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Hypoglicemic and Antioxidant Activity of Yellow Pumpkin (Curcubitamoschata) in Diabetic Rats

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Abstract

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia due to disorders of insulin secretion, insulin sensitivity, or both. The main mode of diabetes control can be achieved with diet, exercise, and insulin replacement therapy nor with various oral hypoglycemic drugs. Diabetes mellitus also results in disorder of the lipid profile of the body so that the cells are more susceptible to lipid peroxidation. Malondialdehyde (MDA) is formed as a result of lipid peroxidation that can be used to measure lipid peroxidized. It has been reported that the polysaccharide from the pumpkin (Cucucrbitamoschata) has hypoglycemic activity by increasing plasma insulin.

This study aims to determine the activity of hypoglycemic, and the effect of hepatoprotective pumpkin in alloxan-induced diabetic rats by 30 Rattus norvegicus, divided into 6 groups of 5 each. Group K (-) was the negative control group without treatment, K (+) of untreated diabetic control group, TC1, TC2, TC4 were grouped with pumpkin flour with 1, 2 and 4 gram/kg bwt for 21 day by gavage, and the diabetic group given glibenclamide 0.45 mg/kg body weight. The hypoglycemic effect was determined by measuring the blood glucose level, the antioxidant effect determined by measuring the activity of AST and ALT enzymes and the serum malondialdehyde levels.

The results of this study showed that the pumpkin flour had the hypoglycemic effect of lowering blood glucose level at 4 g/kg bwt doses equivalent to Glibenclamide 0.45 mg/kg bwt ($p > 0.05$); had antioxidant activity by decreasing the activity of AST and ALT enzymes ($p < 0.05$) and decreasing malondialdehid levels in diabetic rats ($p < 0.05$).

Keywords: *Hypoglycemic; Antioxidant; Pumpkin; Cucurbita.*

Introduction

Diabetes is a serious chronic disease that occurs when the pancreas does not produce enough insulin (a hormone that regulates blood sugar or glucose), or if the body cannot effectively use the insulin it produces. Diabetes is an important public health problem, one of four priority non-communicable diseases (Non Communicable Disseases). Both the number of cases and the prevalence of diabetes have continued to increase over the past few decades. Diabetes of all types can cause complications in many parts of the body and may increase the risk of death as a whole early. Possible complications include heart attack, stroke, kidney failure, leg amputation, loss of vision and nerve damage.[1]

Hyperglycemia in DM increases the risk of microvascular damage (retinopathy, nephropathy and neuropathy) and macrovascular (ischemic heart disease, stroke and peripheral vascular disease). This results in a decrease in quality of life, a decrease in life expectancy, and even death can occur due to these complications.[2]

Diabetes mellitus results in disruption of the body's lipid profile so that cells are more susceptible to lipid peroxidation. Experimental studies show that

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polyunsaturated fatty acids in cell membranes are very susceptible to attack by free radicals because of the many bonds. Lipid hyperperoxide (LHP) through a radical reaction between producing a highly reactive and toxic lipid radical that forms a new LHP.[3] The resulting lipidperoxide often decomposes into radicals, which in turn react with the most biologically important molecules such as proteins and lipids. Further decomposition of this lipid peroxide produces toxic malondialdehyde (MDA).[4] Malondialdehyde (MDA) is formed as a result of lipid peroxidation which can be used to measure lipid peroxide.[3]

Pumpkin is a plant that has often been used as a functional food or medicine. For hundreds of years, pumpkin has been considered as a traditional Chinese medicine used for the prevention and treatment of various diseases such as hypertension, hypercholesterolemia, tumors, immunological disorders and DM. It has been reported that polysaccharides of pumpkin (PP) have hypoglycemic activity by increasing plasma insulin in normal mice and diabetes.[5]

Material and Method

Pumpkin is obtained from traditional markets in Yogyakarta, Indonesia. White rats Rattus norvegicus Wistar strain obtained from Universitas Gadjah Mada Yogyakarta. Ingredients: ethanol, CMC (carboxy methyl cellulose), Glucose reagent kits, AST and ALT from Sigma and tiobarbiturate (TBA) reagents from Sigma. Instruments and tools: syringes, spectrophotometers, automatic pipettes 10 µl, 20 µl, 100 µl, 1000 µl, vial, white tip, yellow tip, blue tip, test tube.

