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Intake Purple Sweet Potato (*Ipomoeo Batatas L*) Extract Reduce Level of Blood Glucose, 8-Hidroxyl-2 Deoxiguanosin on Hyperglycemia Wistar Rats and its Pancreatic Cell Histopathology

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Abstract. Hyperglyemia is a condition with an increase of blood glucose fasting levels (above 109 mg/dL) and 2-hour blood glucose post prandial (above 158 mg/dL). Hyperglycemia increases reactive oxygen species (ROS) through the process of enzymatic reactions. This reaction includes oxidation and phosphorylation and *ADPH*-Oxidase *r*eaction and through non-enzymatic process by forming of Gluco oxidants and Glycation . Hyperglycemia is caused by abnormalities in insulin secretion or action of insulin disorders. The state of hyperglycemia in diabetes lead to increase formation of free radicals, antioxidants and decrease a number of events that eventually occurs is called oxidative stress. Hyperglycemia can induce an increase in free radicals through glucose auto-oxidation, the formation of Advanced Glycation End Products (AGEs), and increase of polyol pathway activity (sorbitol). The process outooxidationnon hyperglycemiaandglycation reactions result in the release of electrons. The release of the electrons will trigger the formation of free radicals (RB) particulary superoxide radicals (O_2), and hydrogen peroxide (H_2O_2) and via Haber-Weis and Feton will form hydroxyl radicals (OH·)**.** These materials are known as oxygen free radicals (RBO), which scan damage cell membranes, a lipid peroxides are known to malondialdehid (MDA). Purple Sweet Potato (*Ipomeo Batata L*) extractrich in anthosianin (colorFlavonoid/ flavonoid sequeatrinis important in the plant). The chemistry anthosianinhave structur aromatic This research aimed to study the effect of Purple Sweet Potato (*Ipomeo Batata L*) extract in inhibiting stress oxidative in hyperglycemic. A randomized pre-test and posttest control group design was performed on 40Wistar rats by analyzing blood glucose,8-Hydroxy-2 Deoxiguanosin (8OHdG) activity and histopathology of pancreatic cell. Extraction of 1 kg purple Sweet Potato (*Ipomeo Batata L*) with water gave 0,710 kg crude extract. Hyperglycemic on Wistar rats was made by inducing hyperglycemic with alloxan 2 week. The experimental was performed on five groups of animals that were Hyperglycemic: P_0 for normal control, P_1 for negative control (hyperglycaemia), P_2 for hyperglycemia rats with intake purple sweet potato extract 2.0 mL/kg body weight; P_3 for hyperglycemia rats treated with intake purple sweet potato extract 4.0mL/kg body weight and P_4 treated with intake purple sweet potato extract 8.0 mL/kg body weight. The result showed that intake of purple sweet patato extract in 8,0 mL/kg body given have decreased the activity of blood glucose 34.90mg/dL, decreased 8-OHdG 1,46 ng/mL.Histopatology profile of pancreatic found in the hyperglycemia rat.

INTRODUCTION

Hyperglycemia is a condition where there is increase blood glucose with fasting blood glucose level above 109 mg/dL and 2-hour blood glucosepp (post prandial) above 158 mg/dL is called diabetes mellitus.[1] Diabetes mellitus is a metabolic disease which indicated the existence of hyperglycemia resulting from impaired insulin secretion and insulin action. Hyperglycemia induces oxidative stress and increased formation of reactive oxygen compound (ROS) through a non enzymatic process by forming gluco-oxidant and glycation.[1,2] Chronic hyperglycemia can worsen the formation of reactive oxygen species (ROS) through avariety of mechanisms, namely; through the polyol pathway, hexosamine pathway, protein kinase-C (ROS) through a variety of

mechanisms, namely: through the polyol pathway, hexosamine pathway, namely: through the polyul pathway, hexosamine pathway, protein kinase-C pathway and AGEs through.[1-3]

