

Conference Paper

The Effect of Bulb Extract from Dayak Onion (*Eleutherine americana* Merr) to the Cholesterol Level and the Triglyceride Level in Mice (*Mus musculus*)

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Abstract

Bawang dayak or *dayak* onion (*Eleutherine americana* Merr) is an endemic plant from West Kalimantan. The bulb extract from *dayak* onion is known to be consumed as traditional medicine by *dayak* ethnic group. *Dayak* onion contained alkaloid, flavonoid, glycoside, saponin, and tannin that are very beneficial for health. This research aims to examine the effect of ethanol extract of *dayak* onion towards the cholesterol level and the triglyceride level in animal testing male mice (*Mus musculus*). This research uses completely randomized design with experimental laboratory methods by doing 5 treatments and 5 times replications. Mice are given mixed food consists of CP 511 concentrate and cow fat using 7:3 comparison for 10 days. Mice are also given treatment with the ethanol extract of *dayak* onion for 7 days with classifications of: negative control treatment, positive treatment, 50 mg/Kg BB, 100 mg/Kg BB, and 200 mg/Kg BB. The research result shows that the treatment of ethanol extract *dayak* onion has significant effect towards the decrease of triglyceride level in mice's blood, but based on LSD test, the differentiation between each treatment is not significant. Based on the research result, ethanol extract of *dayak* onion according to the average result can indeed decrease the level of cholesterol, but the statistics test has not yet shown significant results.

Keywords: ethanol extract of *dayak* onion, male mice, the level of cholesterol, the level of triglyceride

INTRODUCTION

Bawang dayak or *dayak* onion (*Eleutherine americana* Merr) is a plant from Borneo forest that is commonly used by the local tribe as herb or a traditional medicine that can cure various diseases. The bulb of this plant which came from *Eleutherine* genus have been scientifically proven by several studies to contain the secondary metabolite compound in the type of naftokuinon (elecanacin, eleutherin, elutherol, eleutherinon). Up until now, *dayak* onion plants are well-known to be used in the therapy of colon cancer, breast cancer, diabetes mellitus, hypertension, lowering the level of cholesterol, a cure of ulcers, stroke, abdominal pain after childbirth (Galingging, 2009). The results of a research done by Kuntorini (2013) show that *dayak* onion bulb contains different antioxidants depending on the age of the bulb, the antioxidants contained in *dayak* onion bulbs are steroids, tannins, quinones and flavonoids.

Dayak onions contain substances such as alkaloids, flavonoids, glycosides, saponins and tannins that are very useful for maintaining the body's health. The content of these substances are believed to be capable of lowering cholesterol levels and triglyceride levels. The objective of this research is to examine the effect of *dayak* onion extract on cholesterol level and triglycerides level of mice (*Mus musculus*).

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The high level of serum cholesterol or hypercholesterolaemia may increase the risk of coronary heart disease. It is characterized by the concentrations of plasma total cholesterol and a high amount of low density lipoprotein. Hypercholesterolemia can occur due to unbalanced food intake, which is the intake of high-fat and carbohydrate, low fiber foods, along with smoking and drinking alcohol and lack of exercise. If the caloric content of the food intake is not balanced with the needs in the body, there will be accumulation of fat in the body (Hardani, 2008).

Triglycerides in the body are used to provide energy for various metabolic processes. Some lipids, especially cholesterol, phospholipids and triglycerides are used throughout the body to form cell membranes and to perform other cellular functions (Guyton, 1997). The level of triglycerides in the body can be increased because of food intake that contain high amount of fat. The high level of triglyceride also increases the risk of coronary heart disease.

Cholesterol is the fat component found in the blood vessels of all animals, as well as humans. Cholesterol is actually useful as a source of energy, forming the cell in the body, and as the basic ingredient of hormone formation. Cholesterol is needed, but if it is excessive, it can form deposits on the walls of blood vessels. The high level of cholesterol will cause the thickening of plaques in the lumen of blood vessels that can cause damage to blood vessels. Plaque attached to the walls of blood vessels will contain fat and inflammatory components (Kuntorini, 2013).

