ARTICLE / INVESTIGACIÓN

The effect of Cocoa Fruits (*Theobroma cacao* L.) extracts on serum blood glucose levels in white rats (*Rattusnovergicus*)

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Abstract: Checking blood glucose levels supports healthy lifestyle and pattern changes to avoid foods that contain high glucose, which can increase blood glucose levels. This study aims to analyze the administration of cocoa pod extract (*Theobroma cacao* L.) to describe serum blood glucose levels in white rats (Rattusnovergicus). This study used an RCT (randomized controlled trial) design involving five groups of cocoa pod extract samples through an evaporation process using a rotary vacuum evaporator after going through the maceration process for 3 x 24 hours divided into three concentrations of 50 mg/Kg body wt, 100 mg/Kg body wt, 150 mg/Kg body wt. Furthermore, serum blood glucose levels will be measured on the 3rd, 7th, and 14th day after administration of metformin and waste extract of cocoa pod shell (*Theobroma cacao* L.). Analysis of the research data used a paired t-test. The results showed that the average fasting blood glucose levels of K1 rats (Mus musculus) to the average fasting glucose levels in K2, K3, K4, and K5, respectively, were as follows: 0.037 ($p < 0.05$); 0.000 ($p < 0.05$); 0.028 ($p < 0.05$); and 0.015 ($p < 0.05$). This shows that the average fasting blood glucose level is significantly different ($p < 0.05$). The average fasting blood glucose levels of K2 (metformin group) to the average glucose levels in K3 (extract group 50 mg/kg body wt), K4 (extract group 100 mg/kg body wt), and K5 (extract group 150 mg/kg body wt) respectively are as follows: 0.157 ($p > 0.05$); 1.000 ($p > 0.05$); and 0.995 ($p > 0.05$). This shows that the average blood glucose levels between K2, K3, K4, and K5 did not differ significantly ($p < 0.05$) after measuring cocoa pod extract for two days. Based on the results of data analysis from this experimental study, it can be concluded that there is an effect of giving cocoa pod extract (*Theobroma cacao* L.) on fasting blood glucose levels of mice (Mus musculus) with type 2 diabetes mellitus hypercholesterolemia model.

Key words: Diabetes mellitus, Cacao, Blood Glucose, Rats.

Introduction

Diabetes mellitus (DM) is a condition involving decreased insulin activity in quantity and a functional disorder in terms of activity. This condition results in chronic hyperglycemia and carbohydrate, lipid and protein metabolism changes[1,2]. There are two primary forms of diabetes: insulin-dependent diabetes mellitus (type 1 diabetes mellitus, DM1) and non-insulin-dependent diabetes mellitus (type 2 diabetes mellitus)[3-5].

Most DM2 results from interactions between genetic, environmental, and other risk factors. Other conditions correspondingly contribute to the progression of DM2, such as loss of the first phase of insulin release, impaired basal insulin secretion, and increased glucagon levels[6,7]. Nonetheless, there is no exogenous insulin dependence for cases of T2DM. Exogenous insulin is needed when diet or oral hypoglycemic medication poorly controls blood glucose levels. In addition, people with DM2 are often accompanied by complications, such as cardiovascular disease, diabetic neuropathy, nephropathy, and retinopathy. Diabetes and its related complications reduce people's quality of life and generate enormous economic and social burdens[8].

DM2 has been a long-observed global public health problem. Analysis of recent statistical data reveals that DM2 has several new epidemiological characteristics. First, diabetes continues to increase steadily in developed countries like the United States and Japan. It should be noted that DM2 has become a severe problem at an alarming rate in developing countries[9,10].

It has been predicted that DM2 will be on an increasing trend in the next twenty years, with a target of 70% of sufferers aged 45-64 years coming from developing countries. Until now, the highest number of Diabetes Cases has been found in lower-middle-income countries, including India, China, Russia, Brazil, Pakistan, India, and Bangladesh. Among them, the prevalence rates are 12.1% and 9.7% in India and China, respectively[11]. Second, although advancing age is a risk factor for DM2, increasing rates of childhood obesity have resulted in DM2 becoming more common in children and adolescents, a serious emerging epidemic and a new public health problem with a significant proportion[8].

In 2017, the International Diabetes Federation (IDF) reported that Indonesia is the fourth most populous country. It has the sixth largest diabetics worldwide (> 10 million

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people with diabetes). The increased number of diabetics in Indonesia results from increasing modifiable risk factors, including hypertension, abdominal obesity, obesity, pre-diabetes, and smoking[12].

