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Effects of Coffee Consumption In Improving Hyperglicemia In Diabetes-Induced Mice

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Abstract. Diabetes mellitus (DM) is a chronic metabolic disease. It was caused by lack of insulin or cells cannot use insulin so that blood glucose becomes high (hyperglycemia). And it will cause other systemic diseases of the disease (metabolic syndrome) that can increase the factor of death. Coffee is one of the consumed plants that contain antioxidants and chlorogenic acid that have a role as antihyperglycemia. In other hand Jambi as one of the coffee producing regions (arabica, robust and liberica) in Sumatra and it has a potency as a coffee producer. In addition, people's habits in consuming coffee have the opportunity to be one solution in overcoming diabetes. This study aimed to determine the effect of hyperglycemia disease. The method was experimental with 5 treatments (metformin, arabica, robust, liberica and aquades) and 3 replications. Before threatments mice were injected with streptozotosin in order to be hyperglycemia, then mice with blood glucose ≥ 116 mg / dl were measured their blood glucose, weight and made the histology of the liver. Then the datas were analyzed using ANAVA. The results showed that Jambi coffee (arabica, robust and liberica) can lower blood glucose levels hyperglycemia mice until day 16. And the treatment with liberica coffee lowers hyperglycemia with the lowest glucose levels. While the histological features of mice liver showed lower cell degeneration especially in mice by coffee treatment, especially arabica coffee.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder due to lack of insulin or cells cannot use insulin so that blood glucose becomes high (hyperglycemia). Long-lasting hyperglycemia can cause damage to various body systems, especially the nerve and blood vessels. The condition of hyperglycemia due to diabetes is one of the causes of the increase in Reactive Oxygen Species (ROS). The increase in ROS caused by hyperglycemia can occur through various mechanisms,

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either due to increased production of oxidants or decreased antioxidants (Juśkiewicz et al. 2008). In the condition of diabetes, this increase in the number of ROS can cause inhibition of the delivery of insulin signals so that the flow of signals to subsequent proteins is inhibited. Inhibition of the delivery of these signals will prevent GLUT translocation to the cell surface so that blood glucose uptake by cells becomes reduced (Erol, 2007).

This if left unchecked will cause other systemic diseases (metabolic syndrome) to increase mortality. It is known that based on WHO data in 2014, 422 million people die each year due to DM (Global Burden of Diabetes, 2016). Whereas in Indonesia it is known that the number of DM patients is 2,650,340 people and can be more because more than 1 million people have DM characteristics but have never been diagnosed in hospitals or health centers (Ministry of Health, 2013).

Coffee is one of the community consumption plants that are processed both from seeds and leaves as a beverage. Aside from being a coffee drink, it contains various phenol compounds so that they have the potential as medicinal ingredients, including diabetes drugs. It is known that coffee leaf extract (arabica) contains antioxidants that can act as antidiabetic (Retnaningtyas & Si 2016). In addition, research shows that coffee consumption can reduce the risk of DM by improving the condition of hyperglycemia as research by Yamauchi et al. (2017) in diabetic mice. Then coffee which has polyphenol content in the form of chlorogenic acid and has a metabolic form of cafeid acid is known to improve glucose transport in mice skeletal muscle cells (Tsuda et al. 2012). While research on Korean women with diabetes and nondiabetic who consume one or more cups of coffee per day shows better kidney function and also lower blood sugar levels (Kim et al. 2013).

Jambi is one of the coffee-producing areas in Sumatra with a total production of around 13,317 tons per year. The coffee is cultivated in Kerinci, Merangin, Sarolangun, Batanghari, Muaro Jambi, Tanjung Jabung Timur, West Tanjung Jabung, tebo, Bungo and Sungai Penuh districts (BPS Jambi Province 2016). Coffee which is cultivated in Jambi consists of arabica, robusta and liberika coffee. Considerable production potential and interest in community coffee consumption (Bakti 2015) can be developed into one solution to overcome and prevent diabetics. So, based on this, we want to know how the influence of coffee consumption comes from the condition of changes in hyperglycemia in diabetes-induced mice.

2. Materials and Methods

Coffee samples. Coffee samples in the form of coffee beans of the type of arabica, robuska and liberika obtained from coffee sellers "Cafe Coffee" in the form of expressions. The coffee came directly from coffee-producing areas in Jambi province, namely arabica coffee from Kerinci

district, Robusta coffee from Merangin district and liberika coffee from Tanjung Jabung Barat district (Tungkal).

