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Case Report Paper

The Effectiveness of Garlic Extract against Triglyceride Levels of Wistar Rats Induced by 50% Ethanol

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Abstract: Alcohol affects the metabolism of triglycerides. Excessive intake of fat, carbohydrates, and protein can increase triglyceride levels. In addition to fat and carbohydrates, factors that can affect triglyceride levels are age, lifestyle, smoking, alcohol intake, hypertension, and liver disease. Triglyceride levels (> 200 mg/dL) raise the risk of atherosclerosis. The purpose of this study was to determine the levels of triglycerides of Wistar rats before and after induction of ethanol 50% and garlic extract therapy. The method was experimental, with a sample of 25 Wistar rats which were divided into 5 groups. G1, rats were given 50% ethanol from day 9-14, G2, rats were only given distilled water, G3, G4, and G5 treatment groups at doses I, II, and III, after ethanol administration 50% on the 9th – 14th day followed by the administration of garlic extract at a dose of 200 mg/gr BW, 400mg/gr BW and 600 mg/ gr BW, respectively. Rat blood was taken on the 8th day before administration of ethanol and garlic extract and taken again on the 15th day after administration of ethanol and garlic extract, then triglycerides were measured using a photometer. The results showed that there were significant differences in triglyceride levels in G1 with G2, G3, G4, and G5. Triglyceride levels in rats before treatment and after administration of 50% ethanol and garlic extract 200 mg/gramBW increased but were still within normal limits.
The study concludes that garlic extract can reduce triglyceride levels in rats induced by 50% ethanol.

Keywords: Ethanol, Garlic Extract, Triglycerides, Wistar Rat.

1. Introduction

Alcohol is an addictive substance or a substance that causes addiction (addiction) and dependence (dependence). Alcohol abuse is a major health problem as well as a social problem in society. People with alcohol dependence usually have a heavier consumption pattern and resulting in widespread organ damage. The main organs targeted by alcohol are the liver, cardiovascular, neurological, and digestive tracts [1].

Alcohol is known to affect the metabolism of high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C) cholesterol, and triglycerides. Excessive intake of fat, carbohydrates, and protein can increase triglyceride levels in the blood. In addition to fat and carbohydrates, factors that can affect triglyceride levels are age, lifestyle, smoking, excessive alcohol consumption, hypertension, and liver disease. Triglyceride levels in the blood (> 200 mg/dL) pose a risk of atherosclerosis, which is the formation of plaque in blood vessels, and if it clogs it can cause coronary heart disease (CHD) [2].

Excessive alcohol consumption is known to cause damage to fatty tissue and lead to fat deposition which leads to fatty liver. Alcohol consumption is often associated with an increase in plasma triglyceride concentrations and has relevance to cardiovascular disease [3]. In a study stated that triglyceride levels decreased in moderate consumption and increased in heavy alcohol consumption, alcohol consumption can increase fat production in the liver [4].

High triglyceride levels are one of the risk factors for coronary heart disease because most triglycerides are rich in lipoproteins, especially residual chylomicrons and very low-density lipoprotein (VLDL). Remnant lipoprotein is small so that it can enter the subendothelium and cause atherosclerosis [5].

Garlic (Allium sativum) is a tuber of the Allium sativum plant, included in the Amaryllidaceae family, other benefits as a seasoning for canned meat dishes, sauces, soups, and others [6]. Garlic can reduce blood clotting and reduce blood pressure, so it is important in the treatment of cardiovascular disease. Allicin and adenosine are the most important anti-platelet constituents in garlic [7]. Garlic can help improve cardiovascular function because it can overcome atherosclerosis, hyperlipidemia, hypercholesterolemia caused by free radicals. Garlic has antioxidants that can counteract free radicals [8].

In the study, the test animals used were white rats. Rats are one of the test animals in health because they have an organ structure similar to humans [9]. White rats (Rattus norvegicus) have been widely used as experimental animals because they are easy to obtain in large quantities, have a fast response, provide a scientific picture that may occur in humans, and are relatively inexpensive.

