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Activity Test of 96% Ethanol Extract of Water Guava Leaf (Syzygium aqueum) on LDL and HDL Values with Propylthiouracil Induction in Rats

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ABSTRACT

One of the plants that can be thought to have antihyperlipidemic potential is water guava leaves (Syzygium aqueum) because they contain chemical compounds, one of which is flavonoids. The aim of this research is to determine the activity of 96% ethanol extract of water guava leaves (EEDJA) as an antihyperlipidemic agent. Male Wistar rats were induced using propylthiouracil (PTU) for 2 weeks (14 days). Hyperlipidemic mice were divided into 5 test groups, CMC-Na group, simvastatin group, EEDJA group (100, 200 and 400) mg/BW. The solution was given to mice routinely once a day for 2 weeks (14 days) orally. The results of the study showed that there were differences before and after administration of EEDJA (100, 200 and 400) mg/BW in LDL and HDL levels with a significance value of 0.000 (<0.005). The conclusion from this study is that EEDJA has potential as an antihyperlipidemic agent in male white Wistar rats.

Keywords: HDL, hyperlipidemia, LDL, Simvastatin, Syzygium aqueum

1. INTRODUCTION

The use of synthetic drugs is thought to cause some unexpected side effects. For example, undesirable effects on kidney conditions and digestive disorders (Perkeni, 2021). The potential appearance of side effects in synthetic drugs increases the development of drugs from plants as antihyperlipidemia.

One plant that is thought to have potential as an antihyperlipidemia herbal medicine is water guava leaf (Syzygium aqueum). There are several chemical contents in water guava leaves, one of which is flavonoid compounds (Anggrawati and Ramadhania, 2016). Research by Noviani, et al. in 2021 showed that the flavonoid content in water guava leaves can be used as anticholesterolemia, which showed that water guava leaf extract (Syzygium aqueum) showed a decrease in total cholesterol levels by 58.74% in vitro (Noviani *et al*., 2021).

The mechanism of flavonoids in reducing cholesterol levels is by inhibiting the HMG Co-A reductase enzyme. The HMG Co-A reductase enzyme will convert to HMG Co-A mevalonate. Inhibition of HMG Co-A reductase will reduce the formation of mevalonate so that lipid levels will be normal. The mechanism of action of flavonoids is almost the same as the statin group, for example simvastatin (Hasimun et al., 2011).

Flavonoids as antihyperlipidemia can reduce cholesterol biosynthesis by reducing the activity of 3-hydroxy-3-methyl-glutaryl

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coenzyme hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG CoA reductase), a key enzyme in cholesterol biosynthesis. Flavonoids also work by decreasing the need for NADPH, where NADPH is used to synthesize cholesterol and fatty acids. Lipid levels can be improved by flavonoids by modifying lipoprotein metabolism through increasing LDL receptor and lecethin choleterol acyl transferase (LCAT). The role of LCAT is to convert free cholesterol into HDL (Eddouks *et al*., 2005).

Based on the above background, researchers want to test the activity of EEDJA on HDL and LDL levels in rats.

2. METHODS

2. 1 Research Variables

There are 3 variables, namely independent, dependent and controlled variables.

Independent Variable: Dose of water guava leaf extract (Syzygium aqueum)

Dependent Variable: HDL value and LDL level

Controlled Variables: Age, body weight, sex, test animals, type and amount of feed, rearing site and treatment time.

2.2 Preparation of Test Preparations

2.2.1 Preparation of simplisia powder

Water guava leaves are washed and then heated using an oven at 40°-60°C. The purpose of the heating process is to reduce water content. The dried water guava leaves were cut and then pulverized using a blender and obtained water guava leaf powder. The powder was sieved using sieve no 40. After that, the sieved powder was checked for water content using a moisture balance. Silica in the moisture balance tool is heated with an oven, then installed on the tool and the water guava leaf simplisia powder is sprinkled on the silica. Measurements are made by pressing the button on the moisture balance tool and waiting until the moisture content measurement is complete. Water guava leaf simplisia has good quality if the moisture content contained is < 10% (Depkes, 2008).

2.2.2 Preparation of EEDJA

A total of 500 water guava leaf simplisia powders were macerated using 5 liters of 96% ethanol solvent concentration in a ratio of 1:10. The extraction process took 3 days with 2-3 times stirring. The macerate that has been obtained is then filtered and produces a filtrate that will be evaporated. The dregs obtained are then remacerated with ethanol solvent with a concentration of 96% for 2 days, then filtered again and then the filtrate is evaporated at a temperature of 40-60 ° C to obtain a thick extract (Telaumbanua and Kristiani, 2022). The thick extract of water guava leaves was collected and weighed to calculate the yield.

