

The Effect of Dayak Onion Bulb Ethanol Extract (*Sisyrinchium palmifolium* L.) on Triglyceride Level and Aorta Histopathology in Diabetes Melitus White Rat Induced by Alloxan

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ABSTRACT

The high prevalence of diabetes mellitus in Indonesia has made antidiabetic treatment efforts increasingly popular. In addition, the many side effects caused by the use of chemical drugs triggered the development of research on herbal therapy. The purpose of this study was to determine the effectiveness of Dayak onion bulb extract in reducing two-hour postprandial blood glucose, triglyceride levels, and the amount of lipid vacuoles in aorta blood vessels of diabetic rat. The long-term goal of this study is the creation of innovation products in the form of standardized herbs as complementary therapies that can be used daily by the community, so that the risk of morbidity can be reduced. The research design used was randomized control group pretest posttest design. The results of blood glucose analysis showed that there were significant differences between the positive control group with the 400 mg/KgBW and 800 mg/KgBW extract group, with a significance value of 0,000. In the extract group, 400 mg/KgBW with 800 mg/KgBW extract group showed no significant difference with a significance value of 0.390. The results of the analysis of triglyceride levels showed no significant difference between the positive control group with the 400 mg/KgBW extract group, with a significance value of 0.981. However, there were significant differences between the positive control group with the 800 mg/KgBW extract group with a significance value of 0.025. Between the 400 mg/KgBW and 800 mg/KgBW extract groups, there was a difference with a significance value of 0.024. Aorta histopathology results showed that there was a significant difference in the number of lipid vacuoles between the positive control group and the 400 mg/KgBW extract group and 800 mg/KgBW extract group. Based on the research, it can be concluded that the extract dose of 400 mg/KgBW is a dose that is able to influence the decrease in blood glucose levels, triglycerides and the amount of lipid vacuoles in the aorta blood vessels.

Keywords: blood glucose, Dayak onion bulb, lipid vacuoles, triglycerides.

INTRODUCTION

Socio-economic and lifestyle changes have given rise to new habits that are not in accordance with the principles of a healthy lifestyle. This is one of the causes of the increasing prevalence of degenerative diseases and is suspected to be the main cause of death in Indonesia. One disease to watch out for is diabetes mellitus (Cahyono, 2008). According to the International Diabetes Federation (2011), diabetes mellitus ranks in the top 10 causes of decreased productivity and disability. Therefore, action must be taken, if not the number of diabetics in the world will increase from 366 million in 2011 to 552 million in 2030. Based on Diabetes Atlas 6th Edition (2013), Indonesia is ranked as the third most diabetics in the Asia region after China and India.

Various treatment efforts have been used for centuries to treat diabetes. Research on antidiabetic treatment is also growing, both

by using chemical drugs or herbal therapies from natural ingredients. Diabetogenic substances such as alloxan can cause insulin-dependent diabetes mellitus in experimental animals with characteristics similar to type 1 diabetes mellitus in humans. In Type 1 DM mice there is an increase in blood glucose levels and tends to experience increased cholesterol and triglycerides. This is caused because excessive glucose cannot be formed into energy so that energy is taken from lipid, consequently cholesterol formed in the chain of lipid metabolism increases (Fahri, 2005). Prolonged increase in plasma cholesterol levels will cause narrowing or hardening of the arteries called atherosclerosis. In addition, increased plasma cholesterol levels play a role in accelerating the occurrence of vascular atherosclerosis (Fatmawati, 2008).

The increasing development of modern and traditional drug production is influenced by public awareness about the benefits of plants as medicine. Communities increasingly realize the importance of back to nature by utilizing natural ingredients. Some plants are allegedly can be used in treating

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diabetes mellitus, one of which is the Dayak onion bulbs (*Sisyrinchium palmifolium* L.). Dayak Onion is a typical Indonesian plant and has been used for generations as a medicinal plant for the community. According to Febrinda (2013), the Dayak onion bulb contains naphthoquinone compounds and their derivatives, such as eleutherine elecanacine, eleuthernone, and eleutherol. The ethanol extract of Dayak onion bulbs also contains tannin, alkaloids, saponins, phenolics, steroids, flavonoids and triterpenoids. Naphthoquinone is an antioxidant that can reduce excessive levels of xanthine oxidase enzymes; xanthine oxidase enzymes play a role in the formation of free radicals. The decrease in this enzyme will reduce free radicals and thus inhibit the damage of pancreatic beta cells, then the synthesis and secretion of insulin will increase. Increased synthesis and secretion of insulin will increase the work of lipid-breaking enzymes so that lipid absorption is reduced. The broken lipid will be transported and metabolized in the liver, then excessive lipid is released through bile secretion (Nugroho, 2006).

