RESEARCH ARTICLE

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EFFECT ANTHOCYANIN OF PURPLE POTATO GUNUNG KAWI ON MDA LEVELS, EXPRESSION OF CASPASE-3, AND SPATIAL MEMORY FUNCTION ON DIABETIC WISTAR RATS

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Article History:

Received: April 18, 2018

Accepted: May 17, 2018

Published: January 1, 2019

Cite this as:

Prabawati RK, Ratnawati R, Rahayu M, Prakosa AG. Effect anthocyanin of purple potato gunung kawi on mda levels, expression of caspase-3, and spatial memory function on diabetic wistar rats. *Malang Neurology Journal*; 2019;5:34-41. DOI: <http://dx.doi.org/10.21776/ub.mnj.2019.005.01.6>

ABSTRACT

Background: Hyperglycemia condition will decline cognitive function. No basic therapy has been found for this. Purple potato anthocyanins are useful as anti-inflammatory, antioxidant, neuroprotectant, and antidiabetic.

Objective: Evaluate effect of purple potato's anthocyanins on MDA levels, brain's caspase-3 expression, and spatial memory function in diabetic model of Wistar rats.

Methods: This is an experimental study using diabetic model rats. The sample was divided into negative and positive control, anthocyanin dose of 10mg/kg, 20mg/kg, and 80mg/kg groups. MDA levels were measured using spectrophotometer, caspase-3 expression with immunohistochemistry, and spatial memory function using Morris water maze test.

Results: Tukey test showed that anthocyanin 10, 20, and 80 mg/kg lowering MDA levels, caspase-3 expression, and Morris water maze's travel time compared to control positive ($p = 0.000$). But anthocyanin 80 mg/kg make a significant increase on these three variabels compared to 10 and 20 mg/kg groups ($p = 0.010$). Pearson test showed that there no correlation between anthocyanin's dose, MDA levels, caspase-3 expression, and Morris water maze test.

Conclusion: Anthocyanin doses 10 and 20mg/kg lowering MDA levels and caspase-3 expression, also improves spatial memory function on diabetic model of Wistar rats.

Keywords: Anthocyanins, hyperglycemia, MDA, caspase-3, spatial memory function

Introduction

Type 2 diabetes mellitus (DM) is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. In the early stages of this disorder, glucose tolerance remains close to normal. Despite insulin resistance, since pancreatic beta cells compensate by increasing insulin production, in the final stages, pancreatic beta cells damaged by their failure to meet insulin requirements.¹

The number of DM patients over the past three decades has more than doubled globally, making it one of the most important public health challenges for all nations. Type 2 diabetes and prediabetes are increasingly prevalent in children, adolescents and young adults. The causes of type 2 diabetes mellitus are very complex ranging from genetic systems that interact with behavior and environmental influences.² According to the American Diabetes Association, in the United States, the prevalence of DM in 2012 reached 29.1 million people or 9.3% of the population where 1.2 million people suffer from type 1 diabetes, while for prediabetes sufferers reached 86 million people. DM is the seventh cause of death in the United States in 2010.³ According to the data of RISKESDAS 2013, the prevalence of DM in Indonesia based on a doctor diagnosing or

symptoms is 2.1%. East Java itself places in 4th rank (2.1%).⁴

Hippocampus is the centre of integration for cognitive functions such as learning and memory in the mammalian brain. Memory functions are part of cognitive function. Memory is divided into several kinds associated with the location in the brain, including space-related spatial memory, which is organized in the hippocampus. DM is an important metabolic disorder that causes functional and structural changes in the central nervous system. Moderate cerebral atrophy and increased subcortical and brainstem lesions have been reported in uncontrolled diabetes patients. Previous studies have suggested that memory, learning, and cognitive impairment is more common in diabetics than in non-diabetic patients.⁵

