ABSTRACT

Saponins in the suspect can inhibit cholesterol absorption directly and indirectly in the gut so it can reduce plasma total cholesterol and bile acids, the various mechanisms that are still unclear. The purpose of this study is to prove that saponin intake of Cordyline terminalis Kunth leaf, can inhibit cholesterol absorption directly and indirectly, thus lowering plasma cholesterol and total bile acids, by increasing the excretion total cholesterol and bile acids of feces. The study design was a randomized post-test only control group design, conducted in Wistar rats. Twenty-five rats were divided into four groups; Control, treatment 1, treatment 2, and treatment 3, each of 6 rats. The control group was given only the standard diet, treatment 1 was given high-cholesterol foods, treatment 2 was given high-cholesterol foods and Cordyline terminalis Kunth leaf saponin 30 mg / day, and treatment 3 was given high-cholesterol foods and gemfibrozil 30 mg / day. After treatment 30 days and the rats were fasted 18 hours, blood
samples were taken and plasma separated for examination of cholesterol, and bile acids. Three days last treatment the rats feces were collected for examination of total cholesterol and total bile acids. The results showed an increase in total cholesterol, LDL cholesterol, triglycerides, ratio of total cholesterol / HDL and a decrease in HDL cholesterol, and excretion fecal total cholesterol, total bile acids were significantly (p <0.05). In treatments 2 happen otherwise.

Based on the results of this study concluded that the intake of saponins Cordyline terminalis Kunth leaves can inhibit the absorption of cholesterol directly and indirectly in the intestine, thus lowering plasma cholesterol and bile acids by increasing the excretion of fecal total cholesterol and total bile acids.

**KEYWORDS:** saponins, total bile acids, total cholesterol, foods high in cholesterol.

**INTRODUCTION**

Saponins are natural detergents that have surface active properties, in which the molecular structure consists of a steroid or triterpene aglycone called sapogenin and glycon that contain one or more sugar side chains (Osbourn, 2003; Guclu-Ustundag and Mazza, 2007; Vincken, et al., 2007).

Saponin derived from the Latin word 'sapo' which shall mean containing stable foam when dissolved in water. The ability of saponins foam is caused by a combination of hydrophobic sapogenin (fat soluble) and hydrophilic part of sugar side chains (soluble in water) (Naoumkina, et al., 2010). Saponins with their detergents properties can affect substance fat-soluble in digestion, include the formation of a mixed micelles containing bile salts, fatty acids, diglycerides and fat-soluble vitamins as well as saponins are capable of forming complexes with metals such as Fe, Mg, Zn, and Ca (Southon, et al. 1988; Cheeke, 2001). Saponin acts as an emulsifier and can stabilize the interface of oil / water and also has the ability to dissolve monoglycerides. Based on these activities is believed that saponins able to emulsify fats (Cheeke, 2001).

Family Liliaceae known as one of the families that are very rich in saponins. Garlic also includes the family Liliaceae containing steroidal saponins (Matsuura, 2001). Plants Cordyline terminalis Kunth including Liliaceae family which contains saponins. Based on the research results that the Cordyline terminalis leaves contain steroidal saponins but the
Some researchers reported that the saponin from other plants such as garlic steroid glycosides (Matsuura, 2001), and chloragin of *Chlorophytm nimonii* (Lakshmi, *et al*., 2012) and also from the alfalfa triterpene glycosides of *Medicago sativa* L. (Khaleel, *et al*., 2005) soyasaponins (Sun-Ok Lee, *et al*., 2005), Quillaja saponaria (Cheeke, 2001), and ginseng (Ha and Kim, 1984) have activity as hipocholesterolimea which can inhibit cholesterol absorption and lowers plasma cholesterol concentrations that have been tested in animals and humans (Kim, *et al*., 2003; Zhao, *et al*., 2005; Afrose, *et al*., 2010;). However, the mechanisms responsible for these activities is not clear. Saponins could be expected to prevent formation of micelles with cholesterol during digestion in the small intestine, thus reducing the availability of cholesterol for absorption into enterocytes. Saponins are also thought to inhibit the absorption of cholesterol from micelles and inhibits the reabsorption of bile acid and cholesterol synthesis due to the interaction of saponins with bile acids to form large mixed micelles are not soluble so it can not be absorbed by intestines and is excreted through the feces (Zhao, *et al*., 2005; Lee Sun-Ok, *et al*., 2005). Inhibition of reabsorption of bile acids from the intestine stimulate the metabolism of cholesterol in the liver then converts it into bile acids (Jenkins and Atwal, 1994; Shin, *et al*., 2004; Han, *et al*., 2000). Inhibition of cholesterol absorption in the intestine is also suspected due to the saponin compounds form complexes with cholesterol, but its mechanism of action is unclear.

