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The Effect Of Administration Of Green Grass Jelly Cholorophyll (*Premna oblongifolia* Merr.) On Lipid Profile Of Rats (*Rattus norvegicus*).

Novelina*; Tuty Anggraini.

Department of Agricultural Product Processing Technology, Faculty of Agricultural Technology, Andalas University

ABSTRACT

Indonesia has an incredibly vast chlorophyll sources availability of which is green grass jelly leaves (*Premna oblongifolia Merr*). Several studies have proven that consuming foods with high chlorophyll content could improve health quality because it can lower cholesterol levels. The objective of this study was to determine the effect of chlorophyll extract of green grass jelly leaves on blood lipid profile of hypercholesterolemic rats before and after the administration of cholesterol extract. The extraction process was conducted by maceration using ethanol with an addition of 7% $NaHCO_3$. Test results on the administration of chlorophyll extract of green grass jelly leaves into rat's high-fat diet were able to suppress total cholesterol level by 35.80%, low-density lipoprotein (hereafter LDL) by 83.65%, and triglyceride level by 27.51%. In addition, it was able to increase high-density lipoprotein (hereafter HDL) level by 43.22% in rats with hypercholesterolemia and low atherogenic index value.

Keywords: grass jelly leaves, chlorophyll, lipid profile, hypercholesterolemia, atherogenic index

***Corresponding author**

INTRODUCTION

Chlorophyll can be used as natural dye, as several research results revealed that chlorophyll has an important role as source of antioxidants for health. *In vitro* antioxidant capacity assays toward LDL using chlorophyll of suji leaves could withstand LDL oxidation in the body (Prangdimurti, 2007).

According to Gross (1991), the average chlorophyll content of green grass jelly leaves is 1% (on dry basis). Its extensive availability in the nature and its biological benefit makes chlorophyll becomes potential to be developed as a supplement or functional food. Most of chlorophyll-based supplements or functional food circulating in Indonesia are imported and have a fairly high selling price, whereas its sources availability in Indonesia is plentiful. Therefore, it is necessary to discover its sources, one of which is the green grass jelly leaves (*Premna oblongifolia Merr*).

Astawan and Andreas (2008); Mardiah, *et.al*, (2007) stated that grass jelly is known by Indonesian as a traditional beverage. Green grass jelly is a medicinal plant that could be consumed as a functional food, be used as remedies for fever, stomach inflammation, for relieving nausea, and lowering high blood pressure. The results showed that grass jelly extract can reduce cancer cells and work as antioxidant. Several active components found in grass jelly include carotenoids, flavonoids, and chlorophyll.

According to Gross (1991), structurally, chlorophyll is a porphyrin containing basic tetrapyrroles rings, where the four rings bind to Mg^{2+} ion, and possesses the fifth isocyclic ring near the third pyrrole ring. On the fourth ring, propionic acid substituent is esterified by phytol cluster, a hydrophobic diterpene alcohol ($C_{20}H_{39}OH$). If the cluster is removed from its core structure, the chlorophyll turns into its hydrophilic derivatives.

Various contemporary studies have proved that those who consume foods high in chlorophyll have a better health quality. Chlorophyll contains almost all of nutrients needed by the body in a balanced composition. Chlorophyll is also rich in anti-inflammatory, antibacterial, antiparasitic, and other nutritious medicinal substances (Astawan, 2008).

According to Zulfikar (2009), chlorophyll structure is similar to human blood. The difference lies in the central atom where chlorophyll possesses a central atom of magnesium (Mg), while human blood has iron (Fe). Bahri (2007) added that with this structural similarity, chlorophyll is the only molecule in the world that could naturally accepted by the body and becomes a vital nutrient for human body. In metabolism process, human energy is produced from the red blood cells which delivers oxygen to body cells. Hemoglobin is molecule in the red blood cells that binds it. Besides, chlorophyll generates red blood cells formation most rapidly in human body. By consuming chlorophyll, blood cell count could increase significantly so that supply of energy in the body can be assured continuously.

According to Limantara (2009) and Malinow *et al.* (1979), chlorophyll could lower total cholesterol levels in the following ways: a) Prevents smooth muscle cells multiplications, b) Contains saponins which could prevent cholesterol absorption, c) Improves liver function to remove cholesterol from the blood into the bile so that it can lower cholesterol levels, d) Contains phytols which are lipophilic that enable to bind fat and cholesterol, and remove them through the excretion system, e) Absorbs cholesterol from the bile and food by leveraging its cell wall's absorption function, f) Lowers renin levels and dilate blood vessels so that it prevents blood vessel blockage by fat.