Animal Experiments and Diabetes Induction. The experimental animals used were male mice (*Mus musculus* webster strain) aged ± 8-10 weeks with a body weight of 20-30 g obtained from the Animal Health Laboratory in Jambi City. During the treatment, lighting was used for 12 hours of light and 12 hours of darkness with a humidity level of ± 55% and room temperature of ± 25 oC. The induction of mice into diabetes began with mice fastened for 18 hours and measured their blood glucose concentration. The next two hours (after the wound dries) the mice are given a single dose of Steptozotosin injection of 200 mg / kg which has been dissolved in citrat buffer pH 4.5 per body weight intraperitonially. On the tenth day after injection of Streptozotosin, mice were fasted for 18 hours and their blood glucose concentration was measured. Mice with hyperglycemia with blood glucose levels 6116 mg / dL. And then will be given treatment for 30 days (Furman 2015). The treatments in this study were aquades (Q), arabica coffee (A), robusta coffee (R), liberika coffee (L), and metformin (M).

Blood Glucose Level. Measurement of blood glucose concentration was measured using a glucometer tool. Mice have been fasted for 18 hours, then the blood is taken by injuring the veins of the mice's tail, then dropping it on the stripe that has been attached to the glucometer. Measurement of fasting blood glucose levels is carried out every five days. Glucose tolerance test was carried out on the last day of treatment. Measurement of blood glucose concentration was carried out after mice were fasted 18 hours ago 1 hour and 2 hours after oral glucose was given (2 g / kg body weight).

Histology of Liver and Pancreatic Tissues. For histological observations, the liver and pancreas at the beginning and end of the treatment were taken and then fixed with a formalin buffer, then made preparations using the standard method of histology of tissue examination. After that a microscopic examination of the liver tissue was carried out. From each rat, 5 preparations of liver and pancreas were made, each preparation was observed in 5 fields of view, namely at the four corners and the center of the preparation with 1000x magnification. Then in each heart preparation the mean degeneration value is calculated by multiplying the number of cells according to their category with the values in table 1. The targets observed are changes in the histological structure of rat liver (L.A 2009).

Table 1. Assessment Criteria for degenerating cells

Types Degeneration	Score
Cell Normal	1
Cloudy Swelling	2

Types Degeneration	Score
Hydropic Degeneration	3
Necrosis (piknotik cells, karioreksis, kariolisis)	4

3. Result and Discussion

Blood Glucose Level

In this study the induction of streptozotosin is able to make experimental mice experience hyperglycemia. Mice used in the study had blood glucose at a range of 116-217 mg / dL, a condition of hyperglycemia that was valued under 126 mg / dL was not included in the category of mice with diabetes but included in the Impaired fasting glucose (IFG) category. IFG is characterized by fasting blood glucose in the range of 100-125 mg / dl and is included in the Pre-Diabetes condition (Poretsky 2010).
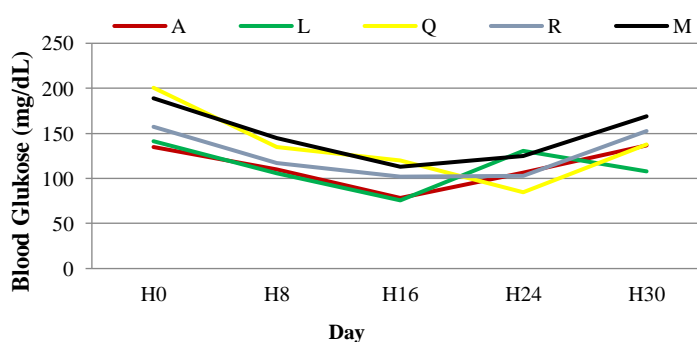

Figure 1. Changes in fasting blood glucose levels of mice during treatment, A: arabica coffee, L: Liberika coffee, R: robusta coffee, Q: Aquades, M: metformin

The treatment that has been given for 30 days shows the same tendency for all treatments (Figure 1). On the graph, there is a decrease in blood glucose levels until the 16th day and again increases until the 30th day, except for the treatment given by liberika coffee. Mice given liberika coffee show lower blood glucose levels than other treatments and tend to be the same as the initial conditions. Blood glucose data analysis was performed using normality test and continued with multivariate analysis. The test results show significant differences between treatments on each day of observation except at H30 at P value <0.05.