Oxidative stress is caused by an imbalance production of ROS at the cellular level. This event is one of the most important factors in the occurrence of vascular complications of diabetes.[1] Hyperglycemia which will trigger the formation of Amadori long. HbA1c and AGE_S. Amadori product and AGE_S are also triggering the formation of ROS/RBO to produce RB, free radicals, radical OHO the most active and dangerous can damage cells through damage to DNA, proteins and cell membranes.[1-5]

Water extract purple sweet potato contain Anthosianin/color flavonoid anthosianin is important in the plant pigment group cause red blue, located in cell polar. Anthosinin compound is derivate of mono or diasetyl 3-(2-glucosyl) glucosyl-5-glucosyl peonidin and sianidin. Anthosianin compound function antioxidant and free radical capture, until prevention of aging, cancer, degenerative deseases, besided anthosianin able antimutagenic and anticarsinogenic, liver dysfunction, antihyphertention and decreases glucose blood.

EXPERIMENTAL

The study was a true experimental design with the randomized pre and posttest control group design is used to prove the effectiveness water extract purple sweet patato in β-cells to repair damaged pancreas by reducing blood glucose, 8-OHdG in rat Wistar hyper glycemia.[9] The study used 40 Wistar rat were divided into five groups, one control group (P_o), rat Wistar hyperglycemia (P_1) and the three treatment extract purple sweet potato 2.0 mL/kg body weight (P_2); rats Wistar hiperglycemia treated extract purple sweet potato with 4.0 mL/kg body weight (P_3), rat to study hyperglycemia, research was conducted to provide a standard formula feed, and fat-rich feed for 7 weeks, while research hyperglycemia induced by alloxan dose of 125 mg/kg bw done for 1 week.[10]

This research was conducted in the Laboratorium of Organic Chemistry Departement of Chemistry and Laboratory of Analytical Science faculty of Udayana University, While checking blood glucose levels and 8-OHdG. The histopathological examination was carried out at the Laboratory of Histology Faculty of Medicine, Udayana University. The study was conducted over eight months, including data analysis and writing of the results.

RESULTS AND DISCUSSION

Purple Sweet Potato extract condensed water maceration 100 ml water with 1 kg purple sweet potato blender. Filtrate and evaporated obtained purple sweet potato extract contain anthosianin 140.23 – 147.0 mg/mL sphectrometrically.[6,7] Anthosianin compound function antioxidan and free radical bebas capture, until prevention of aging, cancer, degenerative deseases, besided anthosianin able antimutagenic and anticarsinogenic, liver disfungution, antihipertention and decreases glucose blood.[6,7]

In this study it was found the purple sweet potato with various doses can lower blood glucose levels in hyperglycemic rats Wistar. Data rates hyperglycemia rat Wistar blood glucose levels are presented in Table 1.The results of statistical analysis showed that the administration of alloxan could cause an increase in blood glucose levels.

TABLE 1 Blood Glucose Levels Before and After Treatment

Treatment	Observation blood glucose levels (mg/dL)		
	Pretest	Posttest	Diference of Blood Glucose
P_0	208.35 ±3.03	109.76 ± 2.25	98.59[a]
P_1	220.33 ± 3.06	118.60 ±3.34	104.40[b]
P_2	217.56 ± 1.19	103.59 ± 1.71	113.45[c]
P_3	I73.41± 2.07	99.91 ± 1.88	74.09[d]
P_4	219.61 ± 2.77	103.69 ± 1.88	117.81[e]

Data mean blood levels of 8-hidroxi-2-dioxiguanosin (8-OHdG) hyperglycemia Wistar rats pre and posttest in table 2.Futhermore, the limit of significance with paired t-test showed a significant difference in the mean decrease in blood glucose and 8-OHdG level between the control group (P_o) with P_1 with $p<0,05$.

