



# The Effect of Coffee Arabica Gayo Leaf Extract (*Coffea arabica* L.) on Increased PI3K Serum Levels in Type 2 Diabetic Rat

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## Abstract

**BACKGROUND:** Coffee arabica gayo leaf extract (*Coffea arabica* L.) contains polyphenols (chlorogenic acid), flavonoids, saponins, tannins, and steroids which improved PI3K serum levels in rats type 2 diabetic rat.

**AIM:** The purpose of the study was to determine the effect of Coffee Arabica Gayo Leaf Extract (*C. arabica* L.) on increased PI3K serum levels in type-2 diabetic rat (*Rattus norvegicus*).

**METHODS:** Thirty-five male Wistar rats with type 2 diabetic induced a combination of feeding a high-fat diet for 5 weeks and intraperitoneal injection of low dose streptozotocin (30 mg/kg). The diabetic rats were randomly divided into seven groups which consist of group without type 2 diabetic, group without type 2 diabetic with dose 250 mg/kg/day Coffee Arabica Gayo Leaf Extract, group with type 2 diabetic without intervention, group with type 2 diabetic with metformin, and group with type 2 diabetic with Coffee Arabica Gayo Leaf Extract doses 150, 200, and 250 mg/kg/day. The extract was administered orally for 30 days. Subsequently, PI3K serum levels were measure by Sandwich Elisa.

**RESULTS:** There were significantly higher PI3K serum levels in the treatment groups than in the control groups ( $p = 0.037$ ) after giving Coffee Arabica Gayo Leaf Extract 200 mg/kg/day and 250 mg/kg/day to rats for 30 days.

**CONCLUSION:** These data suggest that Coffee Arabica Gayo Leaf Extract can improve PI3K serum levels in a rat model type 2 diabetic.

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Keywords: PI3K; Coffee arabica gayo leaf extract; Type 2 diabetic

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## Introduction

According to the International Diabetes Federation in 2019, currently, more than 463 million people in the world live with diabetes mellitus (DM) and about 90% of DM cases in the world are type 2 DM (T2DM). Various epidemiological studies have shown a tendency to increase the incidence and prevalence of T2DM in various parts of the world. From these data, it is predicted that the prevalence of DM throughout the world will increase to 578 million in 2045 [1]. The World Health Organization predicts that the increase in the number of DM in Indonesia will increase 2–3 times from 8.4 million in 2000 to around 21.3 million in 2030 [2]. At present, Indonesia is ranked 7<sup>th</sup> in the world for the highest number of DM sufferers in adults with an age range of 20–79 years with a total of 11.7 million people.

The increasing number of diabetics 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 DM 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. Utilization of the coffee plant is currently still focused on seeds, while coffee leaves have not been widely used either as food products or as natural mixtures for food fortification, let alone medicine. Research by Martina (2019) shows that there is no significant difference between the benefits of coffee bean extract and coffee leaf extract in healthy

rats on reducing blood sugar levels. Both provide the same effect of lowering blood sugar levels compared to placebo [3]. In Sumatra, especially coffee leaves are served as a brewed drink called “Aia Kawa” which is believed to contain polyphenol, flavonoids, and caffeine that can prevent various diseases, one of which is carcinogenic disease [4].

Chlorogenic acid (CGA) is able to inhibit hepatic glucose production by inhibiting hepatic glucose-6-phosphatase translocase from the hepatic glucose-6-phosphatase system [5]. Data on the composition of CGA in beverages and other food ingredients are rarely found in the literature except for coffee, which is one of the best sources of CGA [6]. Types of coffee leaves, especially arabica, show a high accumulation of CGA in young leaves [7].

Where CGA is found, it can stimulate glucose uptake through the activity of PI3K (*Phosphatidylinositol 3-Kinase*) in the body. PI3K activity in the body causes a decrease in glucose production in the liver and fat synthesis so that CGA functions as a good antidiabetic for DMT2 [8], [9]. Therefore, this study was conducted to determine the effect of Coffee Arabica Gayo Leaf Extract on changes in blood sugar levels in T2DM rats.

## Research Methodology

### Research Type and Location

This research was conducted with true experimental study with a post-test control group design. The research was conducted at the Pharmacology and Therapeutic Laboratory of the Faculty of Medicine, Universitas Sumatera Utara. Examination of PI3K serum levels was carried out at the Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara.

### Material

In this study, the material was:

1. Metformin
2. Ethanol extract from Coffee Arabica Gayo Leaf (*C. arabica L.*).

### Animal

This study used male white rats, Wistar strain aged 8 weeks with a body weight of 180–200 g, and the mice used were healthy and had never been tried in other studies. Using Federer's formula (1963), all experimental animals used were around 35 rats [10].

