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The Effect of Gambir's Catechin Isolate (*Uncaria Gambir Roxb.*) on Alanine Aminotransferase and Aspartate Aminotransferase Serum Level at Male Rats (*Rattus norvegicus*) Wistar Strain Induced by High Fat Diet

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ABSTRACT

Background: High fat diet causes inflammation of the liver due to accumulation of fat in the liver cells. Catechin isolates of gambir (*Uncaria gambir roxb*) are an active substance contained in gambir plants which contain flavonoid that have antioxidants activity and potentially as hepatoprotector. This study aims to determine the effect of catechin isolates on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum levels at male rats (*Rattus norvegicus*) wistar strain induced by a high fat diet.

Research Method: This study was true experimental study with post-test only control group design. We use catechin isolates from gambir which are consisting (+)- catechin. This study was conducted on 30 rats divided into 5 groups, i.e., negative control group (K-), positive control (K +) and three treatment groups (P1, P2, P3). The K+, and the three treatment groups were induced by high fat diet. The treatment groups received 10 mg/kgBW/day, 20 mg/kgBW/day, and 40 mg/kgBW/day on P1, P2, and P3 groups of catechin isolate of gambir for 14 days. AST and ALT levels were examined using IFCC method. Data analysis was performed using One way Annova and Post Hoc LSD.

Research Result: High fat diet induction could increase AST and ALT serum on the positive control group compared to the negative control group. There was significant difference in AST and ALT levels after being given by catechin isolates between P3 (40 mg/kgBW/day) and K+. The average level of AST and ALT serum for K- was 26.51 U/L;31.58 U/L, K+ was 38.31 U/L; 67.45 U/L, P1 was 35.90 U/L; 66.35 U/L, P2 was 31.13 U/L; 59.71 U/L, and P3 was 30.36 U/L; 38.21 U/L. The differences of AST levels in each groups were significant with p value=0,02 and ALT levels as well, p value=0.001 ($p<0.05$).

Research Conclusion: The conclusion of this study is catechin isolates of gambir can decrease AST and ALT serum levels.

Keywords: gambir, catechin isolate, AST, ALT, high fat diet

INTRODUCTION

The high consumption patterns of people who consume high fat have an impact on health that is still not realized, namely inflammation of the liver due to accumulation of fat in the liver cells. This liver disease is difficult to treat. It will cause cirrhosis of the liver even liver cancer and lead to death. Though the liver is a central organ in the body's metabolism. Research conducted on the general population in Beijing shows that risk factors for fatty liver include high levels of triglycerides in the blood and consumption of high-fat foods[1]. Potential hepatotoxic mechanisms such as

high energy density and food portion sizes, foods high in saturated fats, high glycemic indexed foods, low in fibre, high in fructose, red meat, and food manufacturer sources of trans fats will cause increased accumulation of free fatty acids in the liver to form triglycerides and inflammation in the liver[2].

The prevalence of abnormal liver tests in the general population has increased[3]. Increased aminotransferases enzymes in both men and women are strongly linked to metabolism, such as central obesity, high plasma calcium levels and dyslipidemia. High serum AST and ALT levels are associated with the risk of future type 2 diabetes[4]. ALT is an acceptable marker for liver steatosis in epidemiological studies[5]. Research conducted in India on mice fed a high-fat diet (68% standard diet, 30% saturated fat and 2% cholesterol) for 2 weeks was found to significantly increase ALT (40.2 ± 1.2 U / L) and AST (23, 3 ± 25 U / L) [6] . This is also supported by studies with a high-fat diet using heated margarine, a significant increase in AST and ALT in the control group with HFD compared to the standard diet[7].

Efforts to protect the liver from damage caused by fat accumulation are more than planned to control risk factors, such as improving insulin resistance and reducing the fat intake to the liver, then only using substances that require hepatoprotection potential. Herbal ingredients have proven to be useful as hepatoprotection, for example, the use of gambir catechin isolates. Catechin isolates are derived from Uncaria plant preparations, which are gambir. Catechin isolates are active compositions derived from Uncaria plants which have the main function as antioxidants[8].

Catechin isolates and their derivatives contain polyphenol compositions. Types of catechin isolates in gambir are (+) - catechin, (-) - epicatechin Gambiriin A1, Gambiriin B1, Gambiriin B2, Catechin (4α-8) -ent-epicatechin, Gambirflavan D1 and Gambirflavan D2. Catechin with its main function as an antioxidant can also help as potential anti-hyperlipidemia, anti-inflammatory, thermogenic, and anti microbiological[9]. The effects of gambir catechins reduce liver damage by reducing oxidative stress, reducing inflammatory cytokines and reducing dysfunction of mitochondria[10].

