Effect of Cocoa Pod Extract (*Theobroma cacao* L.) On Gout Levels of Mice (*Mus musculus*) Hypercholesterolemia Diabetes Mellitus Type 2 Model Through Enzyme Linked Immunosorbent Assay Reader Examination

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Abstract. Hyperuricemia is a condition that describes an increase in uric acid levels in the body, increased uric acid levels in the blood beyond normal limits will cause pain or tenderness. Cocoa pod skin contains a number of antioxidants such as flavonoids, alkaloids, polyphenols and tannins which can be used as an alternative treatment for hyperuricemia. This study aimed to determine the effect of cocoa pod extract on mice's uric acid levels and determine the optimal dose of cocoa pod extract. This research was a quasi-experimental study with a pretest-posttest control group design approach with the independent variable of cocoa pod extract and the dependent variable of mice uric acid levels. Type 2 Diabetes Mellitus in this study was induced using 40 mg/kg BW streptozotocin and hypercholesterolemia was induced with a high-fat diet which was then measured using an ELISA reader. Data were analyzed using a paired t-test and One Way ANOVA test**.** Average uric acid levels between the control group and the 50 mg/kg BW extract, the 100 mg/kg BW group and the 150 mg/kg BW extract group were not significantly different ($p>0.05$). The optimal dose of cocoa pod extract to reduce uric acid levels in mice is a dose of 150 mg/kg BW. Cacao pod extract affects the uric acid levels of mice at a dose of 50 mg/kg, 100 mg/kg, and 150 mg/kg. The mean levels of uric acid between the control group and the 50 mg/kg BW extract, the 100 mg/kg BW group and the 150 mg/kg BW extract group were not significantly different ($p>0.05$).

Keywords: Uric Acid, Cocoa Fruit, High Fat Feed, Streptozotocin, *Theobroma cacao*.

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1 Introduction

Hyperuricemia is a condition that describes an increase in uric acid levels in the body, increased uric acid levels in the blood beyond normal limits will cause pain or pain [1]. The incidence of increased uric acid is often found in cases of increased insulin resistance. The general characteristics of fifty samples of type 2 DM patients had an average serum uric acid concentration of 6.75 ± 1.36 mg/dL and it was stated that the frequency of hyperuricemia reached 30%. In studies on the relationship of hyperuricemia with diabetes including inhibition of glucose stimulation for insulin secretion carried out in experimental animals, it is also stated that inhibition of uric acid excretion in the kidneys can occur due to conditions of insulin resistance, changes in endothelial function and then impaired nitric oxide bioavailability and stimulates hyperinsulinemia.

The treatment used to treat elevated uric acid levels is allopurinol, allopurinol is a uricostatic uric acid drug which is a strong inhibitor of XO which can reduce uric acid levels, but allopurinol has side effects such as hepatitis, nephropathy and allergies so it is necessary to look for XO inhibitors that are from natural sources as an alternative substitute for allopurinol [2]. One of the medicinal plants that can be an alternative to herbal medicine is the skin of the cocoa pod (*Theobroma cacao L.*) [3]. Based on Fitri [4], cocoa pod skin contains a number of antioxidants such as flavonoids, alkaloids, polyphenols, and tannins. The antioxidant effect on cocoa pod skin will directly affect insulin resistance so that it can reduce the risk of diabetes and affect the formation of uric acid [5]. For this reason, an idea is needed that can turn cocoa pod skin into something that can be beneficial to people's lives and not just become waste in the midst of society.

Based on this, researchers were interested in knowing the effect of giving cocoa pod extract (*Theobroma cacao L.*) on uric acid levels in mice (*Mus musculus*) hypercholesterolemia model of type 2 diabetes mellitus.

2 Research Methods

This research is a quasi-experimental design with a pretest-posttest control group design approach. The independent variable was cocoa pod extract (*Theobroma cacao L.*) and the dependent variable was blood glucose, total cholesterol, uric acid levels in mice (*Mus musculus*). This research was conducted at the Experimental Animal Laboratory, Faculty of Medicine, Halu Oleo University in November-December 2022 and has been approved by the Medical Ethics Commission, Faculty of Medicine, Halu Oleo University with No.074/UN29/17.1.3/ETIK/2022.

