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The effect of jengkol (*Archidendron pauciflorum*) fruit peel ethanolic extract to heart histologic of rat induced by streptozotocin

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Abstract. *Abadi SA, Illiyyin Z, Rachmadina JR, Malini DM. 2018. The effect of jengkol (*Archidendron pauciflorum*) fruit peel ethanolic extract to heart histologic of rat induced by streptozotocin. Biofarmasi J Nat Prod Biochem 16: 59-63.* Indonesia is ranked 7th out of 10 countries with the highest diabetic patients in the world. Diabetes mellitus is a disease that can cause heart disorders and adults who suffer from DM are four times more likely to develop heart disease. Jengkol fruit peel has been used traditionally as a drug for diabetes mellitus. The aim of this research was to know the effect of fruit peel ethanolic extract of jengkol (*Archidendron pauciflorum*) (JFPEE) on rat (*Rattus norvegicus*) heart histological structure and to obtain effective dose from JFPEE. This research used experimental method in laboratory with Completely Random Design (CRD) using 6 treatments and 4 replications. Treatment was given for 14 consecutive days consisting of negative control, positive control, comparison (glibenclamide dose 10 mg/kg BW), P1, P2, and P3 (JFPEE dose 385, 770, and 1.540 mg/kg BW). Diabetic induction was performed with streptozotocin dose 65 mg/kg BW in female wistar rat except for negative control group. The parameters that observed were number of necrosis and cell damage score, including fat degeneration, hydropic degeneration, and inflammatory cell. The obtained data were analyzed by ANOVA-Tukey's test with 95% confidence level using SPSS version 21 for Windows. The result of histological structure showed that number of necrosis and cell damage score in group of rats treated with JFPEE dose 385 mg/kg BW (174.25±6.34; 1.25±0.50) were not significantly different from the negative control rats (172.00±7.62; 1.00±0.00). The effective dose of JFPEE that can repair the damage of heart cell's rat induced by streptozotocin was 385 mg/kg BW.

Keywords: *Archidendron pauciflorum,* heart, jengkol fruit peel, rat, streptozotocin

INTRODUCTION

The total population of Diabetes Mellitus (DM) patients in Indonesia is estimated to reach 8.2 million patients over the age of 20 by 2020. Indonesia is ranked 7th out of 10 countries with the highest diabetics in the world (IDF 2015). Diabetes Mellitus is a chronic metabolic disease disorder because the pancreas can not produce enough insulin or the body can not use effectively the insulin production, resulting in an increase in glucose concentration in the blood or hyperglycemia (Kemenkes 2014).

DM can affect all organs of the body and cause various complaints, one of which is heart trouble, such as coronary heart disease (CHD), congetive heart failure, and stroke (Fatimah 2015). According to the American Heart Association in May 2012, less than 65% of people with diabetes die of heart disease or stroke. In addition, adults who suffer from DM are two to four times more likely to develop heart disease than people without diabetes.

One of the organs that has the most important function is the heart. Heart serves as a pumping device to circulate blood, either to the lungs or all other organs of the human body, because of the importance of this heart function, so if there is a disturbance or damage to this organ will result in disruption of all system performance in the body of mammals (Anggraeni et al. 2017).

Streptozotocin (STZ) is a chemical compound in the form of broad-spectrum antibiotics and is a toxic compound for pancreatic beta cells that produce the hormone insulin because it can destruct its cells. The induction of STZ as a diabetic agent is very convenient and easy to use (Abeeleh et al. 2009). Structurally, STZ is an N-nitrosurea and D-glucosamine derivative isolated from *Streptomyces achromogenes* (Raza and John 2013).

Chemical drugs that are often used by people with diabetes mellitus is a type of glibenclamide. According to Mulyanti (2010), diabetes melitus requires serious handling. The adverse effects of synthetic drugs used to treat DM are the main reason for the search for natural antihyperglycemic drugs. Currently, traditional medicine is often used by the community for self-medication. Before modern medicine was discovered and marketed, the use of traditional medicine in Indonesia has been going on for thousands of years. Traditional medicine is widely used to treat chronic diseases such as diabetes mellitus (Pramono 2002). One of the plants that have been used traditionally in some areas in Indonesia as a drug diabetes mellitus is jengkol peel (Syafnir et al. 2014).

Based on the results of research Rahayu and Pukan (2008), disclosed that the content of chemical compounds in jengkol peel are alkaloid, steroid/triterpenoid, saponin, flavonoid and tannin, and jengkol fruit peel also contains protein, vitamin A, vitamin B, phosphorus, and calcium.

Jengkol fruit peel is a good source of protein because it plays a role for the development of the body and can repair back the damaged cells. Therefore, the peel of jengkol fruit (*A. pauciflorum*) is thought to have the potential to keep the heart organ health in diabetics observed through damage to cells in the heart. In this research, the effect of ethanol extract of jengkol peel on histology of heart organ in rat/mouse (*Rattus norvegicus*) induced by streptozotocin was observed.