### Induction DM

Rats were acclimatized for 7 days, given food and drink ad libitum. One mouse was placed in a cage in a room with a temperature of 22–25°C with a light-dark cycle of 12/12 h. The mice were given a standard diet consisting of 12% fat, 60% carbohydrates, and 28% protein for 2 days, then were given a high-fat diet (HFD) consisting of 41% fat, 41% carbohydrates, and 18% protein for 5 weeks. After 5 weeks, the rats were fasted for 1 night, then they were injected with a low dose of streptozotocin (30 mg/kg in 0.1 citrate buffer, pH 4.5) = intraperitoneally. Injection of streptozotocin dose of 30 mg/kg in 0.1 citrate buffer, pH 4.5 was repeated for the following week. After 1 week of the second administration of streptozotocin, blood sugar levels when checked with a glucometer and if more than 200 mg/dl then fasting blood sugar levels and fasting insulin levels are checked to ensure insulin resistance and T2DM have occurred in experimental animals. When measuring blood sugar levels above 200 mg/dl, rats are said to have T2DM.

### Treatment

Wistar rats that had been induced with HFD/streptozotocin (STZ) were then found to have blood sugar levels indicating a value that indicated T2DM on the measurement of blood sugar. Wistar rats were divided into 2 large groups, namely the normal group that was not made the T2DM model (n = 10) and the T2DM model group (n = 25). All experimental animals in the T2DM model group were induced with a low dose of STZ 30 mg/kg (1-week interval) after being given a HFD for 5 weeks to make it a T2DM model. Wistar rats that had become T2DM were divided randomly into 5 groups, namely the T2DM group without treatment (n = 5), the T2DM group with metformin (n = 5), the T2DM group with the administration of Coffee Arabica Gayo Leaf extract at a dose of 150 mg/kg/day (n = 5), the DMT2 group was given the Coffee Arabica Gayo Leaf extract at a dose of 200 mg/kg/day (n = 5), and the T2DM group was given the Coffee Arabica Gayo Leaf extract at a dose of 250 mg/kg/day (n = 5). Likewise, the group of mice that were not made the T2DM model was divided into 2 groups randomly, namely the normal control group without treatment (n = 5) and the normal control group with the administration of Coffee Arabica Gayo Leaf extract at a dose of 250 mg/kg/day (n = 5). Hence, the total number of groups (t) in this study was 7 groups, which consist of 2 Normal groups and 5 T2DM groups. Of these 7 groups, there were 2 groups that did not receive treatment (K1 and K3) and 5 groups that received treatment (K2, K4, K5, K6, K7). The extract was administered at a dose of 150 mg/kg/day, 200 mg/kg/day, and 250 mg/kg/day through a nasogastric tube orally for 30 days.

### Blood sugar check procedure

The measured rat blood was taken from a lateral vein in the rat's tail. The tip of the tail was sheared, then pressed until the blood dropped and finally placed into the glucometer.

### Statistical analysis

The sample in this study was normally distributed, so Shapiro–Wilk test is carried out. Data were presented in the form of mean standard deviation. To show the differences between groups (control and treatment), we used one-way ANOVA test analysis with SPSS computer program and a significance limit of  $p < 0.05$ .

## Results and Discussion

Based on the research, from 35 mice analyzed, there were significant changes in PI3K serum levels before and after the intervention of each group. The results are as follows in Table 1.

Table 1 shows the mean PI3K serum levels in the control group were lower than the treatment group. The order of the mean PI3K serum levels of male Wistar rats after giving Coffee Arabica Gayo Leaf Extract from the largest to the smallest was K4 ( $7.54 \pm 0.08$  ng/dl) > K7 ( $7.35 \pm 0.24$  ng/dl) > K6 ( $7.18 \pm 0.18$  ng/dl) > K2 ( $7.09 \pm 0.36$  ng/dl) > K1 ( $7.08 \pm 0.12$  ng/dl) > K3 ( $6.75 \pm 0.30$  ng/dl) > K5 ( $6.22 \pm 0.08$  ng/dl). It can be seen that there is a decrease in PI3K serum levels by giving Coffee Arabica Gayo Leaf Extract to male Wistar rats with DMT2 model and it is statistically significant. From Table 1, it can be seen that the ethanol extract group of Coffee Arabica Gayo Leaf at a dose of 250 mg/kg/day (K7) and a dose of 200 mg/kg/day (K6) had a mean PI3K serum level higher than the normal group (K1). However, the highest levels were in the group that received metformin (K4).

