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Effectiveness Of Cucumber Extract (*Cucumis Sativus*) In Reducing Cholesterol Levels In Mice (*Mus Musculus*) Induced By Hypercholesterolemia

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Abstract

Hypercholesterolemia is a major global health concern and one of the primary risk factors contributing to cardiovascular disease, which remains the leading cause of morbidity and mortality worldwide. The development of natural cholesterol-lowering agents is considered an alternative strategy to reduce dependence on synthetic drugs that may cause side effects. This study aims to explore the potential, determine the optimal dose, and identify the active compounds of cucumber (Cucumis sativus) ethanol extract as a natural cholesterol-lowering agent. A quantitative approach with a pure experimental method was employed using a pre-test and post-test control group design conducted in vivo on 25 mice (Mus musculus), which were randomly divided into five treatment groups. The main instrument used was the Easy Touch cholesterol checker, and data were analyzed using the One-Way ANOVA test to assess statistical significance. The results showed that cucumber extract significantly reduced cholesterol levels in a dose-dependent manner. The most optimal reduction was observed at a dose of 700 mg/kgBW, with an average decrease of 49.51%, which was close to the effectiveness of the standard drug simvastatin at 54.05%. These findings suggest that cucumber extract contains active compounds with strong hypocholesterolemic activity. In conclusion, cucumber ethanol extract demonstrates considerable potential as a natural cholesterol-lowering agent, and the dose of 700 mg/kgBW may be considered the most effective in providing an effect almost equivalent to conventional therapy.

***Keywords**: Anticholesterol, Cucumis Sativus, Flavonoid, Hypercholesterolemia, Mus Musculus*

INTRODUCTION

Research Phenomenon

Hypercholesterolemia, a condition where blood cholesterol levels exceed the normal range (240 mg/dl), is a serious global health issue and a leading cause of cardiovascular disease. According to the World Health Organization (WHO), heart disease and stroke are the leading causes of death worldwide, accounting for 17.9 million deaths annually (WHO, 2021). This figure is reinforced by data from the 2018 Indonesian Basic Health Research (Riskesdas), which shows that the prevalence of abnormal cholesterol in people over 15 years of age reached 18.3% in men and 24.0% in women (Kemenkes, 2018). This phenomenon highlights the urgency of finding effective, affordable, and safe treatment solutions to reduce the increasing morbidity and mortality rates (Sari, 2016; Putri, 2020).

Hypercholesterolemia is often caused by liver damage, an organ that plays an important role in producing and regulating cholesterol levels in the body (Nofanni & Ruqoyah, 2016). Liver damage due to high cholesterol can be prevented and treated with hepatoprotective compounds. Cucumber (*Cucumis sativus)*, a vegetable that is easily found in Indonesia, is known to contain active compounds, including flavonoids, saponins, and steroids, which have the potential to act as cholesterol-lowering agents (John et al., 2018). Although cucumbers are popular, many people consume the flesh and discard the skin, even though cucumber skin contains very high levels of flavonoids (up to 71%), which act as powerful antioxidants to inhibit LDL oxidation, a trigger for blood thickening and fat deposition on the walls of blood vessels (Aprillyanti et al., 2021; Kusrina et al., 2024).

Research Problems

Although the potential of cucumber as a cholesterol-lowering agent has been documented in several studies, there are still challenges in its use as herbal therapy. One of the main problems is determining the optimal dose that is effective in lowering cholesterol levels. Previous research by Rusmin et al. (2019) showed that cucumber ethanol extract is effective at a dose of 2 mg per 20 grams, but this dose may not fully represent the maximum effect that can be achieved. In addition, there is a need for further exploration of the secondary metabolite compounds contained in cucumber extract, which can provide a deeper understanding of the mechanism of cholesterol reduction (Saini et al., 2022). Therefore, this study aims to fill this knowledge gap, focusing on identifying the most effective dosage and analyzing the bioactive compounds involved.

Moreover, the growing public interest in herbal medicine as an alternative to conventional medicine is an important driver for this research (Putri & Anggraini, 2022). However, the lack of standardization and strong scientific evidence regarding the efficacy and safety of herbal products is often an obstacle. Therefore, this study seeks to provide valid and reliable scientific data on cucumber ethanol extract. This is crucial to ensure that the use of cucumber as an alternative therapy can be carried out effectively and safely, while paving the way for the development of standardized phytopharmaceutical products in the future (Gao et al., 2023).