The making of pumpkin flour is carried out in the laboratory of the Medical Laboratory Technology Department as follows: the pumpkin is peeled, the flesh is taken, shredded and dried in an oven at a temperature of 50-60^{o}C for 72 hours, then mashed with blender and sifted.

The treatment of experimental animals is carried out in the Central Food and Nutrition Laboratory of the Gadjah Mada University, as follows:

1. 30 rats, acclimatized using individual cages for 3 days with room temperatures ranging from 25-280 C and 12 hours daily lighting cycles. During acclimatization and during the study rats were fed standard AD II and drinking water in ad libitum.
2. Rats were divided into 6 groups, each consisting of 5 animals, namely K(-), K(+), TC1, TC2, and TC4.
3. The blood sample were taken from the retroorbital vein, then their blood glucose levels were determined.
4. Group K (-) is a negative control group without treatment, group K (+), TC1, TC2, TC4 made diabetic by giving glibenclamide at a dose of 0.45 mg/kg.
5. On day 3, all rats were determined by their blood glucose levels, AST enzyme activity, ALT and malondialdehyde levels (pre test).
6. The TC1, TC2, TC4 groups were given pumpkin flour with doses of 1, 2 and 4 grams/kg for 21 days using feeding tube, and the diabetic group given glibenclamide 0.45 mg/kg.
7. After 24 hours of the last treatment, rat blood was taken for determination of blood glucose levels, AST enzyme activity, ALT and malondialdehyde levels (post test).

Determination of blood glucose levels using the enzymatic GOD-PAP method, AST and ALT enzyme activity by kinetic enzymatic method, and malondialdehyde levels carried out by the TBA method.

Result and Discussion

Administration of yellow pumpkin flour in diabetic rats can reduce blood glucose levels, presented in the following figure:

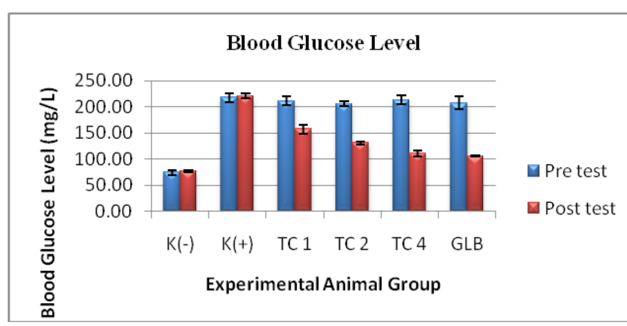

Figure 1. Blood Glucose Level Chart in Diabetic Rats

Administration of yellow pumpkin flour in diabetic rats can decrease AST and ALT enzyme activity, presented in the following figure:

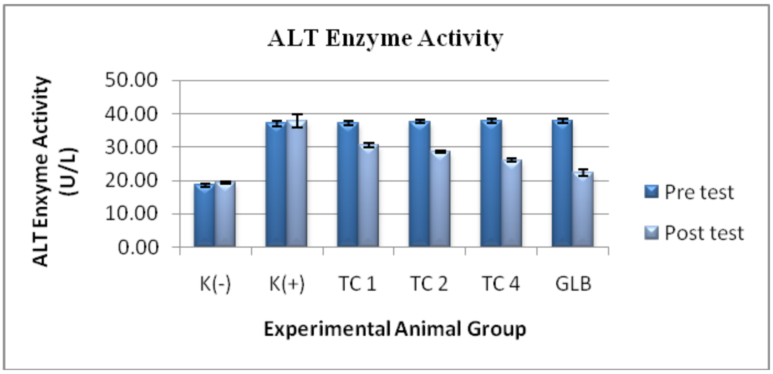

Figure 2. ALT Enzyme Activity Chart

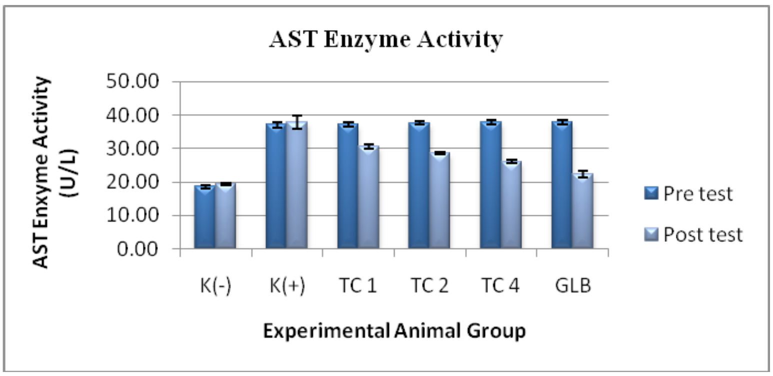

Figure 3. AST Enzyme Activity Chart

Administration of yellow pumpkin flour in diabetic rats can malondialdehyde levels, presented in the following figure:

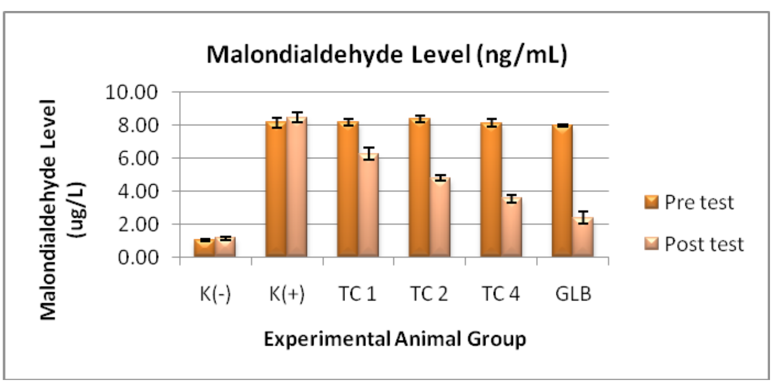

Figure 4. Malondialdehyde Level

The data obtained were analyzed statistically with the following results:

Group	Glukosa mg/dL			ALT U/L			AST U/L			MDA nmol/ml		
K(-)	77.14[a]	±	1.94	19.32[a]	±	0.41	38.35[a]	±	0.77	1.13[a]	±	0.09
K(+)	221.08[b]	±	5.16	37.87[b]	±	1.97	78.26[b]	±	1.70	8.45[b]	±	0.28
TC 1	157.44[c]	±	8.21	30.49[c]	±	0.72	58.16[c]	±	2.56	6.26[c]	±	0.34
TC 2	130.82[d]	±	3.06	28.55[d]	±	0.41	49.13[d]	±	1.43	4.79[d]	±	0.19
TC 4	110.95[e]	±	4.78	26.02[e]	±	0.55	43.31[e]	±	1.21	3.54[e]	±	0.22
GLB	106.10[e]	±	0.62	22.33[f]	±	1.03	40.88[f]	±	1.25	2.39[f]	±	0.37

Annotation: the numbers in the same column with different superscript letters show significant differences ($p < 0.05$)

Administration of yellow pumpkin flour for 21 days with a dose of 1 g/kg, 2 g/kg, and 4 g/kg lowered blood glucose levels in white rat (Rattus norvegicus) that have been made diabetic by giving alloxan. The greater the dose of pumpkin flour, the greater the decrease in blood glucose levels. The results of this study are in accordance with a previous study conducted by Jin et al (2013), which states that pumpkin flour extract in alcohol 95% is hypoglycemic. The mechanism proposed is that pumpkin flour extract does not stimulate β cells on the island of Langerhans to increase insulin secretion, but restore the island of Langerhans, repair damaged islands or act as an insulin sensitizer to increase insulin activity by improving insulin sensitivity in target tissues such as liver, muscle and adipose tissue. Pumpkin polysaccharides can also play an important role in restoring liver function and glucose use.[6]

Pumpkin contains various biologically active components, such as polysaccharides, paraaminobenzoic acid, fixed oils, sterols, proteins, peptides, carotenoids, gninobutyric acid and vitamins (Murkovic et al., 2002).

Pumpkin has antioxidant activity. Antioxidants are substances that protect membrane cells and other components of organisms against damage caused by oxidants. These compounds function by collecting free radicals, transferring electrons to them and ultimately making them inactive.[7]

Therefore, the protective effect of pumpkin pancreas and its hypoglycemic properties are caused, in part, by the antioxidant activity of this fruit. Abdel-Hassan et al. (2000) found that saponins extracted from Cucurbitaceae had high anti-diabetic activity. Flavonoid compounds, including quercetin with antioxidant activity also have a hypoglycemic effect in diabetic rats.[8] In addition, the presence of pectin itself can function as a hypoglycemic agent in pumpkin.[9]