**MATERIALS AND METHODS**

**Diet**

Saponin isolated from the leaves of *Cordyline terminalis* Kunth were grown in Tampak Siring Bali. Saponin extracted by maceration and precipitation and then analyzed by high-performance liquid chromatography (HPLC; Bogoriani, 2001; Bogoriani, 2008).

Saponin dose was given 30 mg / day. Diet control as standard food for rats is CP 551 has the composition of 13% water content; protein from 18.50 to 20.50%; 4% fat; fiber 6%; 8% ash; 0.90% calcium and 0.70% phosphorus. Foods high in cholesterol are the standard diet (60%) and duck egg yolk (40%).

**Animal**

White male Wistar rats as research protocol was taken from laboratory Center Study of Animal Diseases (CSAD) Faculty of Veterinary Medicine, University of Udayana. Twenty-
four Wistar rats, 11-12 weeks old and weight 150-200 g were divided into 4 groups, a control group, three treatment groups. Treatment-1 group was treated rats fed high-cholesterol only, treatment-2 group was treated rats fed a high cholesterol and saponins 30 mg/day. treatment-3 group of rats was fed a high cholesterol and gemfibrozil 30 mg/day, each group of 6 rats. Rats from each group individually caged at room temperature, with a 12:12 hours light:dark cycle. Rats were treated for 30 days and a free drink ad libitum. After 30 days of treatment the rats were fasted to draw all the food and drinks for 18 hours. Blood was taken via the orbital sinus, accommodated in blood tubes containing EDTA solution and then centrifuged at 5000 g for 15 min at 4 °C to obtain plasma then frozen until analysis.

**Analysis of Plasma Lipids and Total Bile Acids**

Determination of plasma total cholesterol CHOD-PAP method by E. Merck. CHOD-PAP method is an enzymatic-colorimetric test is highly specific for the measurement of the area of light that can be seen by the eye, and can be distinguished from the others because of the high fleksibelitasnya. Total cholesterol is calculated as: absorbance of sample divided by the absorbance of standard cholesterol (0.240) multiplied by constant of the standard cholesterol (200mg/dl).

The principle of HDL analysis according to Human (1980), namely the provision of phosphotongstic acid and magnesium ions in the sample so that chylomicrons, VLDL and LDL will settle. HDL levels calculated with the way the sample absorbance multiplied by 318 (mg / dl).

Plasma LDL cholesterol checks were done by reducing total cholesterol with VLDL and HDL, VLDL whereas calculations performed using triglycerides, VLDL which is equal to one-fifth (1/5) triglycerides.

Triglycerides examination conducted by the GPO-PAP method. Triglycerides are determined after enzymatic hydrolysis with lipase. Quinoneimmin indicator formed from hydrogen peroxide, 4-aminoantipirin and 4-chlorophenol under the catalytic influence of peroxide. Triglyceride levels calculated as follows: sample absorbance divided by the standard absorbance triglycerides (0.145) multiplied by a constant of triglycerides (200mg/dl).

Examination of the total plasma bile acids done with the kit rat total bile acids (cristal chem. Inc. USA) in units of μmol / L with a spectrophotometric method. Rat total bile acids kit is
based on enzymatic technology of 3-α-hydroxy steroid dehydrogenase. The presence of NAD, bile acids are converted into 3-keto steroids and NADH. The formation of NADH reacts with blu nitrotetrazolium (NBT) to form color. Color formation was monitored by measuring the absorbance at 540 nm and direct measurement of rat plasma concentration of bile acids.

**Analysis of total cholesterol and fecal total bile acid**

Three days or 72 hours end of the study period. The number of fecal total cholesterol was measured by spectrophotometry 30 IKM. Two grams of feces was extracted with hexane. Extracted while shaken and heated with a water bath. Pipetted 0.3 ml of sample and standard solutions. Then heated in a water bath at 80 ° C for 5 min and then stored in a 105°C oven for 30 minutes. Cool at room temperature and add 4 ml of acetic anhydride, glacial acetic acid and concentrated sulfuric acid, shaken and allowed to stand 35 minutes, then read on a spectrophotometer at a wavelength of 630.

