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Effects of Chlorophyll in Papaya Leaves on Superoxide Dismutation and Blood Glucose Level of Diabetic Rats

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Abstract

Hyperglycemia in diabetes mellitus results in oxidative stress and increases complication development. Experimental studies have shown that chlorophyll has antioxidant activity and papaya leaves contained chlorophyll more than the other green vegetables. This study aimed to evaluate the antioxidant and hypoglycemic role in chlorophyll rich in papaya leaves on diabetic rats. Thirty six rats were randomly divided into 4 groups: without treatment (technique control/TC), diabetic (negative control/NC), diabetic with treatment A (100.3 mg/200 g BW of extract) and diabetic with treatment B (200.6 mg/200 g BW of extract). Diabetic induction was conducted by injecting streptozotocin 40 mg/kg BW intraperitoneally. Extract was given by nasogastric tube. Blood glucose level was measured using enzymatic colorimetric GOD-PAP test at before, after 20 and 40 days of treatment. Hepatic superoxide dismutation (SOD) level was measured after 40 days of treatment. Blood glucose levels in 3 diabetic groups were significantly raised after seven days of induction. In Anova and post hoc LSD analysis, both treatments had lower hepatic SOD level than TC (p = 0.01), and blood glucose level also decreased after given the treatment (p = 0.01). Treatment B had a better antioxidant and hypoglycemic role than treatment A.

Abstrak

Efek dari Klorofil di Daun Pepaya pada Dismutasi Superoksida dan Kadar Glukosa Darah Tikus Diabetes. Hiperglikemia pada pasien diabetes melitus menyebabkan stres oksidatif dan meningkatkan pengembangan komplikasi. Berbagai penelitian eksperimental telah memperlihatkan bahwa klorofil memiliki aktivitas antioksidan dan daun pepaya mengandung klorofil lebih banyak dari sayuran hijau lainnya. Penelitian ini bertujuan untuk mengevaluasi peran antioksidan dan hipoglikemik pada kandungan klorofil di daun pepaya pada tikus diabetes. Tiga puluh enam tikus secara acak dibagi menjadi 4 kelompok: tanpa pengobatan (kontrol teknik/TC), diabetes (kontrol negatif/NC), diabetes dengan pengobatan A (100,3 mg /200 g BB ekstrak), dan diabetes dengan pengobatan B (200,6 mg/200 g BW ekstrak). Induksi diabetes dilakukan dengan menyuntikkan streptozotocin 40 mg/kg BW secara intraperitoneal. Ekstrak diberikan melalui tabung nasogastrik. Kadar glukosa darah diukur dengan menggunakan uji enzim kolorimetri GOD-PAP di sebelum, setelah 20 dan 40 hari pengobatan. Tingkat dismutasi superoksida (SOD) hati diukur setelah 40 hari pengobatan. Kadar glukosa darah pada 3 kelompok diabetes meningkat secara signifikan setelah tujuh hari induksi. Dalam analisa Anova dan uji lanjut LSD, kedua kelompok dengan pengobatan memiliki tingkat SOD hati lebih rendah dari TC (p = 0,01) dan kadar glukosa darah juga menurun setelah diberi pengobatan (p = 0,01). Pengobatan B memiliki peran antioksidan dan hipoglikemik lebih baik daripada pengobatan A.

Keywords: blood glucose, chlorophyll, diabetes mellitus, papaya leaves, superoxide dismutation

Introduction

Diabetes Mellitus is a non-communicable disease that is closely related to a variety of oxidative activities. It leads to an autoxidation of glucose, glycosylated proteins, and activation of polyol metabolic pathway that accelerates the formation of reactive oxygen compounds. Furthermore, the formation of reactive oxygen compounds reduces the antioxidant enzyme superoxide dismutase including catalase, ceruloplasmin, and glutathione which initiated the formation of oxidative stress by increasing the risk of complications.[1] In experimental animal studies, the state of diabetes can be performed using streptozotocin-induced rats.

Increased consumption of phytochemicals that have antioxidant activity can suppress the development of diabetes. In contrast to other phytochemical compounds such as phenolic components, chlorophyll is present in large quantities in plants (± 1% dry weight); therefore, it is potential to be one of functional food.[2] The role of chlorophyll as hypoglycemic agent occurs through inhibition of free radical mechanism. Chlorophyll is an antioxidant chain breaker which donors its electrons to free radicals and form complexes with peroxy radicals and generated stable product.[3] Besides chlorophyll, chloroplasts also contain exogenous superoxide dismutase[4] and ascorbic acid[5] which also have antioxidant capacity.