The conditions experienced from observations are related to glucose tolerance, namely the ability of experimental mice to absorb glucose circulating in the blood into the cell. In this study the condition of hyperglycemia did not affect the condition of blood glucose tolerance except in mice given arabica coffee (Figure 2). This is in contrast to the research of Matsuda et al. (2014),

which showed an increase in glucose tolerance in diabetic mice that had been given coffee and caffeine for 16 weeks compared to diabetic mice. However, when viewed from the difference in the value of fasting blood glucose and 2 hours of Post Prandial (PP) shows that the highest difference is owned by mice that consume liberika coffee, which can be assumed that cells in mice can absorb more glucose than other treatments. So it can be expected that the consumption of liberika coffee can improve blood glucose tolerance in conditions of hyperglycemia and can reduce blood glucose better than other coffees. But for more accurate results it is necessary to conduct research using more replications with initial blood glucose values more heterogeneous.1. *Rattus tiomanicus* Miller. 1900

Classification according to Wilson & Reeder (2005), this animal belongs to the Muridae Family with the genus Rattus. This species has a characteristic dorsal gray-brown hair, black body hair with white ventral hair and a dark brown tail. The average morphometric size of adult mice, length from nose tip to tail tip 29.2 cm, tail length 14.3 cm, hind leg length from heel to the longest toe toe 3.0 cm and ear length 2.1 cm. Rattus tiomanicus is a thicket rat and spreads easily in various habitats, has a total length ranging from 14-18.8 cm, tail length 12-18.1 cm, hind legs 2.8-3.5 cm and ear length 1.1-1 2.8 cm (Cunningham & Moors, 1996; Aplin et al., 2003; Payne et al., 2000 and Yasuma et al., 2003).
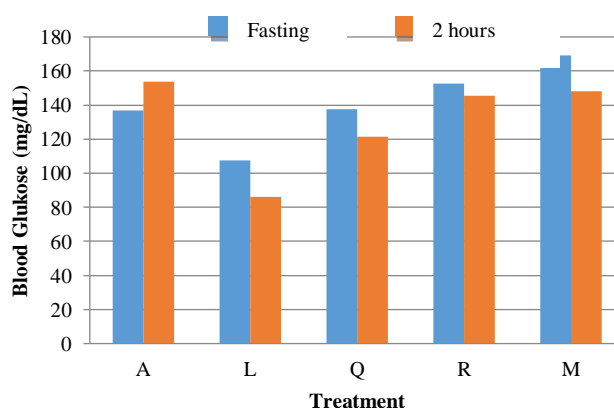

Figure 2. Mice blood glucose tolerance value after treatment, A: arabica coffee, L: Liberika coffee, R: robusta coffee, Q: Aquades, M: metformin (* the figure above the graph shows the difference in blood glucose levels)

Relationship of Hyperglycemia Conditions with Changes in Body Weight

Mice treated with hyperglycemia tend to gain weight after 30 days of treatment (Table 2). An increase in hyperglycemic animal body weight was also seen in studies conducted by Matsuda et al. (2014) that at the 4th week of treatment of diabetic mice and given treatment experienced

changes in body weight but did not experience changes in the amount of food and drink consumed. Weight gain in diabetic animals can be caused by damage to lipid metabolism due to hyperglycemia conditions that occur (Kurniawan 2010). However, the statistical test using multivariate analysis at P-value < 0.05 showed the results were not significantly different from weight gain for all treatments.

Table 2. Changes in body weight of treatment mice

Treatment	Initial Weight (gram)	Final Weight (gram)
Aquades (kontrol)	29,1 ± 5,31	33,7 ± 2,75
Arabika	28,1 ± 3,25	32,3 ± 0,52
Robusta	33,5 ± 3,86	31,9 ± 3,03
Liberika	32, 9 ± 2,69	32,6 ± 2,12
Metformin	28,7 ± 3,9	33,2 ± 1,91

Liver Cell Histology (Hepatocytes)

Cell histology can be measured quantitatively by looking at the degeneration value of the cells. The value of cell degeneration influences coffee consumption in hyperglycemic mice seen from the number of normal cells, cloudy swelling cells, hydropic degeneration and nexrosis in the liver tissue histology (Figure 5.3) and the pancreas. However, pancreatic preparations are damaged during isolation because they are so small that they cannot be observed. Whereas degeneration of liver cells is seen starting from the lightest change / damage, the cell that experiences cloudy swelling. Cloudy swelling is characterized by a larger cell size (swelling) and less absorption of hematoxylin dyes than normal cells so that the cytoplasmic color fades more. The condition of hydropic degeneration is characterized by cystoplasmic conditions which are more turbid than cloudy swelling. The most severe condition at the degeneration stage is characterized by necrosis. Cells classified as necrosis can be found in histological incisions under picnotic conditions, karyorrhexic and cariolysis (Figure 3.b).

