

THE EFFECT OF ETHANOL EXTRACT DRAGON TAIL LEAVES (*EPIPREMNUM PINNATUM* (L.) ENGL.) AGAINST ATHEROSCLEROSIS OF MALE QUAIL

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ABSTRACT: Empirically dragon tail leave is used to decrease lipid. Lipid is one of the risk factors trigger atherosclerosis. Atherosclerosis is a disease that shows thickness and less elasticity (hardening) of the arteries. This research aimed to determine the effect of ethanol extract dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) against atherosclerosis of male quail. The preparation was administered orally for 28 days with a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg. The results were analyzed using one way ANOVA followed by Duncan test. The results showed improvement in the histopathology of the aorta has increased aortic blood vessel lumen area and a decrease in endothelial cell damage score was significantly ($p < 0.05$). The best dosage was indicated by the ethanol extract of dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) was 100 mg/kg. Then the duration of the factors did not show a significant effect on organ weight ratio of heart and liver ($p > 0.05$) but showed a significant influence on the weight ratio of the kidney ($p < 0.05$).

Keywords : Dragon tail Leave, atherosclerosis, histopathology

1. INTRODUCTION

Atherosclerosis is a disease that shows thickening and loss of elasticity (stiffness) in large arteries and small which is characterized by the accumulation of fatty deposits, platelets, macrophages and other white blood cells depths of the intima (lining endothelial cells) and ultimately to the tunica media (smooth muscle layer). Artery most often affected is the aorta, coronary arteries and cerebral arteries [1].

Coronary heart disease is a risk factor for atherosclerotic disease when the reduction in coronary blood flow [2]. Globally, the number one cause of death is cardiovascular disease each year, which is due to malfunctioning of the heart and blood vessels, such as coronary heart disease. Data-Driven Health Research Association, the Ministry of Health and Development Agency of the Republic of Indonesia and Target Population Data, Media Center of the Ministry of Health of the Republic of Indonesia, the prevalence of coronary heart disease in Indonesia is based on the doctor's diagnosis estimated at about 883447 people, while the basis of symptoms is estimated at about 2650340 million people. A number of patients with coronary heart disease in the province of West Sumatra by the doctor's diagnosis of about 20567 people, while based on the symptoms of about 41133 people [3].

The high prevalence of these diseases can be caused by changes in people's lifestyles are sedentary or sedentary and tend to choose foods high in cholesterol and saturated fats [2]. The intake of cholesterol and saturated fats are high in these foods can raise levels of cholesterol *low-density lipoprotein* (LDL) and lower levels cholesterol of *high-density lipoprotein* (HDL) thereby increasing the risk of atherosclerosis [4].

Various measures for the prevention of atherosclerosis treatment have been widely promoted, among others, by using traditional medicines. Community use of plant dragon tail by taking leaves boiled water to treat various diseases including lowering body fat, cancer, hypertension and stroke therapy [5].

Results of previous studies showed that the leaves contain flavonoids dragons of 26.7137 $\mu\text{L/mL}$ manifold flavonoids that have antioxidant activity [6]. Isolation of flavonoid compounds extracts dragon tail leave has antioxidant activity against the radical DPPH (1, 1-Diphenyl-2-Picrylhydrazyl [7]. The ethanol extract dragon tail leaves

(*Epipremnum pinnatum* (L.) Engl.) Have significant effect as anti-lipid, anti-inflammatory and analgesic in albino Wistar rats and Swiss albino mice, as well as LD₅₀ is greater than 500 mg / kg [8]. Ethanol extract dragon tail leaves may also lower total blood cholesterol levels significantly male rats, which is best demonstrated by a dose of 100 mg/kg [9].

Dragon tail leaves suspected to contain secondary metabolites of the flavonoid. Other studies also show the results that secondary metabolite flavonoid contained in saurian leaves have antioxidant activity by administering a dose of 50, 100 and 200 mg/kg. The dose of 50 mg/kg give effect to the best protection in atherosclerosis, with an improvement in wall thickness aorta, the area changes the lumen of the aorta and the state of the cells endothelia in hypercholesterolemia quail induced by feeding high fat (MLT) and *propylthiourasil* (PTU) [10].

Based on the above, the author intends to raise the issue in a study with the title of the effect of ethanol extract dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) Against atherosclerosis of male quail.

