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### The Effect of Ethanol Extract of Kersen Leaf (*Muntingia Calabura L.*) on Reducing Triglyceride Levels in Hypercholesterolemic Rats

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#### ABSTRACT

**Background:** Kersen leaf has the potential to lower triglycerides because they contain flavonoids and tannins. **Methods:** This study was an experimental laboratory study with a pre and post-test with a control group design. The subjects were 30 white male rats (*Rattus norvegicus*), bodyweight  $\pm 200$  grams, aged 3-4 months, and divided into 5 groups with random sampling. The groups were negative control (K-) was given a high-fat diet, and PTU without any treatment, positive control (K+) was given a high-fat diet, and PTU with gemfibrozil treatment, (P1),(P2),(P3) was given a high-fat diet and PTU with ethanol extract of Kersen leaf 100, 200, and 400 mg/kgBB. This study was held for 4 weeks. Triglyceride level was measured using the GPO-PAP method. The data was analyzed using Shapiro-Wilk, Levene test, and ANOVA test. **Results:** The ethanol extract of Kersen leaf dose 100, 200 and 400 mg/kgWB are found effective on reducing blood triglyceride levels with a mean reduction in the first treatment of 112,83 mg/dl, 98,20 mg/dl, 89,92 mg/dl and in the second treatment of 106,71 mg/dl, 93,95 mg/dl dan 76,87 mg/dl. Statistical analysis using one-way ANOVA showed  $p < 0,05$ , which determines a significant difference in triglyceride levels among each group. **Conclusion:** Extract dose of 400 mg/kgWB is found to be the most effective dose in triglyceride reduction.

#### 1. Introduction

Hypercholesterolemia is a degenerative disease in which cholesterol metabolism in the blood is disrupted, causing blood cholesterol levels to rise above the normal limit of 200 mg/dL. Hypercholesterolemia has no symptoms and can only be detected through a blood cholesterol test. The risk of cardiovascular disease and obesity increases as cholesterol rises.<sup>1</sup> Hypercholesterolemia is a common health problem in the community whose prevalence is rising, and its complications are extremely dangerous.

Cholesterol levels in the blood that are too high and excessive can harm the heart and blood vessels, as well as cause other diseases associated with blood

vessel blockages. According to data from the Basic Health Research (Riskesdas), in 2018, 15 out of 1000 Indonesians suffered from cardiovascular disease, with a total population of 4.2 million. North Kalimantan (2.2%), Yogyakarta (2%), Gorontalo (2%), Aceh (1.6%), West Sumatra (1.6%), DKI Jakarta (1.9%), West Java (1.6%), Central Java (1.6%), East Kalimantan (1.9 %), North Sulawesi (1.8%), and Central Sulawesi (1.8%) had the highest prevalence (1.9%).<sup>2,3</sup>

Excess carbohydrate consumption can cause heart or cardiovascular disease because high carbohydrate consumption raises triglyceride levels and lowers cholesterol levels.<sup>3</sup> In the study, triglyceride levels

ranged from 139.97 to 142.24 mg/dl. Another study stated that the carbohydrate content of the feed has a significant impact on blood triglyceride levels. The liver produces triglycerides by converting carbohydrates into free fatty acids. Because they are inhibitors of the enzyme Hydroxy-methylglutaryl-CoA reductase, statins are used in the management and treatment of hypercholesterolemia. Simvastatin is a cholesterol-lowering drug that is used to treat hypercholesterolemia. The starting dose of the drug is 5-19 mg per day, with a maximum dose of 40 mg per day. Simvastatin has a side effect called myopathy. Myopathy is a rare disease that affects only 1% of people. Simvastatin should, however, be considered in patients who are at high risk for muscle disorders.<sup>4-7</sup>

Because Kersen leaves (*Muntingia calabura* L.) contain flavonoids and tannins, they are good for your health. Plant secondary metabolites, which have a polyphenolic structure and are found in certain fruits, vegetables, and beverages, contain flavonoids. Flavonoids have a wide range of health-promoting properties and are used in a variety of nutraceutical, pharmaceutical, and medicinal products. Because flavonoids have antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties, as well as the ability to modulate key cellular enzyme functions, this is the case. Tannins are a class of polyphenolic compounds with large molecular weights that contain hydroxy groups as well as other groups like carboxyl groups to form strong complexes with proteins and other macromolecules, allowing them to act as antioxidants.<sup>8-10</sup>

Alternative therapies for lowering triglycerides using herbal plants have been discovered in several studies, one of which is a Kersen leaf (*Muntingia calabura* L.). Kersen leaves contain secondary metabolites such as flavonoids, polyphenols, alkaloids, saponins, tannins, steroid-triterpenoids, monoterpenes, and sesquiterpenes, which have antioxidant properties and can counteract free radical compounds.<sup>9</sup> Based on this description, This study aimed to determine the effects of an ethanol extract of

the Kersen leaf (*Muntingia calabura* L.) on triglyceride levels in hypercholesterolemic male Wistar rats.

