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Hepatoprotective Effects of Balinese Grape Extract (*Vitis vinifera L.*) on Hepar Histology and Reducing Blood Sugar Levels

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Abstract: This study aimed to determine the hepatoprotective activity of grape skin extract (*Vitis vinifera* L.) (BGE) on hepatic histology and blood sugar level reduction. This study was conducted in four stages: extraction of grape skin using 96% ethanol, phytochemical screening, antihyperglycemic activity test and hepatoprotective activity test. The experimental animals were divided into five groups consisting of negative control (Na CMC 0.5%), positive control (Curcuma), TI (100 mg BGE/kg BW), TII (300 mg BGE/kg BW), TIII (500 mg BGE/kg BW). Phytochemical screening showed that BGE positively contains terpenoids, flavonoids, tannins, saponins and carotenoids. The results of antioxidant analysis on BGE showed an IC_{50} value of 80.77 ppm, which included intense antioxidant activity. BGE and negative control can provide hepatoprotector effects on hepatic cells from hepatocyte cell damage due to paracetamol and can reduce blood sugar levels.

Keywords: Antioxidant; Hepatoprotective; *Vitis vinifera* L.

Introduction

Hepar is the largest organ and gland of the human body after the skin. It is involved in the synthesis, storage and metabolism of many endogenous and exogenous compounds, including the metabolism of drugs. It plays a significant role in detoxifying toxins from the body [1]. Judging from its function, it can be predicted that the liver is very vulnerable to disease attacks that can be caused by various factors such as viral infections and toxic substances (e.g. alcohol, certain drugs), one of which paracetamol can cause damage to the liver in overdose use. Although paracetamol is declared safe at therapeutic doses, high doses of paracetamol can cause liver function failure and trigger pathological inflammation, apoptosis, and oxidative DNA damage [2]. The presence of antioxidant compounds can reduce toxicity to the liver. In the research of Fotschki et al. [3], it has been shown that some phytochemicals such as phytosterols, carotenoids, polyphenols and betalains, which are antioxidant compounds found in vegetables and fruits can affect the health status of humans and animals. One of the fruits that contain antioxidants is Bali grapes.

Uncontrolled hyperglycemia in patients with diabetes mellitus (DM) can cause severe damage to body tissues, such as nerves and blood vessels. Patients with DM require lifelong care to reduce symptoms, prevent disease progression and prevent the development of complications. DM is a metabolic disease characterized by abnormally elevated blood glucose levels (hyperglycemia) and impaired carbohydrate, fat and protein metabolism associated with a lack of insulin sensitivity and secretion and progressive changes in the structure of pancreatic beta cells. Hyperglycemia or high blood sugar levels play a role in cell damage by increasing reactive oxygen species (ROS) that can cause tissue oxidative stress and lead to cell injury through the mechanisms of lipid peroxidation and protein oxidative damage. In addition, high levels of ROS will produce oxidative stress and antioxidants that cannot reduce oxidant levels, causing damage to cells, tissues and organs. Flavonoid compounds present in grape plants have pharmacological properties, and high levels of flavonoids can act as antioxidants. These antioxidants can be used to help in antidiabetic treatment. Previous research also states that some plants that are proven to contain flavonoid compounds have activity as antioxidants, antivirals, antibacterial, anti-inflammatory, anticancer and antiallergic [4].

Antioxidants can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. Free radicals can damage cell-forming macromolecules that can lead to degenerative diseases. Humans have endogenous antioxidants in their bodies that can reduce free radicals. However, if the amount is smaller than free radicals, exogenous antioxidants are needed to minimize the negative effects of free radicals [5]. One of the factors affecting the decrease in blood glucose levels is compliance with undergoing diabetes mellitus treatment therapy. Therefore, compliance is closely related to blood glucose levels. The

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higher the level of patient compliance, the lower the blood sugar levels.