Hyperglycemia has been shown to cause inflammation in the brain. Evidence suggests that there is accumulation of inflammatory mediators in diabetic brains. Hyperglycemia in DM causes elevated levels of free radicals. The autooxidation process in hyperglycemia triggers the formation of free radicals. Free radicals can damage cell membranes into lipid peroxide or Malondialdehyde (MDA). If it continues, it will cause damage to the cell membrane system and cell death.⁵

It is mentioned that the nerve inflammation caused by diabetes mellitus plays an important role in the production of A β and hyperphosphorylation of the tau protein.⁶ In rat model of Diabetes Mellitus, MDA increased significantly, and concentrations of SOD and GSH decreased drastically in the cerebral and hippocampal cortex.⁷ Another study found that the escape latency of rat-induced diabetes mellitus by streptozotocin, using Morris water maze test method, showed a decrease in spatial memory function. The results of this study show that DM can reduce the learning and memory function of rats, destroying the hippocampus and synapses neuron structure, and promoting neuronal apoptosis.⁸ There has been no basic dementia therapy, although there are several therapies to treat the symptoms. Therefore, it is necessary to develop dementia therapy including brain protection such as increased neurotropic factors associated with neurotransmission, synaptic plasticity, and elimination of β -amyloid from the brain.^{5,8} Anthocyanin belongs to the flavonoid group, where there is a phenolic group in its chemical structure and gives color to some flowers and fruits.⁹ Anthocyanin also has a role as anti-inflammatory and neuroprotectant.¹⁰ Research to examine the antioxidant and anti-inflammatory effects of anthocyanins in Alzheimer's model rat suggests that anthocyanins have an effect on the cognitive function of the rat.⁷ Research to observe the effect of anthocyanin on cognitive function of diabetic model rat has not been done. Pursuant to that matter, we would like to know the influence of anthocyanin in cognitive function of rat model of diabetes with parameter of spatial memory function and MDA level as marker of inflammation in hippocampus cell, and expression of caspase-3 in brain hippocampus cell.

Methods

Research Design

This study used a laboratory experimental design, post-test only control group using diabetes-induced wistar rats. Treatment was divided into 5 groups, namely: group A (negative control), group B (positive control), group C (anthocyanin 10mg/kgBW), group D (anthocyanin 20mg/kgBW), and group E (anthocyanin 80mg/kgBW). Surgery is performed at 7 weeks after a high-fat diet. The research was conducted in Pharmacology Laboratory, Pathological Anatomy and Physiology Laboratory of Faculty of Medicine, Brawijaya University, Malang. The study sample was, wistar rats, male, weight 125-175 grams, age 6-8 weeks, randomization obtained from Eijkman Institute Jakarta. The research was conducted in January 2016- April 2016 and approved by the ethics committee of Faculty of Medicine Brawijaya University Malang.

Induction of Diabetes

Wistar rats were given a high-fat diet for 6 weeks and intraperitoneal streptozotocin injection with a dose of 35mg/kgBW at the end of six weeks and 35mg/kgBW 24 hours after the first injection, if hyperglycemia had not occurred. A high-fat diet consisted of 50% PARS, 25% flour, 1% cholesterol, 0.1% cholate, 2.5% pork, and 21.4% water. A high-fat diet is given at 1-6 weeks.

Giving Anthocyanin of Purple Potato (*Ipomoea batatas* L.)

The administration of anthocyanin is using anthocyanin that was purified from of sweet potato (*Ipomoea batatas* L.),

derived from purple sweet potato cultivar of Gunung Kawi, conducted by Dr. Ciptati, MS., in Chemistry Laboratory of Mathematics and Natural Sciences Faculty, ITB, Bandung using modified flash column chromatography with polyamide CC-6 stationary phase and dynamic phase using water and ethanol. Anthocyanin extract was administered once orally every day for 5 weeks at doses of 10, 20 and 80 mg/kgBW.¹¹