Figure 1 shows that the mean PI3K serum levels of male Wistar rats after intervention were higher than PI3K serum levels except in the untreated T2DM group (K3). The highest PI3K serum levels were in the T2DM group that received metformin (K4), while the highest PI3K score was found in the DMT2 group who received ethanol extract of Gayo Arabica Coffee Leaves at a dose of 250 mg/kg/day. In addition, we can also see that the ethanol extract dose of 200 mg/kg (K6) has not been able to match the results of the PI3K

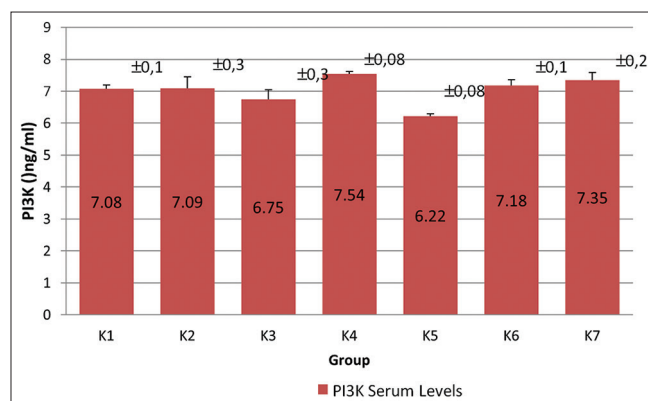


Figure 1: Comparison of mean PI3K serum levels and expression in muscle membrane before and after intervention in seven groups

serum levels score on metformin (K4) administration. In Figure 1, it can also be seen that the PI3K serum levels in the normal group (not T2DM) who received an ethanol extract dose of 250 mg/kg (K2) had the highest value of all groups.

## Discussion

The experimental animals used in this study were 35 healthy male Wistar rats (*Rattus norvegicus* sp.) who had met the inclusion and exclusion criteria. Wistar rats were divided into 2 large groups, namely the normal group that was not made the T2DM model ( $n = 10$ ) and the T2DM model group ( $n = 25$ ). All experimental animals in the T2DM model group were induced with a low dose of STZ 30 mg/kg (1-week interval) after being given a HFD for 5 weeks to make it a T2DM model. Wistar rats that had become T2DM were divided randomly into 5 groups; namely, the T2DM group without treatment ( $n = 5$ ), the T2DM group with metformin ( $n = 5$ ), the T2DM group with the administration of Coffee Arabica Gayo Leaf extract at a dose of 150 mg/kg/day ( $n = 5$ ), the DMT2 group was given the Coffee Arabica Gayo Leaf extract at a dose of 200 mg/kg/day ( $n = 5$ ), and the T2DM group was given the Coffee Arabica Gayo Leaf extract at a dose of 250 mg/kg/day ( $n = 5$ ). Likewise, the group of mice that were not made the T2DM model was divided into 2 groups randomly, namely the normal control group without treatment ( $n = 5$ ) and the normal control group with the administration of Coffee Arabica Gayo Leaf extract at a dose of 250 mg/kg/day ( $n = 5$ ). Hence, the total number of groups (t) in this study was 7 groups consisting of 2 normal groups and 5 T2DM groups. Of these 7 groups, there were 2 groups that did

Table 1: The Mean PI3K serum levels after treatment orally coffee arabica gayo leaf extract in the control and the treatment groups within 30 day

| Groups                    | K1 (n = 5)  | K2 (n=5)    | K3 (n = 5)  | K4 (n = 5)  | K5 (n = 5)  | K6 (n = 5)  | K7 (n = 5)  | p      |
|---------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------|
| PI3K serum levels (ng/mL) | 7.08 ± 0.12 | 7.09 ± 0.36 | 6.75 ± 0.30 | 7.54 ± 0.08 | 6.22 ± 0.08 | 7.18 ± 0.18 | 7.35 ± 0.24 | 0.037* |

not receive treatment (K1 and K3) and 5 groups that received treatment (K2, K4, K5, K6, K7).

Insulin resistance generally involves glucose metabolism in the PI3K pathway. Insulin simultaneously activates the PI3K pathways. Tannin, Saponins, and mostly CGA in extract coffee leaves have been observed to increase glucose uptake by mediators of insulin signaling pathways, such as PI3K. CGA, which is a polyphenol with large amounts in coffee, known to be able to activate glucose utilization through PI3K activation [11], [12]. The antidiabetic effect of flavonoids is mainly due to their modulation effect on blood sugar transporters by increasing insulin secretion, reducing apoptosis, increasing pancreatic  $\beta$  cell proliferation, reducing insulin resistance, inflammation and oxidative stress, and increasing GLUT-4 translocation through the PI3K pathway [13], [14].

Intervention of CGA over a long period of time was able to increase PI3K serum levels. In this study, the group which given the Coffee Arabica Gayo Leaf extract at a dose of 150 mg/kg/day, 200 mg/kg/day and 250 mg/kg/day have  $p = 0.037$ . One-way ANOVA test value that shows a  $p < 0.05$  so that it can be concluded there are effect ethanol extract of Coffee Arabica Gayo Leaf in increased PI3K serum levels.

## Conclusion

In this study, it was found the ethanol extract of Coffee Arabica Gayo Leaf Extract (*C. arabica L.*) can increase PI3K serum levels greater than the normal group without T2DM and the T2DM group without treatment but not greater than the T2DM group that received metformin in Wistar rats. These data suggest that Coffee Arabica Gayo Leaf Extract can improve PI3K serum levels in a rat model type 2 diabetic.

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