## 2. Methods

The test animals used in this study were male Wistar rats aged 3-4 months and weighing less than 200 grams, as well as the reagent for measuring triglyceride levels, namely the Triglyceride Reagent Kit with the Colorimetric method. The filter used 96 percent ethanol, gemfibrozil, PTU, and high-fat feed (raw chicken egg yolk) to induce hypercholesterolemia in test animals, as well as standard feed and drink.

### Preparation of extract

The Kersen leaves are aerated or dried in indirect sunlight for 14 days. Kersen leaves are pounded and then sifted after they have been dried. Add 96 percent ethanol solvent to 150 grams of Kersen leaves and 600 ml of solvent to make a Kersen and ethanol mixture (dry Kersen leaves: ethanol as much as 1:4). The maximum yield of a Kersen-ethanol mixture was precipitated over seven days, with one stirring per day. After that, the mixture is filtered. The extract obtained with the ethanol solvent is then separated using the evaporation process. After that, the extract was turned into a stock solution.

### Animal model

Male Wistar rats weighing 200 grams and aged 3-4 months were used in this study. Wistar rats were acclimatized for three days before being fed a standard diet of pellets and water on a daily basis. The goal of acclimatization is to help Wistar rats adjust to their new surroundings. Wistar rats were housed in wire-covered cages and fed husks. Every three days, Wistar rat cages are cleaned. PTU (Propylthiourasil) 12.5 mg/kg BW/day in one dose was used to induce male Wistar rats. PTU induction should be combined with a daily dose of 1 ml raw chicken egg yolk. The ethanol extract of a Kersen leaf (*Muntingia calabura* L.) was weighed to obtain a stock solution concentration of 5 mg/ml, which was then dissolved in 100 ml of distilled water. This study used doses of 100 mg/kg BW, 200

mg/kg BW, and 400 mg/kg BW. The concentration of simvastatin stock solution was 0.2 mg/ml after dissolving two 300 mg gemfibrozil tablets in 100 ml distilled water. In this study, the dose was 1 mg/kg BW/day. 35 male Wistar rats were used in this experiment. Wistar rats were divided into five groups, with the P1 group receiving a 100 mg/kg BW ethanol extract of a Kersen leaf (*Muntingia calabura* L.), the P2 group receiving a 200 mg/kg BW ethanol extract of a Kersen leaf (*Muntingia calabura* L.), the P3 group receiving a 400 mg/kg BW ethanol extract of a Kersen leaf (*Muntingia calabura* L.), and the K (+ Each Wistar rat was given 1 mg/kg BW of simvastatin suspension, while group K (-) Wistar rats were not treated.

### Examination of triglyceride levels

Triglyceride levels was measured on four occasions: day 0, day 14, day 21, and day 28. On day 0, after administration of PTU and high cholesterol feed, on the 14th day, one week after treatment with Kersen leaf ethanol extract, on the 21st day, and two weeks after treatment with ethanol extract, baseline triglyceride levels were measured before the test animals were given treatment. On the 28th day, there were Kersen leaves. The Reagent Kit with the

Colorimetric Enzymatic Test (GPO PAP) method was used to determine triglyceride levels. A total of 10µL serum reacted with triglyceride response 1000µL. The samples were then incubated for 10 minutes at 250°C or 5 minutes at 37°C before being examined with a spectrophotometer at a wavelength of 500 nm.

### Data analysis

The statistical methods used were the Shapiro-Wilk normality test, the Levene homogeneity test, and the ANOVA test to determine the efficacy of Kersen leaves ethanol extract on lipid profile levels.

### 3. Results

There are differences in the test animals' triglyceride levels in the negative control group, positive control, extract treatment group 100 mg/kg BW, extract treatment group 200 mg/kg BW, and extract treatment group 400 mg/kg BW, which were measured in the first and second weeks after treatment. Because each treatment group received different therapy, there was a difference in triglyceride levels (Table 1).

