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Grape (*Vitis vinifera L.*) skin extract reduced levels of SGPT and SGOT and improved the liver tissue structure of Wistar rats (*Rattus novergicus*) fed a high-cholesterol diet

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ABSTRACT

Background: This study determined the effect of adding grape skin extract (*Vitis vinifera L.*) to the diet of Wistar rats fed with high cholesterol on levels of SGPT and SGOT and the structure of liver tissue.
Methods: This study used 5 groups, namely control (standard diet), P1 (high cholesterol diet), P2 (high cholesterol diet and extract of grape skin as much as 100 mg/200 gBW/day), P3 (high cholesterol diet and extract of grape skin as much as 250 mg/200 gBW/day) and P4 (high cholesterol diet and extract of grape skin 500 mg/200 gBW/day). The study began with the production of extracts, the extracts dosage determination, high cholesterol diet preparation, sample (animals) preparation, extract addition to animals, SGPT and SGOT levels measurement, rat liver histopathology preparation and histopathological features observation, then ended with data analysis.
Results: The results showed that administration of grape skin extract in Wistar rats fed a high-cholesterol diet affected the levels of SGPT and SGOT, as well as parenchymatous degeneration of hepatocytes. Administration of grape skin extract as much as 500 mg/200 g BW/day had the lowest mean levels of SGPT and SGOT and the lowest parenchymatous degeneration of hepatocytes.
Conclusion: Thus, the extract of grape skin (*Vitis vinifera L.*) has the potential of hepatoprotector because it contains anthocyanin, which functions as an antioxidant and protects against liver damage.

Keywords: anthocyanin, grape skin extract, hepatoprotector, high-cholesterol diet.
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INTRODUCTION

The diet pattern of millennial society is changing due to significant lifestyle changes. The four-healthy five-perfect diet, especially the consumption of vegetables and fruits as a complement to staple food, has become less attractive. Fast food is the main choice for millennials today because it is practical and tastes good, but its nutritional value and hygiene are not guaranteed. On the other hand, many fast foods contain high levels of fat and cholesterol, which can cause fat and cholesterol levels in the human body to exceed normal limits,[1] which is called dyslipidemia, which is a lipid metabolism disorder characterized by an increase in total cholesterol, triglyceride, and Low-Density Lipoprotein (LDL) levels and decrease in High-Density Lipoprotein (HDL) levels.[2]

Dyslipidemia is a process of lipid metabolism disorders in humans. In this condition, total cholesterol levels increase in the blood, which exceeds normal levels. The state of dyslipidemia is caused by an unhealthy lifestyle, an unbalanced diet and a lack of physical activity from humans themselves. Unhealthy lifestyles include consuming foods high in fat, carbohydrates, and lacking fiber content in food.[3]

Dyslipidemia is not only caused by high cholesterol levels but can also be caused by Non-Alcoholic Fatty Liver Disease (NAFLD).[4] NAFLD is a liver disorder with a characteristic feature of macrovesicular fat that appears in patients who do not consume alcohol 20 grams of ethanol per week.[5] Starting from the accumulation of hepatic triglycerides (steatosis), which increases the vulnerability of the liver, mitochondrial dysfunction and oxidative stress, which leads to steatohepatitis and fibrosis. The impact of NAFLD causes liver cell damage so that there is an increase in the levels of enzymes found in the liver, such as SGOT and SGPT.[6]

In other conditions, such as high cholesterol levels in the blood, the body will neutralize it by converting cholesterol into bile acids. Increased synthesis of bile acids has an impact on increasing the production of free radicals. The presence of excess free radical production means that antioxidants in the body cannot cope with the free radical, and oxidative stress occurs.[7] Oxidative stress will cause membrane and cytosolic lipid peroxidation reactions that reduce fatty acids that damage cell membranes and cell organelles. Cell membranes are very important for receptor function.

Membrane lipid peroxidation will result in total loss of cell function; if this continues, it can cause liver cell damage. Damage to liver cells will cause transaminase enzymes, namely Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) in the liver cells to enter the blood circulation due to changes in cell membrane permeability so that the levels of SGOT and SGPT enzymes in the blood will increase.[8] The presence of this enzyme is an indicator of impaired liver function.[9]

Grapes (*Vitis vinifera L.*) are one of the natural ingredients that contain anthocyanins, especially in the fruit's skin.[10,11] In addition, grape skin contains total phenols (168.55 mg/100 g), flavonoids (61.10 mg/100 g) and IC_{50} of 1,065 ppm.[11] In another study, it was also found that grape skins and grape seeds, which are wastes in the wine-making process, can reduce hyperlipidemia,[12] which is caused by antioxidants present in grape skins and grape seeds. The existence of grapes in Indonesia, especially in Grokgak District, Buleleng Regency, Bali Province, is sufficient to support the manufacture of white wine; grape skins are not included, so this situation can be used as the use of grape skins as an herbal therapy in the treatment of liver function damage.[13] Based on this background, this study aims to determine the effect of grape skin extract on reducing SGPT and SGOT levels and reducing damage to the liver tissue structure of Wistar rats fed a high-cholesterol diet.