Brain Sampling

The wistar rats were anesthesia by ether inhalation. After the rat was confirmed unconscious (not showing spontaneous movement), surgery were performed to take rat brain tissue. Surgery is done by cutting the cranium with the sagittal direction from the caudal (occipital) to the rostral (frontal), right between the two rat brain hemispheres. Furthermore, rat brain liberation was performed in the basal region of adjacent connective tissue. The left-sided hemisphere of the brain is taken and inserted into a bottle filled with 10% formalin solution, while the right hemisphere is inserted into the plastic for MDA examination. Bottles containing brain tissue and formalin solution are then closed tightly. Furthermore, the brain tissue was sliced and we can make a paraffin block (the incision of brain preparation and making the slide was done at Pathological Anatomy Laboratory, Faculty of Medicine, Brawijaya University).

Deparaffinisation

The brain tissue was inserted in 10% formalin tube and cutted off with a microtome rotary as thick as 4 microns and placed in poly-L-lysine and then left at room temperature. Furthermore, deparaffinisation was done, but before it, the slide was first heated at 60 °C for 60 minutes. And then we will insert the solution sequentially, xylol (2x10 min), absolute ethanol (2x10 min), ethanol 90% (1x5 min), ethanol 80% (1x5 min), ethanol 70% (1x5 min), sterile aquades (3x5 minutes).

MDA Level Inspection

Brain samples were taken as much as 100mg, then homogenized with 2cc of phosphate buffer. After that, the homogenate were added by EDTA 200 μ L, trichloroacetic acid (TCA) 40% 250 μ L, HCl 200 cc, and thiobarbituric acid (TBA) 250 μ L. Then from that mixture, was taken for 200 μ L and augmented by aquabidest 0.5 cc. Then was heated by 1000 in waterbath for 25 minutes and centrifuge for 3000 rpm. After centrifugation, the supernatant part was then added H₂O 3 cc. MDA level was measured with λ 532 nm spectrophotometer.

Caspase-3

Caspase-3 expresion was measured by immunohistochemistry method. The first step was Antigen Retrieval process with citrate buffer. The slide is immersed in a chamber containing a pH 6.0 citrate buffer then heated in a temperature waterbath of 95°C for 20 minutes. Slide removed from the waterbath, wait until room temperature ($\pm$ 20 minutes) then washed with PBS (3x2 minutes). The immunohistochemical painting process was then performed: the slide was sterilized with 3% H₂O₂ in methanol and incubated for 15 minutes, then washed with PBS for 2 minutes 3 times. After that, make a blocking unspecific protein with background sniper, incubated 15 minutes at room temperature then washed with PBS for 2 minutes 3 times. Dropped primary antibody (caspase-3

dissolved in PBS buffer with ratio 1:50 and 5% FBS) overnight at 4°C. The slides were then incubated with secondary antibody for 30 minutes at room temperature then washed with PBS for 2 minutes 3 times. After that, the slide was incubated by the enzyme SA-HRP for 20 minutes at room temperature, then washed with PBS for 2 minutes, 3 times, and washed with aquades. DAB and buffer DAB was dropped with ratio of 1:50 and incubated 3-10 minutes at room temperature, then washed with PBS for 2 minutes 3 times and washed with aquadest for 2 minutes 3 times. Then dropped Mayer and tap water with a ratio of 1:10 and incubated 5-10 minutes at room temperature then rinsed with tap water, dried and observed under a microscope with 400 times magnification.

Evaluation Method

Caspase-3 expression was observed and measured by immunohistochemical examination of brain samples of wistar rats using a 400x magnification microscope. Cells expressing caspase-3 show a brown cytoplasm in all cell types. Measurements were made by calculating the average of cells expressing caspase-3 from 10 fields of view.

Morris Water Maze¹²

Wistar rats were treated in introduction to the examination method in the form of exercise twice a day with a moveable place, whereas the position of the intended base remains (in quadrant five) according to quadrant division. There are five routes that must be taken each rat, that is: I - 5; II: 2 - 5; III: 8 - 5; IV: 3 - 5 and V: 7 - 5 with maximum travel time in the process of finding the intended ground was 120 seconds and allowed to be on the runway for a maximum of fifteen seconds.