Research Objectives, Urgency, and Novelty

This study has three main objectives: first, to explore the potential of cucumber (*Cucumis sativus*) ethanol extract as a cholesterol-lowering agent; second, to determine the optimal dose of cucumber ethanol extract that has a significant effect on cholesterol levels; and third, to identify the secondary metabolite compounds contained in cucumber ethanol extract that play a role in this activity. The urgency of this research lies in the increasing prevalence of hypercholesterolemia in Indonesia, which requires the development of scientifically proven herbal-based alternative therapies. The novelty of this research lies in the scientific validation of the optimal dose of cucumber ethanol extract, which has not been fully identified previously, as well as an in-depth analysis of the secondary metabolite compounds responsible for its pharmacological effects, providing a strong scientific basis for the use of cucumber as a natural hepatoprotector.

RESEARCH METHODS

Type and Method of Research

This study used a quantitative approach with a pure experimental method (*True Experimental*). The research design applied was a pre-test and post-test control group design. According to Sugiyono (2023), this design is effective for measuring the effectiveness of a treatment by comparing the initial (*pre-test)* and final (*post-test)* conditions between the group given the treatment (experimental group) and the group not given the treatment (control group). The main objective of using this method is to prove the causal relationship between the administration of cucumber ethanol extract and a decrease in cholesterol levels. The entire research process was carried out *in vivo* using laboratory animals, namely mice (*Mus musculus)*, to test the pharmacological effects of the extract (Rusmin et al., 2019). This research was conducted at the Pharmacology Laboratory of the Pharmacy Undergraduate Program, Duta Bangsa University Surakarta, Central Java, from April to June 2025.

Instruments and Data Analysis Techniques

The tools used in this study included mouse cages, *blenders*, *ovens*, *rotary evaporators*, analytical scales, and *easy touch* cholesterol test kits and strips. The main ingredients used were cucumbers from Nglebak Village, Karanganyar, as well as other chemicals such as propylthiouracil, 96% ethanol, CMC Na, and simvastatin. The data collected were the results of measuring the cholesterol levels of mice at various stages of treatment. To analyze the data, a One-Way ANOVA statistical test was used to compare the average cholesterol levels between treatment groups. If a significant difference was found (p < 0.05), a Tukey *post-hoc* test was used to identify which groups had significant differences (Emzir, 2021). This analysis technique ensured the validity of the research findings in assessing the effectiveness of cucumber extract.

Population and Sample

The population in this study was laboratory mice (*Mus musculus*). The research sample consisted of 25 male mice weighing between 20 and 30 grams, which were divided into five treatment groups at random, with each group consisting of five mice. This grouping was important to minimize bias and ensure the validity of the results (Creswell, 2021). The five groups were: negative control group (given 1% CMC Na), positive control group (given 0.052 mg/kgBW simvastatin), and three treatment groups given cucumber ethanol extract at doses of 175 mg/kgBW, 350 mg/kgBW, and 700 mg/kgBW.

Research Procedure

The research procedure included several systematic and logical stages. The initial stage was the collection of cucumber samples from Nglebak Village, Karanganyar, followed by plant determination and *ethical clearance*. Next, sample preparation was carried out, namely the production of simplisia from cucumbers through a drying and grinding process, as well as simplisia standardization to ensure the quality of the raw materials. After that, extraction was carried out using the maceration method with 96% ethanol solvent to obtain a thick extract. The resulting extract then underwent phytochemical screening to identify the content of active compounds such as flavonoids, alkaloids, triterpenoids, steroids, phenolics, saponins, and tannins (Maulida, 2020; Oktavia & Sutoyo, 2021).

The next stage was treatment of the test animals. The mice were observed for 5 days and their initial cholesterol levels (*pre-test*) were measured on day 0 (T_0). Next, the mice were induced with propylthiouracil orally for 7 days to trigger hypercholesterolemia. Cholesterol levels were measured again on day 7 (T_1) to ensure successful induction. After that, each group was given treatment according to the dosage for 7 days. Cholesterol levels were measured on day 14 (T_2), day 21 (T_3), and day 28 (T_4) to evaluate the effectiveness of the extract over time. The entire procedure was designed to comprehensively evaluate the effectiveness of cucumber ethanol extract in lowering cholesterol levels.

