results And Discussion

Dandang gendis powder was macerated with 96% ethanol solvent for 5 consecutive days, then fractionated to get the water fraction of dandang gendis leaves. Next, the water fraction of Dandang Gendis leaves was tested for phytochemical screening to determine the active compounds contained in the water fraction which were thought to play a role in decreasing blood glucose levels. The results of the chemical screening test of the water fraction of dandang gendis leaves can be seen in Table 1.

In Table 1 the results of phytochemical screening tests showed that the water fraction of dandang gendis leaves contained flavonoids, alkaloids, saponins, steroids, and tannins.

Table 1. Results of Qualitative Testing of Ethanol Extracts and Water Fraction of Daun Dandang Gendis (*Clinacanthus nutans*).

Group of Compound	Reactor	Positive results (reference)	Research Result Water Fraction
Fenolic compound	FeCl ₃ 1%	Green, red, purple, blue/ black solution (Robinson, 1995)	(+) Black solution
Polifenol	Kalium Heksasionat (III) + FeCl ₃ 1%	Blackish blue solution (Hanani dan Mun'im, 2005)	(+) Blackish blue solution
Tanin	FeCl ₃ 1%	Black or dark blue (Sa'adah, 2010)	(+) Black or dark solution
Flavonoid	Powder Zn + HCl (p)	Orange colored solution (Depkes RI, 1995)	(+) Orange colored solution
Alkaloid	HCl + Dragendorff	Brown/ red brown sediment (Depkes RI, 1995)	(+) Red brown sediment
Saponin	Sharken + HCl 2N	A stable foams formed (Depkes RI, 1995)	(+) Stable foam
Steroid	Ether+ acetic acid anhydrous + concentrated sulfuric acid	Blue or Green solution (Harbone, 1987)	(+) Bluish green color

Information :

(+) Indicates the presence of a test compound

(-) Indicates no test compound

Based on the results of the qualitative test it was found that the water fraction of dandang gendis leaves contained flavonoids, steroids, alkaloids, saponins, phenolic compounds, tannins, and polyphenols. According to Yuana (2008; 101) that the extract of dandang gendis leaves contains phytochemical compounds such as alkaloids, steroids, and tannins. Determination of the dose of the water fraction of dandang gendis leaves was based on a previous test conducted by Ramlan (2011; 259) that extracts of ethanol at a dose of 100 mg / kgBW of mice can reduce blood glucose levels in mice. From this dose, it is converted first to the dose of the mouse with a multiplier factor of 7.0. Then it is converted again from the extract dose to the dose of the water fraction so that it gets a dose of 31.78 mg / kgBW mice. Furthermore, from the dose three dosage levels were made, dose 15.89 mg / kgBW, dose 31.78 mg / kg BW, and dose 47.67 mg/ kg BW rat.

Measuring blood glucose levels of mice was done 3 times, namely on day 1, day 31 and day 45. Measurement of the 1st day to determine the blood glucose level of the rats early or before treatment. On day 1 in all groups shows a range of 111-134 mg / dL. This value is in accordance with the literature of Mary and Charles (2008: 8) which states that the normal value of glucose levels in male rats is 70-208 mg / dL. Then on the 31st day, blood glucose levels were measured in all groups to determine the success of induction from high-fat fructose and feed. On the 45th day blood glucose levels were measured in all groups to show the presence of antioxidant activity from the water fraction of the dandang gendis leaves (*Clinnacanthus nutans*) which was given and calculated the percentage reduction in blood glucose levels. The results of measurements of blood glucose levels in the mean value \pm SD can be seen in Table 2.

Table 2. Average Blood Glucose Levels of All Tested Animal Treating Groups and Percentage of Decrease in Blood Glucose Levels

Groups	Blood glucose level (mg/dL)			% difference of day 45 – day 31
	Day 1 X \pm SD	Day 31 X \pm SD	Day 45 X \pm SD	
Normal	115,2 \pm 27,82	103 \pm 19,17	92,4 \pm 34,02	-10,73 ^b
Control (-) (CMC-Na 0,5%)	111 \pm 34,55	226,4 \pm 61,97	232,4 \pm 57,05	+7,78 ^b
Control (+) (metformin)	113,2 \pm 18,34	225,8 \pm 78,64	85,2 \pm 24,89	-57,96 ^a
Dosage 1	121 \pm 14,98	207 \pm 52,63	114,4 \pm 23,80	-41,39 ^a
Dosage 2	134,2 \pm 11,76	201,2 \pm 29,24	79,2 \pm 18,86	-59,87 ^a
Dosage 3	115,6 \pm 36,69	2235 \pm 47,43	88,4 \pm 28,31	-60,38 ^a

a Different letters show significant difference values with negative control
 b Different letters show significant difference values with positive control

