

BLOOD ANTHOCYANIN LEVELS OF HEALTHY AND DIABETIC RATS AFTER FEED WITH A SINGLE DOSE OF PURPLE SWEET POTATO TUBERS AQUEOUS EXTRACT

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Background: Aqueous extract of purple sweet potato tuber shown to decrease the blood glucose levels and has antioxidant properties in rats with streptozotocin-induced diabetes. The purpose of this study is to prove that an increase in anthocyanin levels in the blood of rats after administration of aqueous extract of purple sweet potato tubers in healthy and diabetic rats. **Methods:** This is an experimental study with randomized post-test only control group design. The subject of experiments were 72 rats, divided into two major groups, namely diabetic rats (treatment group 36 rats) and control group (36 healthy rats). Each major group was divided into 6 small groups each 6 rats. The control group was given aqueous extract of purple sweet potato tuber with a dose of 4 ml single dose. The diabetic group were given streptozotocin and followed by aqueous extract of purple sweet potato tuber 4 ml single dose. Observed variables include: blood glucose levels to define diabetes. To determine the levels of anthocyanin, the blood were taken and the level of anthocyanin were examination by HPLC method, in healthy and diabetic rats after 30 min, 1 hours, 2 hours, 4 hours, 8 hours and 16 hours of giving single dose of aqueous extract of purple sweet potato tubers. **Results:** The results showed an increase in blood sugar levels were significant ($p < 0.05$) in group given streptozotocin compared to control. Anthocyanin levels in the blood of two groups were significantly different from 30 min after administration of the extract to 8 hours after administration of the extract ($p < 0.05$). Higher anthocyanin levels ($p < 0.05$) was observed in control group (healthy rats) compared to rats with streptozotocin-induced diabetes. Peak levels of anthocyanin in blood were achieved after 2 hours of feeding. **Conclusion:** The conclusion of this study is the absorption of anthocyanin in aqueous extract of purple sweet potato tuber better in healthy rats compared to diabetic rats. The peak levels of anthocyanin was reached after 2 hours of aqueous extract of purple sweet potato tubers single dose consumption either in healthy rats and in diabetic rats.

Keywords: blood, glucose, anthocyanin, rats, diabetic.

INTRODUCTION

Diabetes mellitus (DM) is a health problem in almost all countries around the world, including Indonesia. Hyperglycemia that occurs in diabetic patients will increase the formation of advanced glycation end products/AGEs resulting in oxidative stress.^{1,2} Several studies have shown that administration of antioxidants in diabetic patients can overcome macrovascular, microvascular complications, cope tissue damage due to oxidative stress.^{1,3} Further research proved that oxidative stress can be prevented by various types of food, because micronutrient which is phytochemical group of various food ingredients, derived from

plants have a protective effect against oxidative stress.^{4,6} There are various types of natural antioxidants derived from plants belonging to flavonoids, one of which is the pigment anthocyanin, which the pharmacokinetic profiles mostly unknown.⁷

Purple sweet potato in Bali have been studied and relatively has high levels of anthocyanin,⁸ and has antioxidant effects on the blood and various organs in oxidative stress of mice.^{9,10} Aqueous extract of purple sweet potato tuber can maintain blood sugar levels and increase the total antioxidant in rats given high doses of glucose load.¹¹ Anthocyanins in addition with antioxidant properties also have blood sugar lowering effect as anthocyanin can improve insulin secretion by pancreatic beta cells.^{7,12} Study in the year of 2012

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has proven that the aqueous extract of purple sweet potato tuber prevent oxidative stress and protects the pancreas in rats with streptozotocin- induced diabetes. That suspected the anthocyanin content of aqueous extract of sweet potato tuber can be absorbed in the gastrointestinal tract both in healthy mice and diabetic rats. To prove these allegations this study conducted in healthy rats and in diabetic rats induced by streptozotocin. The rats were then given a purple sweet potato tuber water extracts a single dose 4 ml of each rats. The problem is whether the anthocyanin levels in the blood of these rats increase.

MATERIALS AND METHOD

Aqueous extract of purple sweet potato tubers

Aqueous extract of purple sweet potato tubers were made in the following manner: purple sweet potato tuber, aged 3-4 months obtained from farmers at Tabanan, Bali-Indonesia, washed with clean water and then peeled. Once peeled sweet potatoes were cut into pieces (thickness: 2 - 2.5 cm). Pieces are mixed with water in a ratio of 1 kg of sweet potato with 1 liter of water and then blended and filtered with three layers of gauze. The filtrate obtained was heated to boiling for 30 minutes.

Animal Models and Blood Examination

This study applied randomized post-test only control group design. Samples were 72 male rats, aged of 3-4 months obtained from the Laboratory of Pharmacology Faculty of Medicine, University of Udayana, Bali-Indonesia. Samples were divided into 12 groups with 6 rats per group. Group 1, 2, 3, 4, 5 and 6 are only given standard feed as the control group. Group 7, 8, 9, 10, 11 and 12 are diabetic rats group induced by streptozotocine. Rats blood glucose level were checked after 3 days streptozotocine administration to diabetic group. All groups of rats were given water extract of purple sweet potato tuber at a dose of 4 ml with oral. The blood of rats were taken after 30 minutes, 1 h, 2 h, 4 h, 8 h and 16 h for group 1, 2, 3, 4, 5, and 6 as control group and the group 7, 8, 9, 10, 11 and 12 as diabetic group of rats. Blood anthocyanin level was determined by employing HPLC method.

Statistics Test

The data obtained are presented as mean \pm SD and evaluated by independent t test. Results within $p < 0.05$ were accepted as statistically significant results.