RESULTS AND DISCUSSION

Sample Collection and Plant Determination

This study used cucumber (*Cucumis sativus* L.) as the main ingredient. Cucumber samples were obtained from agricultural land in Nglebak Village, Karanganyar Regency, Central Java Province. The part of the plant used in this study was the cucumber fruit. Cucumber plant identification was carried out at the Functional Implementation Unit (UPF) for Traditional Services located in Tawangmangu, Karanganyar, Central Java, to ensure the accuracy of the plant species used. The identification results stated that the samples used were cucumber plants with the scientific name *Cucumis sativus* L., which belong to the *Cucurbitaceae* family. The identification results can be seen in Appendix 2.

A. Ethical Clearance

This study has obtained ethical approval from the Health Research Ethics Committee of Moewardi Hospital, Jl. Kolonel Sutarto No.132, Jebres, Surakarta City, Central Java, with decision letter number: 846 / IV / HREC / 2025, issued on April 26, 2025. All procedures performed on experimental animals in this study have referred to the principles of animal research ethics, including the 3R principles (*Replacement, Reduction,* and *Refinement)* to ensure animal welfare during the research process. The male Wistar mice used were kept in standard laboratory conditions, and the treatment given during the study was carried out with consideration for the ethical aspects, safety, and comfort of the animals. The *ethical clearance* results can be seen in Appendix 3.

B. Preparation of Simplisia

The process of making ethanol extract from cucumber begins with the selection of high-quality cucumbers, namely those that are fresh and free from damage or defects. After that, the cucumbers are washed with running water to remove dirt, so that the material is in a hygienic condition before extraction. After being washed clean, the cucumbers are sliced thinly to expand the surface area to be extracted. The cucumber slices are then dried in an oven at a low temperature, around 40–50°C, or air-dried for 2 days until completely dry. This drying process aims to reduce the water content in the herbal material, thereby facilitating the extraction of active compounds. After drying, the cucumber is crushed and sieved using a number 40 mesh sieve to obtain a uniform powder size and standardize the simplisia.

Table 1 Calculation Results of Cucumber Simplisia Percentage

Wet Weight of Simplisia (g)	Powder Weight (g)	Percentage (%)
3,500 g	150g	4.3

Based on Table 1, it can be seen that there are 3,500g of cucumber. After drying, the cucumber is ground and sieved using a number 40 mesh sieve to obtain a uniform powder size and standardize the simplisia.

Table 2 Calculation Results of Powder Simplisia Percentage Relative to Cucumber Simplisia

Wet Weight of Simplisia (g)	Powder Weight (g)	Percentage (%)
150g	100.19 g	66.7

Based on Table 2, the percentage of powder from the crude drug to the cucumber crude drug is 66.7%.

C. Extract Production

Extraction was carried out using the maceration method. Cucumber powder was placed in a glass container, then 1000 mL of 96% ethanol solvent was added (1:10). The container was then left for 3 days in a tightly sealed condition, protected from sunlight, and stirred occasionally. After 3 days, the macerate was filtered using flannel cloth or filter paper to separate the filtrate from the residue. The resulting powder was then left to stand for another 2 days, with an additional 500 mL of 96% ethanol added, kept covered and protected from sunlight, and stirred occasionally. After obtaining the cucumber extract maserate, the extract is concentrated using a vacuum rotary evaporator at a temperature of 40-60°C and then evaporated using a water bath until a thick extract is obtained.

Table 3 Extract yield results

Simplified powder (g)	Concentrated extract (g)	Extract yield (%)
100.19 g	28 g	28.28

Based on the yield of the crude drug obtained from weighing 100.19 grams of crude drug powder and 28 grams of concentrated extract, the result is 28.28%. The calculation results can be seen in Appendix 10.

D. Standardization of Simplisia and Extract

1. Drying Loss

Each sample was weighed at 2 g and placed in a container that had been calibrated and weighed together using *an oven.* It was then dried at a temperature of 105°C and left for 30 minutes until the moisture content appeared, and the results obtained were weighed and recorded (Ministry of Health, 2017) . The percentage of drying shrinkage of the crude drug is determined by comparing the initial weight before heating with the weight after heating. In the Indonesian Herbal Pharmacopoeia, 2nd edition, 2017, the requirement for drying shrinkage of crude drugs is ≤10% (Ministry of Health of the Republic of Indonesia, 2017) .