MATERIAL AND METHODS

Research type, groups, and location

This research type was experimental laboratory research, with the post-test only a control group design. This study used 5 groups, namely control (standard diet), P1 (high cholesterol diet), P2 (high cholesterol diet and grape skin extract as much as 100 mg/200 g BW/day), P3 (high cholesterol diet and grape skin extract 250 mg/200 g BW/day), and P4 (high cholesterol diet and grape skin extract as much as 500 mg/200 g BW/day). This research was conducted at the Food Analysis Laboratory, Faculty of Agricultural Technology, Universitas Udayana; Histology Laboratory, Faculty of Medicine, Universitas Udayana; Mantra Medika Clinical Laboratory, Ketewel, Gianyar; and Denpasar Veterinary Center.

Materials and instruments

The materials and instruments used were grape skin, 95% ethanol, goat fat, egg yolk, standard feed (550), Wistar rats, cotton and filter paper, rat cage, probe, blender, rotary evaporator, Erlenmeyer tube, balance, drop pipette, hematocrit tube, serum vacuum tube, and cool box.

Procedures

This study began with the production of grape skin extract, dose determination of the extract, preparation of a high cholesterol diet, preparation of test animals, treatment of test animals, measurement of SGPT and SGOT levels, preparation of rat liver histopathology preparations and observation of histopathological features.

The manufacture of grape skin extract began with sorting the skin of the grapes, aerated to constant weight and crushed into powder. The powder was macerated with 95% ethanol in a ratio of 1:7 for 3 days; then, the immersion was filtered to obtain extract 1. The residue was macerated with 95% ethanol in a ratio of 1:4 for 3 days, filtered and obtained extract 2. Extracts 1 and 2 were mixed and evaporated using a rotary evaporator to obtain a thick grape skin extract.

Determination of the dose of grape skin extract was based on research conducted by Yusmadri (2016), with doses of 100 mg/200 gBW, 250 mg/200 gBW and 500 mg/200 gBW. Thus, in this study the doses were 100 mg/200 gBW/day, 250 mg/200 gBW/day and 500 mg/200 gBW/day.

A high cholesterol diet was produced by mixing 200 g of goat fat, 100 g of egg yolk, and 700 g of standard feed. The amount of fat given to rats is based on the recommended fat consumption for humans with a body weight of 70 kg, which is 100 g/day. This dose was then converted to a rat's weight of 200 g so that the high cholesterol diet given was 2 g/day.[14]

Test animals were prepared by selecting 2-3 months old, wistar rats 150-200 g in weight and healthy condition. The total sample was 25 Wistar rats divided into 5 groups (control, P1, P2, P3, and P4).

The treatment of the test animals was carried out in each research group. Before the study began, the rats were acclimatized with standard feed (550) and distilled water for one week. After that, the control group was given standard feed for 15 days, while the P1, P2, P3, and P4 groups were given a high-cholesterol diet for 15 days. Then, grape skin extract was given to groups P2, P3, and P4 with doses of 100 mg/200 gBW/day, 250 mg/200 gBW/day, and 500 mg/200 gBW/day, respectively. The control group was continued with standard feeding, and the P1 group was given a high cholesterol diet. On the 31st day, SGPT and SGOT levels were measured as post-test.

Measuring SGPT and SGOT levels was carried out by taking 1-2 mL of blood samples from the orbital veins of Wistar rats; the blood in the tube was allowed to clot. After freezing, it was centrifuged at 3000-4000 rpm for 10 minutes to obtain serum. The serum was placed in the cup as a material ready to be examined. The examination uses the Erba XL 100 tool, which consists of running controls and samples.