On the contrary, the lower the patient's compliance with treatment, the blood glucose levels cannot be controlled, which means that blood glucose levels will remain high [6]. People with diabetes who take antidiabetic drugs in the long term or for life can experience side effects, which are only used to relieve symptoms and prevent complications, so alternative treatment of medicinal plants such as extracts and herbal medicines is needed to reduce the side effects of drugs. People affected by diabetes mellitus often take synthetic drugs and are even highly dependent. Synthetic drugs consumed by people tend to have fewer therapeutic effects than side effects, and most synthetic drugs cause side effects that are unacceptable to the body [7].

One of the plants that can be used in the community as an antidiabetic is grape. Grapefruit skin has strong antioxidant activity due to very high polyphenolic compounds, namely flavonoids. Flavonoids and antioxidants are believed to be able to prevent several diseases, one of which can protect cells and DNA damage by cleaning cells from ROS, which causes complications from diabetes mellitus. Community knowledge in the utilization of medicinal plants is very diverse, including the processing method, how to use it, the parts used, and the efficacy of each type of plant in curing diseases. People know that the flesh of salak fruit can be used as processed food [8]. Public knowledge about the benefits of grape skin waste is minimal, and how to process it as a medicine is still rarely known. Another potential that can also be developed from grape skin extract products is as an antidiabetic. Some active compounds that are usually believed to be drugs for preventing and treating diabetes include active compounds such as alkaloids, flavonoids, saponins, steroids and triterpenoids, phenolic hydroquinones, and tannins [9]. Grapefruit skin contains active elements that work simultaneously on the patient's body to cure the disease. The active elements in salak skin that can cure diabetes are ferulic acid and proline, cinnamic acid derivatives, arginine and pterostilbene [10]. DM management is effective in the early stages before symptoms or prediabetes appear. One way to control blood glucose levels is by using natural ingredients.

This study aims to determine the hepatoprotective activity of Balinese grape extract (*Vitis vinifera* L.) (BGE) on hepatic histology and blood sugar level reduction. This study is also helpful in providing information to the public about the potential of a processed grape product for health.

Research Method

Extract of Bali Grapes and Phytochemical Screening

The dried Balinese grape (*Vitis vinifera L.*) was cleaned and then crushed. The powder is then put in a container, macerated with ethanol solvent and allowed to stand for a certain period. The macerate is separated by filtration and repeated at least twice with the same amount and type of solvent until the solvent is explicit. All the macerates were collected, and the solvent was evaporated using a rotary evaporator to obtain a thick extract. Balinese grapes that have been dried and reduced in particle size are then extracted using 96% ethanol [11]. The extractant was filtered and then thickened using rotary vapour to produce an extract of Balinese grape [12]. BGE were then screened for their phytochemical content.

Antioxidant Activity (DPPH Method)

Preparation of DPPH solution by weighing 5 mg of DPPH and then adding methanol p.a up to 50 mL. Determination of the maximum absorption wavelength of DPPH by determining the optimum wavelength, measuring the absorbance at a wavelength of 510-525 nm. The mother liquor was made in series with 4, 8, 12, 16, 20 and 100 ppm concentrations. Determination of % Inhibition and IC_{50}.

$$\% \text{ Inhibition} = \frac{\text{Abs. blank} - \text{Abs. sample} \times 100\%}{\text{Abs. Blank}}$$

Preparation of 0.5% Na CMC, Curcuma and Paracetamol suspension

A total of 5 mg/ml Na CMC was sprinkled into a mortar containing 10 mL of hot distilled water. Let it stand for 15 minutes until a transparent mass is obtained, crushed to form a gel, diluted with a small quantity of distilled water, poured into a 100 mL volumetric flask, and added distilled water to the limit mark. This suspension was used as a carrier for grape ethanol extract and paracetamol. One Curcuma® tablet contains 20 mg of Curcuma xanthorrhiza. Paracetamol suspension in 0.5% Na CMC suspension was prepared by weighing the equivalent, 1,294.86 mg of paracetamol powder that had been weighed into 0.5% Na CMC suspension.