The potential of Dayak onion bulbs as a medicinal plant is very large, but scientific studies regarding the efficacy of onion Dayak are still limited. This is the background of the research on the effectiveness of ethanol extract of Dayak onion bulbs (*Sisyrinchium palmifolium* L.) on blood glucose levels and triglycerides, as well as the aortic histopathology in type 1 diabetes mellitus white rats induced by alloxan.

METHODOLOGY

Materials and tools

The materials used in this study were Dayak onion bulbs obtained from Kubu Village, Bangli Regency, Bali Province, aquadest, 80% ethanol, alloxan, glibenclamide, CMC Na, ketamine, xylazine, HCl 2N, reagent Dragendorff, Mayer reagent, iron (III) 10% chloride, amyl alcohol, chloroform, anhydrous acetic acid, and concentrated sulfuric acid.

The tools used in this study include glassware, hot plates, ovens, vacuum rotary evaporators, Olympus BX51 light microscopes, digital scales, Buchner funnels, and Blood glucose Gluco-D® Test Meters.

Test animals

The test animals used in this study were white Wistar rats, which were 2.5-3 months old and weighed 190-200 grams. The rats are kept in the laboratory, given food according to laboratory standards and drinking water in ad libitum.

Experimental animals are conditioned of diabetes mellitus by induction using alloxan.

Extract making

Dayak onion bulbs were determined in advance at the Indonesian Institute of Sciences (LIPI), UPT Eka Karya Botanical Gardens Plant Conservation Center, Bali. Extraction was carried out by the maceration method using 80% ethanol solvent in a beaker glass-covered and protected from light. Macerate is stirred constantly for 60 minutes using a stirring rod. The second and third days, macerate is stirred again for 60 minutes, closed and stored again. On the fourth day, macerate was filtered with the help of a Buchner funnel (vacuum) to obtain the first filtrate. The pulp is macerated again with the same type and amount of solvent as the procedure above, maceration is carried out until the sixth day. The filtrate was evaporated with a rotary evaporator at 40°C, so that a thick extract was obtained.

Phytochemical screening

Making test solutions for phytochemical tests was carried out by dissolving as much as 300 mg of ethanol extract of Dayak onion bulbs in 30 ml 80% ethanol.

Alkaloid test

A solution of 2 ml test extract was evaporated on top of the porcelain dish until the residue was obtained. The residue is then dissolved with 5 ml 2N HCl. The solution obtained is then divided into 2 test tubes. The first tube was added with 3 drops of Dragendorff reagent. The second tube was added to the Mayer reagent by 3 drops. Orange deposits in the first tube were formed and yellow to yellowish deposits in the second tube showed alkaloids (Farnsworth, 1966).

Flavonoid Test

One gram of powder plus 50 mL of hot water, boiled for 5 minutes, filtered. Then 5 mL of the solution is added with Mg powder, plus 2 mL of chlorhydric alcohol solution and amyl alcohol. The solution is shaken strong and allowed to separate (if the flavonoids are positive, the amyl alcohol layer is yellow) (Ditjen POM RI, 2000).

Tannin Test

The 1 ml test extract solution was reacted with a 10% iron (III) chloride solution, if occurs dark blue or greenish black color showed the presence of polyphenol and tannin compounds (Robinson, 1991; Jones and Kinghorn, 2006)

Saponin test

The test extract as much as 0.5 gram is put into the test tube, added 10 ml of hot water, cool and then shaken vigorously for 10 seconds. Formed solid foam for no less than 10 minutes as high as 1-10 cm. At the addition of 1 drop of 2N HCl, froth is not lost (Depkes, 1989).

Test steroids and terpenoids

A 2 ml test solution was evaporated. The residue obtained was dissolved in 0.50 mL of chloroform, then added with 0.5 ml of anhydrous acetic acid. Next, this mixture was dripped with 2 ml of concentrated sulfuric acid through the tube wall. If bluish green is formed, it indicates sterols. If the results obtained are in the form of a brownish or violet ring on the border of the two solvents, indicating the presence of a triterpenoid (Ciulei, 1984).

Implementation of Research

All rats were adapted for 30 days before being treated and on the 31st day blood rats were taken through tails to be examined for post-prandial 2-hour blood glucose levels (before treatment).