In addition, the protective effect of PP on island cells damaged by STZ is through increased levels of SOD and MDA and reduced production of nireogen oxide (NO). PP inhibits apoptosis in damaged STZ island cells through modulating Bax/Bcl-2 expression. Given the significant protective effect of PP on small island cells, we suspect it will potentially improve the therapy of diabetes mellitus, which is used as a functional and nutraceutical food ingredient.[5]

The apoptotic signal meets the common cell death pathway, where caspase carries out apoptosis and the Bcl-2 family protein regulates it. There are two classes of regulatory proteins in the Bcl-2 family that support the opposite effect of apoptosis: anti-apoptotic members include Bcl-2 and Bcl-xL which protect cells against some forms of apoptosis, and pro-apoptotic members include Bax, tubs, and Bcl- xs that promote programmed cell death. The balance between pro-and anti-apoptotic signals from this family has a central role in the release of cytochrome C and its main consequences.[5]

In the diabetes model, regulation of decreased bcl-2 expression has been demonstrated. To further explain the protective effect of polysaccharides on pumpkin (pumpkin polysacharide = PP) and its mechanism, the effects of STZ and PP-1 on bcl-2 mRNA expression on Langerhans island cells were tested. The results showed that bcl-2 mRNA expression in Langerhans islet cells treated with streptozotocin (STZ) decreased, implying that bcl-2 was involved in the β cell apoptosis process induced by STZ. Pumpkin polysaccharides play the role of antiapoptosis by increasing the level of bcl-2 mRNA on Langerhans island cells.

Bax protein is a member of the Bcl-2 family that increases apoptosis. In previous studies it was found that bax mRNA expression was higher in the diabetes model. The study further proved that bax overexpression in Langerhans island cells treated (STZ) occurred, indicating that bax was responsible for the development of apoptosis of injured island cells. The role of pumpkin polysaccharides can reduce bax mRNA expression to protect island cells from damage to STZ.[5]

In this study, pumpkin was made flour, in the hope that it could later be used as nutrasetikal. Pumpkin flour is made a solution with the help of carboxymethylcellulose (cmc = carboxymethyl cellulose) then given to the sab diabetic model so that the dose is measured. The results of this study indicate that there was a decrease in blood glucose levels in the group of rats given yellow pumpkin flour for 21 days, the largest decrease occurred in the group with a dose of 4 grams/kg. The decrease in blood glucose levels is comparable to the glibenclamide dose of 0.45 mg/kg ($p > 0.05$), and but has not reached normal blood glucose levels ($p < 0.05$).

The results of testing the antioxidant activity in this study indicate that the administration of pumpkin flour can reduce the activity of AST and ALT enzymes. The AST and ALT enzymes are liver intracellular enzymes. In the diabetic model there is an increase in the levels of AST and ALT enzymes due to the administration of alloxan which is also produced to be hepatotoxic resulting in liver cell damage. The decrease in the activity of these two enzymes shows that there is a mechanism for protecting liver cells from oxidative damage by alloxan.

The results of this study also showed a decrease in malondialdehyde levels in model rats given pumpkin flour. This shows the antioxidant activity in pumpkin. Malondialdehyde is one of the lipid peroxidation products. Lipid peroxidation is greatly increased in diabetic conditions because of an increase in free radicals. In alloxane-induced diabetic model rats also increase malondialdehyde levels because alloxane produces free radicals which subsequently occur lipid peroxidation to produce malondialdehyde.

These results are in accordance with the study conducted by Sopan et al. (2014) showed that Cucurbita maxima (pumpkin) flour contained phenolic compounds. This phenolic compound has many hydroxyl groups including ortho-hydroxy groups which have very strong antioxidant potential.[10]

Conclusion

Pumpkin flour has a hypoglycemic effect that is lowering blood glucose levels in diabetic mice, at a dose of 4 grams/kg BW equivalent to 0.45 mg/kg BB Glibenclamide, has antioxidant activity by reducing the enzyme activity of AST and ALT in diabetic mice ($p < 0.05$) and reduce malondialdehyde levels in diabetic mice ($p < 0.05$).Subsequent research can examine pancreatic cells to find out the mechanism of blood glucose reduction, whether through its effects on pancreatic beta cell repair, further investigating the effects of improvement on lipid profiles in diabetic conditions.

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Conflict of Interest: All authors state that there is no conflict of interest.

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