2. METHODOLOGY

2.1 Tools

The tools used are sonde, 3 cc syringe (Terumo), analytical balance (Ohaus), the scales of a *triple beam balance* (Ohaus), *infrared moisture balance* (Ohaus), vacuum evaporator (*Ika*), measuring cups (Iwaki), test tubes (Iwaki), filter paper, a water bath (Mettler), funnel (Iwaki), rod stirrer (Iwaki), micropipette (Bio-rad), pycnometer (Iwaki), photometer 5010 V5 (Riele), watch glass, cover glass, glassy objects (*Sail Brand*), plate TLC silica gel 60 F₂₅₄ (Merck), porcelain crucible, spectrophotometric UV-Vis spectrophotometer (Shimadzu), lamp UV 254 (Camag) *tissue processor* (Network processor Automated Histology T-TSGAJH), *tissue embedding center* (Dispensing Console EC350-1), *rotary microtom* (Biosystems Leica RM2125 RTS) microscope (Olympus BX 51 DP2-BSW DP20).

2.2 Raw

materials used are the dragon tail leaves, feed quail (Hi-Pro-Vite Medicated 324-2) (PT Charoen Pokphand Indonesia), egg yolks, beef tallow, *propylthiourasil* (PTU) (Dexa), 70 % ethanol (PT Bratachem), 96 % ethanol (PT Bratachem), distilled water (PT Bratachem), *sodium*

carboxy methyl cellulose (Na CMC) (PT Bratachem), copper sulphate (Merck), iodine (Merck), potassium iodide (Merck), potassium sodium tartrate (Merck), bismuth subnitrat (Merck), sodium chloride (PT Bratachem), methanol (PT Bratachem), lead acetate (Merck), acetic acid (Pt. Bratachem), acetic acid anhydride (PT Bratachem), chloroform (PT Bratachem), mercury chloride (Merck), potassium hydroxide (Merck), picric acid (Merck), ferric chloride (Merck), sulfuric acid (PT Bratachem), sodium hydroxide (PT Bratachem), hydrochloric acid (PT Bratachem), alpha naphthol (Merck), gelatin (Pharma Lab) ammonia (PT Bratachem), n-butanol, physiological 0.9% sodium chloride (NaCl physiological 0.9%) (PT Widatra Bhakti), formalin (PT Bratachem), dye *haematoxyllin* (SPA. Chem), *eosin* (The Science Company), *xylol* (Merck), *mayer's albumin* (He Sys), paraffin (Merck), adhesive *etellan* (Merck).

2.3 Sampling

The samples used in this study are the dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) Fresh as much as 3 kg taken in Jalan Sawahan III, Sawahan, District East Padang, Padang, West Sumatra.

2.4 Plant Identification

Identification extract dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) is performed at Andalas University Herbarium Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang.

2.5 Preparation Animal Experiments

Animal experiments used were male quail were 2-3 months old weighing 100-150 g as many as 25 animals. Were randomly Animals divided into 5 groups. Each group consisted of 5 mice. Before treatment, the first animal experiments in acclimatization for 1 week (before and after acclimatization the animals were weighed weight) are fed by feeding quail and drinking enough water. Each group received a different treatment for 28 days. then the animals were sacrificed by dislocation of his neck, then do surgery incision lengthwise on the midline of the abdomen to the chest and abdominal cavity contents moved to the right by using a wad of cotton, so the heart can be seen clearly, then cardiac, liver and kidney are separated from connective tissue by using tweezers to be weighed. The aorta of the heart organ is taken, used for examination of atherosclerotic lesions.

2.6 Dose Planning

Dose extract dragon tail leaves (*Epipremnum pinnatum* (L.)Engl.) given to animals orally is 50 mg/kg, 100 mg/kg, and 200 mg/kg.

2.7 Preparation of Test preparations

Extracts made by maceration using 70 % ethanol. A total of 300 g of powder simplicia dragon tail leaves inserted into the two dark bottles, each 150 g plus 1.5 L of ethanol 70 % in each of the bottles of dark, soaked for 6 hours while occasionally stirring, then allowed to stand for up to 24 hours. Maserat separated by filtration using a flannel cloth and pulp macerated repeated twice with the type and amount of the same solvent. All master collected and evaporated in a vacuum evaporator to obtain a thick extract.

2.8 High-fat food manufacture

High-fat food is made by means of beef tallow heated to melt, add the feed quail stirring until evenly, then add egg yolks stirring until homogeneous high-fat foods.