Based on the results of a previous study entitled the effect of consuming alcoholic beverages on triglyceride levels, it was found that only 1 person (5%) had normal triglyceride levels and 19 people (95%) had triglyceride levels more than normal (hypertriglyceridemia). Based on the criteria for the risk of coronary heart disease, 1 person (5%) has triglyceride levels with ideal criteria, 4 people (20%) are included in the high limit criteria for the risk of coronary heart disease, 7 people (35%) are included in the high-risk criteria and 8 people (40%) included very high-risk criteria for coronary heart disease [10].

Based on a study entitled the effect of giving garlic (*Allium sativum*) to dyslipidemia, it was concluded that the content contained in garlic, namely Allisin and adenosine, could be given to patients suffering from coronary heart disease by inhibiting platelet aggregation in vivo [11].

2. Literature Review

2.1. Ethanol

Ethanol, also known as ethyl alcohol, pure alcohol, or simply alcohol, is a volatile, flammable, colorless, and toxic liquid. The alcohol most often used in everyday life is ethanol which belongs to a single chain alcohol with the chemical formula C2H5OH and the empirical formula C2H6O and is a constitutional isomer of dimethyl ether. Ethanol is often shortened to EtOH, where "Et" stands for the ethyl group (C2H5). The fermentation of sugar into ethanol is one of the earliest organic reactions that humans have ever carried out. The effects of consuming intoxicating ethanol have also been known for a long time [12].

Alcohol includes addictive substances or substances that can cause addiction, namely addiction and dependence (dependence). Alcohol abuse is a major health problem as well as a social problem in society. People with alcohol dependence usually have a higher consumption pattern severe and result in widespread organ damage [13].

Alcohol is known to have an effect on the metabolism of high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C) cholesterol, and triglycerides. Excessive intake of fat, carbohydrates, and protein can increase triglyceride levels in the blood. In addition to fat and carbohydrates, factors that can affect triglyceride levels are age, lifestyle, smoking, excessive alcohol consumption, hypertension and liver disease. Triglyceride levels in the blood (> 200 mg/dl) pose a risk of atherosclerosis, namely the formation of plaque in blood vessels and if they clog it can cause coronary heart disease (CHD) and stroke [14].

High triglyceride levels are one of the risk factors for coronary heart disease, because most triglycerides are rich in lipoproteins, especially residual chylomicrons and very low density lipoprotein (VLDL). Remnant lipoproteins are small so they can enter the subendothelium and cause atherosclerosis.

Atherosclerosis is a condition where there is narrowing and hardening of the arteries due to the deposition of cholesterol and other fatty substances. Blockage occurs slowly. The inside of the artery is lined with endothelium, a thin layer of cells that keeps the artery tight and smooth. Smooth arteries make blood circulation to the heart smooth. When the endothelium is damaged due to high blood pressure, smoking, or high cholesterol, that's when plaque begins to form. Atherosclerosis can occur in all parts of the body. If it occurs in the walls of the heart, coronary heart disease will occur. Better lifestyle changes such as avoiding fatty foods, limiting daily alcohol consumption, exercising and quitting smoking, can help prevent the worsening effects of atherosclerosis. Atherosclerosis cannot be eliminated .

2.2. Triglycerides

Triglycerides are a type of fat that can be found in the blood and fat cells. The body gets most triglycerides from food, such as butter**,** cooking oil, fatty meats, cheese, and cream. Triglycerides can also come from sugar and alcohol. Fat from the food you eat is broken down and converted into energy. Any fat that is not used by the body will be converted into triglycerides and stored in fat cells. When needed, triglycerides are released to be used as energy. Increased levels of triglycerides in the blood occurs when the intake of triglycerides from food exceeds the amount needed by the body. This condition can lead to thickening of the walls of blood vessels, which increases the risk of stroke, heart attack, and heart disease.

