Effect of Gambir Catechin Isolate (Uncaria Gambir Roxb.) Against Rat Triacylglycerol Level (Rattus novergicus)

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Abstract. Catechin isolate is an active substance contained in gambir consisting of epicatechin compounds, epigALLOCATEchin-3-errs and epigALLOCATEchin which have the potential effect to reduce triacylglycerol levels. This study aims to determine the effect of catechin isolates on rat triacylglycerol levels induced by a high fat diet. We use catechin isolates which is an active compound from gambir extract. This study was conducted on 25 rats divided into 5 groups, namely, negative control group (K -), positive control (K +), and 3 treatment groups (P1, P2, P3) given a high-fat diet of cow's brain for 14 days. The treatment group was then given catechin isolates with a dose: 10 mg, 20 mg, and 40 mg/kg body weight/day for 14 days. Triacylglycerol levels were examined using Glycerylphosphate Oxidase (GPO) Method. Data analysis was performed using One way Annova and Post Hoc Tukey HSD. The results showed a decrease in triacylglycerol levels after being given by catechin isolates. Triacylglycerol levels in positive control mice (K +) 147.8 ± 8.5 mg / dL were higher when compared to the negative control group and the treatment group, ie 104.6 ± 15.3 mg / dL, 101.4 ± 15.7 mg / dL, 106.4 ± 17.6 mg / dL, and 110 ± 3.2 mg / dL. There were significant differences in the P1, P2, and P3 groups with the positive control group p = 0.014 (p <0.05). The conclusion of this study is catechin isolates can affect triacylglycerol levels.

Keywords: gambir, catechin isolate, triacylglycerol

1. Introduction
Catechin isolates are active compounds derived from uncaria plants which have the main function as antioxidants. The isolates of the catechins are obtained from uncaria plant material, namely gambir. Gambir will then be extracted and the active ingredient is taken, namely catechin isolates.

Catechin isolates and derivatives contain polyphenols [1,2]. It consists of epicatechin, epicatechin-3-gallate, epigallocatechin-3-gallate and epigallocatechin. The main function of catechin isolates are as antioxidants, but it also has many potential benefits such as anti-cardiogenic, antihyperlipidemic, anti-inflammatory, thermogenic, probiotic and antimicrobial agents [3].

Catechins can increase thermogenesis and reduce fat formation in the body. Catechins can reduce TNF-α so that it inhibits the synthesis of free fatty acids (FFA) and increases the regulation of enzymes that play a role in beta-oxidation in the liver and increase insulin sensitivity. Insulin sensitivity will increase the work of the lipoprotein lipase enzyme and reduce FFA, and inhibit absorption and increase fat secretion through feces. Catechins contain anti-oxidants which have the potential to inhibit oxidative stress, so they can reduce fat and triacylglycerol levels in the body [4,5].

Triacylglycerol is a compound consisting of glycerol and three fatty acids which are connected through an ester bond. Triacylglycerol has an important role in lipid transport and storage. Lipid storage occurs at adipose and liver tissue. Triacylglycerol has a function as an energy source in the body. The level of triacylglycerol in the blood is affected by the level of fat consumed. Excessive fat intake will cause the development of non-communicable diseases. Non-communicable diseases are the number one cause of death in the world. Every year there are 40 million deaths caused by non-communicable diseases and constitute 70% of the total deaths in the world. Non-communicable diseases are based on the presence of metabolic syndrome and obesity. Basic Health Research in Indonesia (2013) states that 19.7% of men and 32.9% of Indonesian women are obese [6,7]. Because
of that, it is important to overcome obesity problem, one of them by using isolates catechin from Gambir.

National Cholesterol Education Adult Treatment Panel III Program (NCEP-ATP III) states that the diagnosis criteria for metabolic syndrome are an increase in blood triacylglycerol levels> 150 mg / dL and a decrease in HDL cholesterol <40 mg / dL in women and <50 mg / dL in men male, increased blood pressure> 130/85 mmHg, and an increase in fasting blood glucose> 100 mg / dL[7,8]. Increased triacylglycerol plays an important role in increasing body weight, and a high diet of hypertriasilglycerol is the basis of the pathophysiology of the disease.