Nowadays, there are a lot of imported food supplements based on chlorophyll. Moreover, papaya leaves which has the highest chlorophyll content among green vegetables, are also very common in Indonesia.[6] The leaves also contains saponins, vitamins, minerals.[7] A research showed papaya leaves increase hypoglycemic effect of rats treated with metformin.[8] The purpose of this study was to analyze the effect of chlorophyll in carica papaya Linnaeus leaves on superoxide dismutase and fasting blood glucose levels of diabetic rats.

Methods

The extraction procedure of chlorophyll in papaya leaves was based on research conducted by Setiary and Nurchayati (2009) using 85% acetone solution with a ratio of the sample and acetone 1:100. The extract was filtered by filter paper and analyzed using UV-Vis spectrophotometer at a wavelength of 644 nm and 663 nm. Setiary and Nurchayati found that the chlorophyll content in papaya leaves is 29.5975 mg/g.

Recommendation of 120 g/day of fruit and vegetable consumption (WNPG) resulted in 150 g wet papaya leaves after conversion with Food Processor II and equivalent to 78 g dry leaves. After being extracted with acetone, the weight was reduced to 10% or 7.8 grams. The calculation of the dose conversion factors for human was based on Gosh.[9] A safe dose for human weighed 50 kg is equivalent with (7.8*0.018*50/70)/200 g of rat body weight, or 100.3 mg/200 g body weight for treatment A, and twofold higher for treatment B. The extract was given 1 ml orally.

A randomized controlled trial over 41 days was conducted at PAU Center for the Study of Food and Nutrition, Gadjah Mada University. After 7 days of adaptation period fed with Comfeed AD II and provided with drink *ad libitum*, a total of 36 Sprague Dawley rats were randomly divided into 4 groups: 1 group as control with no treatment, other groups are negative control (diabetic with no treatment), treatment A, and treatment B (twofold higher). Streptozotocin was administered intraperitoneally with a dose of 40 mg/kg body weight, which were given to 3 groups. Rats were categorized as having diabetes mellitus when the blood glucose levels were higher than 200 mg/dL.[10]

The examination of blood glucose levels was conducted quantitatively for 3 times: pretest (7 days after induction), post-test I (day 21 of treatment), and post test II (day 41 of treatment) with Enzymatic Colorimetric Test method "GOD - PAP". Blood was taken via the orbital sinus. After the second post test of blood glucose level, all rats were terminated using intramuscular ketamine hydrochloride with a dose of 60 mg/kg body weight.[11] The measurement of superoxide dismutation levels from rat liver was based on Marklund and Marklund's research,[12] which found that superoxide dismutation enzyme inhibits autoxidation process of pyrogallol into purpurogalin by capturing the oxygen.

Analyses of variance (Anova) was used to assess the difference in superoxide dismutation level of rat liver and the changes of pre and post blood sugar level among 3 groups (negative control, treatment A and treatment B). If the differences were present, Post Hoc LSD were used to assess which groups were different. Data were presented as means±SD with all statistical comparisons made at the 0.05 level of significance (Table 1).

This research passed the study of health research ethics committee, Faculty of Medicine, University of Diponegoro, with ethical clearance number 220/EC/FK/RSDK/2012 dated June 18th 2012.

Results and Discussion

Weight, blood glucose and liver weight. The mean weight at baseline, before induction, and before treatments showed no difference. The streptozotocin induction in 3 groups resulted in some unique characteristics of diabetes such as not physically active, polyuria, weight loss, but without polyphagy. The intakes were relatively the same for all groups (isocaloric) at 17.6 gram/day for 40 days, equivalent to 286 kcal/day. After 40 days of treatments, the best growth and development occurred in treatment B (0.93 g/day), while the biggest weight loss presented in negative control group (-0.36 g/day or 6.8%). Without induction (control techniques), the weight gain could reach 1.27 g/day.

Blood glucose levels increased after 7 days of induction and exceeded Braunwald's (2008) criteria (higher than

200 mg/dL). The treatments (21 and 40 days) resulted in lower blood glucose level; even treatment B reached the normal range of 50-125 mg/dL[13]on day 41, although not as good as normal rat without induction (Figure 1).

The induction also raised the liver weight by 61% (negative control), 44% (treatment A), and 14% (treatment B) compared to control technique. Group treated with a dose of 200.6 mg/200 g body weight have liver weight closer to normal (control technique).