In addition to excessive fat intake, high levels of triglycerides in the blood are also influenced by several factors, namely:

- Genetic disorders
  A person who has a family member with a history of familial genetic disorder hypertriglyceridemia is at risk of having an abnormal increase in triglycerides in the body
- Consumption of excess carbohydrates
  Poor diet and consumption Polafood with carbohydrates too much can trigger an increase in calories and sugar in the body. Excess sugar and calories will be converted by the liver into triglycerides to be stored in fat cells.
- Consumption of alcoholic beverages
  Alcohol contains a lot of calories and sugar so it can affect triglyceride levels in a person's body.
- Smoking habit
  Compounds in cigarettes can cause the body to be unable to respond insulin hormone. In fact, the hormone insulin has an important role in regulating sugar and triglyceride levels. As a result, triglyceride levels in the body continue to increase.
- Rarely exercise
  When you exercise, your body breaks down fat cells for energy. If a person rarely exercise, fat will accumulate so that blood triglyceride levels will increase.
- Certain diseases or conditions
  Certain diseases or conditions can cause a person to experience an increase in triglycerides in the blood. These diseases include: Diabetes, kidney illness, liver disease, Low thyroid hormone levels (hypothyroidism), Obesity, and Menopause.
- Certain drugs
  There are several types of drugs that can cause side effects in the form of increased triglycerides.

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2.3. Garlic

Garlic or garlic comes from the Old English "gar" which means spear or spearhead, and "lic" which means tuber or daffodil. Sometimes garlic is also called Allium sativum which comes from the Celtic language "All" which means bad smelling, and "sativum" which means to grow . Garlic (*Allium sativum*) is the tuber of the Allium sativum plant, included in the Amarylidaceae family, other benefits as a seasoning for canned meat dishes, sauces, soups, and others garlic can also reduce blood clotting and reduce inflammation, so it is important in the treatment of cardiovascular disease. Allicin and adenosine are the most important anti-platelet compounds in garlic.

As a traditional medicinal plant, garlic has many benefits. The main part of garlic that is efficacious as a medicine is the tuber. Various studies have been carried out by scientists to examine the potential of garlic bulbs and their biological activities related to pharmacology, including antidiabetic, antihypertensive, anticholesterol, antiathero-sclerosis, antiplatelet aggregation, fibrinolysis booster, antiviral, microbial, anticancer, and antioxidant.

Garlic can help improve cardiovascular function because it can overcome atherosclerosis, hyperlipidemia, hypercholesterolemia caused by free radicals. Garlic has antioxidants that can counteract free radicals .

3. Methodology

This research is experimental research conducted at the Integrated Laboratory of Poltekkes Kemenkes Bandung for the manufacture of garlic extract using the maceration method and the tool used is a rotary evaporator, while for the examination of triglyceride levels it is carried out at the Pharmacology Laboratory of UNPAD using a photometer.

The population in this study was Wistar rats (Rattus norvegicus) with a total sample of 25 male Wistar rats weighing 200 gr which were divided into 5 groups with different treatments, each group consisted of 5 Wistar rats.

The number of rat samples used in this study was calculated using the Federer formula:

$$(t-1)\,(r-1) \geq 15 \qquad [1]$$

Description:
t = number of treatment groups, and
r = replication

$\rightarrow (t-1)(r-1) \geq 15$
$\rightarrow (5-1)(r-1) \geq 15$
$\rightarrow 5r-5-r+1 \geq 15$
$\rightarrow 4r-4 \geq 15$
$\rightarrow 4r \geq 19$
$\rightarrow r \geq 19/4$
$\rightarrow r \geq 4.75 = 5$ rats

The results of the data that have been obtained are presented in the form of an illustration. Then analyze the data systematically. The data were then analyzed statistically with descriptive methods, in the form of information from researchers and several library sources.