All groups of mice were given a single dose of alloxan injection 24 mg / intraperitoneal 200g rat.

All diabetic rats with post prandial 2-hour blood glucose levels <120-140mg / dL. then divided into 4 groups. Group 1 as a positive control group that DM induced and treated with glibenclamide 5 mg/kgBW). Group 2 as negative control group that DM induced and given placebo (water + CMC Na), group 3 was a group DM induced and treated with Dayak onion bulbs extract dose of 400mg/kgBW, group 4 was DM induced group and treated with Dayak onion bulbs extract dose of 800mg/kgBW.

The treatment was carried out for 30 days by giving Dayak onion bulbs extract.

After 30 days of administration of extracts, blood was taken to measure blood glucose levels and triglycerides, as well as surgery for test animals to see the aortic histopathology.

Data analysis was performed to compare the results of the four groups.

Examination of Blood Glucose Levels 2 Hours Post Prandial

Blood collection was carried out twice, namely before administration of extracts and after administration of extracts (30th day). Blood collection is carried out through a rat's tail. Blood is dropped on the blood glucose measuring strip Glucose-D® Blood glucose Test Meter.

Examination of Triglyceride Levels

Examination of triglyceride levels using spectrophotometric tests. A serum sample of 10µL plus 1 ml triglyceride reagent was put into a test tube, incubated for 10 minutes at 37°C. The results of each sample were read on a spectrophotometer with a wavelength of 546 nm.

Making Aortic Blood Vessel Preparations

The vascular aortic organs of rats were put into a 4% para-formaldehyde (PFA) solution. The making of histopathological preparations includes dehydration, clearing, embedding, sectioning, attachment to the object glass, staining of hematoxylin eosin. Histopathology of aortic blood vessels was observed using an Olympus BX51 light microscope with a 400x magnification and was divided into five visual fields to identify the histopathological picture of aorta experiencing atherosclerosis. Histopathological observations that observed were changes in aortic tissue in the form of lipid infiltration in tunica adventitia. Histopathological analysis of aorta was carried out at the Pathology Laboratory of the Faculty of Veterinary Medicine, Udayana University.

Data Analysis

Data obtained in this laboratory study were statistically analyzed to determine the characteristics of the sample. The data normality test was carried out by the Shapiro-Wilk Test because of the number n <30. Test the homogeneity of data using Levene's Test. Comparative tests were carried out through analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening on Dayak onion bulbs ethanol extract showed that the extract contained tannin, triterpenoids and flavonoids (Table I).

Measurement of Blood Glucose Levels

Blood glucose levels were measured twice, before (pretest) and after treatment (posttest). The results of measuring the average blood glucose level (Figure 1).

Figure 1. shows that in the positive control group and extract group (Dayak onion bulbs extract 400 mg/kgBW and 800 mg/kgBW) there was a decrease in blood glucose levels after treatment (posttest), in contrast to the negative control group where glucose levels increased blood from an average of 155mg/dL (pretest)

Table I. Phytochemical screening result

| Secondary Metabolites | Result | Conclusion |
|---------------------------|--|------------|
| Alkaloids | Orange deposits are not formed. Yellow deposits are not formed. | (-) |
| Sterols and Triterpenoids | No bluish green ring (Sterol) is formed. A brownish and violet (Triterpenoid) ring is formed. | (-) (+) |
| Saponin | No solid foam is formed for no less than 10 minutes as high as 1-10 cm. | (-) |
| Tannin | A greenish black color indicates the presence of tannin compounds | (+) |
| Flavonoids | A yellow amyl alcohol layer is formed. | (+) |

