The Effect of Gayo Arabica Coffee Leaf Extract on Blood Sugar Levels in Rat with Type 2 Diabetes Mellitus

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ABSTRACT

Purpose: *To understand the effect of ethanol extract of Gayo Arabica coffee leaf (Coffea arabica) on changes in blood glucose levels in type-2 diabetes mellitus rats (Rattus norvegicus).* **Research Methodology:** *To understand the effect of ethanol extract of Gayo Arabica coffee leaf (Coffea arabica) on changes in blood glucose levels in type-2 diabetes mellitus rats (Rattus norvegicus).* **Results:** *The study showed a decrease in blood glucose levels which was significant with a value of p=0.035 after giving Gayo Arabica extract 150 mg/kg/day to rats for 13 days.* **Limitations:** *This research provides treatment for 13 days.* **Contribution:** *Gayo Arabica coffee leaves show had chlorogenic acid which can stimulate glucose uptake in skeletal muscles with AMPK activity, which leads to a decrease in glucose levels in the blood. This supports Gayo Arabica coffee leaves as a good anti-diabetic for type 2 diabetes mellitus.*

Keywords: Diabetes mellitus, Metformin, Gayo Arabica coffee leaf extract

INTRODUCTION

Based on the results of data collection, it was found that more than 425 million people live with Diabetes Mellitus (DM), in which most of the cases are Type 2 Diabetes Mellitus (T2DM) [1]. In 2017, Indonesia was ranked sixth in the world for the highest prevalence of diabetes sufferers in the world followed by China, India, the United States, Brazil, and Mexico with an estimated number of people with diabetes of around 10 million people [1].

The increasing number of diabetes and the weak progress of disease cure encourage the world of health to further develop treatment in treating diabetes so that it does not cause microvascular and macrovascular complications and shows a good prognosis. However, the economic problem that often becomes a problem in diabetes mellitus treatment causes a low number of patients who get a good prognosis, so that alternative medicine is sought after by the public. Of the various alternative treatments that have emerged in the community, coffee leaves are one of the alternative treatments for which its efficacy is still little researched. So far, coffee consumption generally only focuses on the seeds, but lately, coffee leaves, especially Arabica coffee leaves, are often served as brewed drinks by the community. In Sumatra, especially coffee leaves are served as a brewed drink called "Aia Kawa" which is believed to contain flavonoids, caffeine, and polyphenols that can prevent various diseases, one of which is a carcinogenic disease [2].

This drink resembles tea from coffee leaves is also called Kawe tea by the people of Pagar Alam, in the South Sumatra area. Kawe tea is believed to be a drink that has refreshing and healthy properties for the body [3]. One of the phenolic compounds found in coffee leaves is chlorogenic acid [4]. Chlorogenic acid can inhibit hepatic glucose production by inhibiting hepatic glucose-6-phosphatase translocase from the hepatic glucose-6-phosphatase system [5]. Data on the composition of chlorogenic acid in beverages and other food ingredients are rarely found in the literature except for coffee, which is one of the best sources of chlorogenic acid [6]. Types of coffee leaves, especially Arabica, show a high accumulation of chlorogenic acid in young leaves [7].

Chlorogenic acid can stimulate glucose uptake in skeletal muscles through the activity of AMPK (AMP-Activated Protein Kinase) in the body. AMPK activity in the body causes a decrease in glucose production in the liver and fat

synthesis. So, chlorogenic acid functions as a good antidiabetic for DMT2 [8]. Therefore, this study was conducted to determine the effect of Gayo Arabica coffee leaves extracts on changes in blood sugar levels in type 2 diabetes mellitus rats.

RESEARCH METHODOLOGY

Research Type and Location

This research was conducted with experimental type research. This research was conducted at the Animal House Biology research site, Faculty of Mathematics and Natural Sciences, University of Northern Sumatra.

