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The Pharmacological Mechanisms of Anthocyanin in Aqueous Extract of Purple Sweet Potato as Antihyperglycemic Herbal Remedy

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Conclusions/interpretation: The acetylated anthocyanin on purple sweet potato tuber could protected the damaging of pancreatic β -cells by induced streptozotocin, reduced the blood glucose into normal level.

Keywords: purple sweet potato, pancreatic β -cell, diabetic rat.

I. INTRODUCTION

The diabetes mellitus (DM) is a chronic disease, which need lifelong therapy. Herbal remedies are most popular choice for this condition. Purple sweet potatoes (*Ipomoea batata* L) tuber is an interlude snacks for Balinese people. The aqueous extracts of purple sweet potatoes, which are ingested into rats, introduced a hypoglycemic effect and prevent oxidative stress [1]. This extract could reduce a blood glucose level on rats, which were administrated high dose

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glucose [2]. The antioxidant agents of this potatoes increased antioxidant enzymes, which is superoxide dismutase (SOD) [3]. The pharmacological mechanism of this hypoglycemic effect is still studied. One of the proposed mechanisms is a protection of pancreatic β -cells by the antioxidant agents of this sweet potato.

The consequence of hyperglycemia on DM patients is a vulnerable pancreatic β -cells to the reactive oxidative stress (ROS). In this condition, the β -cells are not able to compensate the insulin resistance. The hyperglycemia can be a trigger for the free radical production [4]. This state can also lead to glycation reactions, which could be increase, the β -cell apoptosis [5], and also lower levels of antioxidant enzymes in pancreatic tissue such as: catalase, SOD and glutathione peroxidase [6]. This condition introduces the β -cell dysfunction.

On in vitro and in vivo studies were reported that the acetylated anthocyanins in purple sweet potato tubers are the potential antioxidant agent to overcome ROS reaction (7- 10). The aqueous extract of purple sweet potatoes, which were harvested in Bali, contained relatively high anthocyanin [11] and evinced a as prevent oxidative stress and anti-hyperglycemic effect [1,10]. The aim of this study was to describe the mechanism of the acetylated anthocyanins in purple sweet potato tubers to prevent the ROS on pancreatic β -cells of hyperglycemia rats.

II. MATERIALS AND METHODS

Materials: The male Wistar rats obtained from animal house facility of Pharmacology Laboratory, Medicine Faculty, Udayana University, Denpasar-Bali-Indonesia, streptozotocin (Sigma, St. Louis, Mo, USA), animal laboratory, citrate buffer (pH 4.5), sweet potato tubers from Balinese farmers, 5% glucose solution, formalin, ethanol, xylene, aldehyde, phosphate buffered (the chemicals were analysis grade from Merck, Germany), distilled water, commercial pellet diet.

Instruments: Histoplast (Thermo Scientific, UK), microtome Leica 820 (Germany), Gomori's Aldehyde Fuschine stain and counterstaining with Nuclear Fast Red. Briefly (Sigma-Aldrich, USA), blood glucose level control (Roche, Germany), Olympus CX41 Microscope and Optilab camera (Optilab, Indonesia) for five

Langerhans Island each slide at 400X-600X magnification. Quantification was done by ImageRaster software (Optilab, Indonesia).

Aqueous Extract of Purple Sweet potato: The washed and peeled purple sweet potato tubers from Balinese farmers were trimmed into cubical a form 2.0-2.5 cm3. One kg of these cubical forms was mixed with 1 liter water and then was blended. This doughy was filtered through three layers of gauze. The filtered aqueous extract was boiled up for 30 minutes. The extract was keep sterile till the administration.

The male Wistar rats: The study was proved and allowed by the ethic commission of Medicine Faculty - Udayana University on number:792/UN.14.2/Litbang 2012. The 15 male Wistar rats were 3-4 months, 175-225 g weigh. They lived under standard laboratory conditions at $25 \pm 2^\circ\text{C}$, relative humidity ($50 \pm 15\%$) and normal photoperiod (12-hour light-dark cycle). Commercial pellet diet and water were provided ad libitum. The rats were divided into 3 groups and each consisted of 5 rats. Group 1 was a diabetic group, which's induced by intraperitoneal injection single dose of 40 mg/kg streptozotocine in freshly dissolved of citrate buffer (pH 4.5). After injection, the rats had free access on food and water and were administrated 5% glucose solution to drink overnight to counter hypoglycaemic shock. Group 2 was the treated group. The rats on this group were administrated orally aqueous extract of purple sweet potato tuber at the dose of 4 ml/day for 2 weeks along, before intraperitoneal injection single dose of streptozotocin. The administration of aqueous extract of purple sweet potato tuber was continued till 60 days after diabetic induced. Group 3 was a control group, which were administrated a placebo (citrate buffer injection) and normal diet. This study was conducted on randomized control group post-test design.