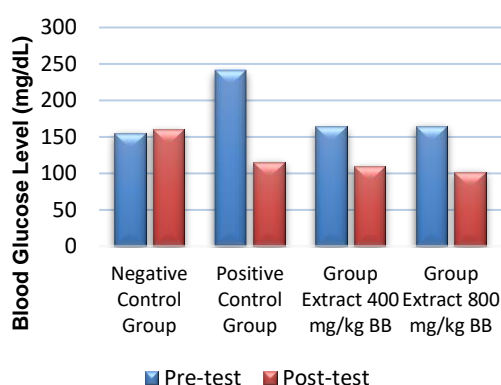


Figure 1. Average blood glucose levels of rats at pretest and posttest

to 160mg/dL (posttest). The decrease in blood glucose levels in the test animals was thought to be due to the presence of secondary metabolites in Dayak onion bulbs extracts. Flavonoids have hypoglycemic activity or lower blood sugar levels (Dhamuri, 2014). Research conducted by Arjadi and Susatyo (2010) states that flavonoids can reduce blood sugar levels by stimulating pancreatic β cells to produce more insulin. Tannin is known to stimulate glucose and lipid metabolism so that the accumulation of these two sources of calories in the blood can be avoided. Tannin also has hypoglycemic activity namely by increasing glycogenesis (Daliamartha, 2005). Triterpenoids are indicated as therapeutic agents that can be utilized in the management of diabetes mellitus, because triterpenoids have been shown to be effective in controlling the symptoms of glycosuria (Raissa, 2014).

Statistical analysis

The results of the normality test data after the Shapiro-Wilk test showed that blood glucose

levels (pretest and posttest) in each group were normally distributed with a significance value of $p > 0.05$. In the homogeneity test obtained a significance value of 0.283 (pretest) and 0.245 (posttest), so it can be concluded that the data variant is homogeneous.

The results of Paired T-Test in the negative control group showed no significant difference in blood glucose levels pretest and posttest, with a significance value of 0.061 ($p > 0.05$). In the positive control group and extract group (extract 400mg/KgBW and 800mg/KgBW) significant values were obtained in 0,000; 0,002; 0,000 ($p < 0.05$), this shows that there is a significant difference in blood glucose levels before and after treatment.

The results of One-way ANOVA analysis of pretest and posttest data obtained a sig value of 0,000 ($p < 0.05$). This shows that there are differences in all treatment groups. The LSD test results showed that there was a significant difference between the positive control group and the Dayak onion bulbs extract group 400mg/KgBW and a dose of 800mg/KgBW, with a significance value of 0,000 ($p > 0.05$). In the group of Dayak onion bulbs extract 400 mg/KgBW with 800mg/KgBW extract obtained a significance value of 0.390. This shows that there were no significant differences between the two groups. Comparison of blood glucose levels of each group (Table II).

Measurement of Triglyceride Levels

Diabetes mellitus can cause an increase in plasma lipids. According to Widyastuti (2000), that the increase in lipids in diabetics is caused by lack of insulin. Insulin increases lipoprotein lipase activity on the surface of endothelial cells in catalyzing the reshuffle of triglycerides from kilomycron and lack of insulin will reduce this

Table II. Test results for LSD analysis of the mean blood glucose levels

| Group | Group | Sig. | Information |
|----------------------------|----------------------------|-------|-----------------------|
| Negative Control Group | Positive Control Groups | 0,000 | There is a difference |
| | Group Extract 400 mg/Kg BW | 0,000 | There is a difference |
| | Group Extract 800 mg/Kg BW | 0,000 | There is a difference |
| Positive Control Group | Negative Control Group | 0,000 | There is a difference |
| | Group Extract 400 mg/Kg BW | 0,000 | There is a difference |
| | Group Extract 800 mg/Kg BW | 0,000 | There is a difference |
| Group Extract 400 mg/Kg BW | Negative Control Group | 0,000 | There is a difference |
| | Positive Control Groups | 0,000 | There is a difference |
| | Group Extract 800 mg/Kg BW | 0,390 | No difference |
| Group Extract 800 mg/Kg BW | Negative Control Group | 0,000 | There is a difference |
| | Positive Control Groups | 0,000 | There is a difference |
| | Group Extract 400 mg/Kg BW | 0,390 | No difference |

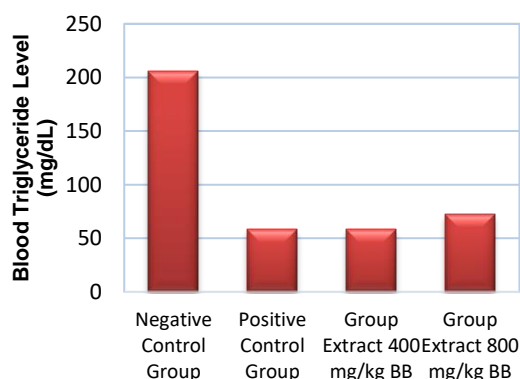


Figure 2. Average triglyceride levels after treatment

enzyme (Fatmawati, 2008). Diabetes mellitus is a disease that is often associated with free radical activity that causes oxidative damage in some tissues (Fatmawati, 2008; Jung, 2006). The results of the average level of triglycerides in each group (Figure 2).

