Research Article

The Effectiveness of Green Grape Extract (*Vitis vinifera*) on Decreasing White Rat (*Rattus novergicus*) Triglycerides Levels

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ABSTRACT

**Background:** Grapes (*Vitis vinifera*) are a source of antioxidants with high content of polyphenols and anthocyanins. Grape skin is rich in flavonoids. Anthocyanins and flavonoids have the effect of lowering blood triglyceride levels. **Purposes:** This study aimed to determine the effect of using green grape extract (GGE) in lowering triglyceride levels in the blood. **Methods:** Five groups of white male rats (*Rattus novergicus*) were divided into a negative control group (KN), positive control (KP), rats with a GGE dose of 100 mg/200g BW as P1, rats with a GGE dose of 250 mg/200g BW as P2, and rats with GGE dose of 500 mg/200g BW as P3. Each group was given a hypercholesterolemic diet for five weeks. Groups P1, P2, and P3 were given GGE according to their respective doses for 14 days, then measured triglyceride levels in the blood. **Results:** The results showed that GGE 500 mg/200g BW significantly reduced triglyceride levels in all treated mice. **Conclusion:** These results indicate that GGE has a great potential to treat dyslipidemia by lowering triglyceride levels in the blood.

**Keywords:** dyslipidemia, green grape extract, triglyceride level

INTRODUCTION

A lifestyle that tends to consume foods high in fat and cholesterol can cause fat and cholesterol levels in the blood to be higher than average (1,2). The composition of a healthy body is that the amount of protein must be more significant than fat. The role of protein is vital. One of them is supporting the immune system (3).

Excessive consumption of carbohydrates and saturated fats can stimulate the release of cytokines such as TNF-α (tumor necrosis factor-alpha); increased TNF-α levels can lead to insulin resistance, suppress the oxidation of fatty acids in the liver, and improve cholesterol synthesis by hepatic cells. This can cause problems and abnormalities in the heart, such as coronary heart disease (4,5).

Coronary heart disease (CHD) can occur due to the accumulation of lipid plaques in the artery walls and cause narrowing of arteries (atherosclerosis). High levels of total cholesterol, LDL (Low-Density Lipoprotein), triglycerides, and low HDL levels (High-Density Lipoprotein) result in high levels of lipids (6,7). One of the drugs that can reduce lipid levels is statins. Statins are a class of drugs used to lower lipid levels. Statins work by
inhibiting cholesterol synthesis and increasing the number of LDL receptors on liver cell membranes (8). Statins have serious side effects such as myopathy and hepatotoxicity. Therefore, new treatment treatments are needed to lower lipid levels (9).

The trend of “back to nature” in society in maintaining the health of the body raises the concept of “functional food” that can cure or eliminate the harmful effects of certain diseases and does not cause toxins at specific doses. Traditional medicines derived from plants also have side effects that are much lower in danger than chemical drugs (10).

Grapes (Vitis vinifera) are one of the fruits in Indonesia which is a source of antioxidants with a high content of polyphenols and anthocyanins (11). In Indonesia, grapes are widely consumed because of their sweetness and have many health benefits. Several types of grapes that thrive in Indonesia include Probolinggo grapes, Caroline grapes, and Balinese grapes, more commonly referred to as Buleleng grapes because they are widely grown in the area and are a type of fruit known as the typical Buleleng fruit (11).

Grape skin is rich in flavonoids. It is generally known that flavonoids have antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor, and antioxidant activity with higher power than vitamin C (12). Grape seed extract is known to have strong antioxidant properties, especially the content of proanthocyanidins which have greater antioxidant power when compared to other antioxidants such as vitamin C, vitamin E, and β-carotene in protecting cells from DNA damage and lipid peroxidation due to free-radical chain reactions (13).

