


## Antidiabetic Effects Of Robusta Coffee Bean Ethanol Extract (Coffea Canephora) In Diabetic Mice With Alloxan Induction

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Article Info	ABSTRACT
<b>Keywords:</b> Coffea canephora, blood glucose, alloxan.	Non-communicable diseases are a group of diseases which in themselves become a public health burden because they are widespread, one of which is diabetes mellitus (DM). Robusta coffee bean ( <i>Coffea canephora</i> ) is one of the medicinal plants that can be efficacious in lowering blood glucose levels. The purpose of this study was to determine the effect of Robusta Coffee Beans ( <i>Coffea canephora</i> ) on blood glucose levels in alloxan-induced mice. This study used a type of laboratory experimental study using male mice ( <i>Mus musculus</i> L.) Bulb/C strain. There were 6 groups randomized and treated Robusta Coffee Bean Ethanol Extract (RBEE) with dose 100 mg/kg.bw, dose 200 mg/kg.bw, dose 400 mg/kg.bw, CMC Na 0.5% and metformin 195 mg/kg.bw. The treatment is given orally for 14 days. Blood glucose level data was analyzed using the ANOVA one-way parametric test with a confidence level of 95%. After being given treatment, the blood glucose level in negative control group (545.7 mg/dl), positive control (130.25 mg/dl), RBEE 100 mg/kg.bw (296.5 mg/dl), RBEE 200 mg/kg.bw (211.5 mg/dl), RBEE 400 mg/kg.bw (147.25 mg/dl), so that the percent decrease in blood glucose levels in RBEE 100 mg/kg.bw (27.10%), RBEE 200 mg/kg.bw (50.14%), RBEE 400 mg/kg.bw (68.00%), CMC Na (-53.60%), metformin (71.11%). The results showed that there was an effect on reducing blood glucose levels in mice significantly compared to the negative control group. Robusta coffee beans ( <i>Coffea canephora</i> ) have the activity of lowering blood glucose levels so that it can be developed as an antidiabetic.
This is an open access article under the <a href="https://creativecommons.org/licenses/by-nc/4.0/">CC BY-NC</a> license 	<b>Corresponding Author:</b> Sholihatil Hidayati Universitas dr. Soebandi Jember <a href="mailto:sholihatilhidayati@yahoo.co.id">sholihatilhidayati@yahoo.co.id</a>

### INTRODUCTION

Disease no infectious disease is group disease that becomes burden health public Because spread broad, spreading to all over the world, and become reason main enough death difficult controlled. Awareness disease no infectious disease will increase day always increase Because frequency what happens to society. Enhancement this especially occurs in diabetes, stroke and hypertension which causes disease No infectious become problem main health public (Wadja et al., 2019). According to data from the International Diabetes Federation (IDF) in 2021 stated that Indonesia has amount most people with diabetes fifth in the world. This figure increase almost double only in two years' time which compared to 2019 was 10.7 million. Amount diabetes attacks in Indonesia rose to 18 million in 2020. At the moment that

prevalence case the happen enhancement by 6.2 percent compared to 2019. Regency Jember is city biggest third in East Java to have prevalence of diabetes mellitus based on a doctor's diagnosis amounting to 6,092 patients in 2018 (Riskasdas, 2018)

Diabetes mellitus sufferers need treatment period long for reduce symptoms and prevention progressiveness disease. use drug the show a number of weakness that is can give rise to effect side No desired like disturbance channel digest that is nausea, vomiting and diarrhea (Kurniawaty & Lestari, 2016). Lately this is a diabetes mellitus sufferer try control or control his blood sugar levels through treatment traditional use herbal plants, because considered relatively more safe, easy obtained and minimal effect beside him (Rahmadyta Syafitri, 2020). One of plants that can used public for treatment traditional antidiabetic that is robusta coffee beans.

Coffee beans contain compound active like sour chlorogenic, caffeine, alkaloids, flavonoids, terpenoids and polyphenols. By empris Robusta coffee lots used as drink traditional for healthy and usable as treatment of diabetes mellitus. In a number of study on animal, coffee beans containing sour chlorogenic show activity in metabolism glucose and lipids like for hypoglycemia, as drug diabetes, increase secretion insulin and reduce vulnerability to oxidation LDL (Furqan & Nurman, 2020). Besides that coffee also contains enhancing antioxidants insulin sensitivity so can prevent or hinder development of DM type 2 (Rangkuti, 2022).