The histopathological preparations were made by taking the liver organs and washing them with water and 0.9% physiological-NaCl, then they were put in formalin 10 for initial fixation. Then the liver was processed into histopathological preparations with tissue fixation and paraffination (fixation, dehydration, clearing, impregnation, embedding, blocking, and trimming), tissue cutting, and tissue staining (fixation, dehydration, clearing, impregnation, embedding, blocking, dewaxing, hydration, hematoxylin-eosin staining, dehydration, and mounting). After that, the histopathological picture of the rat liver was seen. In the liver tissue, an examination was performed by observing every 20 hepatocytes in the central vein. From these 20 hepatocytes, there were normal hepatocytes and damaged hepatocytes (parenchymatous degeneration, hydropic degeneration, and necrosis).

Data analysis

Data analysis of SGPT and SGOT levels and histopathological structure were carried out through the one-way Anova test and continued with the Least Significant Difference (LSD) test. Statistical analysis

is assisted by computer data processing program.

RESULTS

The Statistical Analysis Result

The mean levels of SGOT and SGPT, as well as hepatocytes parenchymatous degeneration of Wistar rats with a high-cholesterol diet, had the lowest values in the P4 group, which was given ethanol extract of 500 mg/200 g BW/day (according to Table 1, Table 3, and Table 5). This study's results were then analyzed for normality, homogeneity, and comparability tests.

In the normality test of SGPT and SGOT levels and parenchymatous degeneration of hepatocytes, the results showed that the data were normally distributed ($p>0.05$, according to Table 1, Table 3, and Table 5). The homogeneity test also showed that the levels of SGPT and SGOT, as well as parenchymatous degeneration of hepatocytes in this study, were homogeneous ($p>0.05$, according to Table 1, Table 3, and Table 5). Because the data were normally distributed and homogeneous, a comparability test was carried out in the form of a one-way ANOVA test, and a significant difference was obtained ($p<0.05$, according to Table 1, Table 3, and Table 5) between the treatment groups.

LSD test was conducted to determine the difference between the two treatment groups. In SGPT levels, the control group and P1 had significant differences ($p<0.05$, according to Table 2), which indicated a significant difference in SGPT levels in rats with standard diets and rats with high cholesterol diets without grape skin extract. The LSD test results of groups P1 and P3 as well as groups P1 and P4 had significant differences ($p<0.05$, according to Table 2), which indicated that there was a significant difference in SGPT levels in rats with a high cholesterol diet without grape skin extract and rats on a high cholesterol diet with grape skin extract of 250 mg/200 gBW/day and 500 mg/200 gBW/day.

In the levels of SGOT and parenchymatous degeneration of hepatocytes, the results of the LSD test showed significant differences between groups ($p<0.05$, according to Table 4 and 6). This result indicated that the treatment groups significantly differed in SGOT levels and hepatocyte parenchymatous degeneration.

Table 1. Statistical analysis of SGPT levels in each treatment group.

Treatment group	Mean ± SD	Normality value	Homogeneity value	One-way Anova test
Control	67.54 ± 6.04	0.457		
P1	107.02 ± 7.42	0.264		
P2	96.84 ± 8.71	0.078	0.484	0.000
P3	91.52 ± 9.53	0.941		
P4	81.28 ± 13.64	0.791		

Table 2. LSD test of SGPT levels in each treatment group.

Treatment group	Mean difference	p-value
Control and P1	39.48	0.000
Control and P2	29.30	0.000
Control and P3	23.98	0.001
Control and P4	13.74	0.032
P1 and P2	10.18	0.103
P1 and P3	15.50	0.017
P1 and P4	25.74	0.000
P2 and P3	5.32	0.383
P2 and P4	15.56	0.017
P3 and P4	10.24	0.101

Table 3. Statistical analysis of SGOT levels in each treatment group.

Treatment group	Mean ± SD	Normality value	Homogeneity value	One-way Anova test
Control	139.88 ± 5.86	0.912		
P1	237.02 ± 16.59	0.537		
P2	192.38 ± 4.69	0.748	0.091	0.000
P3	179.02 ± 1.35	0.507		
P4	156.00 ± 3.39	0.817		

Table 4. LSD test of SGOT levels in each treatment group.

Treatment group	Mean difference	p-value
Control and P1	73.14	0.000
Control and P2	56.64	0.000
Control and P3	70.00	0.000
Control and P4	101.02	0.006
P1 and P2	16.50	0.000
P1 and P3	3.14	0.000
P1 and P4	27.88	0.000
P2 and P3	13.36	0.019
P2 and P4	44.38	0.000
P3 and P4	31.02	0.000

The histopathological structure of liver tissue

In the histopathological structure of the liver of Wistar rats in the control group (Figure 1) and the group with high cholesterol diet (Figure 2), it can be seen that there are normal hepatocytes and liver tissue damage in the form of parenchymatous degeneration.