The sample for this study was male mice (*Mus musculus*) weighing 25-30 grams with a minimum number of samples needed was 5 for each treatment group and the total number of male mice in the test group was 25. To prevent dropping out in the middle of the study, one sample was added for each group. Thus, the total sample required for 5 groups is 30 rats.

The tools in this study were Vacuum rotary evaporator (Buchi Rotavapor) (Buchi®), Elisa Rader BIORAD, Sonde gastric, Digital scales (Fujitsu ®), Centrifuge, Micropipette, Tip, 50 mL and 300 mL beaker, Oven, Counter, Hot plates, clear jars, stirring rods, experimental animal cages, vials, Glucose Cholesterol Uric acid meter device (Easy Touch ®), Microtube, Pestle mortar.

The ingredients in this study were 30% sucrose, STZ, duck egg yolk, AD 2 feed, Simvastatin, 70% ethanol, HCl, dragendorff reagent, FeCl3, NaOH, cocoa pod shell (*Theobroma cacao L.*), glucose strips, Na-CMC, syringe, EDTA tube.

Test animals were euthanized using chloroform [6] by placing mice in a jar containing cotton or cloth that had been moistened with chloroform, then closing the jar and waiting for their breathing to stop. After breathing has stopped, confirm death by physical methods such as cervical dislocation or decapitation. Furthermore, mice blood will be taken intracardially for the final measurement in the study. After that, the mice will be buried.

3 Results

Based on Table 1, it shows the difference in mean before and after treatment using the Paired T-Test, it was found that the K2 and K5 groups showed a significant difference ($p < 0.05$)

Table 1. Differences in average uric acid levels of mice (grams) before and after treatment.

Group		Uric acid (mg/dL) (Mean ± SD)	P-value*pre and post test
K1	Before Treatment	6,4±0.2	0.049
	After Treatment	7.1±0.5	
K2	Before Treatment	6.7±0.3	0.001*
	After Treatment	2.1±0.7	
K3	Before Treatment	6.5±1.3	0.095
	After Treatment	6.0±1.2	
K4	Before Treatment	6.1±1.0	0.143
	After Treatment	5.4±1.6	
K5	Before Treatment	6.5±0.8	0.001*
	After Treatment	5.0±0.8	

This illustrates that there are significant results between the average uric acid levels before and after treatment. In K2, the difference between before treatment and after treatment was +4.6 mg/dl, indicating a decrease in uric acid.

At K5, the difference between before treatment and after treatment was +1.5 mg/dl which indicated a decrease in uric acid levels between before and after treatment.

In the cocoa pod extract test group, namely K3, K4, and K5, the difference in mean uric acid levels between before and after treatment were: +0.5 mg/dl; +0.7mg/dl; +1.5 mg/dl which indicates that there was a decrease in uric acid levels between before treatment and after treatment, so it can be stated that cocoa pod extract had an effect on uric acid levels in mice (*Mus musculus*) so that Ha was accepted and H0 was rejected.

Based on Table 2, it shows that the analysis of the One Way ANOVA test obtained a p-value of 0.000 (p <0.05), which means that there was a significant difference in the average uric acid levels of mice between groups K1, K2, K3, K4, and K5. Thus, it is said that Ha is accepted and H0 is rejected.

Table 2. Differences in average uric acid levels of mice.

Group	n	Difference in average uric acid levels	P-value*
K1	5	+0.7	0.000*
K2	5	-4,6	
K3	5	-0.5	
K4	5	-0.7	
K5	5	-1.5	

Table 3. Differences in average uric acid levels of mice for each experimental group.