Several studies have shown that red grapes contain active substances, such as anthocyanin, proanthocyanidin, procyanidin, flavonoids, polyphenols, and resveratrol (14,15). Resveratrol is also found in grape skins; it can inhibit the accumulation of lipids in the body. Pterostilbene is one of the ingredients in grapes that have pharmacological effects (16). Pterostilbene has shown many protective benefits against atherosclerosis (17).

METHODS

Experiment Design

The study carried out was an experimental study using a pre-test and post-test control group design using 25 adult male white rats (Rattus norvegicus). The sample was divided into five groups. The five sample groups were tested for triglyceride levels before and after treatment. The results of the triglyceride levels examination were then analyzed and presented using descriptive analysis, data normality test, data homogeneity test, comparability test, and treatment effect test.

The dose of the green grape extract (GGE) given in this study was determined based on research conducted by Saputra, Frans, et al (18). The research was carried out on white male rats (Rattus norvegicus) according to the sample determined in the study, which was conditioned in a state of > 150 mg/dL triglycerides, using ethanol extract of green grapes to triglyceride levels. To determine the number of samples, used Federer’s formula as follows: 

$$(t-1)(n-1) < 15,$$  
where $t =$ number of groups;  
n = number of subjects per group (19). 

Based on the results of sample calculations, it is known that the number of samples for each treatment group is five individuals.
This research was conducted in several places, including Bio Pesticides laboratory, Faculty of Agriculture, University of Udayana – Bali, to manufacture GGE. Animal Laboratory Unit of the Pharmacology Section of the Faculty of Medicine, Universitas Udayana, for the treatment of experimental animals, and in the Clinical Pathology Laboratory of Sanglah Hospital-Bali, to check blood triglyceride levels in samples.

Homogeneous male rats that qualify as samples based on inclusion criteria were then sampled with a simple random sampling technique; white rats were divided into five groups. KN group is a negative control, KP group as a positive control, while group P1, P2, and P3 is the treatment group. The sample size for each group was determined based on Federer’s calculation formula, which resulted in a minimum of 5 rats per group (5 groups). The study sample inclusion criteria are: (1) White Rat (Rattus norvegicus), 3-4 months olds; (2) male sex; (3) weighting 180-200 g; (4) Blood triglyceride levels >150mg/dL.

Drop-out criteria are rats in a state of illness/death while the study is ongoing. The study was approved by the Health Research Ethics Commission of Polytechnic of Health Denpasar (LB.02.03/EK/KEPK/0161/2019).

**Preparation of GGE**

The grapes were washed and then thinly sliced, and the grapes were dried in the shade until dry. Dried grapes are then crushed to form a powder. Grapes that have been powdered are then filtered to obtain a fine powder. The fine powder was then soaked in 96% ethanol for 24 hours. Ethanol-soaked grape powder 96% with a ratio of 1:7 for 3-4 days, the marinade is filtered through a glass funnel has been coated with filter paper and obtained extract wine 1, residue re-macerated with ethanol 96% with a ratio of 1:4 for 3-4 days, the marinade is filtered through a glass funnel has been coated with filter paper and obtained extract grape 2, grape extract one mixed with grape extract 2, liquid grape extract evaporated by using a rotary evaporator until thick extract obtained.

**Hypercholesterolemic Feed Making**

Hypercholesterolemic feed with a composition of 100g (10%) lard, 50g (5%) egg yolk, 10g (1%) cooking oil, and up to 100% standard feed. The fat was heated until it melted, and the yolk was taken from the boiled egg and then mixed with oil and common feed, which was given ad libitum for 35 days (18).

**Preparation of Simvastatin Solution**

Simvastatin is made in the form of a solution by dissolving 10 mg (1 tablet) of
Simvastatin in 100 ml of solvent (distilled water) so that 1 ml contains 0.1 mg of simvastatin. The dose used for rats with an average weight of 200g is 0.2 mg/day.

