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## Antihipercolesterolemia Effects and Acute Toxicity Test of Pheophytin on Green Tea

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#### Abstract

Hypercholesterolemia is a condition characterized by increased levels of total cholesterol in the blood. Pheophytin is the result of degradation of chlorophyll which is the dominant pigment in green tea, and has been shown to have the same antioxidant properties as  $\beta$ - carotene. This study aims to determine the antihypercholesterolemia effect and effective dose of pheophytin isolates and acute toxicity tests to determine the safety level of pheophytin isolates. Antihipocolesterolemia test uses induction of high-fat feed that is a mixture of standard food, pork oil and quail egg yolk (16: 3: 1). While the acute toxicity test is carried out based on OECD 423, Clinical symptoms that arise for 3 hours are observed intensively. Histopathological examination data of liver and kidney organs were performed on day 14. Data obtained in the form of LD 50 and clinical symptoms that arise as well as histopathological results were analyzed qualitatively. Statistical test results of percent reduction in total cholesterol levels showed a significant difference between the negative control group and the pheophytin isolates of the three doses. This proves that pheophytin isolates can have antihypercholesterolemic effect with an effective dose of 10 mg/kgBW. Histopathological examination results of aorta and liver of rats showed that the higher the dose of pheophytin isolates, the lighter the degree of fatty acid. While the toxicity test results showed that the LD50 value of pheophytin isolates was more than 2000 mg/kgBW (non-toxic category). However, pheophytin isolate with LD50 is more than 2000 mg/kgBW which has shown the presence of pancreatic degeneration and hydropic degeneration, but necrosis has not occurred yet.

Keywords: pheopytin, green tea, antihypercholesterol, acute toxicity

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#### Introduction

Cholesterol is a yellowish fat, like wax produced by the body, especially in the liver [1].

Cholesterol is actually one component of fat that is needed by the body in addition to carbohydrates, proteins, vitamins and minerals. Cholesterol is needed by the body especially to form the walls of cells in the body. Cholesterol is also a basic ingredient in the formation of steroid hormones. But if excessive cholesterol in the body (hypercholesterolemia) will accumulate in the walls of blood vessels and cause a condition called atherosclerosis, which is narrowing or hardening of the arteries. Hypercholesterolemia is a precursor to heart disease and stroke [2,3]. In addition, increased levels of lipids can also cause nonalcoholic fatty liver diseases (NAFLD). NAFLD is a liver disease which is shown by the infiltration of fat in organs [4].

Tea (Camellia sinensis (L) Kuntze) is one of the most popular health drinks in the world. Tea contains nutrients and non-nutrients that are needed by the body [5, 6]. There are 3 types of tea, namely black tea, oolong tea, and green tea. Green tea is chosen because it is believed to reduce weight. The compounds contained in green tea are caffeine, polyphenols, essential oils, cellulose, crude fiber, protein, theobromine, tannins, amino acids. manganese. potassium. phosphorus. vitamins and chlorophyll [6, 7, 8]. Chlorophyll is the dominant pigment in green tea [7], but chlorophyll is easily degraded into its derivatives due to the effects of heating and storage [9]. Some examples of chlorophyll degradation products that are generally formed due to these effects are pheophytin and pheeophorbide [7, 9, 10]. The content of pheophorbide in green tea according to [7] is less than that of pheophytin. This fact occurs because the activity of the chlorophyllase enzyme in the tea is deactivated due to the steaming process after picking, while the formation of pheophytin is caused by the storage process [7].

Pheophytin from green tea has antioxidant activity [11]. Based on these studies produced that pheophytin has the same antioxidant activity as  $\beta$ -carotene. Antioxidants are compounds that can inhibit the process of free radical oxidation reactions [12, 13]. Free radicals are very dangerous because they can damage body tissues which can cause degenerative diseases such as cancer, heart disease, cataracts, premature aging, obesity, etc. [14].

Based on this, research needs to be done to see the antihypercholesterolemia effect of pheophytin isolates in green tea. An acute toxicity test also needs to be done in order to determine the safety of pheophytin isolates so that they can be applied to the community as antihypercholesterolemia.

#### Materials ad Methods

#### Materials

The main ingredients are green tea leaves (Camellia sinensis (L) Kuntze), acetone, aquadest, CaCO<sub>3</sub>, diethyl ether, hexane, methanol, Na<sub>2</sub>SO<sub>4</sub> anhydrous, HCl, silica gel G-60, NaOH, H<sub>3</sub>PO<sub>4</sub>, aquadest, pork oil, duck eggs, Na CMC, Orlistat, male white rats of Wistar strain, Cholesterol reagent solution from DIASYS (*Diagnostic Systems Holzheim Germany*) consisting of Good's buffer pH 6-7 50 mmol/l, fenol 5 mmol/l, 4-aminoantipirin 0,3 mmol/l, cholesterol esterase 200 U/l, cholesterol oxidase 50 U/l, peroxidase 3 U/l, and standard 200 mg/dl (2,3 mmol/l).