Group (n=5)		P-value*
K1	K2	0.000
	K3	0.499
	K4	0.133
	K5	0.046
K2	K3	0.000
	K4	0.000
	K5	0.001
		0.003
K3	K4	0.499
	K5	0.000
		0.906
		0.629
K4	K5	0.133
		0.001
		0.906
		0.981

Based on Table 3 of Tukey's Post Hoc follow-up test, the HSDp-value of the average K1 uric acid level to the average uric acid levels in K2, K3, K4, and K5 respectively is as follows: 0.000 (p <0.05); 0.499 (p>0.05); 0.133 (p>0.05); 0.046 (p<0.05).

This shows that the average uric acid level between the K1 and K2, K3, K4, and K5 groups was significantly different (p<0.05). The p-value analysis of the average K2 total cholesterol level to the total cholesterol level in the K3, K4, and K5 groups respectively was as follows: 0.000 (p<0.05); 0.001 (p<0.05); 0.003 (p<0.05).

This shows that the mean total cholesterol levels between groups K2 and groups K3, K4, and K5 differ significantly (p>0.05).

4 Discussion

Based on Table 3, it can be seen that the p-value analysis of the average K1 uric acid level to the average uric acid levels in K2, K3, K4, and K5 respectively is as follows: 0.000 ($p < 0.05$); 0.499 ($p > 0.05$); 0.133 ($p > 0.05$); 0.046 ($p < 0.05$). This shows that the mean uric acid levels between the K1 and K2 and K5 groups were significantly different ($p < 0.05$). This shows that cocoa pod extract has the ability to reduce uric acid levels in mice. However, the resulting decrease is not optimal. Thus, it can be said that the ability of cocoa pod extract in reducing uric acid levels has not matched the ability of the control drug allupurinol in reducing uric acid levels. In addition, the increase in uric acid that occurred after STZ induction did not result in mice in a state of hyperuricemia indicating that the accumulation of uric acid in mice had not occurred. Thus, the decrease in uric acid that occurs after administration of cocoa pod extract cannot be concluded with certainty whether the extract is able to reduce uric acid levels in mice or mice uric acid levels decrease as a physiological form of the body, considering the ability of cocoa pod extract is still significantly different from the ability allupurinol in reducing uric acid levels in mice.

Nevertheless, there are studies that support the ability of cocoa pod extract to reduce uric acid levels because it is influenced by the metabolites contained therein, namely flavonoids, tannins, and saponins. Flavonoids can also inhibit several enzymes, such as xanthine oxidase, cyclooxygenase, lipoxygenase and phosphoinositide 3- kinase. Xanthine oxidase is oxidative in damage to living tissue and can cause hyperuricemia. This is due to the ability of cocoa pod extract to reduce uric acid levels in mice due to the influence of alkaloids, flavonoids, and tannins [7]. Flavonoids catalyze the oxidation of hypoxanthine and xanthine for uric acid and based on the research of Nagao et al. [8], flavonoid compounds have the greatest inhibitory activity against the xanthine oxidase enzyme with the largest inhibitory value of 0.44μM.

Tannins in mangosteen rind can reduce uric acid levels by binding to free radical compounds during the conversion of purines to uric acid, in a study conducted by Sa'idah and Sumiwi [9], a decrease in uric acid levels was found by 49.231% after administration of 8μg/mL extract mangosteen skin. Saponins can reduce uric acid levels by their mechanism of action by inhibiting the activity of the xanthine oxidase enzyme. In a study conducted by Rachmania et al. [10], a decrease in uric acid levels of 58.992% was found after administration of 1000 ppm of secang bark extract.

5 Conclusion

Based on the average decrease in blood uric acid levels in the administration of the extract at a dose of 50 mg/dl, a dose of 100 mg/dl, and a dose of 150 mg/dl, the difference in the decrease in blood uric acid levels before and after treatment was sequentially as follows: +0.5 mg/dl; +0.7 mg/dl; +1.5mg/dl. Thus, it was found that the optimal average decrease in uric acid levels was at a dose of 150 mg/dl.

5.1 Suggestion

Future researchers can carry out phytochemical testing for polyphenols, quinones, monoterpenoids and sesquiterpenoids in cocoa pod extract, can perform assays to determine the amount of each metabolite compound contained in cocoa pod extract, and can analyze the effects of cocoa pod extract. on the nutritional status of mice

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