Although research on the effects of gambir catechin extract as a hepatoprotection on AST and ALT levels has been carried out, no one has yet discussed the isolation of gambir catechin as a hepatoprotection by inducing a high-fat diet. While a high-fat diet is becoming the lifestyle and eating pattern of today's society. Based on these facts, this study was conducted to see the optimal dose that can be used to reduce levels of liver enzymes in plasma that indicate cell damage.

LITERATURE REVIEW

A number of chylomicrons transport triglycerides to adipose cells when consuming a high-fat diet and if excess they will be transported by the liver. The increase in fatty acids brought to the liver is not only caused by excessive food intake but is also related to obesity which causes insulin resistance which results in lipolysis of adipose tissue[11]. This free fatty acid will ne transported to liver. An overload of FFAs into liver causes an increase in beta-oxidation. However, over time this causes mitochondrial dysfunction and induces disruption of the electron transport chain which results in electron leakage. These electrons interact with oxygen to form radical oxidative species (ROS)[12].. The treatment of high-fat diets increases the number of cells in liver tissue which causes excessive production of free radicals in these tissues[13].

Fat accumulation will cause an increase in TNF-α. TNF-α will increase NADPH oxidase which will again produce super reactive radicals which are very reactive because they have two unpaired electrons, especially very reactive to lipids, causing lipid peroxidation[12]. Lipid

peroxidation will cause changes in permeability in liver cells, causing enzymes produced by liver cells such as AST and ALT to increase in the blood. When damaged cells are repaired, these cells will release enzymes to the blood. The enzyme can be used as an indicator of cell damage[14,15,16].

There are nine types of catechins in gambir namely (+)- catechin, (-)-epicatechin, Gambiriin A1, Gambiriin A2, Gambiriin B1, Gambiriin B2, Catechin-(4α-8) -ent-epicatechin, Gambirflavan D1 and Gambirflavan D2 (Taniguchi *et al.,* 2008). Catechins obtained in gambir can reach 98%[17,18]. Catechins are polyphenol derivatives. Gambir catechins reduce liver damage by reducing oxidative stress, reducing inflammatory cytokines and reducing dysfunction of mitochondria[10]. As a hepatoprotection, gambir catechins which are a class of polyphenol compilations have the ability to be anti-free radicals and prevent the development of free radicals in the body that can repair damaged body cells and are able to produce liver enzymes in the blood[8.19].

Catechins as antioxidants can prevent free radicals and biological cells are not responsible for inflammation. Catechin as an anti-inflammatory can reduce TNF-α so that it will reduce lipolysis and increase the enzymes needed in beta-oxidation in the liver and increase insulin sensitivity[20]. The suppression of the production of free radicals and inflammatory cytokines can ultimately reduce mitochondrial damage so that the beta-oxidation process can work well in ensuring energy savings for cells to function and repair the damage.

MATERIALS & METHODS

Animals, diets and experimental design

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. 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 30 rats divided into 5 groups, namely the negative control group (given normal diet, n =6), the positive control group (given high fat diet + melted margarine 1,7gr, n = 6), treatment 1 (given high fat diet + melted margarine and catechin isolate 10 mg / kgBW / day, n = 6), treatment 2 (given given high fat diet + melted margarine and catechin isolate 20 mg / kgBW / day, n = 6), and treatment 3 (given high fat diet + melted margarine and catechin isolate 40 mg / kgBW / day, n = 6). Acclimatization was carried out for 7 days. Rats are kept in clean cages with adequate lighting and ventilation. The animal enclosure is cleaned 3 times a week so that rats can be healthy and avoid dirt that can cause infection. *Measurement of body weight was done every week.* Positive control and treatment groups were given a high-fat diet ad libitum and an oral round of melted margarin 1,7 gr/day. Negative control group given normal diet ad libitum and an oral round of water. After five weeks, treatment groups 1, 2, 3 were then given catechin isolates with multilevel doses for 14 days. At the end of the treatment, the blood samples were collected from retro orbita vein using capillary pipes.