Table 1. Average triglyceride levels of male Wistar rats (mg/dL)

Group	Pre induction	Post induction	1 <sup>st</sup> week	2 <sup>nd</sup> week
K (-)	67,25 ± 1,22	121,67 ± 2,89	132,83 ± 2,88	136,89 ± 1,97
K (+)	66,19 ± 2,04	122,05 ± 1,67	93,88 ± 2,74	86,59 ± 1,79
K 100mg/kgBW	67,96 ± 1,12	120,40 ± 2,41	112,83 ± 1,76	106,17 ± 2,51
K 200mg/kgBW	66,31 ± 1,25	120,78 ± 2,87	98,20 ± 1,37	93,95 ± 2,55
K 400mg/kgBW	65,62 ± 2,42	122,30 ± 1,65	89,93 ± 1,53	76,87 ± 1,37

An independent sample t-test was used to determine the difference in the triglyceride levels of the test animals between the treatment groups after the first week of treatment, with P<0.05 indicating that the triglyceride levels of the test animals between the treatment groups after the first week of treatment were significantly different, while to determine the decrease in Triglycerides of test animals between postinduction and a week after treatment, an independent sample t-test was used. The Anova test was also used to

determine which treatment group had the lowest triglyceride levels. The research data were first tested for homogeneity and normality to see if they met the requirements for the one-way ANOVA statistical test before being analyzed. The results of the normality test using Shapiro Wilk obtained P>0.05, indicating that the data is normally distributed (symmetrically). The data were then tested for homogeneity using the Levene statistic, which yielded a P>0.05, indicating that the data came from populations with similar

variance, allowing for a one-way ANOVA statistical test. When compared to the other treatment groups, the group that received ethanol extract of a cherry leaf at 400 mg/kg BW had the lowest triglyceride levels, according to the results of the one-way Anova test.

An independent sample t-test was used to determine the difference in triglyceride levels of the test animals between the treatment groups after the second week of treatment, with results of  $P > 0.05$ , indicating that the triglyceride levels of the test animals between the treatment groups after the second week of treatment were significantly different, while to determine the decrease in triglycerides of the test animals between postinduction and two weeks of treatment, an independent sample t-test was used. The Anova test was also used to determine which treatment group had the lowest triglyceride levels. The research data were first tested for homogeneity and normality to see if they met the requirements for the one-way ANOVA statistical test before being analyzed. The results of the normality test using Shapiro Wilk obtained  $P > 0.05$ , indicating that the data is normally distributed (symmetrically). The data were then tested for homogeneity using the Levene statistic, which yielded a P-value  $> 0.05$ , indicating that the data came from populations with similar variance, allowing for a one-way ANOVA statistical test. In comparison to the other treatment groups, the group that received ethanol extract of the cherry leaf at 400 mg/kg BW had the lowest triglyceride levels, according to the results of the one-way Anova test.

A paired sample t-test was used to determine the difference in triglyceride levels in the experimental group between the first and second weeks after treatment, with  $P > 0.05$  in the negative control group, indicating that the triglyceride levels in the test animals were between the treatment groups between the first and second weeks.  $P > 0.05$  in the group that received the extract 100 mg/ kg BW, which means the triglyceride levels of the test animals between the treatment groups between the first and second weeks after treatment were significantly different, P-value 0.05 for the positive control group, which means the

triglyceride levels of the test animals between the treatment groups between the first and second weeks after treatment were significantly different,  $P > 0.05$  in the group that received the extract 100 mg/ kg BW, the difference in triglyceride levels test animals between treatment groups between the first and second weeks after treatment was not significant,  $P > 0.05$  in the group receiving 200 mg/kg BW extract and the group receiving 400 mg/kg BW extract, which means triglyceride levels test animals between treatment groups between the first and second weeks after different treatment meaning