#### **Pigment Extraction**

A total of 100 g of green tea was extracted with acetone: methanol (7: 3 v/v) for 15 minutes. The extracted pigment is filtered and then the residue is extracted again until all the pigment is removed. The result of pigment extract is added Na<sub>2</sub>SO<sub>4</sub> anhydrous to bind H<sub>2</sub>O and partitioned with ether solvent. The obtained pigment is then dried with a rotary evaporator [15].

#### Antihypercholesterolemia Test

Prepared 30 white male rats of Wistar strain weighing 150-250 grams. Before treatment, rats were first adapted in laboratory conditions for one week by being given adequate food.

Experimental animals were divided into 6 groups, each group consisting of 5 animals, each group was treated as follows:

Group I (Normal) :	feed standard days 0-67
	and aquadest by oral
Group II (Control -) :	HFD days to 0-60, and
	suspension Na CMC
	0,5% days to 61-67
Group III (Control +) :	HFD days to 0-60, and
-	orlistat 10 mg/kgBW
	days to 61-67 by oral
Group IV, V and VI :	HFD days to 0-60, and
	suspension of
	pheophytin isolates 10;
	20; 40 mg/kgBW days
	to 61-67 by oral
Description :	High Fat Diet (HFD) is a
-	standard food mixture of
	rats with 15% lard and
	5% duck egg yolk

On day 0 (before being given a high fat diet), 61(after induced by a high fat diet) and 68 (after a treatment), all experimental animals were blood drawn through the optimal vein of the eye. Then the blood is allowed to stand for 15 minutes, centrifuged at a speed of 3000 rpm for 15 minutes, and a clear part, called a serum, is taken to determine the total cholesterol level.

#### Acute Toxicity Test OECD 423

- a. Swiss sex strain female mice aged 2-3 months confirmed their quality and adapted to the laboratory environment for at least 1 week.
- b. Mice are grouped according to a predetermined dose rating. The test group was divided into 5 groups, each group consisting of 3 test animals.
- c. Mice were treated with oral pheophytin isolates according to the dose determined by each group. In Group I the CMC Na was 0.5% (as a negative control). Group II, III, IV, and V are treated with suspension of pheophytin isolates 5 mg/kgBW, 50 mg/kgBW, 300 mg/kgBW and 2000 mg/kgBW.
- d. The toxicity test was started from the highest dose of pheophytin isolate 2000 mg/kgBW given to 3 mice.
- e. An intensive observation period during the first 4 hours by physical observation of toxic symptoms (scratching the nose and body, standing with two legs behind, and decreased activity).
- f. The observation period is carried out for 24 hours up to 14 days.
- g. Observation is stopped when there are mice that die
- h. If there are mice that die, the dosage of pheophytin isolates is reduced according to the dose rating determined by OECD 423.
- i. If no mice died until 14 days of observation, the experiment was repeated once again at the same dosage of pheophytin isolates.
- j. On Day 15 mice were sacrificed to observe histopathological changes in the liver and kidney.

#### Data analysis

Data on total cholesterol levels obtained were then calculated as a percent decrease to see

the magnitude of the decrease in the test compound. Percent decrease in total cholesterol levels were analyzed statistically with SPSS version 16.0, 95% confidence level.

#### Results and Discussion

This study aims to determine the antihypercholesterolemia effect and the acute toxicity test of pheophytin isolates in green tea. Green tea (*Camellia sinensis* (L) Kuntze) is obtained from a tea plantation in the Medini Boja area of Central Java.

Pheophytin isolates that have been obtained are then tested on white Wistar strain of rat animals to determine the antihypercholesterolemia effect. Induction of hypercholesterolemia in test animals uses a high-fat diet. A high-fat diet is a food mixture consisting of standard feed, 15% pork fat and 5% duck egg yolk. This induction is carried out for 60 days. The results obtained were significant differences in the total cholesterol levels of all test animals between day 0 (initial) compared to day 60 (induction), except for test animals that were not induced (normal controls). This shows the induction of high fat and fructose diets which can cause a hypercholesterolemia significantly.

Based on the [16], the fat content in lard is 100 grams and in the egg yolk portion is 31.9 grams. In addition, the fatty acid content in pig oil consists of 39 grams of saturated fats, 45 grams of monounsaturated fat, 11 grams of polyunsaturated fat and 95 mg of cholesterol [17]. While the fat in the volk is bound in the form of lipoprotein which consists of 85% fat and 15% protein. Fat from phospholipids lipoprotein consists of 20% (lecithin, phosphatidyl serine), 60% neutral fat (triglycerides) and 5% cholesterol [18]. High saturated fatty acids in lard and triglycerides in chicken egg yolks and cholesterol in both can cause an increase in cholesterol levels in the blood.