Measurement of serum parameters

Blood was placed into a sterile Vacutainer plastic tube (BD Vacutainer, Plymouth, UK). Serum was separated by centrifugation (4000 rpm, 10 min) and transferred to Eppendorf tubes. The concentrations of aspartate aminotransferase and alanine aminotransferase in serum were measured with commercial kits (DiaSys Diagnostic System, Germany). Examination was carried out using the IFCC (International federation of Clinical Chemistry and Laboratory Medicine) method.

Statistical Analysis

The statistical analysis of AST and ALT results were carried out by one way analysis of variance (ANOVA) and Post Hoc LSD tests to obtain significant difference of the result at 0.05 level of confidence ($p<0.05$). Normality data test using Shapiro-Wilk. The statistical analyses of rats's weight were performed using independent T-test to compare between negative control and each of treatment groups. Differences were considered to be significant at 95% CI.

RESULT

The results of a diet high in fat and margarine on body weight can be seen in table 1. In this table, it appears that a high-fat diet can increase the bodyweight of mice. In addition, there are also significant body differences to negative control after five weeks of giving a high-fat diet. The results of AST and ALT levels can be seen in table 2 and table 3.

Table 1: Average and Normal Weight Test Results of Rats

Group	n	Adaptation Period	p Value	After 5 weeks	p Value	After 7 weeks	p Value
		X±SD (gram)		***X***±SD (gram)		***X***±SD (gram)	
K-	6	226.17±4.16	0.99	231.67±4.84	0.07	267.00±11.48	0.63
K+	6	234.83±11.78	0.52	273.00±26.64*	0.84	300.50±50.93	0.85
P1	6	237.17±13.31	0.14	292.00±42.61*	0.33	272.00±18.62	0.39
P2	6	234.50±13.33	0.45	272.50±28.90*	0.26	285.00±48.27	0.31
P3	6	232.33±10.82	0.40	281.33±45.55*	0.46	277.00±20.47	0.79

Note: The p-value indicates the normality of the data after the Shapiro-Wilk test. Signs * show significant differences in the negative control group ($p <0.05$).

Based on table 2, the highest AST average was found in the positive control group of 38.31 U / L, while the AST average among the lowest groups in the P3 group was 30.36 ± 6.06 U / L. Significant influence was obtained on isolates of gambir catechins on AST levels of rats fed a high-fat diet ($p <0.05$). To find out the significant differences in each group, further analysis using Post-Hoc test is the Least Significant Difference (LSD) in table 3. From the analysis results, there was a significant decrease in blood AST in the P3 group towards positive control and when compared with the negative control group there was no significant difference. The administration group with a dose of 10 mg/kg and 20 mg/kg showed a decrease in the average AST but was not significant when compared to the positive control group. These results prove that the administration of gambir catechin isolates at a dose of 40 mg/kgBW can reduce AST levels which are increased due to high-fat diets and approach the average value of the negative control group.

Table 2: Average AST and ALT blood levels of rats that had been given Gambir catechin isolates

Subject Group	n	Average AST+ SD	Average ALT + SD
K-	6	26.51 ± 7.62	31.58 ±10.11
K+	6	38.31 ± 6.05	67.45 ± 20.91
P1	6	35.90 ± 3.75	66.35 ± 9.07
P2	6	31.13 ± 6.30	59.71 ±13.22
P3	6	30.36 ± 6.06	38.21 ±11.08

Note: ANOVA analysis of the average AST levels obtained p-value of 0.020. ANOVA analysis of the average ALT levels obtained p-value of 0.001

Table 3: Post Hoc Test LSD Test results in each study group

Group	AST				
	KN	KP	P1	P2	P3
KN	-	0.003*	0.013*	0.201	0.284
KP	0.003*	-	0.498	0.052	0.033
P1	0.013*	0.498	-	0.187	0.128
P2	0.201	0.052	0.187	-	0.829
P3	0.284	0.033*	0.128	0.829	-

Note: the * sign indicates a significant average difference

The highest ALT level in the positive control group was 66.35 ± 9.07 U / l, while the lowest ALT level in the P3 group was 38.21 ± 11.08 U / l. Significant influence was obtained on isolates of gambir catechins on AST levels of rats fed a high-fat diet ($p <0.05$). Although the ANOVA test results for AST and ALT levels are equally significant, the significance level of ALT levels is better than AST. ALT is a more specific enzyme because it is synthesized only by the liver (Fraser et al.,

2004; Harrison, 2005). Post Hoc LSD test analysis showed a significant increase in the average ALT levels in the positive control group when compared with the average negative control group (table 4). These results prove that the administration of gambir catechin isolates with a dose of 40 mg / kgBW can reduce levels of ALT which are increased due to high-fat diets and again increase the levels of negative control groups.