The initial recognition test is performed for three days, and after each test, the rat will be placed in a transit enclosure place in open space under the sun for fifteen minutes and dried before being put back into the cage. This way has the purpose to avoid the occurrence of hypothermia. As for the cleanliness of the tool, the water in the vessel was removed and the vessel was washed with disinfectant and dried to avoid infectious organisms.

During the first three days of the experiment, rats will only be fed standard and allowed to adapt to the enclosure. On the fourth day all animals are given an introduction to Morris Water Maze twice a day for three days according to the method of introducing the environment, and at the end of the tool introduction session, we calculated the mean travel time by someone else that not include on the experiment (to remove the research bias). At the end of treatment (at the end of week sixth) we recalculated the average travel time with Morris Water Maze tool on the five groups of animals.

Statistical Analysis

Data that obtained in subsequent research carried out statistical analysis. Statistical analysis used was Oneway ANOVA test to know the difference of MDA level, caspase-3 expression, and time of Morris Water Maze in treatment group. Statistical test results are significant if $p < 0.05$. Then, continued with Tukey test to find the location of significant differences in the treatment group. Pearson correlation test was used to determine correlation between dose of anthocyanin with MDA content, caspase-3 expression, and time of Morris Water Maze.

Results

Brain MDA Levels Evaluation

Evaluation brain MDA levels were shown at Table 1.

Table 1. Brain MDA Levels (average).

Groups	Brain MDA levels
K(-)	0.08 ±0.01
K(+)	0.47 ±0.04
P1 (10mg/kgBB)	0.09 ±0.01
P2 (20mg/kgBB)	0.09 ±0.01
P3 (80mg/kgBB)	0.25 ±0.02

K(-): Negative control group; K(+): Positive control group; P1: Anthocyanin 10 mg/kgBW; P2: Anthocyanin 20 mg/kgBW; P3: Anthocyanin 80 mg/kgBW

Comparison of expression of brain MDA levels showed a significant difference in the five groups ($p < 0.05$), in which the brain MDA levels of wistar rats between K (-) differed significantly with K (+) and P3, but did not differ significantly with P1 and P2. The MDA level of the wistar rat brain between K (+) differs significantly with K (-), P1, and P2, and P3. MDA levels of cerebral wistar rat between P1 differed significantly with K (+) and P3, but did not differ significantly with K (-) and P2. MDA levels of cerebral wistar rat between P2 differed significantly with K (+) and P3, but did not differ significantly with K (-) and P1. The MDA level of cerebral wistar rat P3 differed significantly with K (-), K (+), P1, and P2. MDA levels of brains of wistar rats are highest at K (+) and lowest in K (-).

Figure 1. Comparison of MDA Levels.

K(-): Negative control group; K(+): Positive control group; P1: Anthocyanin 10 mg/kgBW; P2: Anthocyanin 20 mg/kgBW; P3: Anthocyanin 80 mg/kgBW

Based on the results of correlation test showed the correlation coefficient between MDA level of brain of wistar rat and dose of anthocyanin extract was -0.054 with significance value of 0.821 ($p > 0.05$), so it can be concluded that there is no significant relationship between MDA brain level of wistar rat and anthocyanin purple sweet potato dose.

Evaluation of Caspase-3 Expression

The average count of caspase 3 expression in the brains of wistar rats in each group on day 50 was obtained in Table 2 and Figure 2.

Table 2. Caspase-3 expression (average).