METHODOLOGY

Chlorophyll Extraction

Chlorophyll extraction was based on Sukarti *et.al*'s (2008) research with several modifications. Firstly, green grass jelly leaves were cut into ± 1 cm in size, then ethanol was added with 1:3 ratio (w/v) into 500 ml erlenmeyer flask. 0.7% $NaHCO_3$ which had previously diluted into 10 ml of water was also added in. The next step was a maceration process at room temperature for 24 hours in an incubator shaker. Extraction yield was filtered with filter paper using vacuum filtration, then vacuum evaporator was used to evaporate the ethanol in order to obtain a concentrated extract. The obtained concentrated extract was then put in a refrigerator at

8 ^{0}C for 24 hours, then 10 ml of distilled water was added to obtain the final chlorophyll extract to be treated onto rat's diet.

High-fat Diet Preparation

Soehardjono (1990) mentioned that rats to human conversion factor with 200 g equivalents to 70 kg of human body weights (human equivalent dose) is 0.018. Chlorophyll dosage supplied for treatment purpose is 100-300 mg (Limantara, 2009). Thus, chlorophyll dosage calculation for rat is as follows:
Dosage for rat = 0,018 x 100 mg / 200 g BB = 1,8 mg / 200 g BB

Experimental Animals Preparation and Lipid Profile Test

Research was conducted using 15 male white *Spraque Dawley* rats with body weight range from 190-220 g. Prior to treatment, rats were adapted for a week at the laboratory. Rats were made hypercholesterolemia by feeding diets containing 1% cholesterol (w/w). They were divided into three groups, each group consisted of five rats as follows: Group I was the normal control group (P1), Group II was the hypercholesterolemic (positive) subject (P2), and Group III was the hypercholesterolemic group administered with appropriate dosage of chlorophyll extract (P3). The blood samples from all treated rat were taken from the tails to get total cholesterol levels analyzed to monitor hypercholesterolemia. Experiments conducted over 4 weeks and by the end of treatment, all rats were euthanized using 0.1 mL ketamine HCl (100 mg/mL), then the blood was drawn through orbital veins and collected using tubes containing EDTA to obtain the plasma. Plasma were then analyzed to find levels of lipid profile, covering total cholesterol levels, triglycerides, High-Density Lipoprotein (HDL cholesterol), Low-Density Lipoprotein (LDL cholesterol), and atherogenic index calculation.

Lipid Profile Analysis and Atherogenic Index (AI) Calculation

Lipid profile analysis involved the analysis of plasma total cholesterol, HDL cholesterol level, and triglyceride level using Fluitest REF 4241 LOT D393, Fluitest HDLCHOL REF 410 LOT D312, Fluitest TG REF 5748 LOT D716 kits (Human, *Geselischaft fur Biochemica und Diagnostica,* mbH-Germany) respectively. Enzymatic colorimetric assay was used as the method of analysis and absorbance values were read at a wavelength of 546 nm. LDL-cholesterol level was calculated using the Friedewald formula with this following equation: (total cholesterol - HDL - TG/5). Atherogenic index was calculated using this equation: atherogenic index (AI) = (total cholesterol - HDL) / HDL

RESULTS AND DISCUSSION

Phytochemical extraction of concentrated chlorophyll conducted by Novelina *et al.* (2015) yielded that it contains phenolics, flavonoids, triterpenoids, steroids, alkaloids, and coumarin. Flavonoids are one of polyphenolic compounds that are widely available in nature, particularly in the green vegetation that can be found in plant extracts. According to Astawan (2008) polyphenols are divided into two major parts, namely flavonoids and phenolic acids. Among the two compounds, flavonoids are the largest group. Furthermore, this study also found total polyphenols from chlorophyll extract of green grass jelly leaves at 1191.70 ppm.

In Vivo **Assessment of Extracted Chlorophyll Administration toward Rat's Lipid Profile**

Research that has been conducted covers the provision of high-fat diet which was rat feed supplemented with beef fat with 4:1 in ratio (feed:beef fat). In addition to the provision of high-fat diet, rats were also administered with 1 ml/day of beef fat 30 minutes before supplementing chlorophyll extract. A high intake of saturated fatty acids and cholesterol caused an increase in body cholesterol concentration (Murray *et al.*, 2006). Beef fat that was orally administered was 19.87% in content. Compositions of the high-fat diet administered during the study were as follows: water (7.46%); minerals (6.38); proteins (11.98); fats (19.87), and carbohydrates (54.31). Results of lipid profile analysis (cholesterol, TG, HDL, and LDL) of rat plasma during four-week treatment are presented in Table 1.