Treatment of Experimental Animals

Male white mice that had been induced by alloxan were divided into five groups and given intake of Curcuma® (positive control), 0.5% Na CMC (negative control), Balinese grape extract 100 mg/Kg BW (BGE1), 300 mg/Kg BW (BGE2) and 500 mg/Kg BW (BGE3) induced until 7 days. Blood glucose content was measured in mice on days 7:7-14 and 21:7-21. On day 8, mice were induced with toxic doses of paracetamol orally for 3 days. After 24 hours, mice were sacrificed by intracardiac embolization. On the 10th day, the hepar of mice was taken through a surgical process [13]

Preparation of Histological Incision of Hepar

In mice hepar, the fixation process is carried out for at least 1x24 hours, then dehydration by putting the sample into alcohol of graded concentrations, namely 70%, 80%, 90% and 95% alcohol each for 24 hours and continued with 100% alcohol for 1 hour which is repeated three times rinsing. Then, paraffin infiltration was carried out for 4 hours, followed by the embedding process, which is the process of planting organs in block media. Furthermore, the sample size of 6 um was cut to be attached to the glass object. Enter into the dye (water-based), namely

Hematoxilyn-Eosin. Hematoxylin 10 minutes, wash with tap water, enter into acid ethanol to remove excess hematoxylin, rinse with distilled water. Dehydrate again with graded alcohol, each stage for 5 minutes. 70% - 80% - 96% - 100%. Then, the mice's hepatic tissue was viewed under a light microscope.

Result and Discussion

Phytochemical Screening and Antioxidant Activity

Before analyzing the effect of ethanolic extract of grape extract on blood glucose levels, grape vine extract was screened for phytochemicals on the extract. The results of the phytochemical screening analysis are shown in Table 1.

Table 1. Phytochemical Screening Analysis

No.	Identification of compounds	Result
1.	Flavonoids	+
2.	Tannins	+
3.	Terpenoids	+
4.	Saponins	+
5.	Carotenoids	+

According to the results of this study, it can be seen that the ethanolic extract of grapes contains several phytochemical compounds such as terpenoids, saponins, flavonoids, carotenoids and tannins. This study's results align with research conducted by others that contain phenols, flavonoids, tannins, saponins, and carotenoids. Another study also reported that snake fruit skin extract contains flavonoids, saponins, phenol, tannins, and terpenoids. The phytochemical content of the ethanolic extract of grapes provides various benefits such as antioxidants or skin lightening. Phytochemical screening tests from powdered and grape leaf ethanol extract contain flavonoids, polyphenols, quinones, steroids, and triterpenoids. This flavonoid compound can lower blood glucose levels because of its ability as an antioxidant. Antioxidants can improve insulin sensitivity. In lowering blood sugar levels, these flavonoids work by repairing the regeneration of damaged pancreatic beta cells and stimulating more insulin release [14]. In addition to the quality of the extract, the antihyperglycemic effect of grapefruit skin extract is also related to its phytochemical content. Saponins and flavonoids have antioxidant and inhibitory effects on glucosidase enzymes. The inhibitory effect of glucosidase enzymes can absorb glucose in the digestive tract, thus reducing postprandial blood glucose levels. In addition, the antioxidant effect of ethanol grape skin extract also contributes to the antidiabetic effect. This is related to the mechanism of pancreatic damage caused by alloxan. Alloxan will be reduced by GSH, which forms unstable dialuric acid and can undergo autoxidation to form alloxan radicals.

Table 2. IC_{50}

Material	IC_{50} (ppm)
Balinese grape extract	80.77

The amount of antioxidant activity is indicated by the IC 50 value, which is the concentration of sample solution required to inhibit 50% of DPPH free radicals. Table 2 shows that the IC50 value of grape skin extract is 80.77 ppm, which is included in the strong antioxidant activity.