Groups	Caspase-3 expression
K(-)	2 ±0.4
K(+)	17 ±1.3
P1 (10mg/kgBB)	8 ±0.9
P2 (20mg/kgBB)	4.25 ±0.4
P3 (80mg/kgBB)	12.5 ±0.6

K(-): Negative control group; K(+): Positive control group; P1: Anthocyanin 10mg/kgBW; P2: Anthocyanin 20mg/kgBW; P3: Anthocyanin 80mg/kgBW

The comparison of the expression of brain caspase 3 of the wistar rats showed a significant difference in the five groups ($p < 0.05$), in which the expression of caspase 3 of the wistar rat brain between K (-) was significantly different with K (+), P1, and P3, but not significantly different with P2. Expression of caspase 3 of the wistar rat brain between K (+) was significantly different with K (-), P1, P2 and P3. Expression of caspase 3 of the wistar rat brain between P1 differed significantly with K (-), K (+), P2 and P3. Expression of caspase 3 of the wistar rat brain between P2 differed significantly with K (+), P1, and P3, but did not differ significantly with K (-). Expression of caspase 3 of the wistar P3 rat brain differed significantly with K (-), K (+), P1 and P2. Expression of caspase 3 of the wistar rat brain is highest at K (+) and lowest in K (-). Based on correlation test result, showed that correlation coefficient value between expression of caspase-3 brain of wistar rat and dose of anthocyanin extract equal to 0.207 with significance value equal to 0.381 ($p > 0.05$), so it can be concluded that there is no significant relationship between expression of brain caspase 3 of wistar rat and dose of anthocyanin extract.

Figure 2. Comparison of Caspase-3 Expression.

K(-): Negative control group; K(+): Positive control group; P1: Anthocyanin 10 mg/kgBW; P2: Anthocyanin 20 mg/kgBW; P3: Anthocyanin 80 mg/kgBW

Spatial Memory Functions

In each group, spatial memory function was measured through the time taken to complete the Morris Water Maze, on day 3 and day 50 (see Table 3 and Figure 4). Comparison of the time of Morris Water Maze on the 3rd day there was no significant difference between groups in all quadrants. The lowest and highest travel time also varies each quadrant. The comparison of time of Morris Water Maze on the 50th day on all quadrants showed significant differences in some groups ($p < 0.05$), where the time of Morris Water Maze's day on the 50th day between K (-) was significantly different from K (+), but did not differ significantly with the P1, P2, and P3 groups. The travel

time of Morris Water Maze on the 50th day between K (+) differs significantly with K (-), P1, P2, and P3. The travel time of Morris Water Maze on the 50th day between P1 differed significantly with K (+), but did not differ significantly with K (-), P2, and P3. The time of Morris Water Maze on the 50th day between P2 differed significantly with K (+), but P2 did not differ significantly with K (-), P1, and P3. The time of Morris Water Maze on the 50th day between P3 differs significantly from K (+), but P3 is not significantly different from K (-), P1, and P2. Morris Water Maze travel time on the 50th day on all the highest quadrants at K (+) and lowest on K (-) and P2.

Figure 3. Expression Brain Caspase-3 with IHK.

K(-): Negative control group; K(+): Positive control group; P1: Anthocyanin 10mg/kgBW; P2: Anthocyanin 20mg/kgBW; P3: Anthocyanin 80mg/kgBW

Based on the results of correlation test shows the value of correlation coefficient between the time of Morris Water Maze and the dose of anthocyanin extract in each quadrant of 0.247, -0.232, -0.008, -0.221 with a significance value greater than 0.05, so it can be concluded that there is a

relationship that is not significantly between the time of Morris Water Maze and the dosage of anthocyanin extract, where the dose change did not affect the travel time of Morris Water Maze.

Figure 4. Graph Morris Water Maze time.

K(-): Kontrol negatif; K(+): Kontrol positif; P1: Anthocyanin dosis 10mg/kgBB; P2: Anthocyanin dosis 20mg/kgBB; P3: Anthocyanin dosis 80mg/kgBB

Correlation Test between MDA Levels, Caspase-3 Brain Expression, and Spatial Memory Function of Wistar Rat