Blood glucose levels: The blood samples were collected from the retro-orbitalis sinus and the blood glucose level was determinated by orthotoluidine method. The blood glucose levels were controlled in every week of treatment.

Pancreatic β -cell Examination: This test was done at histology labor of medicine faculty of Udayana University, Denpasar, Bali, Indonesia. After the treatment all rats were sacrificed and the pancreas was immediately removed for the examination of β -cell structure. The removed pancreas were immersed in phosphate buffered-formalin 10% for 24 hours, after that dehydrated using serial ethanol 50%, 70%, 80%, 95%, 100% for 2 hours each phase respectively. The Clearing was done using xylene two times for 1 hour respectively. Embedding was done using Histoplast (Thermo Scientific, UK) at 60°C two times for 30 minutes respectively and finally blocking was done. The specimen was trimmed using rotary microtome Leica

820 for 5 um sections and mount on glass microscope slide. Staining for β -cells was done by Gomori's Aldehyde Fuschine stain and counterstaining with Nuclear Fast Red. Briefly, the paraffin section were rehydrated by using xylene two times for 5 minutes each phase, 100% ethanol one times for 2 minutes, 95% ethanol two times for 2 minutes each phase, 70% ethanol for 2 minutes and distilled water for 2 minutes. Immerse slide in filtered aldehyde fuschin stain for 10 minutes and wash in 95% ethanol two times for 30 seconds - 1 minute each phase. Wash slide again briefly in 70% ethanol and distilled water. Immerse slide in Nuclear Fast Red for 5 minutes then washed with distilled water. Dehydrated the slide by immersing in 70% ethanol 20 seconds, 95% ethanol two times each phase 20 seconds, 100% ethanol 20 seconds, xylene two times each phase 2 minutes. Coverslips were mounted on sections using xylene-based mounting medium (DPX). Cells with pink nucleus and purple granules in their cytoplasm are pancreatic β -cells. Photomicrograph was done using Olympus CX41 Microscope and Optilab camera (Optilab, Indonesia) for five Langerhans Island each slide at 400X-600X magnification. Quantification was done by ImageRaster software (Optilab, Indonesia).

Statistical analysis: Statistical analysis was carried out using SPSS for Window 15.0. All data were expressed as mean \pm SD. Groups of data were compared with one way ANOVA. Values were considered statistically significant, when $p < 0.05$.

III. RESULTS

The body weight of rats before treatment was statistically similar ($p > 0.05$). The body weight outgrowth of rats along observation is presented on Fig. 1. The diabetic group rats were losing their body weight significantly compared to other groups ($p < 0.05$).

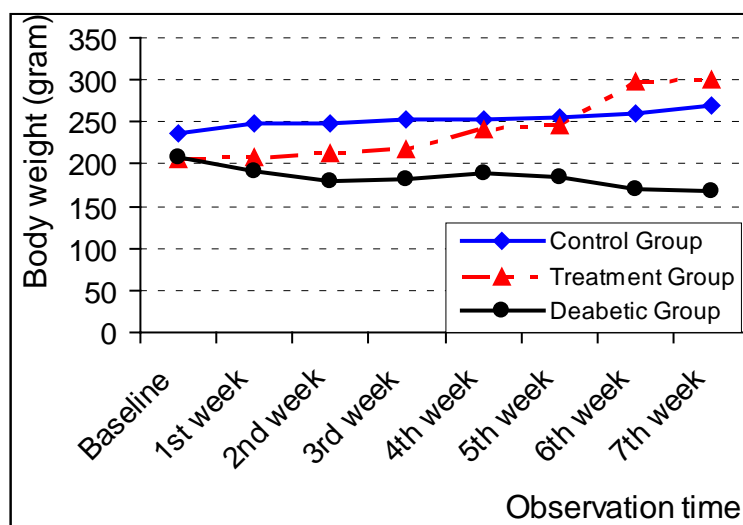


Figure 1 : The outgrowth of rat-body weight during observation

The blood glucose levels of rats before treatment were ranged 115-117 mg/dl. These outgrowth levels are presented on Fig. 2. The blood glucose levels of diabetic group were ranged between (200 - 600) mg/dl with average 422 mg/dl, between (102 - 361) mg/dl with average 152 mg / dl for administrated aqueous purple sweet potato extract group, and

between (113 - 118) mg/dl with average 116 mg/dl for control group, respectively. The blood glucose levels of diabetic group were significantly higher then other groups. The treatment group was presented a significantly lower blood glucose levels then diabetic group ($p < 0.5$).