MATERIALS AND METHODS

Material preparation and diabetes induction

Production of ethanol extract of jengkol fruit peel was done by maceration method using ethanol 70%. The obtained maseration was then filtered and then concentrated with a rotary evaporator at a temperature of 40 ^{o}C (Khan et al. 2012) to obtain an extract in the form of a paste.

The test animal was acclimatized in Biology Department animal cage for seven days with temperature 22-30^{o}C. Test animals were given feed and drink with tap water by ad-libitum (IACUC 2015). Replacement of the chamber cage is done twice a week.

The test animal was checked for its glucose level and fasted for 12 hours. The test animals were then induced with streptozotocin which was dissolved in 0.1 M citrate buffer (pH 4.5) with a single dose of 65 mg/kg BW intravenously. After 72 hours of STZ induction, blood glucose levels of test animals were examined. Rat used as test animals were rat possesing blood glucose >250 mg/dl on day 3 of the test (72 hours after STZ induction) (Furman 2015).

Ethanol extract of jengkol fruit peel given orally every day for 14 days in a row according to dose of each treatment. The extract was administered on day 4 after STZ injection and was considered to be the 1st day and lasted up to 14 days (Sajedianfard 2014).

Histological incision preparation

After administration of the extract for 14 days, the rat was dislocated the neck, dissected, and isolated the heart organ. The organ was washed with 0.9% NaCl to remove blood residue and dried with filter paper. The histological incision of the cardiac organ is made by isolating cardiac organ fixed in Bouin solution for 24 hours. Then the heart organ was cut transversely and washed in alcohol 70% for 24 hours. Afterward, the organ was dehydrated in series alcohol and 100% alcohol-based clarification: xylol. The heart organ was then infiltrated in xylol:paraffin and embedding in paraffin by oven at 60-70^{o}C. Then the organ was cut using microtom with a thickness of 5 microns at a temperature <24^{o}C.

Staining was done using Hematoxylin-Eosin (HE). The stages performed in this staining began with deparaffinization and then rehydration process in series of alcohol, which was then put into Hematoxilin solution for 25 minutes and the histological incision was washed with tap water flowing. The histological incision was then introduced into a solution of Eosin for 10 seconds and dehydrated in a series of alcohols. It was then purified in a solution of xylol series and dried at room temperature and covered with a sliding glass cover.

Histological sample observation

Histological incision of heart dyed in Hematoxylin-Eosin was then observed histological structure using a light microscope. The observation of necrosis was performed by looking at 1000 myocardial cells, which was then be counted. Other changes were observed, such as infiltration of inflammatory cells, fat degeneration, and hydropic degeneration were scored degrees of its severity using the method Karthikeyan et al. (2007), as follows: (0) no change; (1) mild (focal damage to myocytes or small multifocal degeneration with slight inflammatory processes); (2) moderate (broad myofibrillar degeneration and/or inflammatory of the difusa); (3) severe (widespread degeneration with inflammation of the difusa).

Data analysis

The histologic observation of rat heart (*Rattus novergicus*) Wistar was analyzed by One-Way ANOVA parametric statistic and continued with Tukey test using SPSS for Windows version 21 (Sudjana 2012). In One-Way ANOVA H_0 is accepted when $F_{count} < F_{table}$; and H_1 is accepted when $F_{count} > F_{table}$ at the real level ($\alpha = 0.05$). If H_1 is accepted, then the statistical test is continued with the Tukey test.

RESULTS AND DISCUSSION

Observation of cardiac histological structure

Observations of cardiac histological structures include the total of necrotic cell, fat degeneration illustrations, hydropic degeneration, and inflammatory cell infiltration. Histological cross-section of the heart organ of the post-treatment 14-day is presented in Figure 1, with the positive control induced by streptozotocin showed necrotic cells, hydropic degeneration, and inflammatory cell infiltration, whereas on the negative control without the induction of streptozotocin only necrotic cells and hydropic degeneration. Fat degeneration was observed in the treatment of P1 and P3. The average number of normal cells and necrosis cells is presented in Table 1, whereas the illustrations of fat degeneration, hydropic degeneration, and inflammatory cell infiltration are presented in Table 2.

Necrosis cell

The result of ANOVA test for necrotic cell count shows that F_{count} (61,040)> F_{table} (2,77), it means that there is a significant difference at least one treatment group showing jengkol fruit peel extract had an effect on histological structure of female wistar rat (*R. novergicus*) heart organ. This also means that the administration of jengkol fruit skin extract had a significant effect on the number of female Wistar rat (*R. novergicus*) heart necrosis cells. The data analysis was continued with Tukey test to find out the most significant different of all treatments.