Most diabetics in Indonesia are type 2, with an average age of 49.7 ± 6.8 years. Therefore, this study focuses on type 2 diabetes. A slow onset of symptoms characterizes type 2 diabetes. Most Indonesians perceive health as a condition that refers to the ability to carry out daily activities without interference. Thus, people generally come to the doctor when obstructive symptoms develop. Thus, many people are unaware of their diabetes until they have complications. It causes the high prevalence of undiagnosed diabetes in Indonesia[12].

Considering that treating diabetes mellitus patients requires a long time, it is even mandatory to take the drug for life, which has the opportunity to get pharmacological side effects. For this reason, alternative non-pharmacological treatments are needed to reduce blood sugar levels as alternative relatively safe treatments, one of which is herbal medicine or complementary therapy. Cocoa empirically is a plant that has the potential to be an antidiabetic.

Theobroma cacao L. Cocoa is a tree-shaped plant from tropical forests in Central America and northern South America[1,2]. The cocoa pod shell (*Theobroma cacao* L) is the outermost part of the cocoa pod, which covers the most significant part of the whole cocoa pod mass (75.52% of the fresh cocoa pod). Cocoa pod production occurs yearly, so the amount increases and more is wasted[5]. Based on the coffee and cocoa research center, cocoa pod shells have not been utilized optimally, and most of them only become waste in the community environment. For this reason, an idea is needed to help turn cocoa pod shell waste into something that can benefit people's lives and reduce cocoa pod shell waste in today's society[4].

Cocoa (*Theobroma cacao* L.) contains more phenolic antioxidants than most foods. Flavonoids, including catechins and epicatechins, dominate the antioxidant activity. The tricyclic structure of flavonoids determines their antioxidant effects by scavenging reactive oxygen species, chelating Fe2+ and Cu+, inhibiting enzymes, and regulating antioxidant defenses. Cocoa's antioxidant effects can directly affect insulin resistance and reduce the risk of diabetes[13].

Based on the literature search, it is known that research on the utilization of cocoa shells has yet to be carried out. The effect of cocoa shell extract on carbohydrates controlling genes has yet to be observed. Our research has high novelty, especially in treating diabetes mellitus patients using food ingredients other than medical drugs. Based on this phenomenon, the researcher has conducted a study to analyze the Effect of Cocoa Fruit Peel Extract (*Theobroma cacao* L.) on describing serum blood glucose levels in white rats (*Rattus Copernicus*).

Materials and methods

Study Design

This research is a quasi-experimental design study using the pretest-posttest control group design method.

Sample

This research was carried out at the Biomedical Laboratory, Faculty of Medicine, Halu Oleo University, in October-December 2022, involving 25 white rats (*Rattus Copernicus*) that matched the established inclusion criteria, namely male rats, rats aged 3-4 months, rats' body weight 150-200 grams, white fur, healthy body condition (active and not disabled), while sick rats and rats with a weight <150 grams were excluded from the study.

In this study, there were five groups consisting of a control group (-), a control group (+), and a treatment group with different extract doses, extract doses of 50 mg/Kg body wt, 100 mg/Kg body wt, and 150 mg/ Kg body wt. Each group consisted of 5 Rattus novergicus male rats, so the number of samples used was 25 rats.

Variables

The dependent variable in this study was the blood sugar level of white rats, with two criteria: Hyperglycemia if GDS > 200 mg/dL and Non-Hyperglycemia if GDS < 200 mg/dL. The independent variable is the Administration of Cocoa Fruit Peel Extract (*Theobroma cacao* L.).

Intervention

This research was conducted by treating white rats, in this case, white rats (Rattusnovergicus), then measuring blood glucose levels of white rats using the GOD-PAP method (Glucose Oxidase – Peroxidase Aminoantypirin) Serum Samples to get more specific results.

The tools to be used in this study were a glucometer, analytical balance, oven, blender, three cc syringe, mortar and pestle, hot plate, spectrophotometer, and rotary vacuum evaporator. The materials used in this study were white rats (Rattusnovergicus), streptozotocin, 2.5 L acetone, high-fat feed, regular feed for white rats, and cocoa pod skin (*Theobroma cacao* L).