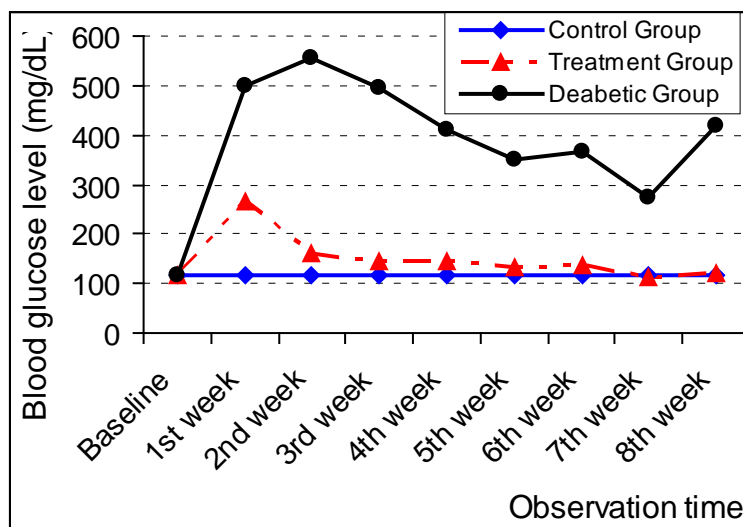


Figure 2 : The outgrowth of blood glucose level during observation

The figure 3 shows the number of pancreatic β -cells of rats after 60 days blood glucose level observation time. The pancreatic histological pictures of all rat-groups are presented in figure 4. The average of β -cell was 50 ± 2.5 cells/5 fields-views for control group, 40 ± 2 cells/5 fields-views for treatment group, and 14.8 ± 0.8 cells/5 fields-views for diabetic group, respectively. The amount of pancreatic β -cells of rats in diabetic group was lowest then other groups and significantly difference ($p < 0.5$).



