

TURNITIN_The_Effects_of_Coloc asia_esculenta

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The Effects of *Colocasia esculenta* Leaf Extract in Inhibition of Erythrocyte Aldose Reductase Activity and Increase of Haemoglobin in Experimental Rats

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Summary Diabetes Mellitus (DM) has reached a number of 382 million in 2013 and expected to rise to 592 million by 2035. Chronic diabetes can lead to impaired formation of erythropoietin in haemoglobin production and may cause anemia. Inhibition of aldose reductase is a key point of diabetes treatment and prevention of complications in diabetes. *Colocasia esculenta* (CE) leaf is one of Indonesian vegetables which has inhibition effect on aldose reductase activity. This research was a true experimental study with post-test only group design. 21 male Sprague dawley rats were divided into: K (control group), P1 (extract CE 200 mg/KgBW) and P2 (extract CE 400 mg/KgBW). Rats were induced to become obese with High Fat Sucrose Diet (HFSD) for 4 wk then extract CE were given for 3 wk. The data were analyzed with independent *t*-test. CE have a significant effect to increase haemoglobin but have no significant inhibition effect to erythrocyte aldose reductase activity. The results of this research found that the mean haemoglobin of control group was 13.14 ± 1.55 , treatment group 1 (P1) was 15.22 ± 0.59 , and treatment group 2 (P2) was 15.77 ± 0.71 . There was significant increase in haemoglobin ($p < 0.05$). The mean of aldose reductase activity of treatment group was lower than control group. However, there was no significant difference found ($p > 0.05$) between the groups. 200 mg/kgBW and 400 mg/kgBW dose of CE could increase haemoglobin and decrease the mean of aldose reductase activity.

Key Words *Colocasia esculenta*, diabetes, aldosa reductase, haemoglobin

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Diabetes Mellitus (DM) is a metabolic disease characterized by high blood sugar levels and impaired carbohydrate, protein, and lipid metabolism as a result of insulin insufficiency. Insufficiency insulin can be caused due to a disruption of insulin production by Langerhans beta cells of the pancreas gland or caused by the lack of responsiveness of body cells to insulin. The estimated number of populations with DM from year to year is predicted to increase. In 2000, there were 171 million people (2.8%) with DM and it is predicted to increase up to 366 million people (4.4%) by 2030 (1, 2).

Obesity is one of the risk factors for abnormal glucose homeostasis that triggers oxidative stress which can reduce sensitivity insulin, resulted in hyperglycemia and hyperinsulinemia. Diabetes Mellitus is characterized by high blood sugar levels (hyperglycemia). On hyperglycemia, there is an increase in glucose input to the polyol pathway, which causes the increase in the mechanism of replication and increased production of reactive oxygen species (ROS). In the polyol pathway, there is a reduction process of glucose into sorbitol which is cata-

lyzed by aldose reductase (AR). Therefore, hyperglycemia condition will cause the increased accumulation of AR and sorbitol. Increased glucose in blood plasma affects red blood cells (erythrocytes) and vascular endothelial cells, including the decrease of oxygen affinity for erythrocytes, decreased Na^+ and K^+ activity, increased sorbitol levels in erythrocytes, decreased erythrocyte life, increased fragile osmotic, and increased membrane viscosity. However, activity of aldose reductase enzyme can be inhibited by Aldose Reductase Inhibitor (ARI) (3–6).

Utilization of AR inhibitors is a key point to prevent DM complications and for DM treatment. ARI has been developed from herbal plants. One of the plants that has inhibition activity of Aldose Reductase is taro leaves (*Colocasia esculenta* L. Schoot). *Colocasia esculenta*, also known as taro, cocoyam, dasheen, or talas. Taro leaves have many phytochemical contents, including flavonoids, such as orientin, isorientin, isovitexin, vicenin-2, orientin 7-O-glucoside, isovitexin 3'-O-glucoside, vitexin X-O-glucoside, and luteolin 7-O-glucoside. Taro leaves are proven to have antioxidant activity, antihyperglycemic, and have inhibitory effect on aldose reductase activity. The aim of this was to examine the effect of

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Table 1. Effect of intervention on taro leaf extract.