Examination of fecal bile acids done by weighing one gram of feces then macerated in methanol: n-butanol 1:2 for 24 hours and then filtered. Take 1 g aliquots enter into a 50 ml tube and added 300 mL 1μg/100 mL cholic acid as an internal standard, and then added a solution of sodium borohydride and shaken for 1 hour to reduce the 3-keto-BAS. Weigh the body weight of rats before and after the three-day gathering. For saponification BAs add 500 mL 2 N HCl and 2 ml of 10 N NaOH, after the process is filtered. Pipette 20 mL of sample and plus Cristal Chem Rat Total bile Acids Kit for measurement of total fecal bile acids with a spectrophotometer.

**Statistical Analysis**

Statistical analysis was performed with the statistical analysis system. Values are expressed as mean ± SD. Results were analyzed by one-way ANOVA, and differences between treatments were determined by a least-significant-difference test (LSD). Alpa 0.05 is used to determine statistically significant differences.

**RESULTS AND DISCUSSION**

**RESULT**

The initial data (control) and after feeding with high cholesterol diet (HC), HC plus saponin (HC+SP) and HC plus gemfibrozil (HC+GB) of plasma TC, LDL-C, HDL-C, TG, TC/HDL-C ratio and bile acid and fecal cholesterol and bile acid can be seen in Table 1 and Figure 1.
The levels of plasma TC (157.83±9.11 vs. 64.83±8.13 mg/dl, p<0.05), LDL-C (95.53±4.27), TG (169.67±4.72), TC/HDL-C ratio (5.48±0.34) were higher significantly; the levels of HDL-C (28.87±1.05) was lower significantly; and no any difference of levels of bile acid in Wistar rat after feeding with high cholesterol compared to control group. Saponin actually could lower and reversed close to control group of the levels of plasma TC, LDL-C, TG, TC/HDL-C ratio and bile acid, and increased the levels of HDL-C in Wistar rat after feeding by high cholesterol. Saponin decreased significantly levels of plasma TC (79.83±9.11 vs. 157.83±9.11 mg/dl, p<0.05), LDL-C (25.57±6.40), TG (36.00±5.55), bile acid (25.10±0.34) and TC/HDL-C ratio (1.69±0.19); and increased significantly the levels of HDL-C (47.50±4.63) in Wistar rat feed high-cholesterol compared to without saponin. The effects of gemfibrozil, well known an active drug for lowering levels of trygliceride and increasing HDL-C levels, as comparison in the study could be noted also in Table 1 and Figure 1.

The levels of fecal cholesterol (212.82±59.84) vs. 576.95±115.33 mg/kg, p<0.05, and bile acid (3.75±0.38) vs. 18.65±0.76 were higher significantly in Wistar rat after feeding with high-cholesterol compared to control group. Saponin increased significantly levels of fecal cholesterol (1401.29±168.38) and bile acid (34.31±0.88) in Wistar rat feed high-cholesterol compared to without saponin.

Table1. Plasma TC, LDL-C, HDL-C, TG, TC/HDL-C ratio, and bile acids, and fecal cholesterol and bile acid in control, HC, HC+SP, and HC+GB in Wistar rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>HC</th>
<th>HC+SP</th>
<th>HC + GB</th>
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</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>64.83±8.13</td>
<td>157.83±9.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.83±9.11&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>105.33±5.43&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>13.67±7.07</td>
<td>95.53±4.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.57±6.40&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>38.77±3.12&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42.27±4.76</td>
<td>28.87±1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.50±4.63&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>58.07±3.97&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>52.67±1.86</td>
<td>169.67±4.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.00±5.55&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>42.33±6.68&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>1.53±0.11</td>
<td>5.48±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.69±0.19&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.82±0.74&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bile acid (μmol/L)</td>
<td>45.79±0.69</td>
<td>46.23±0.84</td>
<td>25.10±0.34&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>47.17±1.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fecal Cholesterol (mg/Kg)</td>
<td>576.95±115.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>212.82±59.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1401.29±168.38&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1151.99±208.52&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bile acids (μmol/day/100 g BW)</td>
<td>18.65±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.75±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.31±0.88&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>26.42±1.18&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglyceride; BW, body weight; HC, wistar rats feed with high cholesterol; SP, saponin; GB, gemfibrozil. <sup>a</sup>Represents significant difference from control at p < 0.05; <sup>b</sup>Represents significant difference from high cholesterol at p < 0.05.
Figure 1. Levels of plasma total cholesterol (A), plasma bile acid (B), fecal cholesterol (C), and fecal bile acid among control and treated groups.