Extraction. The manufacture of garlic extract in this study used the maceration method. The way it works is to peel the skin of the garlic and then weigh as much as 1000 grams. Furthermore, the garlic is washed and dried in an oven at 400°C for 2 days. Then the garlic is mashed in a blender as much as 800 grams. Garlic is then soaked in 2 liters of 70% ethanol solvent for 3x24 hours. The next process is evaporation. Evaporation is the process of changing molecules in a liquid state (for example water) spontaneously into gas (for example water vapor) which is carried out in the laboratory on day 4 using a vacuum rotatory evaporator for 14 hours, in order to obtain a thick extract of garlic as much as 144.282 grams. The dose of garlic extract used for adult humans weighing 50 kg is 7.2 grams/day. The conversion factor from human 70 kg to mouse 200 g is 0.018.

Examination of Triglycerides Level [15] on the 15th day, the rat's blood was taken by looking for the veins in the rat's tail. Blood was collected in a 1 cc syringe and then transferred into a test tube. The tube containing the blood was centrifuged for 15 minutes, then the serum was taken and put into a new Eppendorf tube. The serum obtained was used to measure triglyceride levels using a photometer

with a wavelength of 520 (492-546) nm. Prepare samples, reagents, and standard samples of triglycerides. Prepare three test tubes. Put 1000 l of triglyceride reagent into three tubes. Put a standard sample of triglycerides as much as 10 l into a tube containing 1000 l of triglyceride reagent. Put 10 l of serum sample into the tube containing the reagent. Put 10 l of Aquades into the tube containing the reagent, which was used as a blank. Incubate for 5 minutes at 37oC. Read the absorbance of the sample using a photometer after 60 minutes.

4. Finding and Discussion

4.1. Garlic Extract

The method used is the maceration method. The maceration method has advantages and disadvantages. The advantages of the maceration method include that it does not require complicated equipment, is relatively inexpensive, can avoid evaporation of compound components because it does not use heat, while the disadvantages of the maceration method are that it takes a longer time to extract the active substance in the sample, a lot of solvents so it is not efficient, and the water extract produced will be quickly damaged and smell bad [16].

The garlic extract used in treatment I with a dose of 200 mg was 1.4 grams of garlic extract for five rats used for 7 days, in treatment II with a dose of 400 mg was 2.8 grams of garlic extract for five rats treated used for 7 days. In treatment III with a dose of 600 mg, 4.2 grams of garlic extract for five rats were used for 7 days. Each treatment was diluted with 5 ml of garlic extract using carboxymethyl cellulose (CMC).

4.2. Ethanol

In this study, the ethanol used was 50% ethanol with a dose of 2 ml/mgbb for each rat. Before giving ethanol to rats, the steps taken were diluting ethanol using 125 ml of distilled water with 115 ml of ethanol from all groups. In the negative control, treatment I, treatment II, and treatment III, the ethanol used was 60 ml for 5 rats in each group for 7 days with 2 ml each given

4.3. Triglyceride levels

Measurement of triglyceride levels was carried out before and after treatment using a photometer. The normal value of triglyceride levels in rats is 26-145 mg/dl. Examination of triglyceride levels before treatment was carried out using rat blood taken from the tail, while for examination of triglyceride levels after treatment was carried out using rat blood taken from the heart of rats.

Examination of triglyceride levels was first carried out by centrifuging the blood of rats to obtain serum. After that, the triglyceride reagent was put into the tube according to the number of samples examined (25 samples) of 1000 l each. Then take as much as 10 l of the serum sample using a mucropipette and mix it into a tube that has been filled with triglyceride reagent. Furthermore, it was incubated for 20 minutes at room temperature, then the results were read on a photometer with a wavelength of 456 nm [17].

In this study, triglyceride levels were also examined before and after treatment, namely on the 8th and 14th days. Initial and final triglyceride levels can be seen in Table 1.