Triacylglycerol can be found in foods that are high in fat, one of which is the cow's brain. A high-fat cow brain diet can improve lipid profiles because of the high levels of cholesterol and saturated fatty acids contained in the brains of cattle. The cow's brain contains 2 g of cholesterol and 2.9 g of saturated fatty acids for every 100 g of cow's brain. Cholesterol and triacylglycerol can also be synthesized by the body itself, resulting in high intake fat will increase cholesterol and triacylglycerol.

Intake of saturated fatty acids, poly unsaturated fatty acids (PUFA), and cholesterol in the diet of cow brains will provide a cholesterol level response, each intake of saturated fatty acids 1% of total energy is predicted to increase 2.7 mg / dL cholesterol levels. This situation will also have an impact on other lipid profiles, especially triacylglycerols.

High levels of triacylglycerol will cause adiposapathy, which is a condition of increased intake of fat stored in adipose tissue, over time it triggers an increase in Tumor Necrosis Factor - α (TNF-α). Increased TNF-α will cause insulin resistance and increase blood sugar and increase lipid profiles, especially triacylglycerol. An increased lipid profile will cause an increase in blood pressure [4].

Several studies have examined the effect of giving catechin isolates to rats with a dose of 10 mg / kgBW given to rats for 8 consecutive days after being induced by CCl4, significantly reducing serum malondialdehyde (MDA) by 3.28 nmol / mL. The decrease in MDA will indirectly reduce TNF-α and will further reduce triacylglycerol levels. Subsequent research using multilevel doses of 5 mg / kg body weight, 10 mg / kg body weight, and 20 mg / kg body weight stated that a dose of 20 mg / kg body weight gave a significant difference to positive control compared to other doses. This situation states that catechin isolates can function as good antioxidants, so that it can affect the levels of triacylglycerol [9,10,11]. We aim to determine whether isolate catechin from gambir can affect triacylglycerol level in rat induced by high fat diet.

2. Experimental Method

This type of research design is true experimental research with a post test group design approach that uses experimental animals as research objects. The study group consisted of negative control group, positive control group, treatment group 1, treatment group 2, treatment group 3. This study was conducted after obtaining ethical clearance from the Commission for Research Ethics of the Faculty of Medicine, Andalas University with letter number 439 / KEP / FK / 2017.

The population of this study were male wistar rats (Rattus norvegicus) aged 8-12 weeks, with body weight ranging from 200-250 grams purchased from the Laboratory of the Faculty of Pharmacy, Andalas University. This study used 25 rats divided into 5 groups, namely the negative control group (n = 5), the positive control group (given a cow's brain, n = 5), treatment 1 (given a cow's brain and catechin isolate 10 mg / kgBW / day, n = 5), treatment 2 (given a cow's brain and catechin isolate 20 mg / kgBW / day, n = 5), and treatment 3 (given a cow's brain and catechin isolate 40 mg / kgBW / day, n = 5).

Acclimatization was carried out for 14 days. Mice are kept in clean cages with adequate lighting and ventilation. The animal enclosure is cleaned 3 times a week so that mice can be healthy and avoid dirt that can cause infection.

Positive control group and treatment, rats were given a high-fat diet with an oral round of cow's brain as much as 2 ml / day. The cow's meat was steamed and dissolved with aquadest 1: 1. Cow brain induction is carried out for 14 days [8].

Treatment groups 1, 2, 3 were then given catechin isolates with multilevel doses for 14 days. Day 43 was carried out by taking rat blood through retro orbit using capillary pipes. Mice were previously given ether inhalation. The serum is taken from the blood and triacylglycerol levels are examined. Examination was carried out using the GPO-PAP method Enzymatic Colorimetric Test to
Triglycerides with Lipid Clearing Factor (LCF) The results of the examination were analyzed by computerization with One Way Anova and Post Hoc Tukey HSD tests.

3. Results and Discussion

The measurement of rat serum triacylglycerol levels was only carried out at the end of the study, i.e., after day 43 at the Biochemistry Laboratory of Andalas University. The serum triacylglycerol levels of Rattus norvegicus gratuswistar grooves in each group. The lowest serum triacylglycerol level in the group given standard feed was 83.4 mg / dL. The levels of triacylglycerol in the group induced by high-fat feed were higher than those in the standard-treated group.

This increase in triacylglycerol levels showed an increase in triacylglycerol levels in all treatment groups induced by a high-fat diet for 14 days. The highest serum triacylglycerol level in the group induced by a high-fat diet was 157.1 mg / dL. Triacylglycerol levels in all treatment groups were lower than those in the high-fat diet.