Table 3. Absorbance and % Inhibition of BGE

Material	Concentration (ppm)	Abs. Blank	Abs. Sample	% Inhibition
Balinese grape extract	4	0.67	0.437	12.76
	8		0.683	11.87
	12		0.531	13.36
	16		0.667	12.19
	20		0.587	13.79
	100		0.376	61.78

Table 3 shows that the greater the concentration of the sample solution, the smaller the absorbance value obtained. The smaller the absorbance value, the greater the % elution value. This is because the higher the concentration of the solution, the higher the antioxidant activity. Figure 2 shows that the linear regression equation on the DPPH solution standard calibration curve is y = 15.835x - 22.123 with an R^2 value = 0.736.

Blood glucose levels

The test results showed that the extract has antihyperglycemic activity. Male diabetic white rats experienced a decrease in blood sugar levels on the 14th and 21st days after the administration of grape skin extract in the TI, TII and TIII. The results of the measurement of the decrease are shown in Table 4.

Table 4. Blood Glucose Levels

Group	Day-14 (%)	Day-21 (%)
Positive Control	36.8	57.2
Negative Control	29.7	43.3
BGE1	26.3	47.3
BGE2	27.6	44.5
BGE3	38.6	57.3

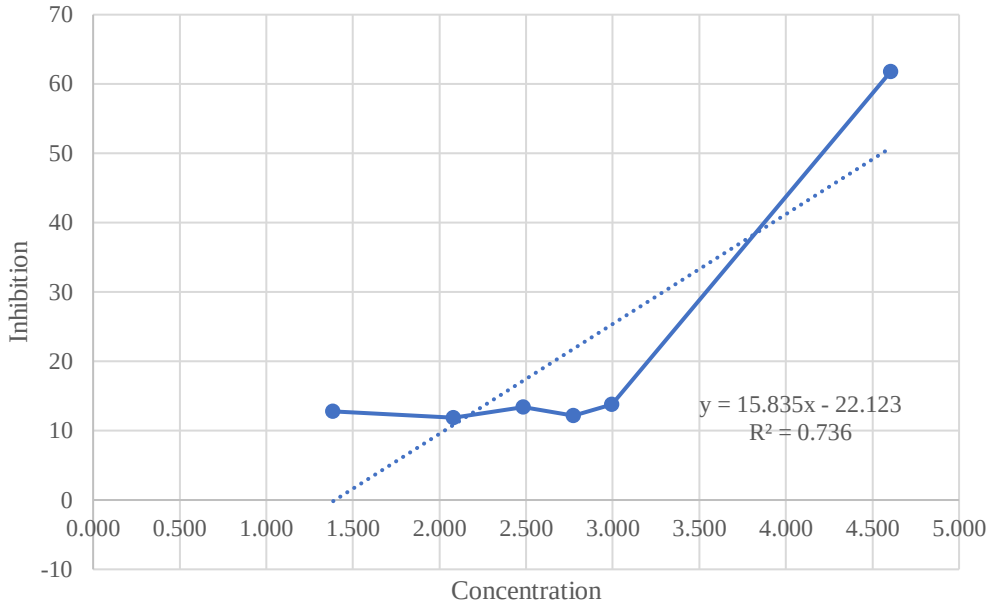

Figure 1. Standard calibration curve of antioxidant activity

Table 4 shows the group's results given Balinese grape extract, which lowered blood sugar levels. The decrease in blood sugar levels in rats is thought to be due to the phytochemical compounds in Balinese grape skin extract. Terpenoids, saponins, flavonoids, carotenoids, and tannins have been shown to have antihyperglycemic properties. Phytochemical compounds found in grape skin extract can modulate metabolic pathways in which glucose can act as a substrate or a product. These phytochemical compounds affect gluconeogenesis, glycogenolysis, pentose phosphate pathway and glycogenesis. These phytochemical compounds also interfere with glucose uptake and inhibit α-glucosidase and α-amylase activity. The mechanism of phytochemical compounds is further thought to be able to reduce triglyceride levels and cholesterol levels, which will have an impact on hyperlipidemia, which is one of the pathophysiological features of DM [15]. Flavonoids and alkaloids can protect against β-cell damage, promote proliferation and stimulate insulin secretion. Flavonoids, alkaloids, tannins and steroids can protect against oxidative stress associated with diabetic complications.