Cocoa pod shell waste (*Theobroma cacao* L.) was cleaned and cut into small pieces measuring ± 3 x 1 cm. After that, the cocoa pod shell waste is then placed on a tray covered with aluminum foil and put into the oven. Cocoa pod shell waste (*Theobroma cacao* L.) was then dried using an oven for three days at a temperature of 40^{o}C. After going through the drying process, the cocoa pod shell waste is weighed to determine the net weight of the sample. Furthermore, waste cocoa pod skin (*Theobroma cacao* L.) is mashed using a chopper to improve results. After going through the refining process, the sample then goes through the maceration process. The maceration process is carried out by immersing the sample in a container using 2.5 L acetone, which is carried out for 3 x 24 hours. After the maceration process, the sample is filtered to obtain the filtrate. The filtrate was then evaporated using a rotary vacuum evaporator and then concentrated in a water bath to obtain a thick extract.

The white rats used in this study were male Rattusnovergicus rats aged ± 3-4 months. After being brought in, the white rats must first undergo an acclimatization or adaptation process. This process was carried out for ± seven days to make the research rats accustomed to their environmental conditions and prevent stress on the white rats.

After going through the acclimatization process, the white rats were then divided into five treatment groups as follows:

1. Group 1, as a negative control (healthy) and given a 0.5% Na CMC colloidal solution.
2. Group 2, as the positive (sick) control, was given high-fat feed in the form of duck eggs for ± 1 week and then 3-5 mL of metformin (according to the weight of the rats in

the group).

3. Group 3 was given high-fat feed in the form of duck eggs for ± one week and then given cocoa shell extract at a dose of 50 mg/Kg BW (Theobroma cacao L.) 3-5 mL (according to the weight of the rats in the group).
4. Group 4 was given high-fat feed in the form of duck eggs for ± 1 week and then given cocoa shell extract at a dose of 100 mg/Kg body wt (Theobroma cacao L.) 3-5 mL (according to the weight of the rats in the group).
5. Group 5 was given high-fat feed in the form of duck eggs for ± 1 week and then given cocoa shell extract at a dose of 150 mg/Kg body wt (Theobroma cacao L.) 3-5 mL (according to the weight of the rats in the group).

After dividing into groups, each white rat's weight, cholesterol levels, and initial blood glucose levels will be measured. It was done to determine white rats' body weight, cholesterol levels, and blood glucose levels before and after a high-fat diet. After assessing these three indicators, the white rats in groups 2, 3, 4, and 5 were given a high-fat diet to increase blood glucose levels and determine the relationship between high-fat conditions and increased blood glucose levels. This feeding was carried out for ± one week. After one week, the white rats will then be measured again for their weight, cholesterol levels, and blood glucose levels to determine the condition of the white rats after being given a high-fat diet. Then, groups 2,3,4, and 5 white rats will be given streptozotocin to trigger a disruption in insulin production in the white rats so that the white rats will be in a state of diabetes.

Furthermore, three days after administration of streptozotocin, the white rats of groups 2, 3, 4, and 5 will again have their blood glucose and cholesterol levels measured. After that, metformin was given to group 2 white rats, and cocoa pod waste extract (Theobroma cacao L.) was given to white rats in groups 3, 4, and 5. Furthermore, serum glucose levels were measured on days 3, 7 and 14 after administration of metformin and cocoa pod waste extract (Theobroma cacao L.).

Outcomes

The output of this study is the visible effect of cocoa shells in lowering blood sugar levels in experimental animals.

Data analysis

Data presentation for categorical variables is in the form of numbers and percentages. At the same time, continuous data is described in the form of mean ± standard deviation (SD) or median with Interquartile Range (IQR). To test the normality or distribution of research data using the Shapiro-Wilk test, and the results are normally distributed data. Then, proceed with bivariate analysis using the ANOVA test. The ANOVA test was used to determine the Effect of Cocoa Fruit Peel Extract (*Theobroma cacao* L.) on the description of serum blood glucose levels in white rats (*Rattus Copernicus*). After seeing the differences in serum blood glucose levels, proceed with the Post Hoc test, which aims to determine which groups have different averages if the ANOVA test results in significant differences (Ho is rejected). All tests with p-value (p) <0.05 were considered significant. Statistical analysis was performed using the SPSS version 16.0 application.