Decrease in triacylglycerol levels in the Wistar strain Rattus norvegicus group after treatment of gambier catechin isolate for 14 days. The lowest serum triacylglycerol level in treatment group 1 with a dose of 10 mg / kg / day was 83.9 mg / dL. The lowest serum triacylglycerol level in treatment group 2 with a dose of catechin isolate 20 mg / kgBW / day was 83.4 mg / dL. The lowest serum triacylglycerol level in treatment group 3 with a dose of catechin isolate 40 mg / kgBW / day was 105.1 mg / dL. Based on the data obtained, the mean triacylglycerol levels between groups in this study were as follows:

Table 1. Average levels of triacylglycerol after induced high fat diets and administration of catechin isolates

| Group | Average of triacylglycerols (mg/dl) ± SD | Decrease (%) |
|-------|----------------------------------------|--------------|
| K-    | 104.6 ± 15.3                           | -            |
| K+    | 147.8 ± 8.5                            | -            |
| P1    | 101.4 ± 15.7                           | 31.3         |
| P2    | 106.4 ± 17.6                           | 28           |
| P3    | 10.1 ± 3.2                             | 25.5         |

(n= 5 mice per each group)

Table 1 shows the mean level of triacylglycerol between groups after induced high fat diet for 14 days and administration of gambier catechin isolate for 14 days. The average level of rat triacylglycerol which was only induced by high fat (K+) diet was 147.8 mg / dL compared to groups K-, P1, P2 and P3. The group of treatment mice 1 (P1) with a dose of 10 mg / kgBW / day had a mean score of more than 100.18 mg / dL compared to the whole group. The treatment group 2 with a dose of 20 mg / kgBW / day and treatment 3 at a dose of 40 mg / kgBW / day had a decrease of 28% and 25.5%. Group 2 treatment rats had lower levels of triacylglycerol levels compared to treatment group 3.

The results of the bivariate test were obtained using a computer program with a 95% confidence interval and a significance level of 0.05 (p = 0.05). The results of the analysis will be described in the data normality test and comparability test. Results of rat triacylglycerol serum levels after treatment in each group were then statistically analyzed. The first test that was carried out was the data normality test using the Shapiro Wilk Test, it was found that the data distribution was normal with p = 0.17 (p > 0.05).

The next test is to find out the significance of the triacylglycerol level of each group, using the One Way ANOVA test. The test results showed a significant decrease in triacylglycerol levels in all treatments (p = 0.014) after administration of catechin isolate gambir for 14 days. To find out the significant differences in each group, further analysis was carried out using the Post-Hoc test, namely Tukey HSD.

The mean level of triacylglycerol in the negative control group was 104.68 mg / dL and the positive control group had a mean triacylglycerol level of 147.88 mg / dL. The difference in mean triacylglycerol levels in the positive and negative control groups showed that there was an increase in triacylglycerol levels due to the induction of a high fat diet in positive control mice. Increased levels of
Triacylglycerol due to high levels of cholesterol and saturated fatty acids contained in the brains of cattle. In 100 g of cow’s brain there is about 2 g of cholesterol, and 2.9 g of saturated fatty acids. Triacylglycerol stored in the body comes from the intake and synthesis of triacylglycerol in the body, so that high fat intake can increase triacylglycerol levels in the blood.

The results of this study are in line with the Hypothesis Heart Diet which states that a diet high in fat, cholesterol, and a diet high in unsaturated fats will increase fat levels in the blood and stimulate the liver to produce fat and inhibit fat removal [13]. The increase in triacylglycerol levels is in accordance with Kerrshaw, namely a high-fat diet resulting in increased levels of triacylglycerol and accumulated by adipocytes and adipose tissue. The accumulation of fat will eventually increase TNF-α. Increased levels of TNF-α will cause insulin resistance. Insulin resistance occurs by stimulating sphingomyelination by TNF-α, thus hydrolyzing into seromide and choline. Seromide will activate the protein kinase C and phosphatase enzymes. This enzyme will increase phosphorylation of serine insulin receptor substrate-1 (IRS-1) and inhibit the enzyme tyrosine kinase, then reduce GLUT 4 translocation so that it causes insulin resistance [14,15].

Insulin resistance in adipocytes decreases the activity of the lipoprotein lipase enzyme, so the VLDL clearance decreases, resulting in increased VLDL levels in the blood. In addition insulin resistance can increase the hydrolysis of triacylglycerol, resulting in an increase in FFA. The FFA will enter the blood circulation and then to the liver. Increased FFA in the liver stimulates secretion from VLDL, resulting in hypertriglyceridemia.