Material

In this study, the ingredients used to reduce Blood Glucose Levels (BGL) were:

- Metformin

- Ethanol extract from Gayo Arabica coffee leaves (*Coffea arabica*)

The making of Gayo Arabica coffee leaf extract was carried out in the pharmaceutical laboratory of the University of North Sumatra. The process starts from the wet weight of the Gayo Arabica coffee leaves measured followed by cleaning the Gayo Arabica coffee leaves in running water until they are clean before drying then followed by drying the Gayo Arabica coffee leaves in the drying cupboard at an ambient temperature of 40°C. The process has been carried out for 2 to 3 days. Then, the Gayo Arabica coffee leaves were blended, then the Simplicia powder was measured again. Furthermore, maceration was carried out for 5 days with 96% ethanol. Then the extract of Gayo Arabica coffee leaves was mixed to get metabolites. After that, the master solution was filtered and then evaporated so that the thick extract was obtained. The making of Gayo Arabica coffee leaf extract was carried out for about three weeks.

Animal

This study used male white rats, Wistar strain aged 2-3 months with a body-weight of 150 g-200 g, and the mice used were healthy and had never been tried in other studies. Using Federer's formula (1963), around 27 rats were used in this experiment.

Induction Diabetes Mellitus

Alloxan injection is given intraperitoneally. After administration of alloxan, it was continued with an administration of Nicotinamide (NA). At the beginning of the experiment to measure the levels of alloxan after induction using a glucometer observation of rat blood glucose was carried out on the 1st day until the 3rd day after alloxan induction. On that day the percentage of diabetic conditions in rats was observed. When measuring blood sugar levels above 200 mg/dL, rats are said to have type 2 diabetes mellitus.

Treatment

Wistar rats that had been induced with alloxan were then found to have blood sugar levels indicating a value that indicated type 2 diabetes mellitus on the measurement of blood sugar. Then Wistar rats with T2DM were divided into three groups, the first group of rats acted as control (-) were given distilled water, the second group of mice namely control (+) was given the drug metformin solution, the third group of mice was given 150 mg/kg/day of coffee leaf extract. The extract was administered orally using a sonde for 13 days. The mice consumed 5 g/day of corn pellets and were given 30 mL of water during the study.

Blood Sugar Check Procedure

The measured rat's blood was taken from a lateral vein in the rat's tail, the tip of the tail was sheared, and then the rat's tail was pressed until it drained a drop of blood. Then put a drop of blood into the auto check.

Statistical Analysis

The research data were analyzed first using the Shapiro-Wilk normality test. Then the Levene test was performed to determine the homogeneity of the data. Homogeneous and normally distributed data were subjected to one way ANOVA parametric test. If the ANOVA test yields a p-value<0.05, then the research is carried out with Post Hoc (LSD) analysis.

RESULTS AND DISCUSSION

Based on the results it revealed that in 23 mice, there were changes in blood sugar levels before the intervention and after the intervention of each group. The reading results are then processed and averaged from each intervention group which produces the following data (Table 1).

Table 1 Mean BGL for each treatment group

Group Type	Average BGL Before alloxan Inj.	Average BGL after alloxan in and before Intervention (mg/dL)	Average BGL after intervention (mg/dL)	Average BGL before and after intervention (mg/dL)	p-value
Negative	91.8	276.1	165	111.1	0.01
Metformin	95.6	299.1	160.7	138.4	0.000
EECL	101.6	424.3	186.2	271.7	0.001
EECL: Ethanol Extract of Coffee Leaf; BGL: Blood Glucose Levels					

The data shown in Table 1 paired t-test results show that the negative control group (distilled water) p=0.01, the positive group metformin p=0.000, and the positive group EECL (Ethanol Extract of Coffee Leaves) Gayo Arabica with p=0.001, is significant because of p-value<0.05. These results indicated that three groups had differences in the mean BGL of rats before and after the intervention of drugs and substances EECL Gayo Arabica in each group.