Ethical Consideration

No economic incentives were offered or provided for participation in this study. In this study, because the subject was still a minor, the researcher had asked for and obtained parental consent so their child could participate. The study was performed following the ethical considerations of the Helsinki Declaration. This study obtained ethical feasibility under the Health Research Ethics Commission of the Medical Faculty, Halu Oleo University and registration number 181/UN29.17.1.3/ETIK/2022.

Results

Based on the results of the cocoa pod (*Theobroma cacao* L) phytochemical test results, the following results were obtained:

Table 1 shows the results of the phytochemical test of cocoa peel extract; it was found to contain alkaloids, tannins, flavonoids and saponins.

Table 2 shows the normality test with data on fasting blood glucose levels of mice (Mus musculus) before and after treatment using the Shapiro-Wilk normality test, with the data results normally distributed p>0.05.

Based on Table 3, the homogeneity test with data on fasting blood glucose levels of mice (Mus musculus) before and after treatment was obtained with a homogeneous distribution of data p> 0.05.

Based on Table 4, a probability value of 0.000 or a p-value <0.05 is obtained. It is interpreted that there is a difference in mice's fasting blood glucose levels (Mus musculus) between K1, K2, K3, K4, and K5.

Based on Table 5, it can be seen that the p-value analysis of the average fasting blood glucose levels of mice (Mus musculus) K1 to the average fasting glucose levels in K2, K3, K4, and K5 respectively, is as follows: 0.037 (p <0.05)); 0.000 (p < 0.05); 0.028 (p < 0.05); and 0.015 (p <0.05). This shows that the average fasting blood glucose level is significantly different (p <0.05).

P-value analysis of average fasting blood glucose levels K2 (metformin group) to mean glucose levels in K3 (extract group 50 mg/kg body wt), K4 (extract group 100 mg/

Compound	Ripe Cocoa Fruit Peel Simplicia	Simplicia of Unripe Cocoa Fruit Skin
Alkaloid	+	+
Tanin	+	+
Flavonoid	+	+
Saponin	+	+

Table 1. Phytochemical Test Results for Cocoa Fruit Peel Extract (*Theobroma cacao* L.).

Sample	Fasting Blood Glucose Levels (Mean ± SD)				
	*P-Value**	*Pre-treatment*	*P-Value**	*Post-treatment*	Average difference
K1	0,399	201,3±13	0,458	204,5±13	+3,2
K2	0,372	330,3±29	0,351	327,0±29	-3,3
K3	0,542	463,1±33	0,992	421,3±39	-41,8
K4	0,702	326,1±31	0,682	332,1±24	+6,0
K5	0,161	347,5±27	0,161	342,5±27	-5,0

Description: *Shapiro-Wilk test, standard if p>0.05

Table 2. Results of the normality test for measuring blood glucose levels in mice (*Mus musculus*) pre and post-treatment.

Sample	*Sig Test of Homogeneity of Variance*
Pre-treatment	0,681
Post-treatment	0,492

Table 3. Results of the homogeneity test for measuring blood glucose levels in mice (*Mus musculus*) pre and post-treatment.

Description: *Homogeneity of variance test, homogeneous if p>0.05

Group	Average Change of Difference	*P-value**
K1	+3,2	
K2	-3,3	0,001
K3	-41,8	
K4	+6, 0	
K5	-5,0	

Table 4. Results of the ANOVA test for measuring blood glucose levels in mice (*Mus musculus*).

Description: *One Way ANOVA test, significant if p <0.05

kg body wt), and K5 (extract group 150 mg/kg body wt) sequentially are as follows: 0.157 (p > 0.05); 1.000 (p > 0.05); and 0.995 (p > 0.05). This shows that the average blood glucose levels between K2, K3, K4, and K5 did not differ significantly (p <0.05) after measuring cocoa pod extract for two days.

Discussion

One of the chemicals widely used for diabetes induction is streptozotocin (STZ). Streptozotocin (STZ,2-deoxy-2-(3-v(methyl-3-nitrosourea)-D-glucopyranose) is a chemical compound synthesized from Streptomyceteschromogens and used to induce both type 1 and type 2 diabetes mellitus[14].

Groups		*P value**
K1	K2	0,037
	K3	0,000
	K4	0,028
	K5	0,015
K2	K1	0,037
	K3	0,157
	K4	1,000
	K5	0,995
K3	K1	0,000
	K2	0,157
	K4	0,199
	K5	0,305
K4	K1	0,028
	K2	1.000
	K3	0,199
	K5	0,999
K5	K1	0,015
	K2	0,995
	K3	0,305
	K4	0,999

Description: *Post Hoc Test, there is a significant difference p <0.05

Table 5. Post Hoc test analysis results.