Table 4. Drying Loss Results of Cucumber Extract

Testing	I	II	III	Average	Requirements
Drying shrinkage	9.5	9.4	9.6%	9.5	≤10%

Based on the results of the drying shrinkage of rambusa leaf ethanol extract, the results obtained in replication I were 9.5%, replication II 9.4%, replication III 9.6%, with an average of 9.5%. These results indicate that the drying shrinkage of rambusa leaf ethanol extract meets the requirement of ≤10% (Ministry of Health, 2017) . The purpose of this drying shrinkage test is to determine the loss of compounds during the drying process. The results of the drying shrinkage calculation for the crude drug can be seen in Appendix 11.

2. **Ash Content**

Weigh 2 g of the crude drug and place it in a preheated and tared silica crucible. Slowly heat until the charcoal is completely burned out, cool, and weigh. If the charcoal cannot be removed using this method, add hot water and filter through ash-free filter paper. Heat the remaining paper and filter paper in the same crucible. Place the filtrate in a crucible, evaporate, calcine until the weight remains constant, and weigh. Calculate the ash content relative to the material that has been dried in air. The acceptable requirement for the ash content of simplisia is no more than ≤ 6.4% (Ministry of Health, 2017) .

Table 5. Ash Content Results of Cucumber Simplisia Powder

Testing	I	II	III	Average	Requirements
Ash content of crude drug	5.5	4.5	3.5	4.5%	<6.4%

Based on the results of total ash content testing on cucumber powder, the average is 4.5%. The high ash content in the simplisia indicates a high internal mineral content in the rambusa leaves themselves. The higher the ash content in the cucumber extract, the higher the mineral content in it (Indonesian Herbal Pharmacopoeia, 2022) . The results of the ash content test calculations can be seen in Appendix 11.

Table 6. Ash Content Results of Cucumber Extract

Testing	I	II	III	Average	Requirements
Ash content of extract	5.5	4.5	3.5	4.5	<6.7%

Based on the results of the ash content test, the cucumber extract showed an average of 4.5%. From these results, the ash content test of the cucumber extract has met the requirements of the Indonesian Ministry of Health's Regulation . The results of the ash content test can be seen in Appendix 11.

3. **Moisture Content**

Each sample was weighed at 2 g and placed in a container that had been calibrated and weighed together using *a moisture balance.* It was dried at 105°C and left for several minutes until the moisture content appeared. The acceptable requirement for moisture content is no more than 10% (Indonesian Ministry of Health, 2022) .

Table 7 Moisture Content Test Results

Test	I	II	III	Average	Requirements
Moisture Content	7.86	6.95	5.59	6.8%	≤ 10

Based on the results of the water content test of cucumber ethanol extract, it has a water content value of 6.8%. These results indicate that the water content test meets the requirement of ≤10%. The water content test aims to determine the amount of water contained in the extract (Indonesian Herbal Pharmacopoeia, 2022) . The results of the water content test can be seen in Appendix 11.

E. Phytochemical Screening of

Table 8 Results of Phytochemical Screening of Cucumber Extract

Compound	Reagent	Positive sign	Results	Description

Alkaloid	Mayer	White or yellow precipitate ( ) (Maulida, 2020) .	Forms a white precipitate	+
	Dragendorf	Orange or reddish-brown precipitate	There is a reddish-brown sediment	+
Flavonoids	Powder and concentrated HCl	Color change to red, yellow, and orange (Maulida, 2020) .	The color turns red	+
Saponin	Aquades + 1N HCl	Stable foam is formed (Oktavia & Sutoyo, 2021) .	Foam is formed	+
Tannin	$FeCl_3$	Forms a dark green or blue color (Oktavia & Sutoyo, 2021) .	Dark green color formed	+
Steroid or triterpenoid	Lieberman-Burchard	Forms a brownish ring (Oktavia & Sutoyo, 2021) .	Brown ring formed	+

Phytochemical screening is the first step in identifying secondary metabolites contained in a plant extract. This test aims to determine the types of bioactive compounds that may have pharmacological effects, including antihypertensive effects in this study. Based on the results of phytochemical tests conducted on cucumber (Cucumis sativus) extract, it is known that the extract contains alkaloids, flavonoids, saponins, tannins, and steroids or triterpenoids, as indicated by positive results for each specific reagent.