METHODS

This research uses a completely randomized design with experimental laboratories method. The research was conducted in Biology laboratory of PGRI University Adi Buana Surabaya, while the test of cholesterol level and triglyceride level was done at laboratory of Bhayangkara Hospital Surabaya. The animal testing that is used are male mice (*Mus musculus*) aged 8 weeks. Before given the treatment, the animal-testing mice were acclimatized in the cage of mice for 7 days according to its treatment group.

The extraction of *dayak* onion is obtained by slicing the *dayak* onion into thin pieces that followed by the process of drying it out in a room temperature without contact with direct sunlight for 5 days. After that, the *dayak* onion is blended until it becomes fine powder. The extraction of *dayak* onion is done by a maceration method. A 100 gram of *dayak* bulb powder was soaked in 1000 ml of 96% concentration ethanol for 3 days, which is followed by a distillation process. The result of extraction was then filtered with Whatman filter paper, and then evaporated using a rotary evaporator to obtain a thick extract. Furthermore, the extract of *dayak* onion is dried in an oven with a temperature of 50^o C to obtain a dry extract for the treatment.

The research was conducted by feeding the mice with cow fat to determine the effect towards cholesterol level and triglyceride level in mice blood. Giving the concentration of *dayak* onion extract refers to the research of Carmelita (2016), with concentrations of 0 mg/Kg BB, 50 mg/Kg BB, 100 mg/Kg BB and 200 mg/Kg BB. Mice are given mixed food consists of CP 511 food concentrate and cow fat using 7:3 ratio of food concentrate: cow fat refers to the research of Shintawati, et al (2011). The provision of cow fat is done by heating the cow fat and turning it into liquid form, then mixing it with CP 511 food concentrate.

This research is accomplished by doing 5 treatments and 5 times replications by providing the mixed of food concentrate CP 511 and fat cow as follows: LBN: negative control group (given no cow fat and no *dayak* onion extract) LBP: positive treatment group 0 mg/Kg BB (given cow fat, but without *dayak* onion extract), LB1: treatment group 50 mg/Kg BB (given cow fat, with 50 mg of *dayak* onion extract), LB2: treatment group 100 mg/Kg BB (given cow fat, with 100 mg of *dayak* onion extract), LB3: treatment group 200 mg/kg BB (given cow fat, with 200 mg of *dayak* onion extract). The treatment groups of LBP, LB1, LB2, LB3 were treated by feeding with the mixed of CP 511

concentrate food and cow fat with the ratio of 7:3 for 10 days which aims to increase the level of cholesterol and the level of triglyceride. While the LBN treatment group is a negative control treatment that is not fed with a mixed of CP 511 concentrate food and cow fat that serves as a comparison.

After 10 days of the induction feeding, each treatment group was given *dayak* onion extract with a dose that has been determined for 7 days by way of feeding. After the treatment stage, blood sampling of mice is obtained through the tail of the mice, the blood samples are then accommodated in the Vaculab tube. Furthermore, the blood samples were tested to determine its level of cholesterol and level of triglyceride at the laboratory of Bhayangkara Hospital Surabaya.

RESULT AND DISCUSSION

The results of the research on the effect of onion *dayak* extract towards the level of cholesterol and the level of triglyceride of mice are presented in Table 1. In Table 1, it can be seen that the provision of *dayak* onion extract on doses of 50 mg/Kg BB, 100 mg/Kg BB and 200 mg/Kg BB are proven to be able to lower the level of cholesterol under the negative control, although is not quite significant yet ($p = 0.122 > 0.05$). Moreover, the provision of *dayak* onion extract on doses of 50 mg/Kg BB, 100 mg/Kg BB and 200 mg/Kg BB are proven to be able to lower the level of triglyceride significantly ($p < 0,05$).