HC, Wistar rats feed with high-cholesterol; SP, saponin; GB, gemfibrozil.

DISCUSSION

In this study, an increase that was significant (p < 0.05) mean total cholesterol, LDL cholesterol, TG and the ratio of total cholesterol / HDL cholesterol and a significant decrease (p <0.05) mean plasma HDL cholesterol blood wistar rats were given a high-cholesterol diet (treatment 1), compared to control rats. Besides cholesterol comes from the food consumed, can also be synthesized by the body itself so that although the control rats not given feed containing cholesterol, but still containing blood plasma cholesterol.

The increase in total cholesterol, LDL cholesterol, TG, ratio of total cholesterol / HDL cholesterol (above 4) and a decrease in HDL cholesterol is a sign of the potential occurrence of dyslipidemia that encourage the formation of atherosclerosis is a risk factor for coronary heart disease (Bhat, et al., 2003; Khaleel, et al., 2005). Dyslipidemia is an abnormal lipoprotein metabolism that lead to atherosclerosis. Atherosclerosis is a condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol and become one of the risk factors for cardiovascular disease (Charlton-Menys and Durrengton, 2006). The results are consistent with studies in rats given a high-cholesterol diet there was
an increase in total cholesterol, LDL cholesterol, TG and HDL cholesterol decrease significantly (p <0.01) (Lakshmi, et al., 2012).

The increase in total cholesterol and LDL cholesterol also occurred in studies with rabbits fed a high cholesterol 28 days (Khaleel, et al., 2005). The increase in total cholesterol, LDL cholesterol and decrease HDL cholesterol in the blood serum of laying hens were given a high-cholesterol diet for 60 days also occur with significant (p <0.05) (Afrose, et al., 2010).

In the test rats given high-cholesterol foods and Cordyline terminalis leaf saponin 30 mg / kg (treatment 2) a decrease in total cholesterol, LDL cholesterol, TG, ratio of total cholesterol / HDL cholesterol and total bile acids and increased plasma HDL were significantly (p <0.05) compared to rats given high-cholesterol diet (treatment 1). The results of this study are supported by the results of the study reported by Al-Matubsi, et al., 2011, that diosgenin saponins can lower total cholesterol, LDL cholesterol and increasing HDL cholesterol with a very significant difference (p <0.01). Karaya saponin can lower total cholesterol, LDL cholesterol and increase HDL cholesterol blood serum of laying hens (Afrose, et al., 2010). Steroidal saponins from Chlorophytum nimonii also can lower total cholesterol, LDL cholesterol, TG and raising HDL cholesterol with a highly significant (p <0.01) (Lakshmi, et al., 2012). Soyasaponin, alfalfa saponins and saponin platycodin also shown to reduce total cholesterol, LDL cholesterol, TG and the ratio of total cholesterol / HDL cholesterol with a significant (p <005) (Khaleel, et al., 2005; Sun-Ok Lee, et al., 2005 ; Zhao, et al., 2005). Intake of Cordyline terminalis leaf saponins were able to reduce total cholesterol, LDL cholesterol, TG, total bile acids and the ratio of total cholesterol / HDL cholesterol and increasing HDL cholesterol with a significant difference (p <0.05) compared with treatment 1 (HC). From the results of these studies indicate that saponins of the Cordyline terminalis leaves have biological activity as anticholesterol or that can prevent the occurrence of atherosclerosis which is one of the risk factors for coronary heart disease.

Based on the literature, saponin is a plant glycosides that has amphiphilic properties and surface active agents. Biological activity is determined by its chemical structure which will determine the polarity, hydrophobicity, and acidity of the compound. Biological activity of saponins increased with increasing number of sugar side chains (number of polar groups) are bound to their aglycone or sapogenin. The strength of the sugar chains also affect the biological activity of both triterpene saponins and steroidal saponins with a single sugar chain is more active than the two sugar chains. Stereochemistry of the terminal sugar chain and the
steroid nucleus is very important to determine the overall activity of the saponin molecule as it affects the overall conformation of saponins. In general, the cluster aglycone and glycon of saponins plays an important role in its biological activity as the polar part. Cholesterol, bile acids, the OH group, the steroid core, the C = C group of saponins interact to form aggregates such as micelles and form insoluble complexes (Siswandono and Soekardjo, B., 1995; Abid Ali Khan, et al., 2012).