DISCUSSION

Based on the research that has been done, it can be said that the administration of 95% ethanol extract of grape skin has a significant effect on the levels of SGPT and SGOT as well as parenchymatous degeneration of hepatocytes of Wistar rats

Table 5. **Statistical analysis of hepatocyte parenchymatous degeneration in each treatment group.**

Treatment group	Mean ± SD	Normality value	Homogeneity value	One-way Anova test
Control	0.80 ± 0.84	0.314		
P1	7.60 ± 1.14	0.814		
P2	5.60 ± 1.14	0.814	0.840	0.000
P3	4.20 ± 0.84	0.314		
P4	2.20 ± 0.84	0.314		

Table 6. **LSD test of hepatocyte parenchymatous degeneration in each treatment group.**

Treatment group	Mean difference	p-value
Control and P1	6.80	0.000
Control and P2	4.80	0.000
Control and P3	3.40	0.000
Control and P4	1.40	0.003
P1 and P2	2.00	0.004
P1 and P3	3.40	0.000
P1 and P4	5.40	0.000
P2 and P3	1.40	0.033
P2 and P4	3.40	0.000
P3 and P4	2.00	0.004

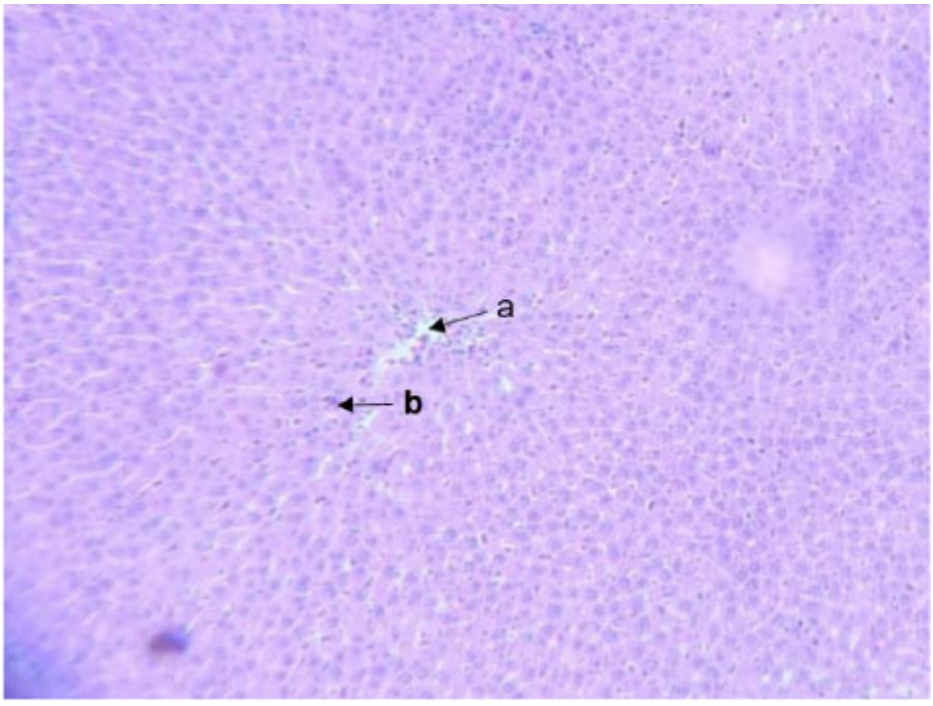

Figure 1. Histopathological structure of the liver of control Wistar rats (standard diet).

fed a high cholesterol diet. The mean levels of SGOT and SGPT, as well as hepatocytes parenchymatous degeneration of Wistar rats with a high-cholesterol diet, had the lowest values in the P4 group, which was given ethanol extract of 500 mg/200 gBW/day (Figure 3). The SGPT parameter was used because this enzyme is one of the enzymes produced in the liver and released into the blood, where the levels are directly proportional to the state of the liver itself; the higher the levels in the blood, the higher the liver damage that occurs.[15,16]

Grape skin contains many anthocyanins, total phenols and flavonoids.[11,17,18] This secondary metabolism has physiological functions, namely as an antioxidant, anticancer and protection against liver damage. In addition to anthocyanins, grape skins contain resveratrol and other flavonoids such as catechins, quercetin and procyanidins.[19] The presence of these antioxidants can reduce or inhibit the increase in the average levels of SGPT and SGOT so that it shows the potential of grape skin extract as a hepatoprotector.[20-23]

This situation followed several previous studies that administering antioxidants as anthocyanins in hypercholesterolemic rats can reduce SGPT and SGOT levels and decrease liver tissue damage.[24-28] This is because antioxidants can neutralize existing free radicals and reduce oxidative stress. Further impact is decreased lipid peroxidation, SGPT and SGOT enzymes and also parenchymatous degeneration of hepatocytes.