The correlation test showed the correlation coefficient value between brain MDA level of wistar rat and spist function of wistar rat on each quadrant (quadrant 1 to quadrant 4) on 50th day of 0.854, 0.861, 0.861, 0.856 with significance value of 0.000 ($p < 0.05$), so it can be concluded that in all quadrants, there is a significant relationship between MDA levels of wistar rat with spist function of wistar rats, whereas higher levels of brain MDA of wistar rats will be followed by decrement spatial memory function of wistar rats, which is indicated by the increase in travel time of Morris Water Maze. Vice versa, the lower the MDA level of the wistar rat brain, it will be followed by the low travel time of the Morris Water Maze of the wistar rat. The result of correlation test between expression of caspase-3 brain of wistar rat and spist function of wistar rat in each quadrant (quadrant 1 to quadrant 4) on day 50 showed the value of correlation coefficient of 0.808, 0.803, 0.856, 0.852 with significance value ($p < 0.05$), so it can be concluded that in all quadrants, there is a significant relationship between the wistar caspase-3 brain expression of the wistar rat with the spist function of the wistar rats, whereas the higher the wistar rat-caspase-3 expression of the brain, it will be followed by improved spatial memory function of wistar rats, which is indicated by an increase in travel time of Morris Water Maze. Vice versa, the lower the expression of caspase-3 of the wistar rat brain, it will be followed by the low travel time of Morris Water Maze of the wistar rat.

Table 3. Morris Water Maze test – travel time recording (average $\pm$ SD)

Morris Water Maze (mean $\pm$ SD) (seconds)		Groups					P value
		K (-)	K (+)	P1	P2	P3	
Day 3	1 st	7.0 $\pm$ 1.78	7.25 $\pm$ 1.25	11.5 $\pm$ 1.19	7.0 $\pm$ 1.78	14.25 $\pm$ 3.90	0.113
	2 nd	9.0 $\pm$ 2.70	23.0 $\pm$ 8.65	18.5 $\pm$ 6.35	17.25 $\pm$ 3.17	9.5 $\pm$ 1.19	0.292
	3 rd	7.75 $\pm$ 3.09	9.75 $\pm$ 0.85	15.5 $\pm$ 1.85	22.25 $\pm$ 5.1	9.75 $\pm$ 2.49	0.025**
	4 th	13.5 $\pm$ 2.95	11.0 $\pm$ 0.91	7.5 $\pm$ 1.19	16.75 $\pm$ 5.07	21.25 $\pm$ 7.84	0.274
Day 50	1 st	7.25 $\pm$ 1.25	53.0 $\pm$ 18.09	25.75 $\pm$ 5.10	16.0 $\pm$ 4.14	46.25 $\pm$ 6.16	0.012**
	2 nd	9.5 $\pm$ 1.19	50.75 $\pm$ 7.48	14.0 $\pm$ 2.73	6.75 $\pm$ 0.63	17.25 $\pm$ 3.63	0.000**
	3 rd	9.75 $\pm$ 0.85	46.0 $\pm$ 5.64	16.13 $\pm$ 1.76	14.25 $\pm$ 1.10	25.25 $\pm$ 3.33	0.000**
	4 th	11.0 $\pm$ 0.91	54.5 $\pm$ 6.44	14.75 $\pm$ 0.48	9.0 $\pm$ 1.08	20.0 $\pm$ 2.65	0.000**

K(-): Negative control group; K(+): Positive control group; P1: Anthocyanin 10 mg/kgBW; P2: Anthocyanin 20 mg/kgBW; P3: Anthocyanin 80 mg/kgBW

Discussion

In this study it was found that in all variables there was a significant difference between treatment group dose 10mg/kgBW and 20mg/kgBW with group of positive control treatment and treatment dose 80mg/kgBW. The results of this study indicate that at doses of 10 mg/kgBW and 20 mg/kgBW can prevent elevated levels of MDA, brain-caspase-3 expression, and time traveled by Morris Water Maze (shown with results approaching negative control), but at doses of 80mg/kgBB increased levels of brain MDA, caspase-3 expression, and travel time of Morris Water Maze wistar rat, with results approaching positive controls.

