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The Analysis of the Chlorogenic Acid in the Ethanol Fraction of Robusta Coffee Beans and Its Effect on Glucose Levels in Wistar Rats

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Abstract

Background: The metabolic disorder caused by high blood glucose levels and pancreatic beta-cell damage is known as diabetes mellitus. Indonesia itself is the 7th country in the world with the number of people with diabetes mellitus. Indonesia occupies rank 7 in the world with the number of people with diabetes mellitus. Apart from that, robusta coffee (*Coffea canephora L*) is one of the most popular drinks globally, including Indonesia. Chlorogenic acid in coffee beans effectively reduces cell damage due to free radicals, including minimizing excessive glucose release from the liver into the blood. **Objective:** This study aimed to analyze the effects of chlorogenic acid in the ethanolic fraction of robusta coffee (*Coffea canephora L*) beans on blood glucose levels in Wistar rats. **Method:** The researchers applied an experimental study with a randomized pretest-posttest control group design. The beans of robusta coffee were extracted using the Maceration method and then fractionated using a hexane and ethyl acetate solvent. The concentration of the obtained remaining fraction was measured using a spectrophotometer. Furthermore, hyperglycemia testing included 30 Wistar rats induced with 20% glucose for 3 – 4 weeks. They were then given the ethanol fraction of robusta coffee with a dose of 400 mg/kg BW and 500 mg/kg BW. Meanwhile, metformin served as a positive control, and NaCMC served as a negative control. **Results:** The chlorogenic acid analysis in the ethanol fraction of robusta coffee on a spectrophotometer with a concentration of 37% indicated a decrease of 16.66% on the negative control and 48.06% on the positive control. Meanwhile, the ethanol fraction of each control was 51.53% and 52.16%, respectively. **Conclusion:** The ethanol fraction of the robusta coffee significantly affects the decrease in blood glucose levels in Wistar rats.

Keywords*:* *Coffea canephora L*., chlorogenic acid, glucose

1. Introduction

The International Diabetes Federation reported that around 382 million people suffered from diabetes mellitus in 2013. Furthermore, it is estimated that there will be 592 million sufferers in 2035 or an increase of 55%. Indonesia ranks seventh highest diabetes mellitus sufferers globally, reaching 8.5 million sufferers in 2013 [1,2].

Diabetes mellitus is a group of metabolism disorders of fat, carbohydrates, and protein because of the damage in insulin secretion, insulin action (sensitivity), or both [3,4]. The increase in diabetes mellitus sufferers in developing countries is due to changes in dietary habits from healthy, high-fiber, low-fat, low-calorie traditional foods to the increased consumption of calorie-containing foods, such as simple carbohydrates, fat, red meat, and low fiber food [5–7].

Robusta coffee bean powder contains chlorogenic acid, a phenolic group compound [8–10]. Furthermore, robusta coffee beans contain the most chlorogenic acid compared to other coffee beans [11–13]. According to Dr. J. Murdoch Ritchie in the book 'The Pharmacological

Basis of Therapeutic,' the caffeine in 1 – 2 cups of coffee can increase heart rate, increase thought and inspiration speed, reduce drowsiness and fatigue, increased sensory stimuli and motor reactions, generate vasodilation, and encourage the flow of fluid secretions and solid secretions from the body so that the body feels fresher (11,14–16).

Chlorogenic acid (CGA) maximizes the potential of insulin action, similar to the therapeutic action of metformin. Chlorogenic acid at a dose of 5mg/kg BW may provide antidiabetic potential in rats [17,18]. Based on the elaboration above, it is necessary to carry out a study on the effect of the provision of the remaining fraction of Robusta coffee (*Coffea canephora L*.) beans to reduce blood glucose levels in Wistar rats (*Rattus norvegicus*) [13,19–21].

2. Method

This study was experimental research with a randomized pretest-posttest control group design. Robusta coffee (*Coffea canephora L*.) beans were taken from Rante Kalua Village, Mangkendek, Tana Toraja, South Sulawesi. This study was conducted from February to April 2021. After providing the negative control, the positive control, and the remaining fraction of robusta coffee beans, the research data were collected and analyzed by the ANOVA using a completely randomized design and further tested using Duncan's test.

The tools used in this study were Erlenmeyer flask, beaker (Phirex), graduated cylinder, glucometer (Elvasense), hot plate, mouse cannula, scale pipette, centrifuge, 5 mL syringe, and stirrer. Meanwhile, the materials used in this study were robusta coffee (*Coffea canephora L*.) beans, a solvent for extraction and partitioning. Furthermore, the chemicals used were distilled water (Aquadest), chlorogenic acid p.a (Sciecewerke), 70% ethanol (One med), ethyl acetate (Merck), glucose (Merck), metformin (Sciencewerke), 1% Na CMC, and n-hexane (C6H14).