2.9 Suspension manufacture PTU

Suspension PTU orally given to quail. PTU suspension award aims to reduce the metabolic function of the quail, which can help increase cholesterol. PTU dose for an adult human 100 mg, converted to quail $100 \times 0.018 = 1.8$ mg/200 g BW, administered daily orally. Prepare a solution of Na CMC 0.5% by means of: 0.5 grams of Na CMC sprinkled over hot water as much as 20 time (10 mL) in a hot mortar leave for ± 15 minutes until puffed, then enter the PTU crushed up to form a homogeneous suspension then add distilled water to a volume of 100 mL.

2.10 Atherosclerosis lesions examination At Heart Aorta Rats Male

Making preparations histopathology (Junqueira *et al.*,1997).

Making preparations for histopathology using paraffin method, which is the heart organ is taken from quail dissected washed first with 0.9 % NaCl solution pphysiologic, fixation with 10 % formalin solution for 48 hours. Dehydrated sequentially with 70 % alcohol-rise; 80 %; 96 %, respectively for 1 hour and then do process *the clearing* with *xylol* 2 times, each for 1 hour. Infiltration into liquid paraffin for 1 hour and incubated for 3.5 hours in an incubator at a temperature of 56-60°C then do the process *embedding* is implanting tissue into the mold with pure paraffin media. Networks that have been planted on a beam made of wood and then cut with a *rotary microtome* thickness of 5 μ m. Put the object glass previously gummed *mayer's albumin* (egg white and *glycerin*), then dry it down and cuts on the slide are placed on a water bath filled with water at a maximum temperature of 40 °C.

Staining dye preparations with *Haematoxyllin-eosin* (Junqueira *et al.*, 1997).

The incision that has been applied to a glass slide with *deparafinisasi xylol* 2 times for 5 minutes, then dehydrated with graded alcohol 96%; 80%; 70% each for 2 minutes wash with running water. Colored with *Haematoxyllin* for 2 minutes and then wash with running water until clean. Then color with *eosin* for 5 minutes. Dehydrated in graded alcohol 70%; 80%; 96% each for 2 minutes using a clearing next *xylol* 2 times, each for 2 minutes after the wind dried. Perform the process *mounting* by providing adhesive *etellan* on preparations and covering it with a *cover glass* to observe under a microscope.

C. Examination of atherosclerotic lesions

1. of the aorta wall thickness of the aortic wall thickness was measured at 6 points that can represent the overall thickness of the aortic wall and then averaged.
2. Examination of the aorta diameter aortic diameter was measured at 3 points that can represent the overall diameter of the aorta and then averaged.
3. The percentage of aortic blood vessel lumen area (% LL)
% LL = (lumen area / wide vessel) x 100%
Size = πr^2 ($\pi = 3,14$)
4. Rate level aortic endothelial cell damage assessment is done by observing the damage to endothelial cells and occurs whether or not the proliferation of aortic smooth muscle cells. Then were scored according to their severity.
 - a. Score 1 for normal endothelial cells (endothelial cells are not damaged).
 - b. Score 2 for severity minor (endothelial cells is slightly damaged, but still regularly).

- c. Score 3 to moderate severity (kontinitasnya endothelial cells have been lost, irregularly shaped and begin the accumulation of fat).
- d. Score 4 for the severity of large (kontinitasnya endothelial cell damage is not clear anymore, irregularly shaped, the accumulation of fat as well as a proliferation of smooth muscle cells). Then calculate the average for each treatment.

2.11 Data Analysis

All data were processed using SPSS 21. Data were analyzed statistically using one-way ANOVA followed by Duncan test.

3. RESULTS AND DISCUSSION

Experimental animals used were quail son because these experiments often used in the testing of cardiovascular disease, especially in diseases of blood vessels, has a vulnerability is very high against the buildup of fat in the blood vessels that are categorized in atherosclerotic lesions and has a diameter of aorta greater compared to mice and rats, so it's easy to see the damage caused to the aorta [11,12]. The occurrence of atherosclerotic lesions that resemble the lesions of atherosclerosis in humans. Tailored to the human physiological, where men are more at risk of developing atherosclerosis than women. This is due to the hormone estrogen in women who can withstand an increase in blood cholesterol, thus preventing atherosclerosis [13]. Inducers used as triggers increased levels of LDL-cholesterol in the blood of experimental animals is a high-fat meal (MLT) with the feed composition of quail, beef fat, egg yolk. The increase in levels of LDL-cholesterol in the blood is a disorder most frequently and importantly as a predisposition to atherosclerosis. High levels of cholesterol circulating endothelial membrane cholesterol content increases, resulting in increased viscosity of the membrane, decreasing the flexibility of endothelial cells and facilitate the occurrence of cell damage. When LDL to oxidation by macrophages in the arterial wall, free radicals can damage cells that occur muscle or tissue beneath the endothelium [14]. Beef fat high in saturated fatty acids, which can be oxidized to acetyl-Co-A as a major source of carbon atoms in the formation of cholesterol so that it can raise blood