Table 1. Rat's average total cholesterol, TG, HDL, and LDL plasma levels during 4-week treatment

	LIPID PROFILE				RATIO	
GROUP	TC	TG	HDL	LDL	AI	TC/HDL
P1	63,98 ± 2,40 a	74,87 ± 2,18 a	39,15 ± 4,99 a	10,13 ± 1,77 a	0,64 ± 0,15 a	1,64 ± 0,15 a
P2	117,31 ± 9,90 b	98,06 ± 3,70 b	35,56 ± 7,23 a	62,16 ± 1,94 b	2,34 ± 0,40 b	3,34 ± 0,40 b
P3	75,31 ± 5,69 a	71,08 ± 1,77 a	50,93 ± 5,76 b	10,16 ± 0,41 a	0,48 ± 0,06 a	1,48 ± 0,56 a

Description: P1: normal control; P2: positive treatment with high-cholesterol diet, P3: treatment with high-cholesterol diet and orally administered green grass jelly chlorophyll extract, twice a day. Figures followed by the same letters toward the column showed no significant difference (P>0.05). TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein; AI = atherogenic index

Total Cholesterol Level

Table 1 presents high cholesterol diet in treated rat could increase total plasma cholesterol. Average total plasma cholesterol in control treatment group (P1) was 63.98 mg/dL and in positive hypercholesterolemic control (P2) was 117.31 mg/dL marking an increase of approximately 83.35% from the normal controls. It indicated that rat treated with high-cholesterol diet had experienced hipercholesterolemia. After treated with the chlorophyll extract, total cholesterol level appeared to be decreased in comparison to the positive control treatment (P2) where significant decrease of around 35.80% appeared during 28-day treatment. It pinpointed that chlorophyll extract of grass jelly leaves could lower total blood cholesterol level in hypercholesterolemic condition.

Triglyceride Level

TG level in negative control treatment (P1) was not significantly different from P3 treatment (P>0.05) but was significantly different from P2 treatment (P<0.05). TG level in positive control treatment (P2) rose up to 31.69% from the control group (P1). TG levels at P3 treatment group was 71.08 mg/dL (decreased by 27.51%). It marked that chlorophyll extract of green grass jelly leaves could lower TG level in hypercholesterolemic condition.

***High-Density Lipoprotein* Level**

HDL level in control treatment (P1) was not significantly different (P>0.5) from P2 treatment but was significantly different from P3 treatment (P <0.05). HDL level in P3 treatment was increased by 43.22% from its positive control (P2). It indicated that chlorophyll extract of grass jelly leaves was able to raise HDL levels in hypercholesterolemic condition.

***Low-Density Lipoprotein* Level**

LDL level in control treatment (P1) was not significantly different (P>0.05) from P3 treatment but significantly different in P2 treatment (P<0.05). LDL level in P3 treatment decreased by 83.65% from its positive control (P2). It indicated that chlorophyll extract of green grass jelly leaves could lower LDL levels in hypercholesterolemic condition.

Total Cholesterol/*High-Density Lipoprotein* Ratio and Atherogenic Index

Atherogenic index and the total HDL cholesterol ratio is a ratio between total cholesterol number divided by HDL. This comparison can be used to determine any possibility of atherosclerotic plaque formation. Rat consuming high-fat diets had elevated total cholesterol levels and decreased HDL cholesterol. It allegedly resulted in increased atherogenic index of rat consuming fatty diets. A desired lower atherogenic index shall be obtained if decreased cholesterol levels were followed by an increase in HDL cholesterol level. Results of the study as showed on Table 1 pinpointed that green grass jelly leaves chlorophyll extract could lower atherogenic index and total cholesterol to HDL ratio in hypercholesterolemic conditions.

DISCUSSION

Cholesterol production that is synthesized in the body is affected by the amount of cholesterol in the diet. When consumed and absorbed cholesterol intake is less than the body needs, it will cause more cholesterol be synthesized by the liver. Cholesterol synthesis occured due to an existing feedback between exogenous cholesterol production derived from foods and endogenous cholesterol in the liver (Linder, 2006). Provision of high-calorie diets, especially those high in carbohydrates did not affect total cholesterol levels in the body, where an increase is influenced by a high-fat diet. Research in rats by providing 0.05% of cholesterol in their diet will cause 70-80% of cholesterol synthesis in the body. However, if the provision of cholesterol intake were leveled up, it will lead to cholesterol synthesis limitation in the body (Marks *et al*., 2000). The statement supported this research by showcasing that the number of food intake containing high-calorie diet is less affective on cholesterol levels where hypercholesterolemia is more influenced by foods containing high cholesterol.