Effect of chlorophyll extract on superoxide dismutation level and blood glucose level. Extract of chlorophyll from papaya leaves was considered to be safe, even with twofold higher dose (treatment B). It tended to have better antioxidant activity, but it still required further histopathologic analysis and toxicity test. Until now, experts have not provided recommended daily dose of chlorophyll.[14] Figure 3 shows that the Negative control group (no treatment) had the highest level of superoxide dismutase (Table 2). The best effect on blood glucose level occurred at day 41th in Treatment B group. Treatment A decreased blood glucose level by 89.83 mg/dL while treatment B by 110.94 mg/dL after 40 days (Figure 2).

Weight, blood glucose and liver weight. There are several implications generated by the induction of streptozotocin. The effects of streptozotocin is irreversible, and causes degranulation and capacity lost in insulin production that leads to hyperglycemia.[15] Streptozotocin also impairs glucose oxidation as well as causing the loss of adipocytes[16] and muscle atrophy accompanied by a decrease in skeletal muscle mass and a loss of structural proteins resulting in weight loss.[17] Structural proteins are proteins that play a role in structural biology support or build living things.[18] Several factors determine the severity of experimental diabetogenic activity, which are the dosage and administration of streptozotocin, animal species, strain, and age of experimental animals.[19]

Other effects are not only limited to the increase in blood glucose levels and weight loss, but also an increase in liver weight. Liver is the main organ in detoxification and oxidative processes. Even at the early stages of the disease, a marker of oxidative stress in the liver began to increase. Free radicals are the results of streptozotocin, liver damage, as well as pancreatic β cells.[20]

Negative control group had the highest level of superoxide dismutation. Higher superoxide dismutation activity means more radicals to neutralized[2,21] and greater volume of superoxide dismutation to inhibit the autooxidation process of pyrogallol compounds into purpurogalin by capturing oxygen.[22] Superoxide dismutase is the highest antioxidant enzymes in the body and are mostly located in liver.[23] This enzyme catalyzes radical superoxide (O2)[24] which in this case comes from streptozotocin.

Table 1. Means of Weight, Blood Glucose Level, and Liver Weight during Study

Indicator	Technique control	Groups		
		Negative control	Treatment A	Treatment B
Weight (g)				
Initial adaptation	206.2±9.74	218.7±10.72	195.4±13.19	199.6±7.43
After induction	213.6±9.64	225.8±10.59	202.6±13.32	205.9±7.66
Initial treatment	224.2±9.54	221.4±10.65	197.9±13.19	201.7±7.73
End of treatment	274.9±9.50	206.4±10.36	221.9±12.69	239.0±7.63
Changes during treatment	50.7±0.47	-15.0±0.78	24.0±0.60	37.3±0.65
Blood Glucose level (mg/dl)				
Baseline (Day 1)	73.0±0.45	223.7±1.16	223.1±0.93	227.2±1.66
Middle (Day 21st)	74.7±0.60	226.2±1.28	170.7±1.03	140.1±1.07
End (Day 41st)	75.7±0.65	228.2±1.10	133.3±2.58	116.3±3.30
Liver weight (g/100 gr BB)	3.6±0.17	5.8±0.44	5.2±0.34	4.1±0.13

Table 2. Effect of Chlorophyll in Papaya Leaves on Superoxide Dismutation and Blood Glucose Level

Indicator	Technique control	Groups			*p*
		Negative control	Treatment A	Treatment B	
Liver superoxide Dismutation level (%)	23.6±0.84^a	57.2±1.17^b	42.9±0.59^c	33.5±0.42^d	0.01*
Changes of blood glucose level (mg/dl)	2.7±0.38^a	4.5±0.45^b	-89.8±2.83^c	-110.9±3.45^d	0.01*

p = Anova in 3 induction groups (negative control, Treatment A and B)
* = *Anova*, significant
The average value followed by different letters in the same row indicates significant differences (p <0.05)