cholesterol [13]. Besides MLT, PTU also is used as an inducer. PTU is an antithyroid agent that affect the role of thyroid hormones in the catabolism of lipids in the blood, so as to increase and maintain the levels of LDL in the blood and accelerate the onset of damage to the blood vessels, especially aortic atherosclerotic lesions [15].

Parameters measured were changes in the structure of the aorta which is characterized by the formation of plaque, or atheroma in the blood vessel wall fat deposition, endothelial cell damage and proliferation of smooth muscle cells of blood vessels. High cholesterol levels will not only cause thickening of plaque in the blood vessel lumen but also easily lead to damage of blood vessels. Plaques were attached to the walls of blood vessels that contain fat and plaque components which thicken the blood vessel wall will further narrow the vessel lumen. Plaques containing cholesterol in the blood vessels can appear anywhere [13].

All animal experiments through the acclimatization phase beforehand, this stage is very important for the adjustment to the new environment. Then the animals are grouped into 5 groups, one group consisted of 5 mice male quail. Each group consists of a negative control, positive control, treatment with a dose of 50 mg / kg, treatment at a dose of 100 mg / kg, treatment at a dose of 200 mg / kg. After treatment for 28 days, on day 29, underwent surgery to take blood vessel aorta. The parameters were observed in the treatment group compared with the control group, namely the negative control group were only fed quail and a positive control group which only MLT and PTU induced without ethanol extract dragon tail leaves.

The results of the study the average aortic blood vessel wall thickness in negative control group of animals; positive control; dose of 50 mg / kg; a dose of 100 mg / kg; dose of 200 mg / kg for 28 days in a row is: 7.46 µm; 9.5 µm; 8.76 µm; 7,82 µm; 6.34 lm. Then research the average diameter of the aorta in a row is 22.2 µm; 24.66 µm; 23.72 µm, 24.2 µm; 21.36 lm. Thus obtained the average area of the blood vessel lumen of the aorta in a row is: 10.8333%; 5.3747%; 6.9174%; 12.5117%; 16.4873% (Figure 1). The average value shows that the ethanol extract of leaves dragons can increase the percentage of lumen area.

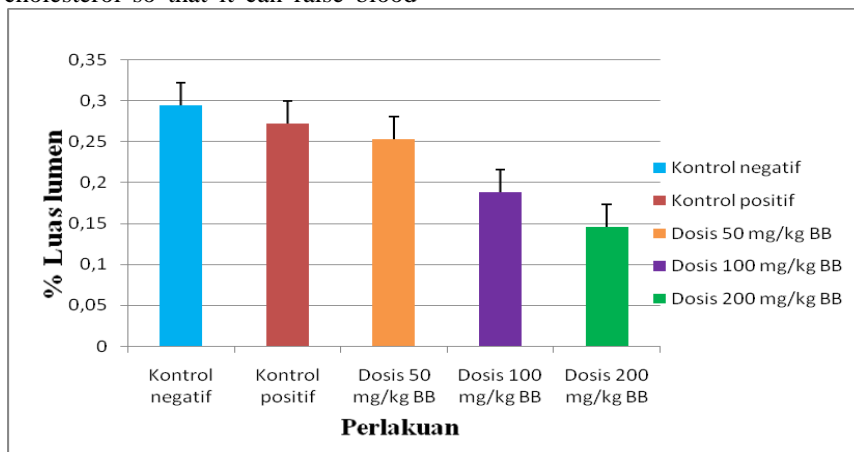


Figure 1: Diagram comprehensive measurement results aorta blood vessel lumen male quail