Chlorophyll derived from grass jelly leaves could suppress cholesterol abundance. It can suppress the formation of cholesterol and LDL, similar to Meynita's (2010) research which testify its ability derived from alfalfa grass to hold down an increase of cholesterol and LDL in rat blood. Chlorophyll can lower cholesterol levels by preventing the smooth muscle cells proliferation, improving liver function to remove cholesterol from the blood to bile and thus generates lower blood cholesterol levels.

According to Limantara (2009), chlorophyll structure has two parts which are porphyrin and phytol. Phytol is a hydrophobic alcohol-like compound which could bind and remove it through the excretion system. It is also lipophilic, enabling it to bind fats and cholesterol as well as remove it through excretion system and absorb cholesterol from the bile and foods by utilizing cell wall's absorption function. It could lead to decreased levels of lipid deposits as well as to prevent inhibition in the blood vessels.

Chlorophyll extract of grass jelly leaves possesses six types of phytochemical compounds, namely alkaloids, flavonoids, phenolics, triterpenoids, steroids, and coumarin (Novelina, *et.al* 2015). Flavonoids could suppress cholesterol by improving endothelial cells function as factor that regulates hemostatis having role of inhibiting blood coagulation (Vita, 2005). Flavonoids can reduce cholesterol levels in the blood by limiting cholesterol synthesis through inhibiting *acyl-CoA cholstrol acyl transferase* (ACAT) in HepG2 cells acting in lowering cholesterol esterification in the intestine and liver, thereby inhibiting the activity of 3-hydroxyl-3-methyl glutaril-CoA enzyme which hampers cholesterol synthesis (Metwally *et al.*, 2009). Furthermore, according to Tian *et al.* (2011) flavonoids can inhibit Fatty Acid Synthase (FAS) which is an important enzyme in fat metabolism. Barriers at FAS directly reduce the formation of fatty acids. Thus fatty acids reduction generates a decrease in triglycerides formation.

Excessive amount of HDL is not needed to meet cholesterol needs in the liver. The function of HDL and LDL are contradictory. Cholesterol is transported by LDL from the liver to peripheral tissues and accumulated there. LDL is atherogenic because it triggers coronary artery calcification, whereas HDL transports cholesterol from peripheral tissues to the liver to prevent calcification. When LDL decreases, more HDL would be needed to meet cholesterol shortage in the liver in order to form bile acids.

High-density lipoprotein (HDL) is known as good cholesterol serves in transporting cholesterol in the blood from the body tissues back to the liver for elimination. A high HDL levels in the blood denote good condition (Uren and Colins, 2008). A research output pointed that HDL levels were decreased due to increased LDL levels caused by too much HDL is not required to meet the cholesterol in the liver. The function of HDL and LDL contradictory. Sent by LDL cholesterol from the liver to peripheral tissues and stockpiled there. LDL is atherogenic because mneybabkan calcification of coronary arteries, whereas HDL cholesterol transport function of the network periphel to the liver to prevent calcification. The decrease in LDL, then HDL will be a lot more is needed to meet the shortage of cholesterol in the liver to form bile acids. Such conditions would stimulate HDL synthesis in the liver that increases HDL levels. Elevated LDL levels will result in decreased HDL levels in the blood.

In addition to focusing on the number of total cholesterol level, TGA, HDL, and plasma LDL, ratio with HDL cholesterol gives essential point whether it will lead to atherosclerotic plaques formation in experimental animals. According to Usoro *et al.* (2006), lower atherogenic index indicated high levels of HDL cholesterol. The risk of various cardiovascular disease can be reduced based on the index. Hara *et al.* (2002) suggested that atherogenic index value above 3 in children means they are at risk for cardiovascular disease. Baraas (1994) argued that an ideal atherogenic index for men is below 4.5 while for women is below 4.0. According to Bhattacharjee and Srivastava (1993), atherogenic index with ≥ 5.0 value is at risk of heart disease.

CONCLUSION

Administration of chlorophyll extract of green grass jelly leaves into rat's high-fat diet was able to suppress total cholesterol, LDL, and triglyceride levels as well as to increase HDL level in rat's blood. In addition to that, the chlorophyll extract also able to lower the atherogenic index, which also means to reduce the risk of coronary heart disease.

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