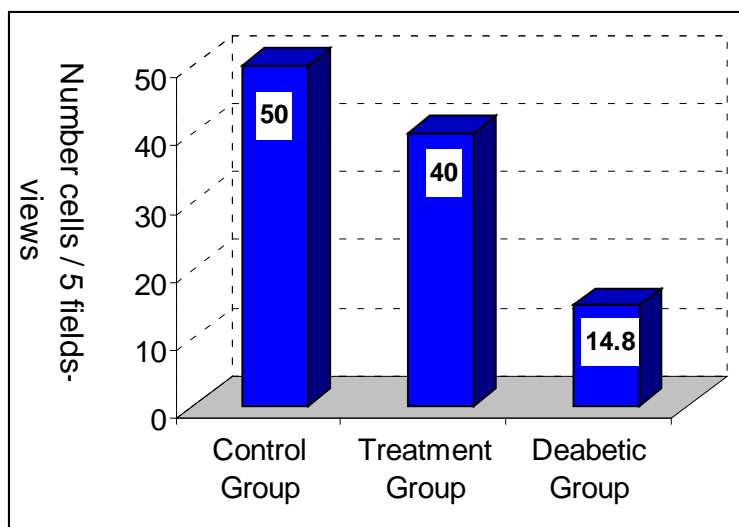


Figure 3 : Comparison of the number of pancreatic β -cells of rats

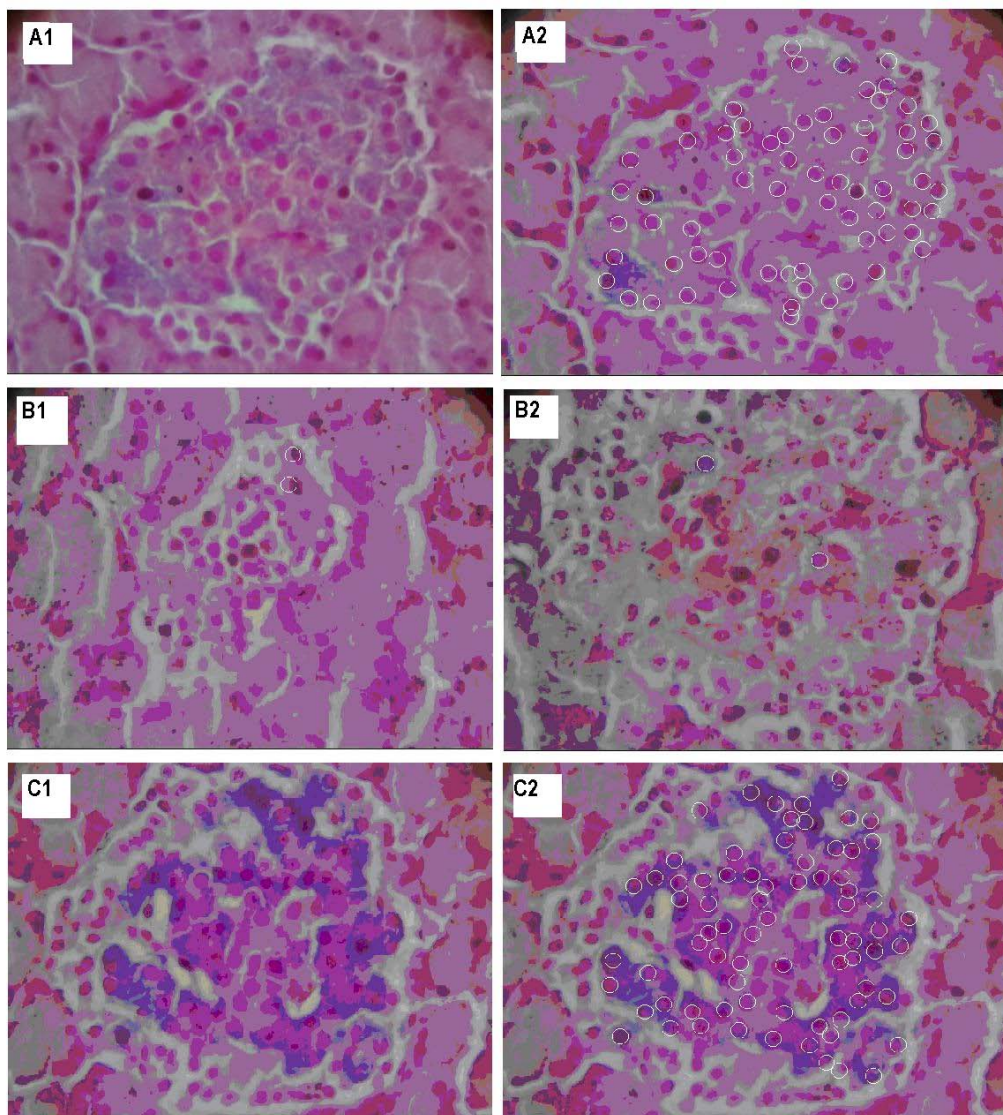


Figure 3 : The pancreatic β -cells of rats in three experimental groups, A: control group, B: diabetic group, C: treatment group, 1: unsigned pancreatic β -cells, 2: the signed pancreatic β -cells, with circle.

IV. DISCUSSION

The induction of intraperitoneal streptozotocin could decrease the amount of pancreatic β -cells and increase the blood glucose level of rats significantly ($p < 0.5$), which are compared to the control group. Administration of streptozotocin induced an oxidative stress in the pancreatic tissue, which due to increased formation of free radical [12]. The streptozotocin was reported has a diabetogenic effect in rats by cytotoxic action on the pancreatic β -cells and increasing free radical generation in pancreatic tissue [13].

The administration of aqueous extract of purple sweet potato tuber before and after induced streptozotocin presented the protection of ROS in pancreatic tissue of rats. The purple sweet potato tuber from Balinese farmers was reported containing a high anthocyanins [11] and has well anti oxidant activity [3]. The protective of ROS on rats-treatment group could due to the contained anthocyanins in aqueous extract of purple sweet potato tuber. The anthocyanin has reported, has ability to prevent apoptosis induced by streptozotocin on pancreatic cells through regulation of caspase-3, Bax, and Bcl-2 proteins [14]. Based on this study, we concluded that the contained anthocyanins on purple sweet potato from Balinese farmers could be covered for ROS after induced streptozotocin in pancreatic β -cells in rats.

The anthocyanin could regulate the blood glucose level by inhibit the alpha-glucosidase [15] and could also increase the phosphorylation of insulin receptor [14]. These effects could prevent the increasing of blood glucose after meal and increase the concerting the blood glucose into glycogen. The administration of the ethanol extract of purple sweet potato tuber from Balinese farmers could maintain the blood glucose level of mice after administrated a high doses glucose load [2]. The high blood glucose level increased intracellular peroxide levels in the pancreatic β -cell, this also will be introduced the intensive ROS in the pancreatic tissue [16]. The study presented, that the blood glucose level of treatment rats group increased to maximum at the first week after induced streptozotocin then decreased along observation time to normal level after the continuing administration of the aqueous extract of purple sweet potato. The study showed that the administration of aqueous extract of purple sweet potato on this study could reduce the blood glucose level into a normal level. The effects of anthocyanin in this potato on damaging protection of pancreatic β -cells, inhibiting the alpha-glucosidase and induction of converting blood glucose into glycogen were the proposed pharmacological mechanisms to the observed outgrowth blood glucose level in the treatment rats group.

The anthocyanin is an important part of the human diet, which can be highly found in purple sweet

potato. This potato can be used as herbal medicine for against ROS-induced degenerative diseases.

V. CONCLUSION

The acetylated anthocyanin on purple sweet potato tuber could protect the damaging the pancreatic β -cells by induced streptozotocin and reduced the blood glucose into the normal level.

Abbreviations

DM	diabetes mellitus
SOD	superoxide dismutase
ROS	reactive oxidative stress
DPX	xylene-based mounting medium
SPSS	statistical package for the social sciences

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