Table 4. Post Hoc Test LSD Test results in each study group

Group	ALT				
	KN	KP	P1	P2	P3
KN	-	0.000*	0.000*	0.001*	0.405
KP	0.000*	-	0.889	0.333	0.001*
P1	0.000*	0.889	-	0.405	0.001*
P2	0.001*	0.333	0.405	-	0.011*
P3	0.405	0.001*	0.001*	0.011*	-

Note: the * sign indicates a significant average difference

DISCUSSION

The increase in the average levels of AST and ALT in this positive control group was due to the administration of a high-fat and atherogenic diet. Delivery of excess fat to the liver will cause fat accumulation in liver cells or steatosis. This condition will cause glucose intolerance of liver cells and allow the release of inflammatory cytokines. Increased free radicals due to mitochondrial dysfunction caused by the oxidation burden of excess fat in the liver coupled with free radicals produced by trans fats from heated margarine. This stimulates the lipid peroxidation process and results in oxidative stress. Increased secretion of inflammatory mediators and the presence of free radicals that attack the lipid membrane will cause hepatocyte lysis cells and excrete enzymes that are included as ALT enzymes[21].

A significant increase in the ALT enzyme from a high-fat diet is supported by research by Asdaq & Inamdar (2010) which provides food in a group of mice on a high-fat diet for 19 days and compared with a group of mice that require standard feed. Other studies supporting mice that were given margarine for 8 days alone were able to increase AST levels to 140.2 ± 1.278 and ALT 62.11 ± 2.28[7]. However, this research was little carried out with research conducted by Ahmed et al (2009). According to this study, a high-fat diet in rats for five weeks did not increase the value of AST and ALT had no steatosis in histopathological examination. According to him, liver damage can develop even though liver enzyme levels are found normal[22].

There are no other research data on studies of gambir catechin isolates on AST and ALT levels in rat blood. However, a study by Fahrudin et al (2015) that only used gambir extract as a hepatoprotection obtained effective gambir extract as a hepatoprotection was a dose of 130 mg / kgB[8]. Research conducted by Alioes et al (2019) shows that the administration of gambir catechin isolates at a dose of 40 mg / kgBW can reduce cholesterol levels[23]. According to Syafitri et al (2015) in non-alcoholic fatty liver found high levels of total cholesterol and triglycerides[24]. While an increase in AST levels can be an indicator of fat deposits in non-alcoholic fatty liver disease. Other studies have also shown an increase in the enzyme transaminase[25,26]. Thus, it can help increase the dose of gambir 40 mg/kgBW can reduce cholesterol and also result in increased AST reduction.

Catechins that contain polyphenols in gambir isolates contain antioxidants and are proven to neutralize free radicals. Catechins have an aromatic ring with more than one hydroxyl (OH) group. The hydroxyl group has a biological activity that is used in enzymatic oxidation reactions as an electron donor substrate from the H atom[27]. Free radical reactions in cells in the body can be neutralized by connecting free metal ions and electron donors from H atoms, so Free radical molecules that have outermost unpaired electrons become stable (not reactive). Antioxidants are protected in the protection of radicals, free and protected, the permeability of liver cell membranes from damage. The process of neutralizing free radicals by antioxidants produces an optimal environment for liver cells to regenerate[28]. Catechin as an anti-inflammatory can reduce TNF-α will reduce

lipolysis and increase enzymes that increase beta-oxidation in the liver and increase insulin sensitivity[20]. The condition of liver tissue that is gradually being improved can restore liver enzymes in the blood[8].

CONCLUSION

The results analysis of liver enzyme levels indicate that the administration of gambir catechin isolate has the potential to be a hepatoprotection which is characterized by a decrease in the level of liver enzymes in the blood. It can repair damaged liver cells that have been induced by high fat diet. The potential of gambir catechin isolate as hepatoprotection is because of the antioxidants and anti-inflammatory it contains so that it can improve mitochondrial dysfunction of liver cells due to a high-fat diet thus preventing liver cell damage. Gambir dosage that is more effective in reducing the increase in AST and ALT enzymes in the blood is a dose of 40 mg / kg BW.

RESEARCH CODE OF ETHICS

This study was approved by the team of research ethics commission of Faculty of Medicine, Andalas University with ethics test number 187/KEP/FK/2019.

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