2.2.3 Preparation of PTU solution

PTU solution was made in the form of a solution, PTU used was 100 mg dissolved into 8 ml of solvent. The dose of PTU used is 1 ml there is a PTU content of 12.50 mg PTU. Administration of PTU in rats amounting to 12.50 mg / day. PTU is given within 14 days every 12 hours. The dose of PTU was injected at 1 cc / day divided into 2 times of administration (Ratulangi *et al*., 2016).

2.2.4 Preparation of CMC-Na solution

The solvent used as well as the negative control in this study was CMC-Na. CMC-Na control solution was made by weighing 0.5 grams of CMC-Na and then put in a mortar containing hot distilled water. The mixture was allowed to stand for 10-15 minutes and then mixed until a transparent mass was obtained, and stirred until homogeneous, then added distilled water, homogenized and put into a measuring flask, added the volume of distilled water with distilled water until it reached 100 ml (Anief, 1999).

2.2.5 Preparation of simvastatin suspension

Simvastatin suspension was prepared by converting the human dose to rat. Simvastatin was put into lumping then Na-CMC solution was added to taste and let the solution expand then stirred until homogeneous. Simvastatin suspension was made as much as 50 (Rusdi *et al*., 2018). Simvastatin stock solution was made as much as 50 mL, using dose conversion, which was changed from humans to rats, where humans (70 kg) to rats (200 g) = 0.018. The

dose of simvastatin used for humans was 10 mg. The dose of simvastatin for rats is 0.018 x 10 mg = 0.18 mg/200 g BW.

2.2.6 Preparation of EEDJA suspension

EEDJA suspension was made by mixing 0.5% CMC Na solution of the test preparation. The doses of water guava leaf extract used are 100, 200 and 400 mg/BB (Mulyana, 2016).

If it is assumed that the body weight of the rat is 200 grams, then for the preparation of EEDJA suspension for a dose of 100mg/BB is 200mg of extract added with 25 mL of solvent, for a dose of 200mg/BB is 400mg of extract added with 25 mL of solvent. For a dose of 400mg/BB is 800mg of extract added with 25 mL of solvent.

2.3 Skrining Fitokimia

To perform phytochemical screening, 2 ml of ethanol extract was taken and 0.5 ml of concentrated hydrochloric acid was added. The result of phytochemical screening is to determine the content of flavonoid compounds in EEDJA. The presence of flavonoid compounds in EEDJA is indicated by the presence of several color indicators, namely red, orange or green, the color that appears depends on the flavonoid structure contained in the sample used (Endarini, 2016).

2.4 Antihyperlipidemia Activity Test of Water Apple Leaf Ethanol Extract (WALEE)

The rats were adapted for 7 days so that the rats were accustomed to the surrounding environment and so that the rats were not stressed during the study. A total of 30 rats were treated with 12.5 mg/BB PTU induction for once a day by oral route, the duration of therapy was for 14 days, then checked for an increase in HDL levels and a decrease in LDL levels using an analyzer used is a Genesys 20 spectrophotometer. Hyperlipid rats were divided into 5 test groups, where each group consisted of 6 rats. Negative control rats were treated with CMC-Na 12.5 ml/BB, positive control was given simvastatin 0.18 mg, EEDJA treated rats were treated with ethanol extract of water guava leaves with concentrations (100, 200 and 400 mg/BB) (Hartono et al., 2020). The administration of the test preparation of each group was given once a day with a duration of 14 days.

Blood sample data on LDL and HDL values were taken before and after treatment in each treatment group. The antihyperlipidemia effect is characterized by a decrease in LDL levels and a significant increase in HDL levels in hyperlipid rats induced with PTU and after being given the test preparation solution routinely within 14 days.

2.5 Data Analysis

The research data obtained data on HDL and LDL values before and after the treatment of the test preparation within 14 days.

LDL and HDL values before and after the preparation were tested using statistical analysis with a 95% confidence level. To see the data entered into the parametric data, normality was first tested using saphiro-wilk, while for the homogeneity test using homogeneity of variance. If the significance is $p>0.05$, it can be concluded that the data used is normally distributed and homogeneous, which is included in the parametric data. If the results show normal and homogeneous distributed data (parametric data), then a different test is carried out using a paired t-test. If the data is included in non-parametric using the Wilcoxon test.