Amount sour chlorogenic in coffee can influenced during the processing process, namely the roasting process. During the roasting process, acid chlorogenic will decomposed become compound volatiles and melanoidins that cause amount from sour chlorogenic experience decline (Furqan & Nurman, 2020; Hamdani & Nurman, 2020). Green coffee is one of processed coffee without through the roasting process, so sour chlorogenic content contained in green coffee more higher than previous coffee roasted. Based on background back above so need done study activity extract robusta coffee beans made with method maceration cold to decline rate glucose blood in mice.

## METHODS

Study This use type study studies experimental laboratory for know influence extract ethanol robusta coffee beans with different doses to rate glucose blood diabetic mice with induction alloxan. In research this use 6 groups of test animals with use design random complete. Study carried out in the Laboratory Biology and Laboratory Pharmacy Clinic and Community Study Program University Pharmacy dr. Soebandi Jember. Research this has get received a Letter of Approval Appropriateness Ethics from Commission University Ethics dr. Soebandi Jember with number 018/KEPK/UDS/II/2023.

### **Making extract ethanol Robusta coffee beans (*Coffea canephora*)**

Powder robusta coffee beans 200 g macerated with 70% ethanol as much as 1000 ml with ratio 1:5 b/v next soaked all over powder until submerged. Receptacle Then closed close and leave in 3 days with occasionally stirred. Soaking results filtered with use paper strain. Dregs results filtering in maceration return use 70% ethanol with Same procedure, remaceration done 3 times, the filtrate obtained thickened with using a rotary evaporator with

pumpkin size 500 ml with 8 repetitions use temperature 50°C up to obtained extract thick. krining phytochemicals to extract obtained done with see contains flavonoids, alkaloids, tannins, polyphenols and saponins (Sulistyawati et al., 2017).

#### **Solution alloxan Preparation**

Alloxan monohydrate weighed as much as 180 mg then dissolved into the 0.9% NaCl solution cold up to 10 ml. Solution the injected to mice in a way intraperitoneal (ip). The dose given in accordance with mouse body weight with provision mice a body weight of 20 g is given as much as 0.2 ml.

#### **Making suspension extract ethanol leaf robusta coffee beans**

Test preparations are made with method suspend extract ethanol robusta coffee beans in 0.5% CMC Na solution. Suspension made with method develop substance suspension CMC Na 0.5% then sprinkle on top mortar filled with hot water, after expand crushed. Then entered extract that has been weighed, crushed until homogeneous. Take each extract ethanol robusta coffee beans as much as 300 mg, 600 mg, and 1200 mg were dispersed to in CMC Na 0.5% to 30 ml for make test solution dose 100 mg/kg.bw, 200 mg/kg.bw and 400 mg/kg.bw orally .

#### **Antidiabetic test**

Mice adapted moreover formerly for 7 days, before induced with alloxan mice fasted moreover formerly for 16 hours but still given drink Then be measured moreover formerly rate glucose mice as rate glucose initial ( $T_0$ ) for ensure mice in normal condition. Mice created diabetes with inject alloxan with dose 180 mg/kg.bw daily intraperitoneal. Mice it is said to be diabetes if rate glucose blood more than 200 mg/dl (Hidayati, 2021). Treatment given for 14 days and evaluate rate glucose blood carried out on the 7th and 14th day post treatment.

#### **Data analysis**

Data analysis was carried out with using statistical tests parametric. Data obtained normality test was carried out use method saphiro wilk and homogeneity test using *Levene's* test. Test differently done with using the one way ANOVA parametric test level 95% confidence and a follow-up LSD ( *least significant differences* ) test was carried out.

## **RESULTS AND DISCUSSION**

Determination plant that is stage initial work done for identify type and ensure truth simplicia before the research process more carry on. This determination is carried out in the laboratory plant Jember State Polytechnic with number 31/PL17.8/PG/2023. In the extraction process started with taking Robusta coffee beans at PUSLIT Rambipuji Then robusta coffee beans smoothed until shaped powder and weighed as much as 200 grams, after. That dissolved use solvent 70% ethanol with ratio 1 : 5 for 3 days, then robusta coffee extracted filtered use paper strain until obtained filtrate . Method maceration chosen because own somen superiority that is more easy and not need use heating, selected solvent based on polarity and solubility material so that separation easy done with a standing soaking process will possible many extracted compounds (Chairunnisa et al., 2019)

**Table 1.** Manufacturing Results Extraction Robusta Coffee Beans

Powder (g)	Extract (g)	Yield (%)
200	39.05	19.52

Objective did it remaceration that is for interesting content still compound left behind at the moment maceration First . After That filtrate concentrated using a rotary evaporator at temperatures of 50°C up to become extract thick, concentration process need 15 hours and results mark possible yield seen in tabel 1.