In Figure 2 shows that the average level of triglycerides in the negative control group is greater than the other groups, which is equal to 206 mg/dL. In the positive control group and extract group (extract of 400mg/KgBW and extract 800mg/KgBW), the average triglyceride levels were sequentially 58 mg/dL, 58 mg/dL, and 72 mg/dL. Flavonoids contained in Dayak onion bulbs extract act as antioxidants by inhibiting lipid oxidation (Kumalaningsih, 2007). Based on research (Wibowo, 2009; Peng and Kuo, 2003),

flavonoids can capture free radicals and prevent lipid peroxidation processes in microsomes and liposomes.

Statistical analysis

The results of normality test data obtained that the data are normally distributed with a significance value in sequence 0.303; 0,313; 0.562; 0.709 ($p > 0.05$). The homogeneity test obtained a significance value of 0.171 ($p > 0.05$), so it can be concluded that the data variants in the study had a homogeneous variety. The results of One-way ANOVA analysis of triglyceride levels obtained a sig value of 0,000 (sig < 0.05). This shows that there are differences in the treatment group. The LSD test results showed that there was no significant difference between the positive control group and the Dayak onion bulbs extract group 400mg/KgBW, with a significance value of 0.981 ($p > 0.05$). However, there was a significant difference with the extract group 800mg/KgBW with a significance value of 0.025. In the group of Dayak onion bulbs extract 400 mg/KgBW with an extract of 800mg/KgBW obtained a significance value of 0.024 indicating that there were significant differences between the two groups. The results of the comparison of triglyceride levels in each group (Table III).

Aortic Histopathology

Damage to the blood vessels of a diabetic diabetes mellitus aorta is characterized by lipid infiltration in tunica media and tunica adventitia (Taylor *et al.*, 2005). Aortic histopathology is done by analyzing lipid vacuoles in the aortic arteries.

Tabel III. Test result for LSD analysis of the mean of triglyceride levels

| Group | Group | Sig. | Information |
|----------------------------|----------------------------|-------|-----------------------|
| Negative Control Group | Positive Control Groups | 0,000 | There is a difference |
| | Group Extract 400 mg/Kg BW | 0,000 | There is a difference |
| | Group Extract 800 mg/Kg BW | 0,000 | There is a difference |
| Positive Control Group | Negative Control Group | 0,000 | There is a difference |
| | Group Extract 400 mg/Kg BW | 0,981 | No difference |
| | Group Extract 800 mg/Kg BW | 0,025 | There is a difference |
| Group Extract 400 mg/kg BW | Negative Control Group | 0,000 | There is a difference |
| | Positive Control Groups | 0,981 | No difference |
| | Group Extract 800 mg/Kg BW | 0,024 | There is a difference |
| Group Extract 800 mg/Kg BW | Negative Control Group | 0,000 | There is a difference |
| | Positive Control Groups | 0,025 | There is a difference |
| | Group Extract 400 mg/Kg BW | 0,024 | There is a difference |

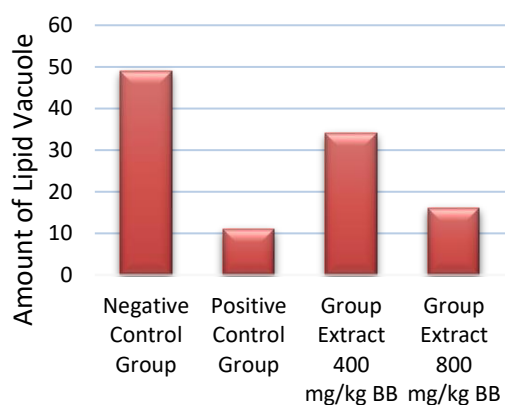


Figure 3. Average amount of lipid vacuole

The amount of lipid vacuole that occurs in the aortic wall of test animals after treatment (Figure 3).

Figure 3 shows that the average number of lipid vacuoles in the negative control group was more than the positive control group and the extract group.