There are several levels of streptozotocin doses used, such as a single injection of high dose streptozotocin (> 65 mg/Kg body wt), repeated injections of low doses (60 mg/Kg body wt) cause massive damage to pancreatic cells, so that it is more towards animal models of type 1 DM and medium-dose streptozotocin (between 40-55 mg/Kg body wt) causes partial insulin secretion disorders such as type 2 DM and a single dose of streptozotocin <35 mg/Kg body wt in normal diet rats does not show hyperglycemia. STZ induction causes insulin resistance, resulting in decreased glucose entry into muscle and adipose tissue so that glucose levels in the blood are high[3].

The mechanism of type 2 DM by administering streptozotocin is entering the pancreatic ß-cell through the GLUT 2 glucose transporter, causing decreased expression of GLUT 2—the results in decreased peripheral insulin receptor sensitivity, increased insulin resistance and increased blood glucose levels[15].

After two days of monitoring, statistical tests showed no significant difference between the metformin group and the group given cocoa pod extract (*Theobroma cacao* L.). However, it was found that after giving the extract for two days, mice's fasting blood glucose levels decreased (Mus musculus). So, cocoa pod extract (*Theobroma cacao* L.) can reduce fasting blood glucose levels in mice.

Based on the results of the phytochemical tests that have been carried out, it was found that the cocoa pod contains alkaloids, flavonoids, and saponins, which can be antihyperglycemic. It also aligns with research conducted by Chusniasih (2019), which states that alkaloids are one of the compounds in cocoa pod shells. Alkaloids are antioxidants that can prevent the oxidation of pancreatic β cells from minimizing the damage that occurs. Administration of STZ to mice is intended to create a state of hyperglycemia by reducing insulin sensitivity by entering the β cells of the pancreas, which can then be corrected by the alkaloid content contained in cocoa pod shells so that blood glucose levels in mice can decrease after being induced by STZ.

In addition, the antioxidant content in flavonoids can also act as a hypoglycemic agent. Flavonoid compounds

can protect against damage and prevent pancreatic β-cell death without changing the proliferation of pancreatic β-cells, thereby increasing insulin sensitivity. Antioxidants in flavonoid compounds can reduce insulin resistance by binding to free radicals[16,17]. Flavonoids are reducing compounds that can inhibit many oxidation reactions. Flavonoids have the ability as antioxidants because they can transfer an electron to free radical compounds. Thus, it can be concluded that after the administration of STZ, the flavonoid compounds contained in cocoa pod extract can help lower blood glucose levels in mice and act as antihyperglycemic agents.

The content of flavonoids in cocoa pod skin can also be reduced when heated, so the extraction method that is safe to use is the maceration process. In addition, in the maceration method, there is no heating in the extraction process, so no temperature factor accelerates the reaction or affects the active compounds in the extract, and the possibility of damage to the chemical components contained in the sample can be avoided[7,18].

In addition, saponins in cocoa pod shells also have antidiabetic properties and compounds that can be developed into new antidiabetic drugs[6,7]. Saponins can reduce blood glucose levels by inhibiting enzymes that break down disaccharides into monosaccharides. This effect affects treating type I and type II DM patients. It can even encourage glycogen storage by the liver and insulin secretion by the islets of Langerhans[2]. In addition, saponins in cocoa pod skin can reduce liver gluconeogenesis, increase liver glycogen synthesis, and increase peripheral glucose oxidation in erythrocytes and adipocytes[9,10]. Therefore, giving cocoa pod extract to mice can cause a decrease in fasting blood glucose levels due to the saponin content in the cocoa pod shells.

The present study is a pilot study involving small-group research subjects. This study cannot provide a comparative picture or differences in the effects of cocoa shells on people with diabetes mellitus who have different characteristics. For this reason, future studies should be tested on human subjects.

Conclusions

Based on the results of data analysis from this experimental study, it can be concluded that there is an effect of giving cocoa pod extract (*Theobroma cacao* L.) on fasting blood glucose levels of mice (Mus musculus) with type 2 diabetes mellitus hypercholesterolemia model.

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Conflicts of Interest

The authors declare no conflict of interest.

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