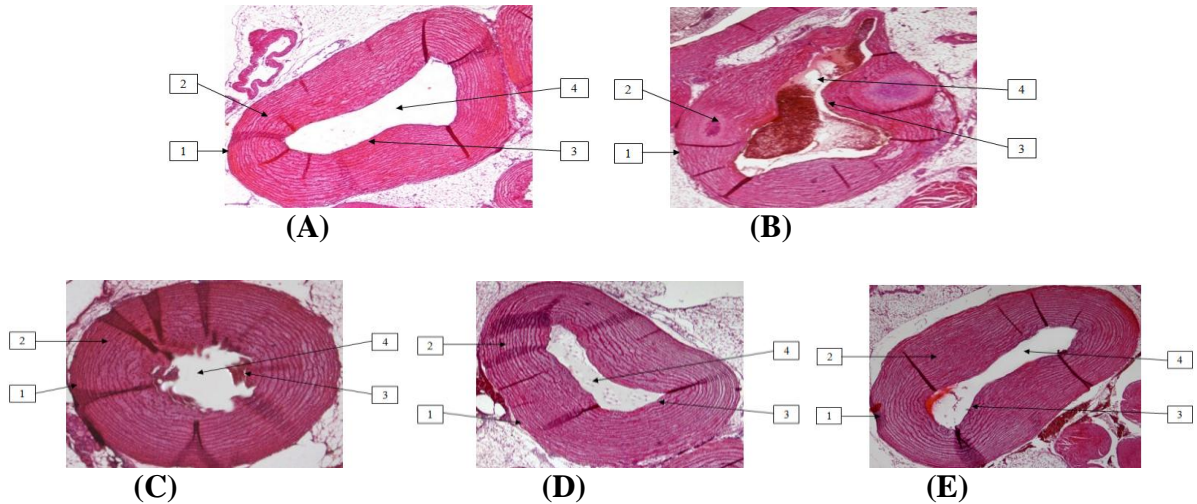


Figure 2: Histopathology aorta negative control group of animals (2A) 1 tunica adventitia, 2 tunica media with intact muscle tissue, 3 intima with regular endothelial cells and 4 large lumens. Group positive control animals (2B) 1 tunica adventitia, 2 tunica media smooth muscle cells proliferate, 3 intima with endothelial cells are irregular and four lumen narrowing. Groups of treated animals a dose of 50 mg / kg bw (2C) 1 tunica adventitia, tunica media 2, 3 intima with interrupted endothelial cells and 4 lumen narrowing. Groups of treated animals a dose of 100 mg/kg bw (2D) 1 tunica adventitia, tunica media intact 2, 3 intima with endothelial cells was irregular and four lumen slightly enlarged. Groups of treated animals a dose of 200 mg / kg bw (2E) 1 tunica adventitia, tunica media intact 2, 3 intima with endothelial cells was irregular and four lumen slightly enlarged, on a 400 x magnification microscope.

Histopathological observations to negative control animals look very wide lumen intima characterized by regular and tunica media with intact muscle tissue (Figure 2A).

On the positive control animals, visible intima damage to the endothelial cells continuity not clear anymore, irregularly shaped, a proliferation of smooth muscle cells, as well as the lumen narrowing due to blood vessel thickening (Figure 2B).

In the treatment with a dose of 50 mg/kg seen intima with interrupted endothelial cells and blood vessel lumen narrowing of the aorta (Figure 2C).

At a dose of 100 mg/kg body weight intact tunica media, tunica intima with endothelial cells that are still regular and slightly enlarged lumen (Figure 2D).

At a dose of 200 mg/kg seen tunica media is intact, the intima with endothelial cells that are still regular and slightly enlarged lumen (Figure 2E).

The observation of histopathology shows wall endothelial cells of the intima have improved relative to the positive control animals, it may be caused due to the ethanol extract of leaves of dragons (*Epipremnum pinnatum* (L.) Engl.)

Can maintain the aortic blood vessel damage caused by high levels of total cholesterol which can lead to atherosclerosis.

The results of normality and homogeneity test data variable percentage of lumen area showed significant results that normality sig. 0.853>0.05 and homogeneity sig. 0.056>0.05, so it can be passed with a one-way ANOVA test. On the matter of the ANOVA results that sig of the percentage of total lumen area with sig. 0.000<0.05, which means that the ethanol extract of leaves of dragons affect the vast percentage of the lumen. Statistical analysis then continued with Duncan test, the results showed value *mean* of each dose is the same subset. A dose of 100 mg/kg was in the same subset with the negative control, the dose of 100 mg/kg is considered effective to prevent the occurrence of atherosclerosis.