Rat treatment group 1 with a dose of 10 mg / kgBW / day, treatment 2 with a dose of 20 mg / kgBW / day, and treatment 3 at a dose of 40 mg / kgBW / day showed the mean level of triacylglycerol was 101.18 mg / dL, 106.44 mg / dL, and 110.1 mg / dL. The decrease in mean triacylglycerol levels when compared with the positive control group were 31.3%, 28% and 25%, respectively. These results indicate there is a decrease in triacylglycerol levels in all treatment groups, but there is a variation in the decrease in each dose. The treatment group 1 had the lowest mean triacylglycerol level compared to the entire treatment group 2 and treatment 3, this showed that the does of 10 mg / kgBW / day of catechin isolates was able to reduce triacylglycerol levels better than the dose of 20 mg / kgBW / day and dose 40 mg / kg / day. The treatment group 2 was able to reduce triacylglycerol levels better than treatment group 3, but not better than the treatment group 1. This decrease in triacylglycerol levels occurred due to various factors, namely, catechin isolate dose, health condition, immunity, and body weight in rats. This variation in weight loss shows that at a dose of 10 mg / kgBW / day is the optimal dose for decreasing levels of triacylglycerol.

The decrease in triacylglycerol levels is influenced by the presence of catechins derived from gambier. Gambir contains about 7-33% of catechins. Catechins are secondary metabolites which are classified as flavonoids and contain polyphenol groups. Catechins contain compounds consisting of epicatechin, epicatechin-3-gallate, epigallocatechin-3-gallate and epigallocatechin. The mechanism of the decrease in triacylglycerol by catechins, one of which is the epigallocatechin-3-gallate compound, among others, by increasing the activity of the lipoprotein lipase enzyme and increasing the VLDL clearance, thereby reducing the state of hypertriglyceridemia in the blood [16].

Epigallocatechin-3-gallate compounds in catechins are known to increase the activity of the enzyme lipoprotein lipase (LPL). This occurs due to EGCG contained in the catechins which can reduce one of the pro-flamation in fat tissue namely TNF-α. The decrease in TNF-α will cause fatty acid oxidation in the liver to increase, inhibit VLDL synthesis by liver cells and increase insulin sensitivity. This increase in insulin sensitivity will trigger an increase in the activity of the lipoprotein lipase [1,16].

Insulin is a precursor which will cause the deconjugation of bile salts. Bile salt hydrolase enzyme (BSH) produces deconjugated bile salts in the form of free colic acid which is less absorbed by the small intestine so that cholesterol absorption will decrease. Bile salts that return to the liver during enterohepatic circulation are reduced, resulting in increased bile salt synthesis from serum cholesterol so that total cholesterol in the body decreases. Low cholesterol returned to the liver, will cause a decrease in the synthesis of triacylglycerol in the liver and reduce triacylglycerol in the blood.

In this study stated that there was a significant difference between positive control with negative control, treatment 1, treatment 2, and treatment 3, namely P = 0.014 (P <0.05). The difference was not significant between treatment group 1, treatment 2, and treatment 3, but showed the best decrease in triacylglycerol levels in treatment group 1. The results of this study stated that at the treatment dose 1
had reached the optimal dose, so that the next treatment dose no longer had an effect against triacylglycerol levels. This research is in line with the research conducted by Dewi in 2016 and Dahlia in 2014, which states that catechin isolates function as anti-inflammatory so that it will suppress cytokine formation, especially TNF-α and reduce triacylglycerol levels [4,17]. The results of this study are also in line with research conducted by Yunarto 2015, which states that at high concentrations will cause the effects of catechins will decrease [18].

4. Conclusion

Based on research conducted on the effect of catechin gambir isolates on rat triacylglycerol levels that have been induced by high-fat diets, it was concluded that the mean levels of triacylglycerol in the negative, positive, treatment 1, treatment 3 and treatment 3 groups were 104.68 mg / dL, 147.88 mg / dL, 101.18 mg / dL, 106.44 mg / dL, and 110.1 mg / dL. There were significant differences in the mean triacylglycerol levels in the negative control group, treatment 1, treatment 2, treatment 3 when compared to positive control. It can be concluded that isolate catechin of gambir influence triacylglycerol levels induced by high fat diet.

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