**Animal Feeding**

According to the study group, the treatment was carried out by feeding orally with a predetermined formulation. KN animals received hyper-cholesterolemic feed for five weeks. KP animals received a hypercholesterolemic meal for five weeks and were provided with 2 ml of simvastatin for 14 days. P1 animals received hypercholesterolemic feed for five weeks and were fed with 1st dose (100mg/200g body weight) of GGE for 14 days. P2 animals received a hyper-cholesterolemic meal for five weeks and were provided with 2nd dose (250mg/200g BW) of GGE for 14 days. P3 animals received hypercholesterolemic feed for five weeks and fed with 3rd dose (500mg/200g BW) of GGE for 14 days.

**Triglyceride Levels Determination**

Triglycerides were estimated by enzymatic methods by using the diagnostic kit TRIGL Cobas Substrates. This method uses a lipoprotein lipase for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dye. The color intensity of the red dye formed is directly proportional to the triglyceride concentration and can be measured photometrically (20). The absorbance was measured at 512/659 nm, and the results were expressed as mg/dl.

**Statistical Analyses**

Triglyceride levels data before and after treatment in each group were tested for normality using a histogram graph with a standard curve. It is known that all groups show a standard curve. They were then continued with the Shapiro-Wilk test. The result showed that the data are typically distributed (p>0.05).

**RESULTS**

Triglyceride level data between groups before and after treatment were tested for homogeneity using Levene’s test. The result showed that the data was homogenous. Comparability Test of Triglyceride Levels. The mean levels of triglycerides in the control group before and after treatment were given a comparative test using the ANOVA test. Data analysis results are presented in Table 3.

**Table 1. Anova Test Results from Mean Triglyceride Levels Before and After Treatment**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>x</th>
<th>S</th>
<th>F</th>
<th>p</th>
<th>x</th>
<th>S</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>KN</td>
<td>5</td>
<td>104.4±37.6</td>
<td>1.41</td>
<td>0.255</td>
<td>108.9±20.6</td>
<td>3.341</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KP</td>
<td>5</td>
<td>122.4±36.5</td>
<td>1.41</td>
<td>0.255</td>
<td>105±42.2</td>
<td>3.341</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>5</td>
<td>106.6±26.1</td>
<td>1.41</td>
<td>0.255</td>
<td>100±26.9</td>
<td>3.341</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>5</td>
<td>106.8±32.3</td>
<td>1.41</td>
<td>0.255</td>
<td>106±32.4</td>
<td>3.341</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>5</td>
<td>77±11.1</td>
<td>1.41</td>
<td>0.255</td>
<td>56.8±18.9</td>
<td>3.341</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the results of the ANOVA test as in table 1, it is known that for all sample groups, the p-value before treatment is 0.255 greater than 0.05. This means that there is no significant difference in the mean levels of triglycerides before treatment. While the triglyceride levels after treatment showed a significant difference with a p-value of 0.03 where p was less than 0.05. This meant that there were differences in triglyceride levels after treatment. Because there is a difference in the mean of triglyceride after treatment, it is
followed by the LSD test, which results were obtained as presented in Table 2.

### Table 2. Post Hoc Least Significant Difference (LSD) Test

<table>
<thead>
<tr>
<th>Groups</th>
<th>p-value</th>
<th>LSD Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>KN – KP</td>
<td>0.020</td>
<td>Sig. dif.</td>
</tr>
<tr>
<td>KN – P1</td>
<td>0.620</td>
<td>No sig. dif.</td>
</tr>
<tr>
<td>KN – P2</td>
<td>0.205</td>
<td>No sig. dif.</td>
</tr>
<tr>
<td>KN – P3</td>
<td>0.007</td>
<td>Sig. dif.</td>
</tr>
<tr>
<td>KP – P1</td>
<td>0.056</td>
<td>No sig. dif.</td>
</tr>
<tr>
<td>KP – P2</td>
<td>0.238</td>
<td>No sig. dif.</td>
</tr>
<tr>
<td>KP – P3</td>
<td>0.620</td>
<td>No sig. dif.</td>
</tr>
<tr>
<td>P1 – P2</td>
<td>0.429</td>
<td>No sig. dif.</td>
</tr>
<tr>
<td>P1 – P3</td>
<td>0.020</td>
<td>Sig. dif.</td>
</tr>
<tr>
<td>P2 – P3</td>
<td>0.101</td>
<td>No sig. dif.</td>
</tr>
</tbody>
</table>