The alkaloid test was conducted using two reagents, namely Mayer and Dragendorff. A positive reaction was indicated by the formation of a white precipitate in the Mayer test and a brownish-red precipitate in the Dragendorff test. This showed that alkaloids were present in the cucumber extract. The reagents reacted with the nitrogen group in the alkaloids to form insoluble complexes or precipitates.

The test for flavonoids using magnesium powder and concentrated HCl produces a red color change. This indicates the presence of flavonoids in the extract. Reduction of the carbonyl group by Mg and HCl produces a red flavilium compound.

In the saponin test, stable foam formed after mixing with distilled water and 1N HCl. This is a positive indication of the presence of saponin in the cucumber extract. Saponin has hydrophilic and hydrophobic groups → acting as a surfactant → forming foam.

The test for tannins was carried out with $FeCl_3$ reagent and produced a dark green color, indicating the presence of tannins in the extract. Tannins (polyphenols) react with Fe^{3+} ions to form colored complexes (Maulida, 2020) .

Meanwhile, the Liebermann-Burchard reagent produces a brown ring on the extract, which is a positive indicator of the presence of steroids or triterpenoids. The reaction between steroids and concentrated sulfuric acid and acetic acid produces a characteristic color due to the formation of stable carbocation ions (Maulida, 2020) .

F.

Initial cholesterol level testing (T_0) was performed after observing the test animals for 5 days. After that, PTU (*propylthiouracil*) and quail egg yolk ( ) were administered orally using a feeding tube for 7 days, and cholesterol levels were retested (T_1) on the 7th day. On day 8, Na CMC was

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administered orally as a negative control, simvastatin as a positive control, cucumber extract at a dose of 175 mg to group 1, cucumber extract at a dose of 350 mg to group 2, and cucumber extract at a dose of 700 mg to group 3 for 7 days with the dosage adjusted according to the body weight of the mice. During the 7 days, the test preparations were given to each group, and cholesterol levels were checked again after administration of the test preparations on day 14 (T_2). Observations continued until day 21, when cholesterol levels were measured again (T_3). As a final step, cholesterol levels were measured again on day 28 (T_4) to determine the optimal dose of cucumber ethanol extract that had a significant effect on cholesterol levels.

Table 9 Cholesterol reduction

Blood in Mice (mg/dl)									
No	Group	replication	T_0	T_1	T_2	T_3	T_4	% decrease	Average %
1	Negative control	1	100	179	176	165	161	10.50%	10.76
		2	100	189	182	176	165	12.70%	
		3	100	165	162	159	150	9.09	
		4	100	177	173	166	158	10.73	
2	Positive control	1	100	283	231	182	134	52.65	54.05
		2	100	234	189	137	100	57.26	
		3	100	209	161	113	100	52.15	
		4	100	242	193	144	111	54.13	
3	Extract 175mg	1	100	159	121	116	105	33.96%	32.83
		2	100	161	132	124	107	33.54	
		3	100	165	150	126	114	30.91	
		4	100	161	134	122	108	32.91	
4	Extract 350mg	1	100	176	150	122	103	41.48%	39.55
		2	100	163	134	107	102	37.42	
		3	100	165	137	113	100	39.39	
		4	100	168	140	114	101	39.90	
5	Extra 700mg	1	100	231	189	150	113	51.08	49.51
		2	100	209	176	137	100	52.15	
		3	100	182	150	120	100	45.05	
		4	100	207	171	135	104	49.75	

Explanation:

T0 = Initial blood cholesterol level test
T1 = Blood cholesterol level test after induction
T2 = Blood cholesterol level test after treatment
T3 = Blood cholesterol level test after treatment
T4 = Blood cholesterol level test after treatment

Cholesterol is a complex fatty compound, 80% of which is produced within the body (liver) and the remaining 20% from outside the body (food) for various functions within the body, including forming cell walls. Cholesterol in the food we eat can increase blood cholesterol levels. However, as long as this intake is balanced with our needs, our bodies will remain healthy. Cholesterol is not soluble in blood fluid, so in order to be transported throughout the body, it needs to be packaged with protein into particles called lipoproteins, which can be considered as 'carriers' of cholesterol in the blood (Utama, 2021)

This study was conducted to determine the effectiveness of cucumber extract (Cucumis sativus L) on mice and to find out which dose of cucumber extract is more significant in lowering cholesterol in test animals induced with propylthiouracil and quail egg yolk. In this study, five groups were used, namely negative control (1% CMC Na), positive control (Simvastatin), 175 mg extract dose, 350 mg extract dose, and 700 mg extract dose.