Endothelial cell damage resulting scores on average in the negative control animals; positive control; dose of 50 mg/kg; a dose of 100 mg/kg; dose of 200 mg/kg 28 days in a row is 1.2; 3; 2.4; 1.6; 1.4 (Figure 3).

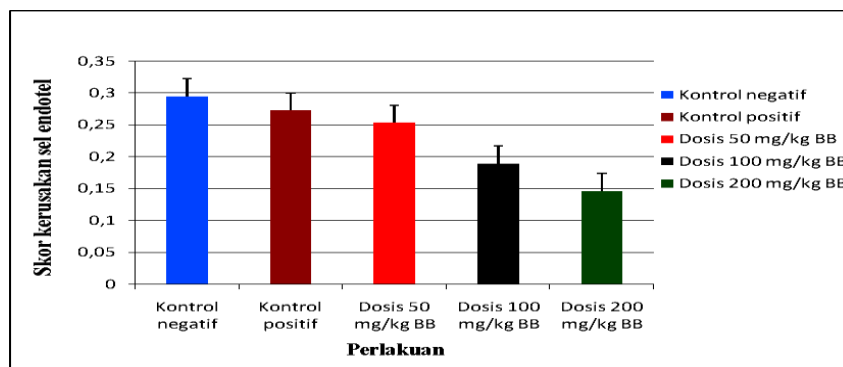


Figure 3. Diagram of the score of the vascular endothelial cell damage aorta of male quail

Histopathologic score observations damage endothelial cells in the positive control showed a great degree of damage. On the negative control indicates the level of

damage that is almost non-existent. In the treatment with a dose of 50 mg/kg showed a score close to the positive control, where there are endothelial cells were

disconnected. At a dose of 100 mg/kg showed a score of more improved than the dose of 50 mg/kg, which is still a little bit damaged endothelial cells in the intima.

The results of normality and homogeneity test score data variables damage endothelial cells showed significant results that normality sig. $0.429 > 0.05$ and homogeneity sig. $0.42 > 0.05$, so it can be passed with a one-way ANOVA test. On the matter of the ANOVA results that sig of the percentage of total lumen area with sig. $0.001 < 0.05$, which means that the ethanol extract of leaves of a dragon's tail effect on endothelial cell damage score. Then the statistical analysis continued by duncan, where the results showed that the ethanol extract of leaves of a dragon's tail at a dose of 100 mg/kg and 200 mg/kg were in a subset of the same with the negative control, it can be concluded ethanol extract of leaves of dragons dose 100 mg / kg and 200 mg/kg body weight can reduce the damage to vascular endothelial cells of the aorta. This may be due to the flavonoids in the leaves of the dragon's tail. Flavonoids have antioxidant properties that can inhibit the oxidation of LDL [16]. Flavonoids are antioxidant potential in the prevention of cardiovascular disease by inhibiting the oxidation of LDL, thereby protecting the cell constituents against oxidative damage and limit the development of athermanous lesions [17].

The research data is also supported by the weight ratio of quail male organ. Organ observed were cardiac, liver and kidney. Where the vital organs play a role in the process of distribution, metabolism and excretion of drugs [18], so that the known effect of MLT, PTU and ethanol extract of leaves of a dragon's tail to the vital organs of test animals for evaluation of drug safety and adverse effects of drugs or toxic compounds in the body (Shargel, et al., 2012), for the treatment long enough (28 days). After statistical analysis test, organ weight ratio with one-way ANOVA ethanol extract of leaves dragons did not affect the ratio of heart and liver organ weights ($p > 0.05$), but affects the kidney ($p < 0.05$). This means that long-term use can affect the kidneys.

CONCLUSIONS

From the study it can be concluded that:

1. Ethanol extract dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) can improve histopathologic aorta with increasing aortic blood vessel lumen area and a decrease in endothelial cell damage score significantly.
2. The increase in aortic blood vessel lumen area and a decrease in endothelial cell damage score is the best demonstrated by the ethanol extract dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) dose of 100 mg/kg.
3. Ethanol extract dragon tail leaves (*Epipremnum pinnatum* (L.) Engl.) did not affect the ratio of heart and liver organ weights ($p > 0.05$), but the ethanol extract of leaves of dragons (*Epipremnum pinnatum* (L.) Engl.) Influence the ratio severe kidney ($p < 0.05$).

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