Table 1. Triglycerides Rate during Treatment

Group	Number of Rats	Triglycerides rate	
		Before [mg/dl]	After [mg/dl]
Negative Control Group [G1]	1	62.2	56.1
	2	45.6	40.7
	3	38.9	26.1
	4	51.1	39.1
	5	45.6	46.6
Average		48.7	41.7
Positive Control Group [G2]	1	36.7	103.1
	2	44.4	75.1
	3	37.8	81.3
	4	32.2	88.9

	5	41.1	63.5
Average		38.4	82.4
Treatment 1[G3] [200mg/ 200grBW]	1	53.3	102.8
	2	47.6	80.4
	3	56.3	45.5
	4	86.7	79.4
	5	100.1	74.1
Average		68.8	76.4
Treatment 2[G4] [400mg/200gr BW]	1	30.9	102.6
	2	80.2	43.4
	3	62.2	51.8
	4	66.7	54.5
	5	46.7	55.1
Average		45.7	61.5
Treatment 3[G5] [600mg/200gr BW]	1	86.7	103.7
	2	31.1	101.3
	3	56.3	92.1
	4	71.1	85.7
	5	91.1	58.2
Average		67.3	88.2

After obtaining data on the results of triglyceride levels before and after. This data was then processed using a computer program. The first step that needs to be done is to test the normality of the data using the Shapiro-Wilk normality test. This test was chosen because the number of samples used was less than 50. After the normality test was carried out. It was found that all data had a normal distribution with $p > 0.05$ so that the test used for this research data is a one-way ANOVA test.

Based on the ANOVA output, it is known that the significant p value in the pre-treatment group is $p>0.05$. i.e. 0.070 so it can be concluded that the average of the five treatments in the pre-treatment group was "SAME" significantly. While the significant value of p in the group after treatment is $p <0.05$ which is 0.004 so it can be concluded that the average of the five treatments in the group after the treatment was "DIFFERENT" significantly between negative control positive control. First treatment, second treatment and third treatment administration of 50% ethanol at a dose of 2 ml/200 gram BW in rats could increase triglyceride levels while solid garlic extract (*Allium sativum*) reduced triglyceride levels in white rats (*Rattus norvegicus*).

The increase in triglycerides after exposure to 50% ethanol at a dose of 2ml/200gr BW [18]. That alcohol contains a lot of sugar and calories. So it can increase triglycerides in the body. Consuming large amounts of alcohol can increase the release of VLDL increase the flow of free fatty acids to the liver from adipose tissue and inhibit the body's ability to break down fat due to a decrease in the metabolic mechanism of breaking down chylomicrons and VLDL residues due to the inhibitory effect of alcohol on lipoprotein lipase activity then alcohol increases the synthesis of large VLDL particles in the liver. Which are the main source of triglycerides in hypertriglyceridemia associated with excessive alcohol intake.

Excessive alcohol consumption will also increase the risk of increasing triglyceride levels. Chronic metabolism of ethanol causes impaired fatty acid oxidation and carbon conversion to fat leads to increased production of triglycerides in the liver. Excess triglycerides in the liver are then released into the blood vessels and there is a buildup of triglycerides in the blood vessels and can progress to Coronary Heart Disease (CHD) [19].

While garlic is thought to be able to reduce triglyceride levels in rats after exposure to 50% ethanol at a dose of 2 ml/200 g BW by inhibiting the production of fatty acids because it contains the compound Allicin causing the allyl chain to be reduced so that the propyl chain becomes saturated and there is a decrease in the levels of NADH and NADPH in the blood thereby reducing the production of NAD+ which causes a decrease in the production of glycerol 3-phosphate. While the active substance allicin in garlic competes with acetate which can reduce the input of acetyl CoA and no glycolysis occurs to form glycerol 3-phosphate so that the production of triglycerides decreases.

5. Conclusion

The conclusion in this study is that 50% ethanol with a dose of 2 ml/200 g BW can increase triglyceride levels in white rats (*Rattus norvegicus*) while garlic extract (*Allium sativum*) can reduce triglyceride levels in white rats (*Rattus norvegicus*) when compared with positive control group that was not treated with garlic extract (Allium sativum).

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