3. RESULT AND DISCUSSION

Water guava leaves after drying were found to be 1,010 g and resulted in a drying shrinkage of 80.44%. Water guava leaf powder has a moisture content of 5.8%. The water content that meets the requirements for good simplisia water content is < 10% (Depkes, 2008). Checking the water content aims to make the simplisia obtained have good quality. The high-water content in simplisia can and can trigger enzymatic reactions, besides that it also causes good growth for molds and bacteria or microorganisms. Therefore, low water content in simplisia can stop enzymatic reactions so as to obtain simplisia that is durable in storage (Prasetyo and Inoriah, 2013).

The results of PTU induction can increase LDL and HDL levels on day 8 to day 22. These results are in line with the research of Sari, et al in 2021 which states that routine PTU induction

within 14 days can increase the HDL and LDL values of rats. The average increase in these values can be seen in figures 1 and 2.

Figure 1 shows the results of the difference in mean values before and after PTU induction on LDL. Figure 2 shows the comparison of the mean values before and after PTU induction on HDL. HDL levels before and after PTU induction within 14 days.

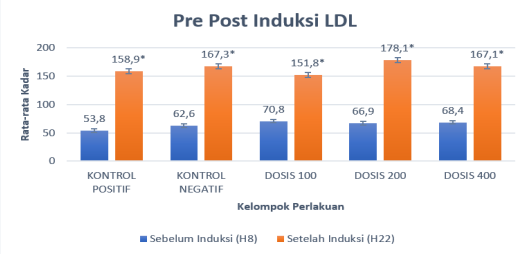


Figure 1. Comparison chart of the average LDL value of PTU induction pre-post

Information:

Control + : Simvastatin
Control - : CMC-Na
Dose 1 : EEDJA 100mg/BB
Dose 2 : EEDJA 200 mg/BB
Dose 3 : EEDJA 400 mg/BB

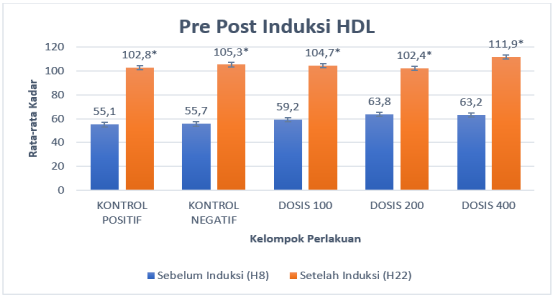


Figure 2. Comparison Chart of the average value of HDL pre-post induction PTU

Information:

Control + : Simvastatin
Control - : CMC-Na
Dose 1 : EEDJA 100mg/BB
Dose 2 : EEDJA 200 mg/BB
Dose 3 : EEDJA 400 mg/BB

Based on figures 1 and 2, day 8 shows that LDL and HDL levels have almost the same levels, in all test animals that have not been given PTU induction. On day 22, the average LDL and HDL levels increased in all test animals given PTU induction given orally. PTU induction works by increasing thyroid levels in rats and will affect lipoprotein metabolism which will increase LDL cholesterol levels, this is due to metabolic pressure on LDL receptors, so as to increase cholesterol in the blood (Nofianti et al., 2015). This is in accordance with the results of this study, where the administration of PTU induction within 14 days routinely can increase the value of LDL and HDL significantly ($p<0.05$).

The results of data comparison of the average levels of LDL and HDL, Simvastatin, CMC-Na, and three groups of EEDJA dose (100, 200, and 400) mg/kg/BB can be seen in Figure 3 and 4.

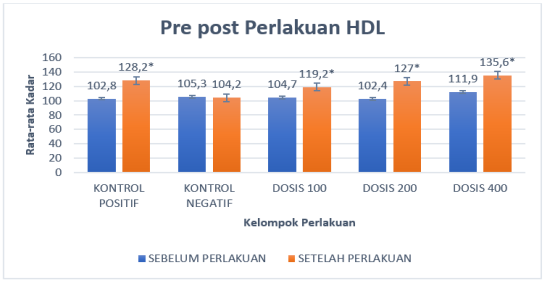
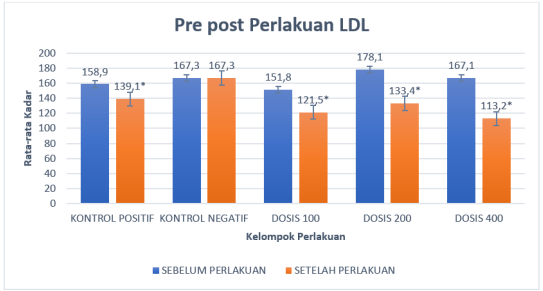


Figure 3. Average comparison chart of pre-post test solution

Information:

Control + : Simvastatin
Control - : CMC-Na
Dose 1 : EEDJA 100mg/BB
Dose 2 : EEDJA 200 mg/BB
Dose 3 : EEDJA 400 mg/BB

Figure 3 shows that the administration of the simvastatin test preparation and the three dose ratings of EEDJA (100, 200, and 400) mg/BB. Figure 4. showed that there was a significant difference in LDL levels before treatment



($p<0.05$).