A total of 25 Wistar mice were divided into 5 groups. Initial blood cholesterol levels were measured by cutting the tips of the mice's tails with a surgical knife, then inducing an increase in cholesterol levels with propylthiouracil and quail egg yolk in the mice for 7 days. The initial cholesterol measurement results were Lo, meaning that the measurement results were lower than the measurable limit. The Easy Touch cholesterol meter cannot read cholesterol levels below 100 mg/dl. Therefore, we used an average of 100 mg/dl for the initial cholesterol level in mice.

To increase blood cholesterol levels in test animals, a combination of high-fat feed as an external trigger and PTU (propylthiouracil) as an internal factor was used to cause an increase in cholesterol in the test animals. PTU works to increase total cholesterol by inhibiting thyroid hormones. Inhibited thyroid hormones reduce LDL receptors, resulting in an increase in blood lipoprotein levels, especially those containing cholesterol. Feeding a high-fat diet also triggers an increase in blood cholesterol. Consumption of a high-fat diet is the main cause of increased total cholesterol levels in the blood.

On day 8, blood was collected from the mice to observe the increase in cholesterol experienced by the mice. After blood collection following induction, each group was treated. The first group was given a 1% CMC Na suspension as a negative control, the second group was given simvastatin as a positive control, the third group was given 175 mg of extract, the fourth group was given 350 mg of extract, and the fifth group was given 700 mg of extract. This study used simvastatin as a positive control in order to compare the effects of the cucumber extract on cholesterol reduction. Cholesterol reduction was measured four times on days 7, 14, 21, and 28 to compare the effects of all treatments.

The cucumber extract was administered orally using an oral syringe or a modified feeding tube. The extract was administered orally through the mouth of the mice using a feeding tube slowly. Blood was collected from the mice by cutting the tip of the tail with a scalpel and then collecting the blood using a cholesterol strip that had been attached. Blood collection through the tail is easier to perform and requires only a small amount of equipment.

This study aims to evaluate the effectiveness of cucumber extract in lowering blood cholesterol levels in mice. Based on the data obtained to date, there appears to be a difference in blood cholesterol levels in each treatment group from time T_0 to T_4.

From the results of the study conducted, the average cholesterol reduction using the strip method was 10.76% for the 1% CMC Na group, 54.05% for the simvastatin group, 32.83% for the 175 mg extract group, the 350 mg extract group obtained a reduction of 39.55%, and the 700 mg extract group obtained a reduction of 49.51%. The method of calculating the decrease in blood cholesterol levels in mice was observed during four treatments after administration, which was calculated using the percentage decrease based on the difference in cholesterol levels after treatment (T_4) compared to cholesterol levels after egg yolk induction (T_1). The formula used was: percentage decrease= $\frac{(T1-T4)}{T1}$ = 100%

The results showed that the negative control group experienced a very small decrease in cholesterol, with an average of 10.76%, indicating that there was no special treatment other than induction and CMC Na as a solvent. Meanwhile, the positive control group given simvastatin showed a significant decrease in cholesterol, with an average of 54.05%, indicating the drug's effectiveness in lowering blood cholesterol. The group given cucumber (Cucumis sativus L.)

ethanol extract showed an increase in cholesterol level reduction with increasing doses. At a dose of 175 mg/kgBW, the average cholesterol reduction was 32.83%. A dose of 350 mg/kgBW showed a higher reduction of 39.55%. Meanwhile, the highest dose, 700 mg/kgBW, resulted in a cholesterol reduction close to that of simvastatin, namely 49.51%.

The negative control group, which was only given 1% CMC Na without additional treatment, showed a very low decrease in cholesterol, with an average decrease of 10.76%. This may be due to the absence of active compounds capable of lowering cholesterol levels in the body, thus reflecting the natural physiological response of mice to the cessation of egg yolk induction.