Blood glucose level before and after treatment

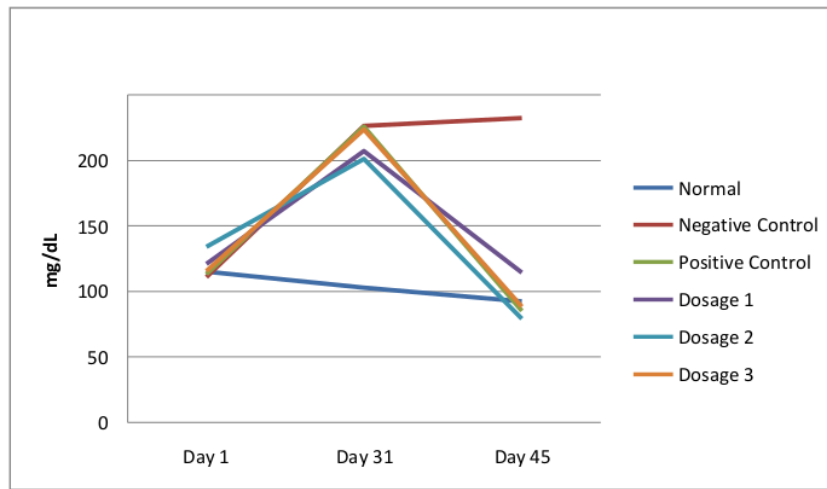


Figure 1. Average blood glucose level before and after treatment

Figure 1 showed that in the normal group there was no improvement in blood glucose levels. While induction groups that were given fructose 1.8 g / kgBW of mice and high- fat feed for 30 days could increase glucose levels of all induction groups. It is also evident from the results of a statistical test of Paired-Samples T Test between days prior to induced after induction in each group had a p-value <0.05, which means there is a significant difference between the blood glucose levels before and after induction for all groups induced. While the average blood glucose level results are shown in Table 2. Based on normality and homogeneity test, the data were normal and homogeneous. Then, the data of percentage of blood glucose levels were analyzed using one-way ANOVA test and Post Hoc Test with results written in Table 3.

Table 3. Recapitulation of Post Hoc Statistical Test Results Percentage of Decrease in Blood Glucose Level

Groups	Sig.	Conclusion
Normal Vs Negative	0,212	Did not differ significantly
Normal Vs Positive	0,003	Differed significantly
Normal Vs Dosage 1	0,044	Differed significantly
Normal Vs Dosage 2	0,002	Differed significantly
Normal Vs Dosage 3	0,002	Differed significantly
Negative Vs Positive	0,000	Differed significantly
Negative Vs Dosage 1	0,002	Differed significantly
Negative Vs Dosage 2	0,000	Differed significantly
Negative Vs Dosage 3	0,000	Differed significantly
Positive Vs Dosage 1	0,262	Did not differ significantly
Positive Vs Dosage 2	0,896	Did not differ significantly
Positive Vs Dosage 3	0,869	Did not differ significantly
Dosage 1 Vs Dosage 2	0,213	Did not differ significantly
Dosage 1 Vs Dosage 3	0,201	Did not differ significantly
Dosage 2 Vs Dosage 3	0,972	Did not differ significantly

The results of the Post Hoc test on Table 3 above between the positive group and the dose rank group showed results ($p > 0.05$), this shows that the positive control of metformin is not as significant as the dose group. Whereas in the test between groups of dose 1 with dose 2 and dose 3 obtained value ($p > 0.05$) this shows that the presence of the dose rating does not show a difference in blood glucose levels. So that the dose of 15.89 mg / kgBW is the most effective dose that can reduce blood glucose levels of fructose-induced mice and high-fat feeds. This is in accordance with the study of Ramlan (2011) which concluded that the percentage of accumulated decreases in blood glucose levels of mice after administering ED₅₀ at 75 mg and 100 mg / kg BW was not much different compared to glibenclamide dose of 1 mg / kg BW. This means that the ethanol extract of the dandang gendis leaves can rapidly decrease the blood glucose level of mice. Yuana (2008) said that the water extract of dandang gendis leaves (Clinacanthus nutans) dose of 150 mg / kg BW can reduce blood glucose levels in alloxan-induced diabetic mice.

The use of biguanid metformin with dose of 0.126 g / kgBW mice, as a positive control because based on the most recent type-2 diabetes mellitus management guidelines from the American Diabetes Association / ADA / EASD and the American Association of Clinical Endocrinologists / American College of Endocrinology (AAACE / ACE) recommends giving metformin as monotherapy first. This recommendation is mainly based on the effect of metformin in reducing blood glucose levels, the price is relatively cheap, efek samping more minimal and does not increase body weight [10].

Flavonoid compounds in plants and fruits are often used as blood glucose levels because they act as glucosidase inhibitors. The glucosidase enzyme is located in the brush border in the small intestine and is needed for the breakdown of carbohydrates before being absorbed as monosaccharides. α -glucosidase inhibitors delay absorption of carbohydrates obtained from food, so they can reduce blood glucose levels after eating [9]. in plants and fruits that are often used as blood glucose levels are flavonoid

compounds. Flavonoids are compounds such as phenols which act as glucosidase inhibitors. The glucosidase enzyme is located in the brush border in the small intestine and is needed for the breakdown of carbohydrates before being absorbed as monosaccharides. Alpha-glucosidase inhibitors delay absorption of carbohydrates obtained from food, so they can reduce blood glucose levels after eating [9].

1 Conclusion

Giving the water fraction of dandang gendis leaves (*Clinacanthus nutans*) can reduce blood glucose levels in male wistar rats induced by fructose and high-fat feed. The dose of 15.89 mg / kg BW is the most effective dose in reducing blood glucose levels and has an effect comparable to metformin

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