Group	Non-Intervention		Intervention				p
	K (x±SD)		P1 (x±SD)		P2 (x±SD)		
	Pre	Post	Pre	Post	Pre	Post	
Aldose reductase (ng/gHb)	39.62±12.02	45.76±5.33	39.62±12.02	43.7±5.79	39.62±12.02	42.82±4.14	0.45**
Haemoglobin (g/dL)	13.6–17.7	12.8 (11.4–16.3)	13.6–17.7	15.4 (14.10–15.7)	13.6–17.7	15.55 (15.10–17.40)	0.00**
Weight (g)	207.86± 26.13	257±33.73	206.5±28.49	235.54±29.14	216.33±30.43	247±29.6	0.00*

Paired t-test ($p < 0.05$).

** Anova One Way ($p < 0.05$).

K=Control.

P1=taro leaf extract 200 mg/kgBW.

P2=taro leaf extract 400 mg/kgBW.

taro leaf extract in inhibiting erythrocyte aldose reductase activity and increase of haemoglobin (7–9).

MATERIALS AND METHODS

Preparation. The following *Colocasia esculenta* leaf extract were used: The extraction of *Colocasia esculenta* (CE) into ethanol 96%. Extracts ethanol of *Colocasia esculenta* (EECE) contained flavonoid 2,041.316 mg/100 g and fenol 3,089.869 mg/100 g respectively. The nutritional studies were conducted using 21 male Sprague Dawley rats, which were divided into three groups. The rats were induced to become obese with high fat sucrose diet (HFSD) for 28 d, then continued with taro leaf extract diet for 21 d. Every day, all animals were fed ad libitum and allowed free access to distilled water. Rats were housed in individual cages in a well-ventilated room.

Animals and diets. The experimental protocol was reviewed and approved by the Ethics Committee of Medical Faculty of Diponegoro University. After the adaptation period, the rats were randomly divided into three groups ($n=7$): the control group (K=HFSD Diet), P1 (HFSD+taro leaf extract 200 mg/KgBW), P2 HFSD+taro leaf extract 400 mg/KgBW. The rats had free access to food and water. Food consumption and weight gain were measured weekly.

Blood biomarkers. Erythrocyte aldose reductase levels were measured by enzyme immunoassay using ELISA KIT. Haemoglobin was measured by hematology analyzer.

RESULTS

The yield of the utilized fresh taro leaves was 81.97% due to the process of leaves and stem separation. From fresh taro leaves, it resulted in leaf powder with yield of 12.57%, because some powder residues were insufficient to the qualified. The water content of taro leaf powder is 6.9%, this is in accordance with the water content which should not exceed 10%. Water content of less than 10% will prevent the rapid growth of fungi.

Extraction with Soxhletation method was chosen because it produced more extracts compared to other extraction methods, this was in accordance with the amount of yield obtained which was 27.24%, or as much as 57.2 grams from 200 grams of taro leaf powder.

Based on statistical analysis in Table 1, there was no significant difference in erythrocyte reductase aldose ($p > 0.05$). There was no difference in erythrocyte reductase aldose levels in the taro leaf extract group with doses of 200 mg/kgBW and 400 mg/kgBW. This was due to the absence of a significant substrate increase on hyperglycemia and the rats were still in sub-acute diabetes mellitus condition. However, there was a significant difference in haemoglobin and body weight ($p < 0.05$).

DISCUSSION

The HFSD (High Fat Sucrose Diet) uses a combination of a high-fat and high-sucrose diet. It consists of high-fat diet using lard and duck egg yolk, 2% myristic fatty acids, 25% palmitic fatty acids, 15% stearic fatty acids, 45% oleic fatty acids, and 9% linoleic fatty acids, egg yolks containing 17 g of protein, 35 g of fat and 884 mg of cholesterol/100 g so high-fat diet can increase body weight (10). It also consists of high sucrose diet by adding sucrose with the dose of 5.625 g/kg body weight to rats (11).