Diagram measurement of rat blood sugar levels by taking data four times the measurement is shown in Figure 1.
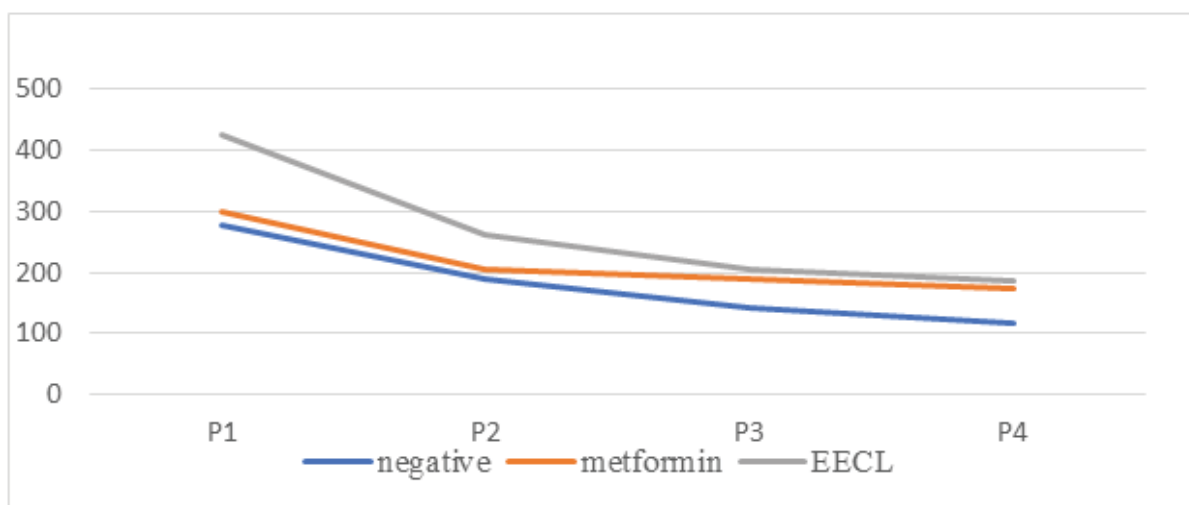

Figure 1 Measurement diagram of Blood Glucose Levels (BGL)

The data in the diagram shows a tendency to decrease in the intervention group after four measurements starting from the measurement before the intervention and re-measurement made after 5 days of intervention until after the intervention was given on the 14th day.

Data analysis in this study used the One-way Anova test because it compared more than two treatment groups. This test was carried out by performing the Shapiro-Wilk test of normality and then continued with the homogeneity test (Test of Homogeneity of Variances) which was carried out simultaneously with the One way Anova test. If the One Way Anova test shows a significant value, the data analysis test is continued with the Post-Hoc test to assess which group is significant. The Shapiro-Wilk normality test was carried out with the Shapiro-Wilk normality test because the sample size was <30.

The data is said to be normally distributed if the p-value is >0.05. The results of the normality test on the data are

p=0.056, p>0.05, then the data is normally distributed. The homogeneity test (Test of Homogeneity of Variances) was carried out with the Levene test, the data was said to be homogeneous if the p-value was >0.05. The data in this study have a value of p=0.224, p>0.05, so the data is said to be homogeneous. The One Way Anova research analysis test was continued by using SPSS, the results were said to be significant if p<0.05, data from the results of this study were p=0.035, p<0.05 then the data were significant.

This test was carried out with SPSS, this section presents further tests to determine differences between groups specifically as well as to find out which of the three groups had the most significant changes in blood sugar levels. For this test, it appears that the significance value between groups is significant if p<0.05. Based on these outputs, the Sig for the Gayo Arabica EECL is p=0.018 for the negative group and p=0.036 for the metformin group which means it shows a significant difference. Thus different blood sugar levels differ in the Gayo Arabica EECL group. T-paired test (T-paired test) was carried out by comparing the data before the intervention and after the intervention to assess the changes before and after the intervention, the value (p<0.005) indicates a significant change (meaning). In the processed data, the value of p=0.000 which means p=0.001 (p>0.005) indicates a significant change (meaning) before and after the intervention (Table 2).