The highest degeration value was seen in the liver of hyperglycemic mice without coffee while hyperglycemic mice given coffee all experienced lower degeneration. And the lowest degeneration was seen in mice given arabica coffee (Table 3). And the results of one-way variance analysis showed that the degeneration value was significantly different at P-value < 0.005 in all treatments.
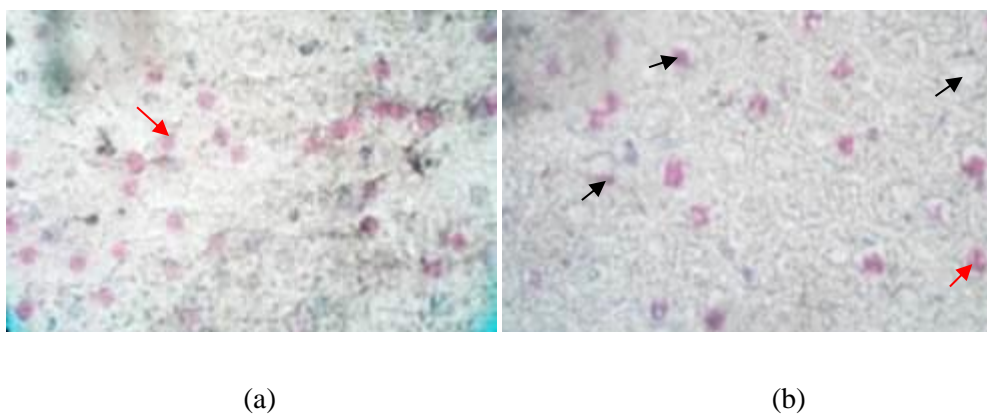
(a)   (b)

Figure 3. Histology of liver tissue of normal mice (a) and hyperglycemic mice (b), magnification 1000x (red arrow: normal cells, black arrows: cell necrosis)

Table 3. Cell degeneration value of mouse liver

Treatment	Degeneration Value
Arabika	$152,5 \pm 32,8^a$
Liberika	$187,5 \pm 53,8^a$
Robusta	$181,3 \pm 22,5^a$
Aquades	$208,8 \pm 13,8$
Metformin	$170,0 \pm 21,2^a$

Hepatocyte degeneration can occur due to the effect of STZ injection which is used as toxic for induction of hyperglycemia. However, changes in liver histology are not directly affected by hyperglycemia but are caused by the effects of STZ (Ozdemir et al. 2009; Guven et al. 2006; Zafar et al. 2009; Amin et al. 2006). Because STZ is a nitrosurea compound that can cause hepatotosic conditions that will affect the appearance of liver histology and also cause a decrease in liver function. This hepatotoxic condition is caused by STZ can increase the activity of superoxide dismutase in the liver, pancreas and kidneys and then affect the amount of malondialdehyde (MDA), reduce antioxidant enzymes such as SOD and GSH-Px, (Guven et al. 2006). In addition, STZ is able to increase the amount of serum alanine aminotransferase (ALT) which causes mitochondrial instability and cell death (nexrosis) (Zafar et al. 2009).

Coffee consumption in hyperglycemic mice showed improvements in liver histology (degeneration value) hyperglycemic mice. This improvement is allegedly caused by the content of chlorogenic acid which acts as an antioxidant in coffee. As well as research (Yukawa et al. 2004) shows that the probandus MDA value that consumes coffee after 7 days has decreased

compared to basal MDA, in addition there is no chlorogenic acid in probandus serum and urine which indicates that the antioxidant is in the form of conjugation with glutathione (cell antioxidant). In addition, consumption of chlorogenic acid is also known to inhibit morphological changes in nucleus condensation and DNA fragmentation which are characteristic of cell necrosis (Cho et al. 2009).

The lowest value of liver cell degeneration is owned by mice that consume arabica coffee, followed by robusta coffee and liberika coffee. However, it cannot be concluded with certainty whether the amount or quality of the chlorogenic acid antioxidant content of each coffee is related to the condition of hepatocyte cell degeneration. So that for further research it is necessary to know the antioxidant content in each type of coffee used

4.   Conclussion

Coffee (arabica, robusta and liberika) can reduce blood glucose levels of mice with hyperglycemia until the 16th day. And at the end of the treatment Liberika coffee provides a lower level of hyperglycemia with the lowest glucose level. While the histological features of mouse liver showed lower cell degeneration in mice given coffee treatment, especially arabica coffee. And it is recommended to conduct further research in the form of measuring plasma insulin levels and HbA1c so that the correlation between the increase and decrease in blood sugar levels is more visible. In addition, the use of larger experimental animals such as mice is recommended to obtain a better sample in terms of quantity.

Acknowledgement

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