TABLE 2 8-OHdG Levels Before and After Treatment

Treatment	Observation of 8-OHdG levels (ng/dL)		
	Pretest	Posttest	Diference of 8-OHdG
P_0	6.35 ± 0.33	3.76 ± 0.15	3.91[a]
P_1	6.75± 0.46	4.60 ± 0.34	2.37[b]
P_2	6.56 ± 0.19	4.59 ± 0.71	2.15[c]
P_3	6.41± 0.07	3.41 ± 0.48	2.59[d]
P_4	6.95 ± 0.47	3.69 ± 0.18	3.55[e]

Structure of histopatology pancreas network Wistar rats, used Gomori-Nuclear fast red staining done to see qualitative changes in the structure of rat pancreatic tissue treatment. Staining is composed of two color components, and the Gomori Nuclear fast red. Gomori an alkaline dye in order to color the cell nucleus that are acidic while Nuclear fast red is an acidic dye that can stain the cytoplasm is alkaline. Histopathological changes in pancreatic tissue morphology Wistar rats with 400 times magnification and staining Gomori Nuclear fast red from the normal state to occur alloxan induced hyperglycemia caused a dose 125 mg/kg bw can be seen in Figure 1.

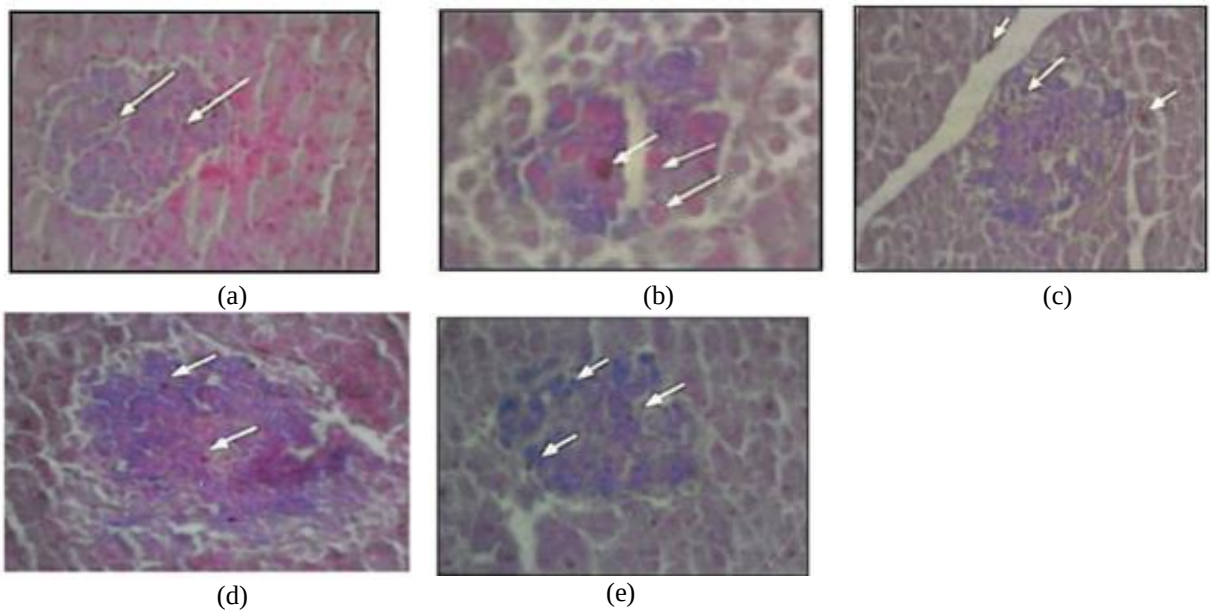

FIGURE 1. Employment purple sweet potato extract

(a) P0 Control indicates normal pancreatic cells
(b) P1 Demage of pancreatic cells (induced by aloxan)
(c) P2 dose of 2.0 mg/kg bw morphological changes
(d) P3 dose of 4.0 mg/kg bw morphological changes
(e) P4 dose of 8.0 mg/kg bw morphological changes

In the structure of the network in the Wistar rat pancreatic island of Langerhans, No visible cytoplasmic granula and clear boundaries between β-cells by α-cells.still seems the cells undergoing necrosis and pancreatic β-cell repair process; Figure 5 dose 8,0 mg/kg bw appear on any cell dageneration to necrosis of the Wistar rat Pancreatic tissue around the island of Langerhans thus showing clear boundaries between β-cells by alpha cells. B-cells were detected by staining with Gomori Nuclear fast red and 100 times magnification images of cells are shown in blue on the islets of Langerhans cell while the other is red.