Table 2 results of Post-Hoc LSD analysis

	Average difference	**CI 95%**		**p-value**
		Minimum	**Maximum**	
EECL *vs.* N	126.5	24	229.1	0.018
EECL *vs.* M	99.5	7.2	191.7	0.036
M *vs.* N	27	-73	127.1	0.579
EECL: Ethanol Extract Of Coffee Leaf; M: Metformin;			N: Negative	

Observation of Clinical Manifestations

Observation of clinical manifestations in mice to see the changes that occur in mice. Changes in signs or symptoms that occur in patients with Type 2 Diabetes Mellitus (T2DM) also appear in the T2DM model mice. Changes in behaviour such as polydipsia, polyphagia, and polyuria appear in a rat of each group when alloxan has been induced as induction for T2DM. These changes can be observed by looking at the frequency replacement of drinking bottles and places to eat which is usually only replaced 2 times a day to be increased and the condition of the cage is wet shortly caused by rat urine so that cleaning the cage must be done 2 times a week. Referring to the signs and symptoms that appear above shows apart from the BGL data >200 mg/dL rats also experience a change in the sign towards T2DM.

DISCUSSION

The research was carried out on 30 mice divided into three groups, namely the negative group with distilled water, the metformin group, and the EECL group. Each group consisted of 10 rats which were grouped based on the BGL value after being induced with alloxan. The negative rat group consisted of 9 rats because during the acclimatization period which was carried out for 7 days one mouse died and during the study period the negative mouse group remained to become 6 mice. In the metformin group, there were 10 rats and during the study the remaining 9 rats, one mouse had died. The EECL 150 mg/kg/day group of rats consisted of 10 rats and during the study 2 rats had died.

This study showed changes in the BGL of rats pre-intervention and post-intervention from each group. Paired t-test showed a significant change (meaning) in the group of rats before and after treatment with a p-value=0.000 (p<0.005). Measurements using the One Way Anova test explain the difference in the average treatment in the three groups. The group of mice with the EECL intervention stated that the decrease in BGL from before and after the EECL intervention showed that EECL was able to reduce blood sugar levels this was due to the polyphenol content in it. A study examining coffee content showed that coffee contains mangiferin, isomangiferin, and chlorogenic acid [9].

Chlorogenic acid, which is a polyphenol with large amounts in coffee, is responsible for most of the antioxidants in coffee [10]. Chlorogenic acid is known to be able to activate glucose utilization by skeletal muscle with AMPK

activation. The intervention of CGA over a long period was able to reduce hepatic G6Pase expression and activity, increase lipid profile and glucose utilization by skeletal muscle in mice. AMPK, which is activated by CGA, can also be beneficial in other processes, such as decreased glucose production by the liver and fatty acid synthesis. Then CGA increases glucose and lipid metabolism, through activation of AMPK [11].

In this study the metformin (+) group with a p-value=0.000 with the (+) EECL group having a p-value=0.001 indicating that the p-value between the positive metformin group and the EECL positive Gayo Arabica group had almost the same value indicating that CGA is a new insulin sensitizer which induces insulin action similar to metformin [12]. AMPK appears by various stressors such as disease, metabolism, metformin, thiazolidinediones, and exercise [13]. AMPK activation was followed by translocation of GLUT 4 on the intracellular membrane to the plasma membrane, which leads to increased glucose transport [14].

Decreased BGL in T2DM rats given the Gayo Arabica EECL intervention were also seen in studies conducted in T2DM rats and given coffee leaf intervention at a dose determined by researchers showed a decrease in blood sugar levels after giving Robusta coffee for 14 days [15].

The results of this study also linked to previous research in the form of the effect of coffee Gayo Arabica leaf extract on reducing blood sugar levels in healthy mice. The study shows that there is no significant difference between the benefits of coffee bean extract and coffee leaf extract in healthy mice on reducing blood sugar levels [16].

One Way Anova test value that shows a p-value<0.05 so that it can be concluded there are differences in average BGL between the negative group, the metformin group, and the Gayo Arabica EECL group. The group that showed significant change was the Gayo Arabica.

CONCLUSION

In this study, it was found the average difference of each negative group, the metformin group, and the Gayo Arabica EECL group before and after treatment and extracts. Then it was found significant differences between treatment groups and showed significant differences. The EECL group showed the group with the most significant difference.

Limitation and Study Forward

This research only provides treatment for 13 days.

DECLARATIONS

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