Structure of *Cordyline terminalis* leaf saponins have a single sugar chain and consists of three sugars (2 L-ramnosa and 1 D-fukosa) are bound to their aglycone with each molecular weight 866 g / mol and 868 g / mol (Bogoriani, 2001; Bogoriani, 2008) so the ability to lower plasma cholesterol in rat blood is higher than gemfibrozil. Based on the data generated in the study showed that the ability of *Cordyline terminalis* leaf saponins to bind cholesterol greater than gemfibrozil with a significant difference (p <0.05). Interaction of the saponins with cholesterol and bile acids is thought to involve hydrogen bonding (OH group), hydrophobic bonding or van der Waals bonds (the steroid nucleus) and electrostatic bonds (C = C) so as to allow the formation of complex stability is quite large and insoluble or formed large mixed micelle thus causing the barrier to the absorption of cholesterol through NPC1L1 in intestinal enterocytes. Saponins including compounds having high molecular weight and then interact with cholesterol and bile acids so that the molecule a large increase resulting in the intestinal absorption barriers that cause a decrease in plasma cholesterol and bile acids, which are then excreted into the feces more thereby increasing the concentration of feces cholesterol and bile acids.

Some researchers reported that saponins with cholesterol in the gut, not absorbed as to form an insoluble complex compounds, so that it can directly reduce cholesterol into the body by NPC1L1. Barriers directly from cholesterol absorption of saponins may cause not only prevent the absorption of cholesterol from the proportion of cholesterol high food, but also proportion of cholesterol a high carried from bile and mucosal cell desquamation (Hostettmann and Marston, 1995; Shin, et al., 2004; Lin, et al., 2005; Sun-Ok Lee, et al., 2005).

In this study also found that the *Cordyline terminalis* leaves saponin intake can lower plasma total bile acids with significant differences (p <0.05) compared with the control and treatment 1 (HC). Some researchers reported that the saponin to form micelles with bile acids, resulting in the ability of bile acids to form micelles with reduced fatty acid, saponin is suspected bile
acid sequestrants (Cheeke, 2001), which is supported by the observation Oakenfull and Fenwick, (1990) that the saponins are capable of binding bile acids in vitro. Based on these results, saponins of Cordyline terminalis leave possibility of forming micelles great with bile acids, thereby blocking or inhibiting the re-absorption of bile acids in the enterohepatic circulation by the apical sodium codependent bile acid transporter (ASBT) or ileal Na + / bile acid cotransporter (Ibat), so causes a decrease in total plasma bile acids directly and decrease plasma cholesterol indirectly and an increase in bile acid excretion via the faeces. Inhibition of bile acid uptake by inhibiting ASBT is a target to increase liver bile acid synthesis and reduces plasma LDL cholesterol. Barriers to uptake of bile acids in the enterohepatic circulation by ASBT can reduce the risk of atherosclerosis (Bhat, et al., 2003). Apical Sodium Bile Acid Transporter codependent (ASBT) is an important component that mediates the enterohepatic circulation of bile acid re-absorption in the terminal ileum (Shneider, 2001). Specific inhibition of ASBT protein will block the re-absorption of bile acids in the terminal ileum and stimulate its excretion via the faeces, thus reducing the amount of bile acids returning to the liver (Bhat, et al., 2003).

**CONCLUSION**

Based on the above results and discussion of a conclusion can be drawn as follows:

1. Intake andong leaves saponin lowered blood plasma total cholesterol of Wistar rats were given a high-cholesterol diets.
2. Intake andong leaves saponin lowered blood plasma bile acids of Wistar rats were given a high-cholesterol diets.
3. Intake andong leaves saponin increased the excretion of feces total cholesterol of Wistar rats were given a high cholesterol diets.
4. Intake andong leaves saponin increased the excretion of feces total bile acids of Wistar rats were given a high-cholesterol diets.
5. Intake andong leaves saponin can reduce blood plasma cholesterol of Wistar rats in two ways: by inhibiting the absorption of cholesterol and bile acids in the intestine thereby increasing the excretion of cholesterol and fecal bile acids.

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REFERENCES