Fig. 1 (a) shows that the number of cytoplasmic granules Wistar rat normally looks still intact, novisible presence of clinical symptoms, and found noβ-cell nuclei and other cell degeration to necrosis around the islets of Langerhans in mikroskopi examination, both qualitatively and quantitatively compared control group (P_0 and P_1) and the treatment group. In contrast to Fig. 1(b) shows that the pancreatic β-cells were detected by staining with Gomori-Nuclear fast red shown in the figure are colored purple cytoplasmic granules. The loss of a number of cytoplasmic granules around the islets of Langerhans. Rupture of a number of β-cell nuclei (karyoreksis), shrinking the cell nucleus and no visible piknosis clear cell boundaries between β-cells and α-cells around the islands of Langerhans.Wistar rat pancreatic β-cell degeneration to necrosis caused by alloxan induced a dose 125 mg/kg bw more than the Wistar rat pancreatic β-cells in the treatment group. This is because alloxan is

selectively destroy pancreatic β-cells through the formation of reactive oxygen species that begin by alloxan reduction and characterized by elevated blood glucose levels (hyperglycemia). Nowhere β-cells around the is lets ofLangerhans β-cell than in normal Wistar rats. Stroma reduced density of Langerhans on the island. There is edema congestion, to undergo necrosis (cell death). In Fig. 1(c) above have been changes in pancreatic tissue morphologyhistopatology in Wistar rat islets of Langerhans due purple sweetof potato extract dose 2.0 mg/kgbw compared with negative control group (P_0), although the amount of pancreatic β-cell degerating to necrosis rather reduced. Similar was also observed for purple sweet potato exctrat 4.0 mg/kg bw (P_3) as can be seen in Fig 1 (d) that the extract repaired pancreatic cell of hyperglycemic rats to become cell pancreatic as control normal (P_0). On the other hand, Fig. 1 (e) shows the effect of purple sweet potato extract in a dose of 8.0 mg/kg bw reveals still damage of pancreatic cells due compare to control. This was also support by the number of β-cells in pancreatic islets of Langerhans in all groups as presented in Table 3.

TABLE 3 The average number of β-Langerhans Levels Before and After Treatment

Treatment	Number of β-cells in the of Langerhans (Cells)
P_0	59.22 ± 4.99^{f}
P_1	5.44 ± 1.83^{a}
P_2	23.83 ± 4.30^{d}
P_3	55.48 ± 4.05^{c}
P_4	14.56 ± 5.68^{e}

The process of solvent extraction with water is intended to get all the component of polar or easily soluble in water from the sample because it has OH bond. Water extract purple sweet potato that has a very high antioxidant capacity and have the ability to destroy cells, dissolcing the bioactive compound as well as to maintain the reactivity properties of a compound. The ability of a solvent to extract the contents of the cell is effected by its ability to loosen the cell walls of cellulose framework and dissolve the active components of the cell contents. This is done in line with the objectives of the study, namely the effectiveness purple sweet potato extract (Ipomoe Batatas L) to prevent damage to pancreatic β-cell in rat Wistar hyperglycemia. In essence, to look for a compound that can later be applied as a remedy, as least these compounds should be easily soluble in water (body fluid) that can provide direct therapeutic effect. Purple sweet potato extract also contains anthosianin compounds derivative that can act as a proton donor, which releases hydrogenatoms and are to scavenger free radical formed from the reaction of non-enzymatic glycosylation of carbohydrates and amino groups proteins.[5-7] Pancreatic tissue histopathology test result on Wistar rats under normal circumstance indicate that the number of cytoplasmic granules seen many and not show clinical symptoms, β-Cell nucleus is still intact, and not degenerate qualitatively and quantitatively compared to the control group as well as the treatment group. After alloxan induced a dose 125 mg/kg bw showed that pancreatic tissue morpologis changes, the cells did not show a clear boundary between β-cell and □-cell to a reduced number of cytoplasmic granules around the islands of Langerhans. Wistar Rat pancreatic □-cell degeneration to necrosis (cell death). This is caused by alloxan can form damaging free radicals and cell membrane permeability resulting in damage to β-cells of the pancreas that produce insulin function. According to Aronson (2008) hyperglycemia can worsen the destruction of β-cells.[1] The reason; chronic hyperglycemia condition tend to increase the formations of free radicals (ROS) such as glucose metabolism through autooksidasi glucose metabolism metilglioksal formation, and oxidative phosphorylation. Toxiaction of alloxan on pancreatic β-cells accept. Increased cytosolic calcium concentration which led to disruption of the oxidation of pancreatic β-cell.[10]