A high-fat diet can cause in weight gain, it can also cause insulin resistance and increased blood glucose levels, and increased risk of diabetes mellitus-2. This due to high-fat diet can cause high levels of fat in the blood so that it can reduce the ability of insulin substrate receptors to activate P1–3kinase and cause GLUT 4 to increase, thus the decrease of GLUT 4 expression will cause the glucose transport into membrane, so the activity of glucose transport decreases, and resulting in the increase of blood glucose levels.

In obesity, glucose tolerance can be disrupted. Increased free fatty acids and glycerol via vein portal due to lipolysis, will affect on the increased oxidation of

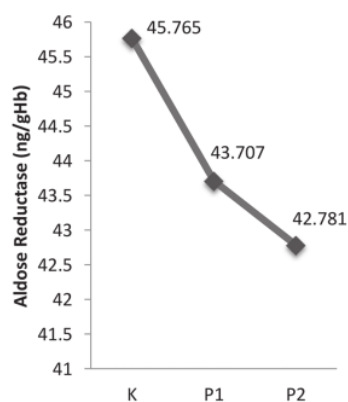


Fig. 1. Graph of Erythrocyte Aldose Reductase.
K=Control.
P1=taro leaf extract 200 mg/kgBW.
P2=taro leaf extract 400 mg/kgBW.

free fatty acids in the muscles and liver which ultimately will be used as alternative energy sources. Glucose that is not used will remain in the blood vessels, so it results in high circulation of glucose levels. A high sucrose diet will increase blood glucose, increase fat deposits, and body weight in rat (12, 13).

Aldose reductase (AR) is an enzyme responsible for the conversion of glucose to sorbitol in glucose metabolic polyols pathway. AR is involved in the pathophysiology of diabetes complications because this enzyme plays an important role in glucose metabolism. Aldose reductase acts to catalyze various substrates and regulate cellular signals initiated by various oxidants. Aldose reductase is found in erythrocytes, retina, lens, cornea, glomerulus, and nervous system (4, 14).

In the path of polyol, Aldose Reductase (AR) catalyzes the reduction of glucose into sorbitol. Sorbitol is converted to fructose with the help of sorbitol dehydrogenase. In both reactions, NADPH and NAD⁺ are needed as cofactors. AR enzyme is the first enzyme on the polyol pathway and it converts glucose to sorbitol in the presence of NADPH as cofactor. The activity of aldose reductase enzyme can be inhibited by Aldose Reductase Inhibitor (ARI) (14).

Erythrocyte aldose reductase has a physiological role, one of its physiological roles is to carry out fat products containing inflammation. Inflammatory signals are induced by cytokines, growth factors, endotoxin, high amounts of growth, allergens, and autoimmune reactions. Aldose reductase will increase in hyperglycemic conditions. Based on figure 1, In group P1 and P2 the mean red cell aldose reductase was lower than the control group. Intervention group had lower erythrocytes aldose reductase than control group. Intervention taro leaf extract can make erythrocytes aldose reductase levels decrease on obese rat. This is related to the effectivity of taro leaf extract which has the ability to inhibit erythrocyte aldose reductase enzyme.

The concentration of sorbitol in the cell is low under

normal conditions, while in hyperglycemia the concentration of sorbitol is high. The formation of sorbitol causes hyperglycemia, since sorbitol is a substrate used by aldose reductase. Inhibition of the aldose reductase enzyme will reduce the buildup of sorbitol in the tissue and this will inhibit the formation of fructose. When fructose formation can be inhibited it will cause the inhibition of the non-enzymatic glycation process that can produce AGE (7).

Aldose Reductase Inhibitor (ARI) from taro leaf extract can prevent or inhibit damage due to hyperglycemic. When hyperglycemic damage occurs, cytokines, growth factor (GF), and lipopolysaccharide (LPS) will produce ROS by forming toxic lipid aldehydes such as HNE through lipid peroxidation process. HNE will conjugate with cellular glutathione (GSH) to Glutathionyl-HNE (GS-HNE). GS-HNE will be reduced to GS-DHN by aldose reductase. GS-DHN will transduce inflammatory signals through cascade of protein kinases that cause activation of Nf- κ B. Activation of Nf- κ B will transcribe the genes responsible for various inflammatory pathologies. Inhibition of aldose reductase will prevent cytokines, growth factor (GF), and lipopolysaccharide (LPS) from inducing cytotoxic signals. In addition, the inhibition of aldose reductase will prevent the activation of inflammatory signals (4, 5, 15).