Anthocyanin Prevents Increased Levels Of MDA Brain Diabetic model Wistar Rat

In this study, there was a significant difference between treatment group of 10mg/kgBW and 20mg/kgBW with positive control treatment group and dose treatment 80 mg/kgBW. The results of this study were consistent with other studies, in which on rats induced hyperglycemia, showed that SOD and GSH activity decreased in rat brain models, and MDA values increased significantly. This suggests oxidative damage to free radicals has occurred in the brains of rat to some extent. However, in animals treated with DM and Anthocyanin, MDA increased significantly, and concentrations of SOD and GSH decreased drastically in the cerebral and hippocampal cortex.⁷ Polyphenol antioxidant mechanisms includes the suppression of Reactive Oxygen Species (ROS) formation with chelating trace elements involved in free radical formation and upregulation of antioxidant enzymes such as

superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).¹³ Activation of these antioxidant enzymes will enhance the clearance of superoxide radicals. This will prevent the formation of hydroxyl radicals and lipid peroxidation and will also prevent the formation of malondialdehyde (MDA).¹⁴

In the positive control group, the highest levels of MDA compared to other groups showed that Reactive Oxygen Species (ROS) were widely produced after induction of hyperglycemia. ROS degrades polyunsaturated fatty acids to produce MDA. High levels of ROS produced due to induction of hyperglycemia, resulting in increased levels of malondialdehyde (MDA). This study also matches the results of a study conducted by Mirshekar, that the administration of anthocyanin injections will normalize glucose levels in the blood and improve serum insulin levels in diabetic rats. Other biomarkers, which also undergo changes are, levels of superoxide dismutase, catalase, malondialdehyde and fructosamine, which decrease, even back to normal levels, in the administration of anthocyanin injections. The possible mechanism is that the anthocyanin will inhibit oxidative damage due to hemoglobin glycation process, so the release of iron (Fe) will decrease and the oxidative damage due to Fe will be inhibited.¹⁵

In this study, anthocyanin dosage of 80 mg/kgBW increased MDA levels. Another study of anthocyanins showed that there were no active ingredient contained total anthocyanins, that had a prooxidant effect. However, there is a theory put forward by Gordon (1990), that large concentrations of antioxidants added can have an effect on the rate of oxidation. At high concentrations, the antioxidant activity of the phenolic group often vanishes, even the antioxidants become prooxidants. The effect of the amount of concentration on the oxidation rate depends on the antioxidant structure, the conditions and the sample to be tested. This is likely to cause at certain doses then the antioxidant effect will be lost on an herbal.¹⁶

Anthocyanin Prevents Increased Brain Caspase-3 Expression

Caspase-3 is an effector of apoptotic processes that play an important role in cell degradation. Caspase-3 expression increased in streptozotocin-induced rat hippocampus compared with diabetic-induced rat using streptozotocin. Hyperglycemia in patients with diabetes mellitus is associated with a process of oxidative stress that will trigger neuron cell and schwann cells death through increased caspase-3 activity.¹⁷ In this study, there was a significant difference between treatment group of 10mg/kgBW and 20mg/kgBW with positive control treatment group and dose treatment 80mg/kgBW. Administration of total anthocyanin may inhibit the increase of caspase-3 expression at doses of 10mg/kgBW and 20mg/kgBW, but at doses of 80mg/kgBW, the total anthocyanin actually increased caspase-3 expression. This study is in line with previous research, that anthocyanin as an antioxidant will protect endothelial cells against oxidative stress by inhibiting cell apoptosis through mitochondrial membrane protection and downregulation of caspase activation 3.¹⁸ In other studies conducted on the retina, anthocyanins have been shown to inhibit retinal degeneration through increased production of antioxidant enzymes, decreased lipid peroxidation (MDA),