Table- 1. The level of cholesterol and the level of triglycerides of mice after being given treatment of *dayak* onion extract

Treatment	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Negative control (LBN)	142 ± 28,636 a	126 ± 18,166 a
Positive control (LBP)	180 ± 62,849 a	262 ± 46,185 b
<i>Dayak</i> onion extract 50 mg/Kg BB (LB1)	136 ± 24,083 a	154 ± 33,615 a
<i>Dayak</i> onion extract 100 mg/Kg BB (LB2)	134 ± 11,402 a	132 ± 27,749 a
<i>Dayak</i> onion extract 200 mg/Kg BB (LB3)	124 ± 11,402 a	136 ± 50,299 a

Description: numbers followed by different letters in the same column show a significant difference ($p < 0.05$)

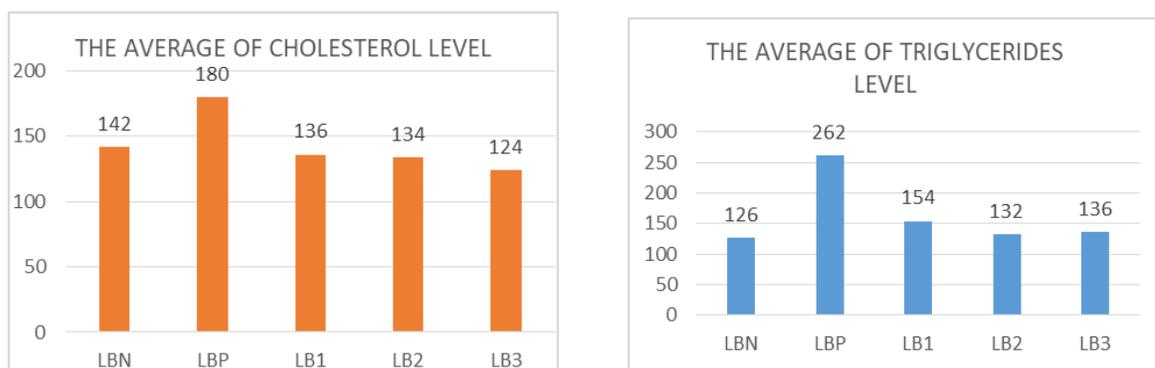


Figure 1. Chart that shows the average ratio of cholesterol level and triglyceride level in the blood of the mice after being given the treatment of *dayak* onion extract

The chart shows that the provision of *dayak* onion extract can significantly lower the level of triglyceride in mice, while the effect of it on the lowering of the level of cholesterol that is not quite significant. The experiment of *dayak* onion extract were given to the animal-testing mice for 7 days, and has not been able to significantly lower the level of cholesterol. However, the average results of the treatments show that the cholesterol levels had lower to below normal rate (140 mg/dL).

In this research, the hyperlipidemia state of mice was obtained by feeding the mice with a mixture of food concentrate CP 511 and cow fat with a ratio of 7:3 for 10 days. The result shows that in positive control treatment group, the level of cholesterol of the mice increased up to 180 mg/ dL. Furthermore, the provision of *dayak* onion extracts can lower the level of cholesterol in mice's blood, although it is not quite significant yet.

The provision of high-fat food for 10 days can increase the level of triglyceride in mice, it can be seen through the positive control treatment group in which the level of triglyceride in mice increased up to 262 mg/ dL. Furthermore, the provision of *dayak* onion extracts can significantly lower the level of triglyceride in mice's blood, although there are no significant differences found between treatment group of LB1, LB2 and LB3. It is possibly because the duration of the *dayak* onion extracts that is given for 7 days.