Figure 4. Comparison Chart of average pre-post levels of test solution

Information:

Control + : Simvastatin
Control - : CMC-Na
Dose 1 : EEDJA 100mg/BB
Dose 2 : EEDJA 200 mg/BB
Dose 3 : EEDJA 400 mg/BB

Statistical analysis of normality test using the saphiro wilk test. The p value was obtained p>0.05, the results of the normality test are shown in Table 1.

Table 1. Before and After Administering Test Preparations on LDL and HDL Values using the normality test.

Group	Treatment	*p value* HDL	*p value* LDL
Simvastatin	Before	0.699	0.532
	After	0.930	0.419
CMC - Na	Before	0.365	0.145
	After	0.767	0.121
EEDJA 100mg/BB	Before	0.952	0.497
	After	0.532	0.160
EEDJA 200 mg/BB	Before	0.616	0.749
	After	0.995	0.366
EEDJA 400 mg/BB	Before	0.907	0.201
	After	0.561	0.555

Statistical analysis of the homogeneity test showed that the data used in this study was homogeneous with a p value of >0.05, the results of the normality test were shown in Table 2.

Table 2. Before and After Administering Test Preparations on LDL and HDL Values using Homogeneity Test.

Groupe	*p value*
LDL	0.328
HDL	0.115

The normality and homogeneity data show a p value of >0.05, so it can be concluded that the data used is included in the parametric data, which is then used to test the hypothesis using the paired t-test. The results of the paired t test can be seen in table 3.

Table 3. Before and after administration of the test preparation on LDL and HDL values using paired t test

Group	Treatment	*p value* HDL	*p value* LDL
Simvastatin	Before and After	0.000	0.000
CMC - Na	Before and After	0.101	0.067
EEDJA 100mg/BB	Before and After	0.002	0.000
EEDJA 200 mg/BB	Before and After	0.000	0.000
EEDJA 400 mg/BB	Before and After	0.000	0.000

The results of this study showed that the administration of simvastatin test preparation and three EEDJA dose ranks (100, 200, and 400) mg/BB was able to increase LDL levels and decrease HDL levels significantly (p<0.05). Simvastatin and three dose ranks of EEDJA in this study proved to have antihyperlipidemia effects on PTU-induced hyperlipid rats. Meanwhile, the negative control group (CMC-Na) did not affect the decrease in HDL and LDL levels with a p value> 0.05, meaning that there was no significant difference in levels.

HDL plays an important role in balancing cholesterol values by taking cholesterol in the tissue which will be taken to the liver, then excreted into bile salts (Murray et al., 2009). The decrease in LDL values is due to the presence of flavonoid compounds in water guava leaf extract. The mechanism of flavonoids in reducing LDL levels is by inhibiting the HMG-CoA reductase enzyme which causes a decrease in cholesterol synthesis. In cholesterol synthesis, cholesterol will be transported from the intestine to the liver, at that time HMG-CoA reductase which functions to convert acetyl-coA into mevalonate will be inhibited by flavonoids so that cholesterol synthesis by the liver will be reduced (Artha, et al, 2017). Research by Harini (2009) showed that flavonoids that have high antioxidant content can reduce LDL oxidation, and cause LDL levels in the blood to decrease (Harini and Astirin, 2009).

CONCLUSION

A significance value of $p<0.005$ was obtained before and after the administration of simvastatin test preparation and three dose ranks of EEDJA (100, 200, and 400) mg/BB. The conclusion of this study is that EEDJA has the potential as antihyperlipidemia in wistar male white rats.

ADVISE

The limitations of this study are the lack of more in-depth phytochemical test screening and the lack of dose variation. Further research is needed regarding checking phytochemical test screening to see other content besides flavonoids in water guava leaves and checking total flavonoid levels. And toxicity test of ethanol extract of water guava leaves is needed. EEDJA toxicity test is needed.

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