#### **4. Discussion**

The test animals were fed a high fat (raw chicken egg yolk) and PTU to induce hypercholesterolemia in them. The fat and cholesterol content of raw chicken egg yolks influences the increase in triglyceride levels. The raw chicken egg yolk contains 60% lipoprotein, which is made up of 85% fat and 15% protein. The fat in lipoproteins is made up of 20% phospholipids (lecithin, phosphatidyl serine), 60% neutral fat (triglycerides), and 5% cholesterol. Fatty foods are primarily composed of triglycerides and cholesterol. An increase in fat stores (triglycerides) both intracellularly and extracellularly causes an increase in triglyceride levels. Giving fatty foods raises total cholesterol and LDL cholesterol and lowers HDL cholesterol. Food-derived triglycerides are emulsified by bile acids before being absorbed by the small intestine.<sup>11</sup> The administration of PTU influenced the increase in triglyceride levels in addition to the high fat and cholesterol content of raw chicken egg yolks. PTU is an antithyroid substance that damages the thyroid gland, preventing thyroid hormone production. The thyroid hormone regulates lipid metabolism, which is one of its many functions. Thyroid hormone induces the coenzyme HMG-CoA, which stimulates de novo hepatic cholesterol synthesis.<sup>12</sup> According to the findings, providing high-fat feed and PTU to test animals increased triglyceride levels. PTU and a high-fat diet were used to induce hypercholesterolemia in 30 test animals, and they all succeeded.

This study used daily doses of cherry leaf ethanol extract of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW. The results of the study after the first week of treatment showed that triglyceride levels were significantly lower before and after administration of cherry leaf ethanol extract in the groups receiving 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW. Similarly, the positive control group receiving gemfibrozil therapy saw a significant reduction in triglyceride levels. Because the negative control group did not receive any treatment, there was no reduction in triglyceride levels. After the first week of treatment, there was a significant difference in triglyceride levels between the treatment groups. This shows that triglyceride levels in male Wistar rats with hypercholesterolemia can drop significantly after just one week of receiving cherry leaf ethanol extract. Similarly, the difference in the dose of cherry leaf ethanol extract given resulted in significant differences in triglyceride levels between the treatment groups. The group that received ethanol extract of cherry leaves at 400 mg/kg BW had the greatest reduction in triglyceride levels after the first week of treatment. The fact that the extract dose in this group was higher than the extract dose in the other groups could have influenced the decrease. Tannins, which are also present in cherry leaves, bind to mucosal proteins and intestinal epithelial cells, preventing fat absorption. Tannin compounds can increase the mechanism of cholesterol into bile acids and increase the excretion of bile acids through feces by preventing an increase in cholesterol levels through an antioxidant mechanism. Low cholesterol in the liver increases cholesterol uptake from the blood to the liver, which acts as a precursor of bile acids. As the liver attempts to replace the lost bile acids, it draws cholesterol from the blood, lowering blood cholesterol levels. The positive control group received simvastatin therapy, which resulted in significant reductions in blood triglyceride levels in the test animals. Simvastatin works by inhibiting cholesterol synthesis and the HMG-CoA reductase enzyme in the liver. The first step in the sterol biosynthesis is mediated by HMG-CoA

reductase. As cholesterol synthesis declines, proteases break down SREBP (Sterol Regulatory Element Binding Protein) in the membrane, which is then transported to the nucleus. The transcription factors will then bind to the LDL receptor gene, causing an increase in the production of LDL receptors.<sup>13-19</sup>

The results of the study after the second week of treatment showed a significant reduction in triglyceride levels in the groups that received the extract dose of 100 mg/kg BW, 200 mg/kg BW, 400 mg/kg BW, and the positive control group that was given simvastatin therapy. The triglyceride levels in the negative control group did not drop. After the second week of treatment, there was a significant difference in triglyceride levels between the treatment groups. This suggests that triglyceride levels in male Wistar rats with hypercholesterolemia can be significantly reduced after the second week of administration of cherry leaf ethanol extract. Similarly, the difference in the dose of cherry leaf ethanol extract given resulted in significant differences in triglyceride levels between the treatment groups. The group that received ethanol extract of cherry leaves at 400 mg/kg BW showed the greatest reduction in triglyceride levels after the second week of treatment.

There were significant differences in triglyceride levels in the positive control group, the group receiving 200 mg/kg BW extract, and the group receiving 400 mg/kg BW extract when the results of the first week of research were compared to the results of the second week of research. This significant difference indicates that the duration of cherry leaf ethanol extract administration is proportional to the reduction in triglyceride levels.

## 5. Conclusion

It was discovered that giving male Wistar rats with hypercholesterolemia ethanol extract of cherry leaves at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW for 14 days could significantly lower their blood triglyceride levels. The best way to lower triglyceride levels was to give cherry leaf ethanol extract at a dose of 400 mg/kg BW/day.

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