Meanwhile, alkaloids can stimulate cellular glucose uptake and reduce insulin resistance. Tannins also have antidiabetic effects with the mechanism of enhancing cell recovery propagation and reducing carbohydrate absorption by inhibiting a-amylase and a-glucosidase activities [16]. Meanwhile, saponins display their antidiabetic activity through mechanisms that might increase insulin resistance, stimulate insulin secretion, and protect pancreatic cells. The antioxidant activity in grapefruit skin peel will complement the body's defence system to counteract free radicals and limit the damage. The antioxidant defence system works by directly interacting with free radicals to prevent the formation of reactive oxygen compounds or convert reactive compounds into less reactive ones [17].

Hepatoprotective Activity Test Results

This study was conducted by giving test preparations in the form of adverse control treatment given Na CMC 0.5%, favourable control treatment given grape vine extract in graded doses, namely doses of 100 mg/kg BW, 300 mg/kg BW and 500 mg/kg BW. The treatment was given for 7 days. On the eighth day, mice were induced with paracetamol at a dose of 1,050 mg/kg BW of mice. After the treatment, the mice were dissected on the 10th day to take the liver and make histopathology preparations. Histopathological examination was carried out by sacrificing mice at the end of the observation, and then the hepatic organs were taken to be observed microscopically. Histology preparations were made using the paraffin block method, and staining was done using Haematoxilin Eosin (HE). Then, microscopic examination using light microscopy with a magnification of 400 times in the entire field of view on each preparation [18]. The results of the observation of the degree of histopathology of hepatic cells that experience parenchymatous degeneration, hydropic degeneration, and necrosis in the treatment group can be seen in Table 6 below.

Table 5. Histopathology Score of Hepar

Treatment Group	Histopathology Score
Negative control	166.6
Positive control	113.2
TI	131.6
TII	152.0
TIII	136.4

Based on the results seen in Table 5, it is known that there are differences in the average value of hepatic histopathology scores of mice in each treatment given. The results observed in the administration of toxic doses of

paracetamol showed changes in the histopathological picture; namely, there were significant differences in the level of damage to the hepatic histopathology structure between the negative control treatment given paracetamol with other treatments because the test animals in this group were not given antioxidants so that a lot of hepatic cell damage occurred in the form of cell death, hydropic degeneration and parenchymatous degeneration. This shows that the administration of toxic doses of paracetamol is enough to cause damage to the liver [19].

This study used data analysis, the Kruskal Wallis test, which is then continued with the Mann-Whitney analysis test because data examination based on the normality test shows that the data is not normally distributed with a Sig = 0.006 value smaller than 0.05. The Kruskal-Wallis test is used to see if a significant difference in each group is indicated by a p-value <0.05. The Kruskal-Wallis test evidences this obtained a significant figure of p = 0.000 so that the data from each treatment of research experiments show significant differences in hepatic cell damage. So, it can be concluded that there is at least a pair of differences in hepatic histology damage scores among the five research treatments. This study was continued by using Mann-Whitney analysis with a post hoc test technique, which aims to determine which treatments experienced differences in hepatic cell damage and which treatments did not experience significant differences in damage by comparing the two treatments [20].

Table 6. Analysis Results Mann-Whitney

Group	Asymp. Sig. (0.005)
Negative control and positive control	0.0016
Negative control and treatment:	
T I	0.009
T II	0.012
T III	0.026
Positive control and treatment:	
T I	0.008
T II	0.008
T III	0.009
Treatment:	
T I & II	0.115
T I & III	0.274
T II & III	0.016