and apoptotic resistance as indicated by decreased expression of caspase-3.¹⁹

The results of this study indicate that administration of anthocyanin doses of 80 mg/kgBW in this study increased the expression of rat brain-3 caspase and approached positive control. In another study, in colon cancer cells, anthocyanins precisely trigger oxidative stress and trigger apoptosis of cancer cells through the activation of caspase-3 at a certain dose. This suggests that at some doses, anthocyanins have a prooxidant and proapoptotic effect, which is consistent with this study. Giving anthocyanin doses of 80mg/kgBW in this study increased the expression of rat caspase-3 and approached positive control. This is likely in line with the rising levels of MDA. Where if an increase in oxidative stress, will trigger the occurrence of apoptosis process. So these two parameters are interconnected. It is also reinforced on the results of the correlation test between MDA levels and caspase-3 expression, which shows a strong positive relationship. So if MDA levels rise, then caspase-3 expression will also increase.

Anthocyanin Prevents Worsening of Spatial Memory Function of Diabetic model Wistar Rats

In this study, there was a significant difference between treatment group of 10mg/kgBW and 20mg/kgBW with positive control treatment group and dose treatment 80mg/kgBW. The results of this study are in accordance with research conducted by Krikorian et al. In a study conducted on humans aged about 76 years, it is said that intake of blueberry juice for 12 weeks will improve memory function²⁰. In another study it was also reported that total intake of plum and blackberry anthocyanins inhibited deterioration of neuron function and restore memory and motor function. The anthocyanin effect is probably mediated by inhibition of the neuron inflammatory process. For example, anthocyanins are known to inhibit upregulation of nuclear factor kB (NFkB) in Fischer mice. The intake of blueberries will inhibit the cognitive and memory-induced function induced by fabricic acid, through IL-1b, TNF α , and k-nuclear factor expression in the hippocampus.¹⁵

Anthocyanin is also known to inhibit the occurrence of insulin resistance and hyperglycemia, thus preventing damage to neurons. Insulin plays an important role in the memory process. In learning and memory, insulin modulates synaptic plasticity by acting on glutamatergic and GABAergic receptors. In the hypothalamus insulin can play an indirect role in the regulation of peripheral glucose metabolism, learning process, and memory especially located in the hippocampus. This role is more likely to be caused by direct modulation of receptor activity in neurons and glial cells. Evidence has shown that insulin signaling plays an important role in synaptic plasticity by acting on both glutamatergic and gamma-aminobutyric acid (GABA) transmissions. The exposure of N-methyl-D-aspartic acid (NMDA) receptors causes insulin to rapidly trigger a potential response of NMDA, which can be mediated by NMDA receptor subtypes. NMDA receptors play an important role in synaptic plasticity in learning and memory formation.²¹

At a total dose of 80mg/kgBW anthocyanin, spatial memory function is not significantly different from positive control, it indicates that the dose is unlikely to inhibit

neuronal damage resulting in worsening of spatial memory function. Research on anthocyanin side effects has not been widely practiced. But the possibility this happens because of the dual effect of anthocyanin.

The limitation of this study is that the use of anthocyanin dose is too far between doses. So as if the increase in doses did not give inhibitory effect. It takes a follow-up study that uses anthocyanin doses between doses of 20 and 80mg/kgBW. It should also be seen the effect of anthocyanin administration with doses greater than 80mg/kgBW, so we can know for certain whether these prooxidant and proapoptotic effects occur only at those doses or indeed subchronic toxicities occur. Effective doses can not be determined yet in this study, since the dose proved to be significantly different for only two doses, while for the dosage effectiveness test of a substance it takes at least 11 doses. in this study also did not check the levels of HbA1c and insulin levels that indicate the presence of pancreatic damage and maintenance of blood glucose levels. However, the mean blood glucose levels we examined at weeks 4, 5, and 6 showed normal results.

Conclusion

Anthocyanin from purple sweetpotato (*Ipomoea batatas* L.) dose of 10mg/kgBW and 20mg/kgBW can improve spatial memory function through decreasing MDA levels and caspase-3 expression in DM-induced wistar rats.

Acknowledgement

This research was carried out with financial support of BPPM (Agency for Research and Community Service) Faculty of Medicine, Brawijaya University.

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