*Sig. dif. = significant difference

**Triglyceride Mean Difference Test Before and After Treatment**

Triglyceride levels before and after treatment [KN: hypercholesterolemic feed for five weeks; KP: hypercholesterolemic feed for five weeks and fed with 2 ml of simvastatin for 14 days; P1: hypercholesterolemic feed for five weeks and fed with 1st dose (100mg/200g body weight) of GGE for 14 days; P2: hypercholesterolemic feed for five weeks and fed with 2nd dose (250mg/200g BW) of GGE for 14 days; P3: hypercholesterolemic feed for five weeks and fed with 3rd dose (500mg/200g BW) of GGE for 14 days] in all groups can be seen in Figure 1.

**DISCUSSION**

Based on the results of qualitative analysis of triterpenoids and phenolics of GGE, it is known that they contain both compounds. Based on the results of quantitative analysis of the active substances contained in GGE, it is known that they have an antioxidant capacity of 24253.40mg/L GAEAC, total phenol 2098.98mg/100g GAE, flavonoids 11500.11mg/100g, 2416.29mg/100g TAE tannin levels, and IC 50 of 39.95 ppm.

The GGE that was made was given to the experimental animals orally. The experimental animal used was adult male white rats conditioned to experience hypercholesterolemia by providing a high cholesterol diet for five weeks.

The subjects used in this study were Wistar strain male white rats (Rattus norvegicus), 2.5-3 months old and weighing 180-200g. The rats at 2.5 months have the same age as young adult humans and have not yet experienced the intrinsic aging process. In this study, the experimental animal used was Wistar strain rats because these rats are more profitable to be used as test animals than mice. Wistar rats are good experimental animals used in genetic research (21,22).
This study used five groups of rats; each group contained five rats. Taking the dosage is based on a previous study. In this study, the treatment group was given GGE orally for 30 days. The taking time of 30 days is based on the results of the previous research.

The result of the analysis after giving GGE was an ANOVA test on triglyceride levels after treatment. Based on the ANOVA test analysis, it was found that there was a significant difference in triglyceride levels p = 0.03 (p<0.05) in all groups after treatment. This showed a decrease in triglyceride levels in all treatment groups.

Based on the statistical test result using the post hoc LSD test, the result showed significant differences in triglyceride levels after treatment in the KP and P3. The calculation of the decrease in blood triglyceride levels in the sample after giving the extract was calculated using the percentage reduction with the formula for the mean level of the negative control post-test minus the mean post-test level in the treatment group then divided by the mean levels of the negative control post-test. From these calculations, the highest reduction in triglyceride levels occurred in the P3 (500mg/200g BW) was 47.70%; then the KP group was 39.78%; P2 (250mg/200g BW) was 20.63%, and P1 (100g/200g BW) was 7.92%. Thus, there is an increase in the percentage decrease in triglyceride levels with the increasing dose of GGE.

The decrease in triglyceride levels is caused by tannin compounds which can lower blood cholesterol levels by binding to bile acids in the intestines and excreting them through feces, thereby decreasing triglyceride levels. The binding of bile acids by tannin resulted in the inhibition of fat absorption so that triglyceride levels were reduced.

Tannins can also inhibit the action of acetyl-CoA carboxylase. Inhibition of the acetyl-CoA carboxylase enzyme activity causes the formation of long-chain fatty acids, which will not turn into triglycerides. Tannin also functioned to prevent oxidation, cholesterol, and LDL in the blood so that it can reduce the risk of stroke and has antimicrobial properties (23,24). Next, anthocyanin levels have the effect of lowering triglyceride levels by reducing the levels of apolipoprotein B (APO B) found in chylomicrons, VLDL, and LDL. The function of APO B is to transport triglycerides in the blood (25).