Aldose reductase is active if intracellular glucose exceeds the limit of hyperglycemia. The increasing flux of aldose reductase causes a decrease in the ratio of NADPH and NADP⁺ cells, which causes disruption in the formation of NO in endothelium. The disruption of endothelial NO formation will cause a decrease in the level of e-NOS which causes NO bioavailability to be decreased. An increase in aldose reductase flux will also cause an increase in the ratio of NADH and NAD⁺ which will cause the production of several inflammatory mediators. Aldose reductase in reducing glucose using NADPH. NADPH besides being needed by Aldose Reductase as a cofactor is also needed in the formation of GSH (Glutathione) from GSSH. The decreasing ratio of NADPH will cause damage to cellular antioxidants and glucose metabolized to sorbitol by aldose reductase, so it will be oxidized to fructose and produce a product in the form of NADH. NADH has the potential to cause an increase in ROS through NADH oxidation (4, 7, 16).

Erythrocyte transports oxygen from the lungs to tissues and carbon dioxide from tissues to the lungs to be excreted. Oxygen cannot dissolve well in water, therefore it needs special transports. Haemoglobin in erythrocytes can carry oxygen per liter (70 times greater than dissolved oxygen). Hb levels can be used to determine anemia. In male Sprague Dawley rats, the normal concentration of haemoglobin is ranged between 13.6–17.7 g/dL (17).

Based on Fig. 2, the results showed that Hb levels of control group can be used to determine anemia, since diabetes is often accompanied by anemia. P1 and P2 groups had higher haemoglobin value than K group. The results showed that treatment groups had higher haemoglobin value than the control group. The results

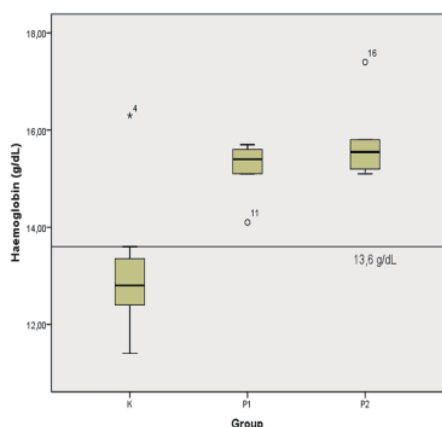


Fig. 2. Graph of Haemoglobin.
K=Control.
P1=taro leaf extract 200 mg/kgBW.
P2=taro leaf extract 400 mg/kgBW.

showed a trend of haemoglobin increase in the treatment groups compared to the control group. CE doses of 200 mg/kgBW and 400 mg/kgBW dose were able to increase haemoglobin. The result showed that extract taro leaf can increase haemoglobin and reduce the risk of anemia. This is due to the compounds in taro leaf extract that can support the process of hematopoiesis. The compounds in taro leaf extract triggers erythropoiesis process, inhibits the process of oxidative erythrocyte damage, reduces the fragility of erythrocyte membranes, reduces the levels of free radicals so that haemoglobin level in the blood is well-maintained. Decreasing Hb levels will cause a reduction in the ability of red blood cells to carry oxygen throughout the body (18, 19).

Haemoglobin is a compound of the complex bonds of protein and Fe. Haemoglobin has an important role in transporting oxygen in the body. The amount of haemoglobin in the blood is influenced by the adequacy of nutrition. The components that make up haemoglobin are amino acids, glycine and minerals. High and low levels of haemoglobin can also be affected by age, species, environment, feed, and state of erythrocyte damage (erythrocytosis), so the more nutrition is consumed, the faster the haemoglobin synthesis process become. (20).

Disclosure of state of COI

No conflicts of interest to be declared.

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