The positive control group, which was given simvastatin, a standard antihypercholesterolemic drug, experienced a very significant decrease in cholesterol, with an average of 54.05%. This demonstrates the effectiveness of simvastatin as an HMG-CoA reductase inhibitor in reducing endogenous cholesterol synthesis.

In the group given cucumber (Cucumis sativus L.) ethanol extract, there was a dose-response relationship to cholesterol reduction. Administration of the extract at a dose of 175 mg/kgBW resulted in a 32.83% reduction in cholesterol, while a dose of 350 mg/kgBW had a higher effect with an average of 39.55%. The highest dose, 700 mg/kgBW, resulted in a cholesterol reduction close to that of the positive control group, with an average of 49.51%. The results of the cholesterol reduction calculations can be seen in Appendix 12.

Thus, cucumber ethanol extract shows potential as a natural cholesterol-lowering agent, especially at a dose of 700 mg/kgBW, which has an effect almost comparable to simvastatin.

Based on previous studies, ethanol extract from cucumber fruit has been proven effective in lowering cholesterol levels at a dose of 2 mg per 20 grams, which is equivalent to 100 ppm of . This is reinforced by research Kusrina et al., (2024) that cucumber (Cucumis sativus L.) is a very high source of flavonoid compounds with a percentage of up to 71%, which is thought to play a role in lowering cholesterol levels. Flavonoids have antioxidant activity by releasing or donating hydrogen ions to free radicals, thereby stabilizing them. This activity inhibits the oxidation of bad cholesterol (LDL), which can cause blood thickening, thereby helping to prevent fat deposits on the walls of blood vessels (Kusrina et al., 2024) .

The research data was analyzed using the one-way ANOVA test method with IBM SPSS software to determine whether there were significant differences between the control group and the treatment group at each observation time point from T0 to T4. The first analysis was to determine normality using the Shapiro-Wilk method, which differs from the Kolmogorov-Smirnov normality test. Kolmogorov-Smirnov is used to test normality in large samples (>50), while Shapiro-Wilk is used for small samples (<50). The normality test in this study was conducted using the Shapiro-Wilk test because the sample size in the treatment was small (Sahrul Ramadhan, 2024) .

The normality test was conducted to determine whether the data in each treatment group was normally distributed. This test is important as a prerequisite for conducting parametric tests, such as ANOVA, which assumes that the data has a normal distribution. In this study , the normality test was performed using the Shapiro-Wilk method on five treatment groups, namely negative control, positive control, 175 mg cucumber extract, 350 mg, and 700 mg. Because the number of subjects in each group was four (n = 4), the interpretation focused more on the results of the Shapiro-Wilk test, as this method is more accurate for small samples (n < 50).

The test results showed that all Shapiro-Wilk significance values (p-values) for each group were as follows: Negative control = 0.767 Positive control = 0.365 175 mg extract = 0.394 350 mg extract = 0.921 700 mg extract = 0.367. This can be seen in Appendix 13.

A homogeneity of variance test was conducted to determine whether the variance between treatment groups was homogeneous or not. This test is an important prerequisite before conducting an ANOVA test, because ANOVA assumes that the variance of each data group must be the same or not significantly different (homogeneous).

In this study, the homogeneity test was performed using Levene's Test, with several approaches, namely based on the mean, median, median with adjusted df, and trimmed mean. The test results showed the following significance values (p-values): Based on Mean: 0.555 Median: 0.740 Median (adjusted df): 0.741 Trimmed Mean: 0.601

All significance values were greater than the significance limit of 0.05 (α = 5%), indicating that there were no significant differences in variance between treatment groups. Thus, it can be concluded that the data met the assumption of variance homogeneity. These results support the validity of using the ANOVA test in this study, because the data is not only normally distributed (based on the normality test), but also has a homogeneous variance. This can be seen in Appendix 13.

All significance values are greater than the specified limit (α = 0.05), which means that the data is normally distributed. Thus, it can be concluded that the data from all treatment groups is normally distributed. These results indicate that the data meets one of the prerequisites for variance analysis (ANOVA), namely the assumption of homogeneity, so that the ANOVA analysis can be continued to determine whether there are differences between treatment groups.