In purple sweet potato extract dose 4.0 mg/kg bw/ day did not appear any cells undergoing necrosis in Wistar rat pancreatic tissue around the island of langerhans. Similarty, the number of β-cell have increased to near-normal conditions that pancreatic tissue repair process can take place quekly. It is played by Anthosianin/color flavonoid compounds through expenditure-insulin by pancreatic β-cell or alter the metabolism of glucose in the cells increases insulin secrection by pancreatic β through mechanism in maintaining a functioning β-cell and anthosianin compounds having reactive groups on the molecular structure, resulting in the ability to capture free radical are also getting stronger, so the pancreatic tissue histopathology in rats Wistar with hyperglycemia can be corrected.

CONCLUSION

It can be concluded that intake of purple sweet potato extract decreased glucose blood and decrase 8-OHdG with hyperglycemic Wistar rats in a dose dependent manner. At a dose of 4.0 mg/KgBw it gave the decreas glucose blood and decreas 8-OHdG, Histopathology analysis found that intake purple sweet potato extract at a dose of 4.0 mg/Kg bw repaired damaged in pancreatic cell tissue to become a normal pancreatic cell.

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REFERENCE

1. D. Aronson, Hyperglycemia and the pathobiology of diabetic Complication. Cardiol, 2008, 45: 1-16.
2. J. L. Evans, L. D. Goldfine, B. A. Addux, and G. M. Grodsky, Oxidative Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type-2 Diabetes, Endocrine Review, 2002, 23: 599-622.
3. T. F. Firdaus, E. Suhartono, and N. Qamariah, Pemodelan Reaksi Glikolisis dan Peran Infus Tapak Dara (C*hataranthus roseus.,L)* sebagai penghambat kerusakan Protein, Berkala Ilmu kedokteran, 2004, 36 (1):1-6.
4. J. S. Johansen, A. K. Harris, D. J. Rychly, and A. Ergul, Oxidative stress and use of antioxidants in diabetes linking basic science to clinical practice. CardiovasculerDiabetol. 2005, April; p 29:4(5). Available from URL: http://www.pubmedcentral. Nih.gov/tocrender.fcgi?iid=1 8041. Accessed 16 April 2008.
5. PERKENI, Konsensus Pengelolahan Diabetes pada Diabetes Melitus tipe 2. PB Perkeni Jakarta, 2012.
6. D. J. Soemadji, The role of free radical in management of 2 diabetic patiens. Disajikan pada Symposium Free Radical in Diabetes and their interaction with Sulphonylurea, Jakarta, 2001.
7. D. N. Suprapta, M. Antara, N. Arya, M. Sudana, A. S. Duniaji, and M. Sudarma, Kajian Aspek Pembibitan Budidaya&Pemanfaatan umbi-umbian sebagai Sumber Pangan alternatif. Laporan hasil Penelitian Kerjasama BAPEDA Propinsi Bali dengan Fakultas Pertanian UNUD-Bali, 2004.
8. A. Ullah, A. Khan, I. Khan, Diabetes Mellitus and Stress Oxidative: A concise Review. Saudi Pharmaceutical Journal, 2016. 24, 547-55.
9. S. J. Pocock, Clinical Trial a Practical Approach Chichester- New York, Singapore: Jon Wiley & Son Ltd, 2008. .
10. D. Watkins, S. J, Cooperstein, and Lazarow. Effect alloxan on permeability of pancreatic islet tissue in vivo. 2008. Available from :http://aiplegacy.physiology.org/cgi/content/abstract/207/ 2/436.