CONCLUSION

The administration of 95% ethanol extract of grape skin (*Vitis vinifera L.*) gave a significant effect on the levels of SGPT and SGOT as well as parenchymatous degeneration of Wistar rats fed a high cholesterol diet, which 500 mg/200 g BW/day had the lowest mean levels of SGPT and SGOT and parenchymatous degeneration in the group with high cholesterol diet.

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None.

DISCLOSURE

Conflict of Interest

There is no conflicts of interest in this study.

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None.

Author Contributions

All authors contributed equally to this research.

Ethical Considerations

This research has been approved by the ethical commission of Universitas Pendidikan Ganesha.

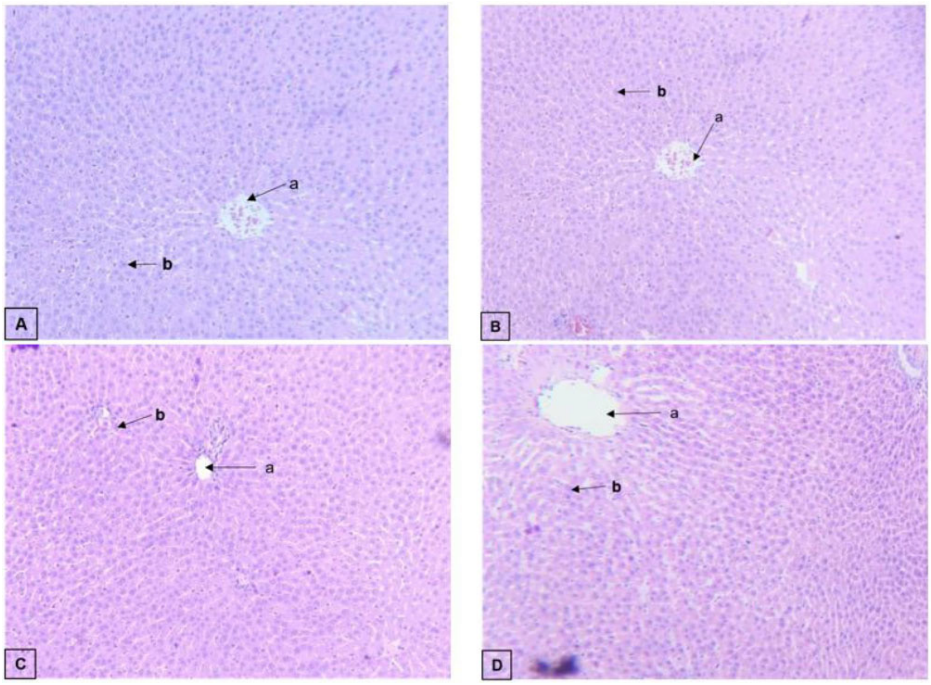

Figure 2. Histopathological structure of rat liver with high cholesterol diet; A. P1, B. P2, C. P3, D. P4; a. central vein, b. parenchymatous degeneration.

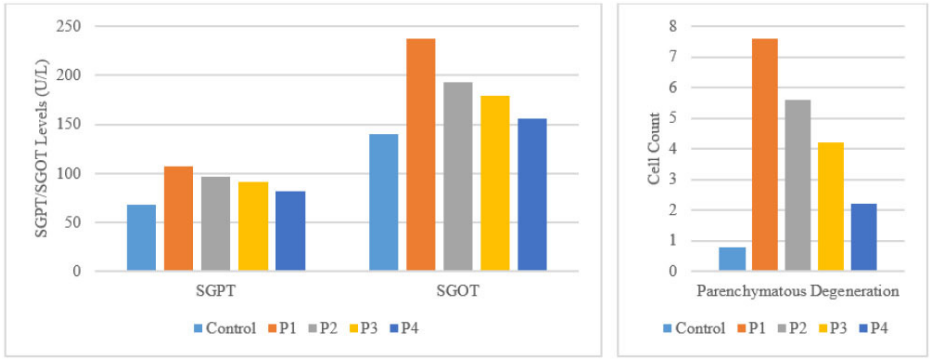

Figure 3. Graph of SGPT and SGOT levels and parenchymatous degeneration of hepatocytes.

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