The comparison of differences between the negative control treatment given paracetamol and the positive control treatment given curcuma obtained a significant value (0.016) <0.05. Thus, H0 can be rejected. It can be concluded that there is a difference in hepatic histology damage score between the negative control treatment and the positive control treatment. This indicates that the damage to hepatic cells caused by the administration of a single toxic dose of paracetamol 1,050 mg/kg BW mice has not been able to repair close to normal by the administration of grape ethanol extract with a dose of 500 mg/kg BW with an average value of 136.4 and obtained a significant value of 0.021 <0.05, a dose of 300 mg/kg BW with an average value of 152 and obtained a significant value of 0.009 <0.05, and a dose of 100 mg/kg BW with an average value of 131.6 and obtained a significant value of 0.009 <0.05. However, on average and statistically, the grape ethanol extract treatment group and the negative control induced by paracetamol showed significant differences. This indicates that grape ethanol extract can provide hepatoprotector effects on hepatic cells from hepatocyte cell damage due to paracetamol exposure given orally. However, the repair effect of grape ethanol extract is not equivalent to the positive control given curcuma.

In the administration of paracetamol, the liver is damaged in the form of parenchymatous degeneration, hydropic degeneration and necrosis. The mechanism of cell necrosis after paracetamol administration is closely related to liver function. Paracetamol forms a free radical group that affects the lipids of the endoplasmic reticulum membrane, causing changes in the morphology of the endoplasmic reticulum membrane. The enzymes of the endoplasmic reticulum will lose their catalytic activity. It cannot synthesize proteins, and lipid conjugates with proteins (lipoproteins) cannot be released from the liver into the blood. Necrosis forms unstable organic peroxides due to the interaction between free radicals from the biotransformation of paracetamol and unsaturated fatty acids that make up the cell membrane. This peroxide will then easily break into new free radicals that can further break down the cell membrane preparation [21]. Hydropic degeneration or swelling is the initial stage of necrosis characterized by swollen hepatocytes, where there are round and pale-coloured vacuoles caused by the paralysis of ion pump activity in the plasma membrane so that it cannot maintain ion and fluid balance [22]. Parenchymatous degeneration is the mildest form of degeneration and is reversible. Parenchymatous degeneration occurs due to oxidation failure that causes water to accumulate in cells so that protein transportation is disrupted [23].

In treating hepatic damage, there is an improvement process by giving ethanol grape extract. Wine can be used as a heat protector with an increase in the supply given to the body in the form of secondary antioxidants that will help GSH (Glutathione) in handling the increasing amount of NAPQI (N-acetyl-p-benzoquinon imine) formed due to the body's metabolic mechanisms directly carried out by the liver. A condition where hepatic cells will be bound by NAPQI (a compound free to bind amino acids) will be damaged. However, it will be overcome by the ample supply of secondary antioxidants provided in advance by grape extract as a protective mechanism. Therefore, hepatic cell damage can be well minimized. Before NAPQI binds to hepatic cell amino acids and damages hepatic cells, most secondary antioxidants are available and bind to NAPQI. Thus, antioxidants can prevent the formation of free radicals and help repair the damage caused by free radicals. Increased amounts of antioxidants supply the availability of glutathione, which will be able to help reduce damage to the liver [24].

The results showed that grape extract contains active ingredients: flavonoids, terpenoids, carotenoids, saponins,

and tannins. Grapes are also the richest source of betalains. Grapes contain many antioxidant compounds, such as polyphenols, betalains, and flavonoids, which can potentially be used as hepatoprotector drugs. Hepatoprotectors are a class of medicinal compounds that can maintain, treat, and restore the liver from damage. Flavonoids act as antioxidant agents by reducing free radicals that damage cell membranes and regenerate cells. Betalain is also one of the water-soluble compounds and a good antioxidant for oxidative stress and inflammation. The red-purplish pigment betaxantin in beetroot is also one of the good detoxifying agents for the body. The antioxidant activity of betalains is greater than vitamin C and polyphenols [25]

Conclusion

Balinese grape extract can reduce blood sugar levels and provide hepatoprotective effects on liver cells from hepatocyte cell damage due to paracetamol. Balinese Grape extract has hepatoprotector effectiveness, so it can be used as a medicinal ingredient that can protect the hepar.

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