Screening phytochemicals done for know type metabolites secondary what only what is contained in each sample. Screening test flavonoids be marked with formation color yellow a bit orange that shows exists flavonoids, to test the formation of alkaloids precipitate show the presence of alkaloids, the formation of foam there is the presence of saponin, the formation color green brownish there is exists tannins and formed blue blackish there is exists polyphenols (Kartikasari et al., 2022). Result of screening phytochemicals robusta coffee beans there is positive exists content flavonoids, alkaloids, saponins, tannins and polyphenols. Screening results phytochemicals shown in table 2.

**Table 1.** Screening results phytochemicals extract robusta coffee beans

Identification carried out	Reagent Name	Changes that occur	Results
Flavonoids	Methanol+H <sub>2</sub> SO <sub>4</sub>	Formed color red	+
Alkaloids	HCl+HgCl <sub>2</sub>	There is precipitate yellow or white	+
Tannin	Water+FeCl <sub>3</sub>	Brownish green	+
Polyphenols	Water+FeCl <sub>2</sub>	Blackish blue / Black bluish	+
Saponins	Water+HCl shaken	Formed foam	+

Next stage that is adaptation test animals mice male strain balb /c with weight between 20 – 30 g. Objective use mice as animal try that is because cycle his life relatively short, amount birth lots of kids, easy handling, characteristics genetics, biology and behavior relatively similar with man (Latief et al., 2021). Mice adapted for 7 days , before treatment mice fasted for 16 hours but still given drink. After adapted stage furthermore mice were induced alloxan (ip) with dose 180 mg/kg.bw. Alloxan can give effect hyperglycemia in test animals (mice) which can achieved in enough time short namely two to three day after induction alloxan (Sukara et al., 2023).

Every group given treatment every day for 14 days. Data is averaged showed in table 2.

**Table 2** Average Glucose Pre and Post Induction Blood Alloxan

Group	Average Blood Glucose Levels of Mice (mg/ dL±SD )	
	Pre induction (H <sub>0</sub> )	Post induction (H <sub>3</sub> )
Normal	91.0±5.6	115.3±12.4
Diabetes	95.8±12.7	427.6±84.0*

\* different significant with H<sub>0</sub>

After adapted stage furthermore mice were induced alloxan (ip) with dose 180 mg/Kg.bw. On manufacture solution induction alloxan done with method that is weigh powder alloxan Then dissolved into the 0.9% NaCl solution then injected in a way intraperitoneal (ip) to in stomach (abdomen) of mice. Alloxan can given in dose double or single through different route namely intraperitoneal, intravenous and subcutaneous, at the intraperitoneal induction dose single is most ways used (Handajani, 2021). Half- life alloxan at 37°C and neutral pH which is 1.5 minutes and can work longer at low temperatures (Djunarko et al., 2022). Alloxan can give effect hyperglycemia in test animals that can achieved in enough time short namely two to three day after induction alloxan (Sukara et al., 2023). Giving This is reversible because done with use dose single and gift no repeated so no capable damage pancreatic  $\beta$  cells in a way irreversible that can be cause pancreatic  $\beta$  cells can regenerate return so that is produced from induction the namely DM type 2.

This diabetes condition caused by mechanisms work substance diabetogen namely in alloxan pancreatic  $\beta$  cells will undergo a reaction process redox becomes dialuric acid and can reoxidized become alloxan Again, reaction redox This will result formation of ROS and radicals superoxide. ROS free radicals will experience dismutation into hydrogen peroxide cause upgrade concentration calcium cytosol Then will be destroyed fast pancreatic  $\beta$  cells, ROS will cause DNA fragmentation resulting in cell DNA experience damage that will damage pancreatic  $\beta$  cells and can lower insulin production that causes hyperglycemia (Dewi et al., 2019).