Statistical analysis

In the Shapiro-Wilk test obtained the number of lipid vacuoles from the four groups was normally distributed with a significance value of 0.591; 0.139; 0.772; 0.294 ($p > 0.05$). The homogeneity test results obtained a significance value of 0.308, this indicates that the data on the number of lipid vacuoles have a homogeneous variety. In the One-way ANOVA test obtained a significance value of 0,000 ($p < 0.005$), this indicates that there are differences in the treatment group. The LSD test results showed that there were significant differences in the number of lipid vacuoles between the negative control group

and the positive control group, Dayak onion bulbs extract 400 mg/KgBW, and extract 800 mg/KgBW with a significance value of 0,000 ($p < 0.05$). This shows that the extract group can improve lipid infiltration better than the negative control group. The flavonoid content of Dayak onion bulbs extract can function as an antioxidant, so it can regenerate aorta that has undergone atherosclerosis (Sulistyoningrum, 2010). The positive control group showed a significant difference in the group of Dayak onion bulbs extract 400mg/KgBW and extract 800mg/KgBW, with a significance value of 0,000 and 0,000 respectively ($p > 0.05$). This shows that glibenclamide is more effective in reducing the amount of lipid vacuole compared to Dayak onion bulbs extract. This is because glibenclamide can increase insulin secretion from pancreatic beta cells and long-term goals in the form of increasing the effect of insulin on peripheral tissue and decreasing liver glucose expenditure (Purwanto, 1994). Insulin deficiency can cause increased mobilization of free fatty acids from adipose tissue which causes an increase in LDL-cholesterol production (Latha and Daisy, 2011). The rapid formation of insulin can reduce lipid vacuoles in rat aorta. The antioxidants in Dayak onion bulbs can oxidize LDL cholesterol which is the main reason for atherosclerosis (Witztum, 1991). The LDL oxidation process that is inhibited causes no inflammation and vasodilation of blood vessels so that there is no inflammatory cells that enter the tunica media of the aorta of rat (Karunia, 2014).

CONCLUSION

Dayak onion bulbs extract dose of 400mg/KgBW is a dose that is able to influence the decrease in blood glucose levels, triglycerides and

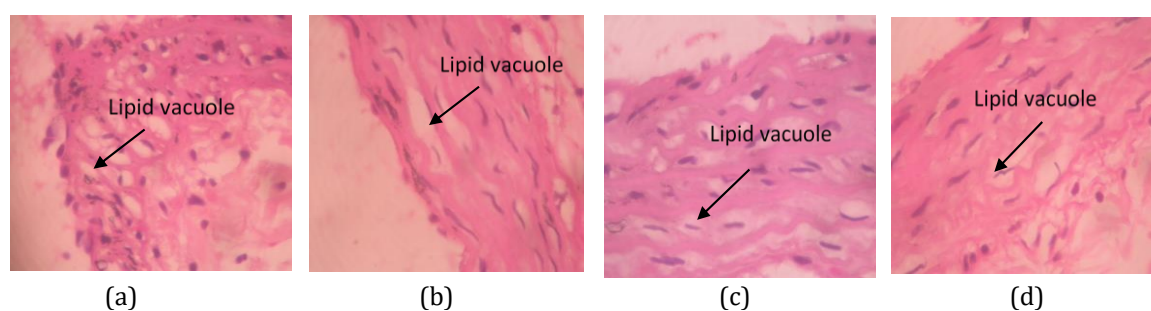


Figure 4. Histopathology of aorta

Information: (a) Histopathological description of aortic negative control; (b) Histopathological description of aortic positive control of glibenclamide; (c) Histopathological description of aorta treatment of Dayak onion bulbs extract 400 mg / kg BW; (d) Histopathological description of aorta treatment of Dayak onion bulbs extract 800 mg / kg BW

Table IV. Test result for LSD analysis of lipid vacuoles number

| Group | Group | Sig. | Information |
|----------------------------|----------------------------|-------|-----------------------|
| Negative Control Group | Positive Control Groups | 0,000 | There is a difference |
| | Group Extract 400 mg/kg BW | 0,000 | There is a difference |
| | Group Extract 800 mg/kg BW | 0,000 | There is a difference |
| Positive Control Group | Negative Control Group | 0,000 | There is a difference |
| | Group Extract 400 mg/kg BW | 0,000 | There is a difference |
| | Group Extract 800 mg/kg BW | 0,000 | There is a difference |
| Group Extract 400 mg/kg BW | Negative Control Group | 0,000 | There is a difference |
| | Positive Control Groups | 0,000 | There is a difference |
| | Group Extract 800 mg/kg BW | 0,000 | There is a difference |
| Group Extract 800 mg/kg BW | Negative Control Group | 0,000 | There is a difference |
| | Positive Control Groups | 0,000 | There is a difference |
| | Group Extract 400 mg/kg BW | 0,000 | There is a difference |

the amount of lipid vacuole in the blood vessels of aortic diabetic diabetes mellitus induced by alloxan.

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