Extraction was conducted by the maceration method. 500 g coffee beans were macerated with a mixture of 1050 mL of Aquadest and 450 mL of 70% ethanol (with a ratio of 7:3) for 3 × 24 hours. After that, it was filtered until the filtrate was obtained. The filtrate was evaporated using a rotary evaporator to produce a thick extract. Furthermore, it was followed by carrying out the re-maceration with the same solvent. The concentrated extract was dissolved in 100 mL of ethanol and partitioned using 3 × 70 mL of n-hexane. The n-hexane fraction was then concentrated. The ethanol fraction was further partitioned using 3 × 70 mL of ethyl acetate as a solvent. The ethyl acetate fraction and the remaining fraction were then concentrated.

There were 20 rats used in this study. They were divided into five groups, consisting of 4 rats (taken randomly) for each. The rats underwent a fast for 8 hours before the treatment was given. After that, their initial fasting blood glucose levels were measured using a glucometer. Furthermore, they were induced with 20% glucose orally. Their blood was drawn again through the lateral vein. Their blood glucose levels were measured again after the induction process. Group I was given 1% Na-CMC solution as a negative control. Group II was given metformin as a positive control. 400 mg/kg BW was given to group III the remaining fraction of robusta coffee beans. Group IV was given the remaining fraction of robusta coffee beans by 500 mg/kg BW. Group V was given chlorogenic acid by 5 mg/kg BW. Measurement of blood glucose levels was carried out on days 4 and 7 using a glucometer.

The blood draw was conducted by wiping the rats' tails with a cotton swab which was first given 70% alcohol, and then the rats' tails were cut using scissors that had been cleaned with 70% alcohol. After that, the tail was held tightly until the blood came out at the tip of the lateral vein. The blood that came out was then dripped onto the strip. Furthermore, the tip of the lateral vein was rubbed with a cotton swab given 70% alcohol so that blood from the lateral vein did not come out.

3. Results and Discussion

3.1. Results

3.1.1. Subsubsection

Data on the average decrease in blood glucose levels after induction and after the provision of the ethanol fraction of robusta coffee bean for group I (1% NaCMC (negative control)), group II (metformin (positive control)), group III (the ethanol fraction of Robusta coffee bean by 400 mg/kg BW), group IV (the ethanol fraction of Robusta coffee bean by

500 mg/kg BW), and group V (chlorogenic acid by 5 mg/kg BW) are presented in the following table.

Table 1. Data of the Average Decrease in Blood Glucose Levels

	Rats	Initial blood glucose level (n)	Blood glucose levels after induction (n)	Blood glucose levels after treatment provision (n)		Decreased (%)
				4	7	
Group I	1	161	162	96	125	22.84
	2	106	138	75	102	26.09
Average		133.5	150	85.5	113.5	24.46
Group II	1	107	147	131	88	40.14
	2	84	184	104	81	55.98
Average		95.5	165.5	117.5	84.5	48.06
Group III	1	77	186	160	74	60.22
	2	87	138	119	100	27.54
	3	86	253	110	86	66.01
	4	71	149	123	71	52.35
Average		80.25	181.5	128	82.75	51.53
Group IV	1	75	166	156	97	41.57
	2	91	185	170	93	49.73
	3	69	219	195	85	61.19
	4	73	203	157	89	56.16
Average		77	193.25	169.5	91	52.16
Group V	1	104	194	155	101	47.94
	2	97	187	99	80	57.22
Average		100.5	190.5	127	90.5	52.58

3.1.2. Statistical Analysis

Because Fcount is > Ftable, H0 was rejected. Therefore, H1 was accepted. In other words, the ethanol fraction of robusta coffee beans affect the blood glucose levels of Wistar rats.

Table 2. Results of Analysis of Variance (ANOVA)

Source of Uniformity	DF	Squared	Middle Squared	F_{count}	F_{table}		Note
					5%	1%	
Correction Factor	1	11696.23	11696.23				
Treatment	4	21866.93	5466.73	17,37*	3.06	4.89	Uniform
Error	15	4719.62	314.64				
Total	20	38282.78					

3.1.3 Further Analysis Using Duncan's New Multiple Range (DNMR) Test

P1 was the group given 1% NaCMC; P2 was the group given metformin; P3 was the group given the ethanol fraction of robusta coffee bean by 400 mg/kg BW; P4 was the group given the ethanol fraction of robusta coffee bean by 500 mg/kg BW; P5 was the group given chlorogenic acid by 5 mg/kg BW. The difference of >1% and >5% indicated high significance; the difference of <1% and <5% indicated no significance; the difference of <1% and >5% indicated the presence of significance.