Test animals are positive suffering from hypercholesterolemia with marked increase in total cholesterol levels above normal cholesterol levels which is 10-54 mg/dl [19]. All hypercholesterolemia test animals were treated with CMC Na 0.1% (negative control), Orlistat (positive control), and suspension of pheophytin isolates in doses of 10, 20 and 40 mg/kgBW for 7 days. There is also a normal group where test

animals are only given aquadest without inducing a high-fat and fructose diet. This group aims to see the total cholesterol level of normal test animals and the influence of the environment as well as stressing on the total cholesterol levels of test animals during the treatment. Days to 68 all treatment groups were measured total cholesterol levels as treatment levels. The results of initial total cholesterol, induction and treatment can be seen in Table 1.

Based on table 1, pheophytin isolates showed significant differences in the negative control group. This proves that the isolate pheophytin can have antihypercholesterolemia effect. Pheophytin isolates also showed insignificant differences in the positive control group. This shows that the antihypercholesterolemia effect of pheophytin isolates is comparable to Orlistat. Therefore, the effective dose of pheophytin isolates as antihypercholesterolemia is 10 mg/kgBW.

Histopathological observations of the aortic and liver organs were also carried out to see whether there was any fat. Fatty found in the aorta and liver can cause atherosclerosis and non-fatty liver diseases. The results obtained are the higher the dose of pheophytin isolates, the lighter the degree of fat in the aorta and liver. Fatty images of the aorta and liver can be seen in Figures 2 and 3.

Pheophytin isolates have an effect on antihypercholesterolemia, presumably because they have the same mechanism of action as Orlistat. The mechanism of action of Orlistat is to inhibit gastrointestinal (pancreatic and gastric) lipase enzymes which can reduce absorption of food fat in the small intestine by about 30% [20].

Table 1. Average ± SD Total Cholesterol Levels (mg/dl), Percentage of Increase and Percentage of Decrease in All Treatment Groups

Group	Total Cholesterol Levels (mg/dl)		0/ In 2022 22	0/ Destasse	
	H0	H51	H58	% Increase % L	% Decrease
Normal	61.39±6.25	53.92±12.69	63.86±9.39	-18.58±27.77	-27.42±54.34 <sup>a</sup>
CMC Na 0,5% (Control -)	69.87±12.42	103.94±15.71	65.47±11.06	32.95±3.34	37.08±3.53 <sup>b</sup>
Orlistat (Control +)	66.85±13.54	132.89±21.69	64.71±8.54	49.41±7.40	50.89±4.66 <sup>a</sup>
Pheophytin 10	65.61±9.21	117.58±15.40	55.77±9.23	$44.02\pm5.88$	51.66±10.89 <sup>a</sup>
Pheophytin 20	69.77±12.41	135.18±14.42	57.42±6.82	$48.44 \pm 6.05$	57.28±5.36 <sup>a</sup>
Pheophytin 40	71.48±11.26	121.93±8.56	59.69±6.01	41.44±7.87	$50.69 \pm 7.70^{a}$

Description:

a: significantly different (p<0,05) to the negative group with the Mann Whitney test

b: significantly different (p<0,05) to positive group with the Mann Whitney test



Figure 1. Graph of changes in cholesterol levels day to 0, 51 (after induction), and 58 (after treatment) of all groups



(c)

Figure 2. Fatty degree of lightening in mice aorta (a), fatty degree moderate in mice aorta (b), and fatty of a severe degree in mice aorta (c)



Figure 3. Fatty degree of lightening in the liver of mice (a), and moderate fat in liver of mice (b)

The toxicity test of pheophytin isolates was also carried out to see the safety level, so that later it could be used by humans. Toxicity test uses the Organization for Economic Cooperation and Development method (OECD) 423, in order to minimize the amount of use of test animals. The results obtained are the value of LD50 isolates of pheophytin that is more than 2000 mg/kgBW. Pheophytin isolates are included in category 5 (unclassified). Histopathological observation of liver and kidney organs was also carried out on the 14th day to see the toxic effects on pheophytin isolates at a dose of 2000 mg/kgBW. The results obtained indicate that parenchymatous degeneration and hydropic degeneration have begun to appear, but necrosis has not occurred yet. This proves that pheophytin isolates are in the safe category, but when used at very high doses of more than 2000 mg/kgBW can cause damage to the liver and kidneys.

#### Conclusion

Pheophytin isolates are proven to have antihypercholesterolemic effects with an effective dose of 10 mg/kgBW. The Pheophytin isolate has value LD50 more than 2000 mg/kgBW (category 5/unclassified).

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