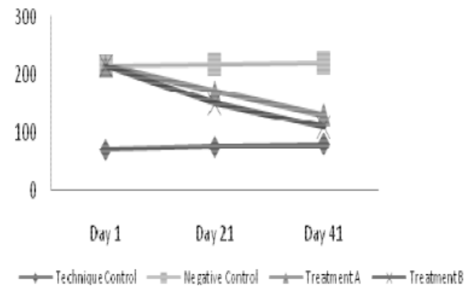

Figure 1. Graphic of Rats Blood Glucose Level

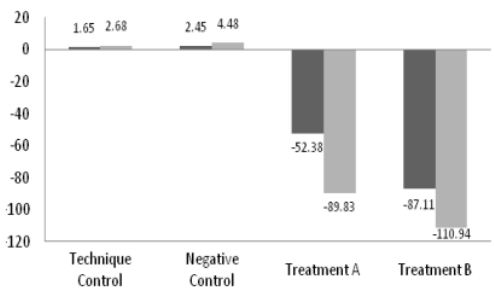

Figure 2. Graphic of Blood Glucose Changing

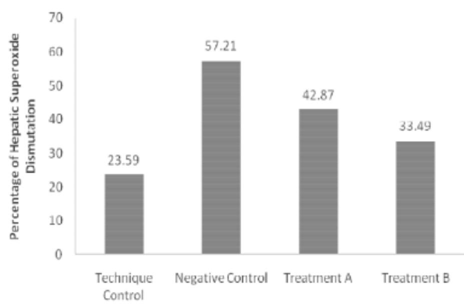

Figure 3. The Effect of Papaya Leaf Chlorophyll Extract on Superoxide Dismutation Level

Higher dose of chlorophyll extract had better antioxidant activity than the lower dose, as indicated from the percentage of inhibition levels were considered to approach the value of control technique. It might be caused by 3 factors. Firstly, the antioxidant properties of chlorophyll. Chlorophyll is a chain breaker antioxidant that works by donating electron to free radicals, especially at the initiating stage before the hydroperoxide is formed. The essential structure of the chlorophyll, which has antioxidant activity, is in the porphyrin structure. This role is supported by magnesium at the core of chlorophyll, primarily in chelates form, not ionic. Secondly, the role of other compound than chlorophyll in chloroplast that have antioxidant capacity, for example exogenous superoxide dismutation, especially Fe-SOD, Cu, Zn-SOD, Mn-SOD and ascorbic acid. Superoxide dismutase is an antioxidant that catalyzes superoxide anion superoxide (O ●) and acts as the first defense against free radicals, whereas ascorbic acid breaks the peroxide radicals chain by giving hydrogen ions to free radicals and change into more stable molecules. And ascorbic acid helps superoxide dismutation role by catalyzing superoxide radicals reaction into H2O2 that more stable.[25] Thirdly, papaya leaves contain saponin, which also have antioxidant capacity. Saponin activates catalase and superoxide dismutation by giving hydrogen ions.[26]

Combination of some antioxidant components in papaya leaves extract, which are chlorophyll, magnesium, superoxide dismutation exogenous, vitamin C, and saponins, have no possible prooxidant effects in experimental animals. Hematic components are prooxidant, while the structure of porphyrins in chlorophyll are antioxidants.[27] Giving oral treatment is considered to be safer than any other way. Saponins have toxic effects when given intravenously, but toxic effects are reduced when administered orally.[28]

The effect of papaya leaf chlorophyll extract on blood glucose level. Both treatment were able to lower blood glucose levels close to normal (50-125 mg/dL) but the best effect occurred in treatment B, although it was not as good as normal rats without induction (technique control). The role of hypoglycemic agent from the extract is thought to be caused by 2 factors. Firstly, the role of some antioxidant components which are chlorophyll, magnesium, exogenous superoxide dismutation, and vitamin C in counteracting free radicals. Secondly, saponin content in papaya leaves also has a role as a hypoglycemic agent. Even saponin content in 22 Chinese herbs has antioxidant activity and high antiglycation, thus it plays a role in the treatment of diabetes mellitus.[29]

Di Naso[30] also found that exogenous administration of superoxide dismutation had no effect on blood glucose levels in diabetic rats group. Exogenous superoxide dismutase in this study was orgotein (generic name Cu-Zn SOD), a pure substance derived from cow liver after going through the process of heating, enzymatic digestion with other proteins, as well as the purification of the homogenate by the method of ionic exchange chromatography. The absence of hypoglycemic effects was thought to be caused by the time and duration of exogenous superoxide dismutase administration. Although there were no effect on blood glucose levels, oxidative stress decreased, and there was increased antioxidant enzymatic activity in the liver of diabetic rats.

Conclusion

Extract of chlorophyll in papaya leaves for 40 days increased the antioxidative status of the body, indicated with low levels of superoxide dismutase and decreased blood glucose level. The higher dose of extract, the lower level of superoxide dismutase and the higher change of blood glucose level. The extract with a dose of 200.6 mg/200 g body weight can lower blood glucose level to normal after 40 days.

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