After the data was declared to meet the assumptions of normality and homogeneity of variance, the analysis was continued with a one-way ANOVA test to determine whether there were significant differences between the treatment groups for the observed variables. Based on the ANOVA test results, a calculated F value of 264.945 with a significance value (Sig.) < 0.001 was obtained. A significance value that is much smaller than the α = 0.05 limit indicates that there are very significant differences between treatment groups in terms of the observed variables.

This means that at least one treatment group had a significantly different average result compared to the other groups. Therefore, this test shows that the treatments (negative control, positive control, and various doses of extract) had different effects on the parameters measured in the study.

However, because ANOVA only shows overall differences, a post hoc test, such as the Least Significant Difference (LSD) test, is needed to determine specifically which groups differ from one another. Next, data analysis was performed using LSD (Least Significant Difference) to determine in more detail which groups differed significantly. The Least Significant Difference (LSD) test was conducted to determine the significant differences between treatment groups in pairs after previously showing significant results in the ANOVA test. Based on the LSD test results in the Multiple Comparisons Table, it can be seen that almost all pairs between treatments show statistically significant differences ($p < 0.05$) in the Mean Difference column.At time T_2, after the treatment began, there were significant differences between the negative control group (1) and the 175 mg cucumber extract group (3) ($p = 0.039$), as well as between the negative control and the 700 mg extract group (5) ($p = 0.045$). This indicates that cucumber extract, particularly at low and high doses, began to show effects on the test parameters at that time. Meanwhile, the other treatment groups did not show significant differences from each other, indicating that the effects of the treatment were not yet fully maximized on observation day T_2.

The negative control group showed very significant differences with all other groups, including the positive control and cucumber extract treatment groups (175 mg, 350 mg, and 700 mg), with p-values < 0.001. This indicates that the administration of treatments, both simvastatin and cucumber extract at various doses, had a significant effect compared to the negative control (CMC Na).

Furthermore, the positive control also showed a significant difference compared to the 175 mg and 350 mg treatment groups, with a p-value < 0.001. However, the difference between the positive control and the 700 mg treatment was also significant, although the mean difference was smaller (mean difference = 4.53250). This indicates that the 700 mg dose of cucumber extract is approaching the effectiveness of simvastatin in lowering cholesterol levels, but is not yet fully equivalent.

The three cucumber extract treatment groups (175 mg, 350 mg, and 700 mg) also showed significant differences from each other, illustrating a dose response. The higher the dose of cucumber extract administered, the greater the reduction effect produced, as seen from the significant mean differences between the three doses (for example, between the 175 mg and 700 mg doses there was a difference of -26.61500 with $p < 0.001$).

Overall, the results of this LSD test reinforce the finding that cucumber extract has a significant cholesterol-lowering effect, and that this effect increases in proportion to the dose administered. However, although the high dose of cucumber extract (700 mg) has an effectiveness

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close to that of simvastatin, there is still a statistically significant difference, indicating that simvastatin is still superior in lowering cholesterol levels in mice in this study. The LSD results can be seen in Appendix 13.

CONCLUSION

Based on the main findings of the study, cucumber (*Cucumis sativus*) ethanol extract shows significant potential as a cholesterol-lowering agent, with effectiveness increasing with increasing dose. The results showed that the positive control group given simvastatin experienced an average cholesterol reduction of 54.05%, while the highest extract dose (700 mg/kgBW) achieved an average reduction of 49.51%. The effectiveness, which is close to that of the standard drug, is supported by the content of phytochemical compounds such as flavonoids, saponins, tannins, and steroids identified in the extract. Statistically, the One-Way ANOVA test showed a very significant difference between treatment groups (p < 0.05), proving that cucumber extract has a real effect in lowering cholesterol levels in mice induced with hypercholesterolemia.

However, this study has several limitations that need to be considered. The main limitation is the small sample size (n=4) per group, even though it meets the Shapiro-Wilk statistical test for data normality. In addition, this study did not measure other parameters related to the lipid profile, such as specific LDL and HDL levels, which could provide a more comprehensive picture of the mechanism of cholesterol reduction. Therefore, for further research, it is recommended to increase the sample size so that the results obtained are more representative. In addition, further analysis of the lipid profile and histopathological examination of the liver are needed to confirm the hepatoprotective role of cucumber extract. Further research could also isolate and identify specific active compounds that play the most significant role in antihypercholesterolemic effects for the development of more standardized phytopharmaceutical products in the future.

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