The cow fat in the mixture of mice's food contains saturated fatty acids that can trigger the formation of triglycerides and cholesterol in the blood. Most tissues can form fatty acids from acetyl-CoA, while some long-chain fatty acid synthesis of short fatty acids can occur in mitochondria. In addition to that, there is also a chain extension system outside the mitochondria. Most of the de novo fatty acids synthesized from acetyl-CoA via different pathways, mainly occur outside the mitochondria, which is in the microsomes (Ganong, 1979).

Dayak onions contain compounds of alkaloids, flavonoids, glycosides, saponins and tannins that are useful for the health of the body because it contains antioxidant. Primary antioxidants follow a mechanism of radical chain breaking reaction by donating a hydrogen atom rapidly to a radical lipid, resulting product that is more stable than the starting product. While secondary antioxidants work by binding metals that act as pro-oxidants, capturing radicals and preventing chain reactions. Secondary antioxidants act as binders of metal ions, oxygen catchers, decomposers of hydroperoxides into non-radical compounds, UV radiation absorbers or deactivation of singlet oxygen. Thirdly, tertiary antioxidants work to repair the biomoleculars damage caused by free radical. The examples of tertiary antioxidants are enzymes that repair DNA and methionine sulfide reductase (Sayuti and Yenrina, 2015).

Flavonoids contribute to their antioxidant activity in vitro by the act of flavonoid binding metal ions such as Fe and Cu. These metal ions can catalyze the reaction that ultimately produces free radicals. Another way that can be used to stop the oxidation process is by adding additives substances. Addition of additives such as antioxidants can be used to inhibit the oxidation process. The mechanism of this inhibition depends on the chemical structure. In this mechanism, the most important thing is the reaction with lipid free radicals, which will form the non-active product (Gordon, et al. 2001 in Sayuti and Yenrina, 2015). Saponin is a natural glycoside bonded to steroids or triterpene. Saponins have extensive pharmacological activities including: immunomodulator, anti-tumor, anti-inflammatory, anti-virus, anti-fungal, can kill shells, hypoglycemic, and hypocholesterol effects.

The ability of *dayak* onion extracts in lowering the level of cholesterol and the level of triglyceride in blood is expected to be due to the role of flavonoids, tannins and saponins. Flavonoids are reducing compounds capable of inhibiting many oxidation reactions both enzymatically and non-enzymatically (Robinson, 1995). This compound is also an antioxidant because it can catch free radicals by releasing hydrogen atoms from its hydroxyl groups (Nurwahyunani, 2006). The inhibition of this oxidation process is expected to be able to inhibit the synthesis of cholesterol and triglyceride. In addition, the content of saponins and tannins are also able to reduce the level of triglyceride by different mechanisms, which is by inhibiting the absorption of triglycerides in the intestine (Nurdewi, 2008). With the triglycerides inhibited from entering the gastrointestinal tract, the triglycerides that enter the blood vessels are reduced. This will result in the decreasing level of triglycerides in the blood. The triglyceride that was not being absorbed then will be excreted with the feces.

Dayak onion is also proven effective to boost the immune system of the body. The results of Carmelita's research (2016) showed that giving the ethanol extract of *dayak* onion orally to mice Balb/ c mice was proven effective to prevent the decrease of germinal center diameter on lymph nodes at doses of 100 mg/ KgBB and the level of serum IgG at doses of 200 mg/ KgBB doses. Furthermore, the results of the Meiliana's research (2016) showed that the provision of ethanol extract of *dayak* onion at doses of 100 mg/ KgBB and 200 mg/ KgBB orally to mice Balb/ c has the effect to prevent the decreasing amount of cell NK and CD 8+.

CONCLUSION

1. The *dayak* onion extract (*Eleutherine americana* Merr) is proved to have significant effect in decreasing the level of triglyceride in the blood of mice (*Mus musculus*), although there are no significant differences between each treatment group.
2. The *dayak* onion extract (*Eleutherine americana* Merr) is proved to have no significant effect in decreasing the level of cholesterol in the blood of mice (*Mus musculus*).

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