**Table 3** Average levels glucose after treatment

Treatment	Average levels glucose blood Mice post treatment (mg/ dL $\pm$ SD )	
	(H-7)	(H-14)
Control Healthy	123.7 $\pm$ 10.9*	124.2 $\pm$ 5.4**
Control negative	440.5 $\pm$ 51.1	545.7 $\pm$ 28.2
Control positive	263.7 $\pm$ 82.5*	130.2 $\pm$ 11.5*
RBEE 100 mg/kg.bw	331.0 $\pm$ 95.0#	296.5 $\pm$ 65.8*
RBEE 200 mg/kg.bw	307.2 $\pm$ 73.9#	211.5 $\pm$ 25.8*
RBEE 400 mg/kg.bw	319.7 $\pm$ 55.0#	147.2 $\pm$ 17.0*#

\* different significant with control negative

#no different significant with control positive

From the results percent (%) decrease rate glucose blood in mice between 7th day post treatment and 14th day post treatment obtained results decline can seen in table 4.

**Table 4.** Percent decline rate glucose blood

Group	% Decrease
Normal	-8.62*
Negative	-53.61
Positive	71.12*
RBEE 100 mg/kg.bw	27.11*
RBEE 100 mg/kg.bw	50.15*#

Group	% Decrease
RBEE 100 mg/kg.bw	68.00*#

\*different significant with control negative

#no different significant with control positive

Based on table happen increase the highest level in the group CMC Na treatment with an average of -53.61%. The group that experienced it decline biggest found in the metformin group with an average of 71.12% After That giving RBEE 400 mg/kg.bw with an average of 68.00% ago followed RBEE 200 mg/kg.bw with an average of 50.15%, after it was the least at a RBEE 100 mg/kg.bw with an average of 27.11% whereas For the normal group did not given treatment anything and nothing experience significant decrease with an average of -8.62. Based on table show average level results glucose blood mice that show increase on the 3rd day and that's it exceeds 200 mg/dl, so can given treatment For know benefits extract robusta coffee beans in glucose blood, then done calculation percentage decline glucose blood with method disaccord rate glucose blood on the 3rd day after induction alloxan with inspection glucose blood on the 17th day, then shared rate glucose blood 3rd day.

Percentage decline glucose blood in mice can seen that the greatest decrease was in the RBEE 400 mg/kg.bw by 68.00% and for RBEE 200 mg/kg.bw almost the same percent its decline with RBEE 400 mg/kg.bw from second dose almost equal percent decline control positive namely metformin. Decline rate glucose blood in mice that have been given extract ethanol robusta coffee beans can decrease because there is compounds that play a role as antidiabetic. One of compounds found in coffee that can lower rate glucose blood is polyphenols and caffeine. Caffeine can lower risk suffer from type 2 diabetes mellitus with method guard sensitivity body to insulin via a number of mechanism and caused by influence caffeine to enhancement rate epinephrine in plasma (Fahmi, 2022). Polyphenols is antioxidants that have function prevent various disease like cirrhosis liver and diabetes mellitus type 2. Compound polyphenols can also slow down expenditure glucose to circulation blood after eat. Polyphenols own anti-inflammatory effect and can influence glycemia through different mechanisms, inhibition absorption glucose in the intestines and increases insulin resistance (Furqan & Nurman, 2020a).

Compound the most dominant polyphenol contained in coffee that is sour chlorogenic. Sour chlorogenic consists from class main compound especially phenolics originate from esterification trans-cinnamic acid, can stimulate production glucagon-like peptide 1 (GLP-1). GLP-1 ie hormone peptides and there are in the channel digestible stimulating insulin secretion from gland pancreas, has effect protection to beta cells- pancreas, as well hinder secretion hormone glucagon so that can control rate glucose blood. GLP-1 secretion can stimulated quick after consume containing foods carbohydrates and proteins. GLP-1 is next will stimulate pancreatic beta cells secrete insulin and suppress alpha cells that result production glucagon will decrease (Rangkuti, 2022).

## CONCLUSION

This study show that treatment RBEE 100 mg/kg.bw, RBEE 200 mg/kg.bw and RBEE 400 mg/kg.bw have influence to decline rate glucose blood mice in a way significant compared group control negative, with decline rate glucose blood mice amounting to 27.10%, 50.14% and 68.00%.

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