The mechanism of action of antioxidants such as flavonoids reduces plasma cholesterol levels by inhibiting cholesterol absorption in the intestine and increasing the reaction of bile acids from cholesterol to be excreted in the feces (26). The flavonoid content in the GGE increases the activity of lipoprotein lipase (LPL) so that it affects the decrease in serum triglyceride levels (27). Based on a previous study, flavonoids can reduce triglyceride levels by increasing the activity of LPL, which functions as an antioxidant (28). In addition, a study has also shown that flavonoids act as free radical scavengers that have a hydroxyl group (OH-) on the aromatic ring and stop lipid peroxidation chain reactions by protecting cells and chemicals in the body (27).

GGE also contains bioactive components of phytosterols which are helpful as hypocholesterolemic substances or can lower blood cholesterol levels. Phytosterols reduce cholesterol by inhibiting the absorption of cholesterol...
from food and inhibiting bile acid cholesterol reabsorption by modifying Acetyl Co-A carboxylase and 7α-dehydroxylase activity, resulting in increased excretion of bile acids through feces. This causes the number of bile acids to decrease and will increase the formation of new bile acids from cholesterol in the blood (14).

Resveratrol is another content in the green grape that causes a decrease in triglycerides. The reduction in triglyceride levels occurs by inhibiting the accumulation of blood fats in three ways: increasing cAMP, decreasing lipogenesis, and increasing AMP-activated protein kinase (20).

The comparison between P1, P2, and P3 has the most significant effect in reducing triglyceride levels is the P3. This occurs because the higher the dose given to the sample, the greater the content of active substances received by the sample, there were phytosterols, tannins, resveratrol, and anthocyanins. So that shows the ability to lower triglyceride levels is also getting better.

A previous study using red grape extract to reduce triglyceride levels showed decreased blood triglyceride levels in the treatment group doses 1, 2, and 3 were 10%, 17.5%, and 40.7%, respectively. This shows that the result of this study is in line with the study conducted that giving grape extract can reduce triglyceride levels with the highest reduction at 500mg/200g BW. The difference between this study and a previous study conducted is in the type of grapes used and the conditioning of the experimental animals (18). Previous studies used red grape and experimental animals with hyperlipidemia with hypercholesterolemia feed and induction of Triton X-100.

CHD is a disease of the heart's coronary arteries due to narrowing, blockage, or other blood vessel abnormalities. This condition can be caused by spasms, atherosclerosis, or both. Obstructed flow can cause the supply of oxygen and nutrients to the myocardium to decrease, causing pain and impaired heart function. Dyslipidemia is a risk factor for CHD. Meanwhile, triglyceride levels are one of the markers of dyslipidemia. Lowering triglyceride levels reduce the risk of dyslipidemia, thereby reducing risk factors for CHD. This risk reduction will also reduce mortality and increase health status, productivity, and quality of life. The result showed a decrease in triglyceride levels after administration of GGE in the P3 group (500mg/200g BW). Thus, it is suggested that GGE can be used to treat dyslipidemia by reducing triglyceride levels. The result of this study can be used as a reference for study in humans so that the effectiveness of GGE in human blood triglyceride levels can be seen.

CONCLUSION
This study concluded that GGE has the effect of reducing triglyceride levels; this is evidenced by the difference in triglyceride levels before and after giving GGE. GGE at a dose of 500mg/200g BW has a more significant effect in lowering triglyceride levels than dose of 250mg/200g BW and 100mg/200g BW.

Variations in the dosage of GGE in determining the toxic effect, effective dose, and duration of the most effective in reducing triglyceride levels. As well as the effect of GGE activity in lowering triglyceride levels in humans. The author
suggested that analyzing the active compounds found in green grapes can reduce triglyceride levels.

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CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

REFERENCES