Table 3. Analysis of treatments on Wistar rats in the confidence levels of 1% and 5%

Treatments	Difference	Significance		Note
		5%	1%	
P1 – P2	8.07	26.73130856	36.95732	Not significant
P1 – P3	34.86	28.02618947	38.54488	Significant
P1 – P4	35.5	28.82440372	39.58256	Significant
P1 – P5	29.44	29.37428	40.32755807	Significant
P2 – P3	26.79	26.73130856	36.95732	Significant
P2 – P4	27.43	28.02618947	38.54488	Not significant
P2 – P5	21.37	28.82440372	39.58256	Not significant
P3 – P4	0.64	29.37428	40.32755807	Not significant
P3 – P5	5.42	26.73130856	36.95732	Not significant
P4 – P5	6.06	28.02618947	38.54488	Not significant

3.2. Discussion

This study was conducted to determine the effect of the remaining ethanol fraction of Robusta coffee (*Coffea canephora L*.) beans by employing 20 rats divided into five groups. Blood glucose checks were carried out four times using a glucometer. The principle of the test with this instrument is based on a small electric current generated by the reaction of blood sugar with a test strip reagent. The glucometer calculates and converts this current into a numerical value for blood sugar and then displays the results on the screen.

Based on Table 1, it was found out that after the provision of 20% glucose, blood glucose levels in the rats' bodies increased above normal limits compared to their initial blood glucose. There was an increase in blood glucose in each treatment group because glucose given orally would be absorbed from the small intestine into the blood. However, some rats in groups 1, 2, and 5 (2 cases each group) did not experience an increase in blood glucose after glucose induction, so that the rats were eliminated. This can be caused by several factors or conditions, such as stress, increased activity, or error during glucose induction [22,23].

Stress conditions in rats may cause disturbances in controlling blood sugar levels by hormones so that the body will produce the epinephrine hormones and cortisol, which cause blood sugar levels to increase automatically [24,25]. Another factor is the increased activity. Physical activity can control blood sugar by converting glucose into energy during physical activity. Physical activity causes insulin to increase so that blood sugar levels will decrease. In less mobile rats, food substances that entered their bodies were not burned but stored as fat and sugar [26–28]. According to Malole (1989), normal blood glucose levels in rats were 50 – 135 mg/dL.

The provision of NaCMC as a negative control resulted in the lowest percentage reduction in blood glucose levels, namely 24.46%. It was because they were only given NaCMC, which had no activity in reducing blood glucose levels. Meanwhile, the treatment with metformin as a positive control group showed a decrease in rats' blood glucose levels by 48.06%. This was because metformin is one of the biguanide antidiabetic oral drugs that works by increasing the body's sensitivity to the insulin produced by the pancreas, decreasing hepatic glucose production through activation of the AMP-activated protein kinase enzyme, and increasing stimulation of glucose uptake by skeletal muscle and fat tissue [12–14].

The provision of the remaining fraction of robusta coffee beans resulted in a decrease in blood glucose levels. The decrease was 51.53% at a dose of 400 mg/kg BW and 52.16% at a dose of 500 mg/kg BW. Meanwhile, the provision of chlorogenic acid with a dose of 5 mg/kg BW decreased blood glucose levels by 52.58%. The decrease in blood glucose levels due to the provision of the remaining fraction of robusta coffee beans and chlorogenic acid was higher when compared to that of metformin (positive control). This was because coffee contains chlorogenic acid compounds, which can reduce intracellular hyperglycemia by regulating fat and glucose metabolism through AMPK activation [29]. Chlorogenic acid can inhibit G6Pase expression and increase fasting glucose, glucose tolerance, and insulin sensitivity [30].

Data from the results of statistical analysis using the ANOVA with a completely randomized design (CRD) shown in Table 7 indicated that the F count was <F table with a confidence level of 5% and 1%, meaning that the provision of the remaining fraction of robusta coffee beans has an insignificant effect on blood glucose levels in Wistar rats. The statistical analysis results also indicated a uniformity coefficient of 64.6%, which is greater than 10%. Therefore, the test was followed by Duncan's test. The results of Duncan's test are presented in Table 3.

Based on the difference in 1% and 5% confidence levels, group I (negative control) showed a significant difference with those given metformin (positive control). Those given the remaining fraction of robusta coffee beans with doses of 400 mg/kg BW and 500 mg/kg BW, and those given chlorogenic acid with a dose of 5 mg/kg BW, in which the effect of the decrease in the percentage of blood glucose in each dose was more significant than the negative control, indicating that the fraction was able to reduce blood glucose levels. Group II (positive control) showed insignificant differences with the groups given the remaining fraction of Robusta coffee beans and chlorogenic acid. This means that the remaining fraction of robusta coffee beans and chlorogenic acid has the same effect as metformin circulating in the market.

4. Conclusion

Based upon the results and discussions aforementioned can be concluded that the ethanol fraction of Robusta coffee beans (*Coffea canephora L*.) has effects on decreasing blood glucose in Wistar rats. Furthermore, the group given the ethanol fraction of robusta coffee beans at a 400 mg/kg BW dose showed a significant difference with that given metformin as the positive control.

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