RESULTS

Blood Glucose Levels

The fasting blood glucose level of streptozotocin-induced diabetic rats was measured

after 3 d of streptozotocin-induced. The results of blood glucose level in both groups of rats are presented in Figure 1. Average blood glucose in the control group was 100.93, whereas in the group given streptozotocin was 358.94. Statistically, the average is significantly different ($p < 0.05$).

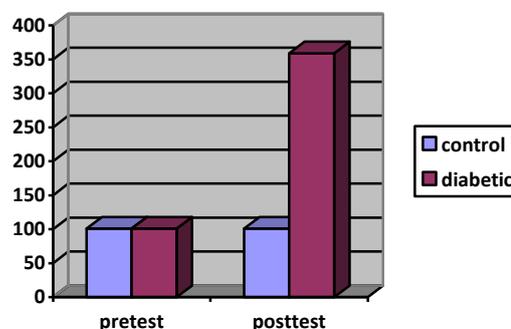


Figure 1

Average blood glucose levels of control groups and diabetic group of rats Blood Anthocyanin Level

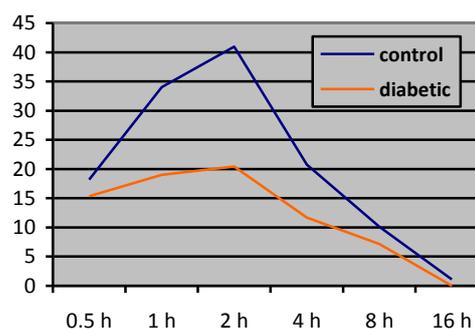


Figure 2

Blood levels of Anthocyanins in Rats

Note: Controls are healthy rats and diabetic are induced diabetic rats with streptozotocin. A number of 0.5 h is the anthocyanin levels in the blood after 30 minutes of administration aqueous extract of purple sweet potato tuber. One hour anthocyanin levels in the blood is 1 hour after administration of the aqueous extract of purple sweet potato tuber. Two hours is the anthocyanin levels in the blood after 2 hours of administration of aqueous extract of purple sweet potato tuber. Four hours is anthocyanin levels in the blood after 4 hours of administration of aqueous extract of purple sweet potato tuber. Eight Hours is the anthocyanin levels in the blood after 8 hours of administration of aqueous extract of purple sweet potato tuber. Sixteen hours is the anthocyanin levels in the blood after 16 hours of administration of aqueous extract of purple sweet potato tuber .

As shown in Figure 2, the anthocyanin levels in the blood of control rats were higher compared

with diabetic rats ($p < 0.05$), after administration single dose of aqueous extract of purple sweet potato tuber. Peak levels of anthocyanin in the blood is achieved after 2 hours after administration single dose of aqueous extract of purple sweet potato tuber. Anthocyanin peak levels in the blood were higher in the control group. Statistically, the average rate was significantly different ($p < 0.05$) with a mean difference 20.49 $\mu\text{g} / \text{ml}$. The levels of anthocyanin in the blood from 30 minutes to 8 hours after administration of single dose aqueous extract always significantly higher in control group ($p < 0.05$). Anthocyanin levels in the blood was very low after 16 hours of administration of both the control and the diabetic rats .

DISCUSSION

The results of this study indicate that anthocyanin levels in the blood of control rats was higher when compared with anthocyanin levels in the blood of rats with streptozotocin- induced diabetes (Figure 2). The peak levels of anthocyanin in the blood is reached after 2 hours of administration in healthy rats and diabetic rats, with peak levels of 40.94 $\mu\text{g}/\text{ml}$ in control rats and 20, 49 $\mu\text{g}/\text{ml}$ in diabetic rats. Anthocyanin levels close to zero after 16 hours of administration . This study proves that the absorption of anthocyanins from the aqueous extract of purple sweet potato tubers in healthy rats and in diabetic model of rats were in different rates significantly ($p < 0.05$). Anthocyanin absorption of aqueous extract of purple sweet potato tuber faster in healthy rats.

In general, the absorption of flavonoids from various plants occur in the small intestine. Generally flavonoid can be absorbed if that flavonoid is binding with the sugar in the form of glucoside, because the glucoside form will be hydrolyzed in the small intestine by β - glucosidase, forming the aglycone form that can be absorbed in the small intestine. While most of the flavonoids bind to the sugar in the form of β - glycosides are not absorbed in the small intestine, but it can be absorbed in the colon in very low amounts after being broken down by bacteria in the colon, resulting in lower blood levels.¹³ It seems that the increase in blood sugar will interrupt the absorption of anthocyanins in the intestine. The results are consistent with studies with cranberry juice (2 cups with a single dose of 94.47 mg total anthocyanin content: tdd 6 types of anthocyanins) was found to reach peak levels in the plasma after 1.5 hours of administration, with very low concentrations of between 0.56 to 4,64 nmol/L after administration of a single dose of juice.¹⁴

Anthocyanins from acai berries in Brazil, which has been used as an antioxidant, administration with single dose of 7 mL/kg in healthy individuals can reach peak levels after 2

hours, with a value of 1138 ng/L.¹⁵ Anthocyanins from various sources appeared to have peak levels between 1.4 to 592 nmol L, after 30 minutes to 4 hours after ingestion. While the metabolism of anthocyanin largely unknown. Although an estimated 68-80 % of the metabolism of anthocyanins can be found in the urine.¹⁶

CONCLUSIONS AND RECOMMENDATIONS

From these results it can be concluded that the aqueous extract of purple sweet potato tuber may increase blood levels of anthocyanin in healthy rats and diabetic rats , with a good pharmacokinetic profile.

There are differences in the pharmacokinetic profiles of aqueous extract of purple sweet potato tubers in healthy rats and diabetic rats, in which the administration of the same dose can achieve higher anthocyanin levels in the blood of healthy rats compared to diabetic rats.

Further research need to be carried out in order to determine the effect of this tube on human.

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