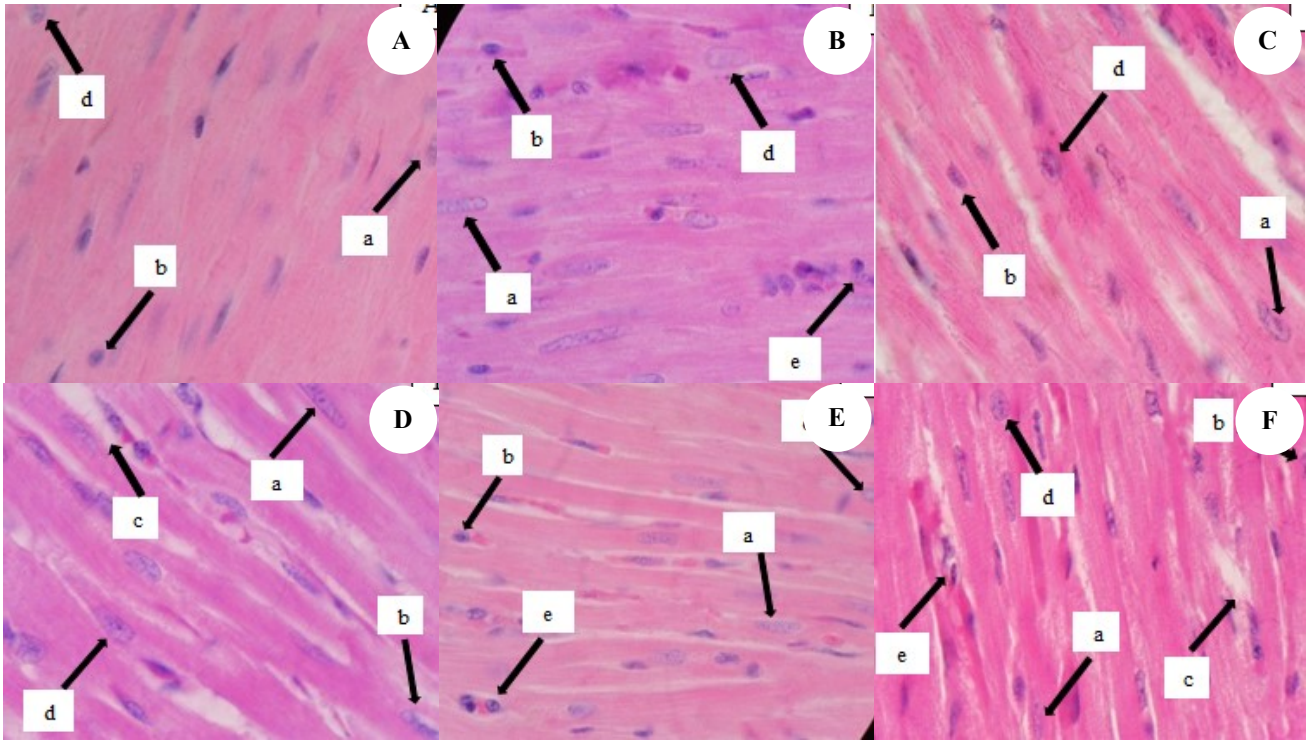

Figure 1. Histological cross section incision of rat heart. Note**:** (A) NC (CMC 0,5%); (B) PC (STZ 65 mg/kg BW + CMC 0,5%); (C) Pb (STZ 65 mg/kg BW + CMC 0,5% + Glibenklamid 10 mg/kg BW); (D) P1 (STZ 65 mg/kg BW + CMC 0,5% + Jengkol Fruit Peel Extract 385 mg/kg BW); (E) P2 (STZ 65 mg/kg BW + CMC 0,5% + Jengkol Fruit Peel Extract 770 mg/kg BW); (F) P3 (STZ 65 mg/kg BW + CMC 0,5% + Jengkol Fruit Peel Extract 1.540 mg/kg BW). [a] normal cell; [b] necrosis cell; [c] fat degradation; [d] hydrophic degradation; dan [e] inflammatory cell inflammation

Table 1. Average number of necrosis cell in rat heart myocardium post-treatment

Treatment group	Average of necrosis cell
Negative control	172.00 ± 7.62^{a}
Positive control (STZ)	234.50 ± 6.56^{b}
Pb (Glibenklamid)	185.50 ± 6.76^{c}
P1 (385 mg/kg BW)	174.25 ± 6.34^{ad}
P2 (770 mg/kg BW)	184.25 ± 6.18^{cd}
P3 (1540 mg/kg BW)	222.75 ± 7.04^{e}

Note: Data were analyzed using ANOVA and Tukey test with 95% confidence level. Different letters in one column show a real difference ($p<0.05$).

Table 2. Average of scoring damage to mouse heart post-treatment miocardium

Treatment group	Average of scoring
Negative control	1.00 ± 0.00^{a}
Positive control (STZ)	2.75 ± 0.50^{c}
Pb (Glibenklamid)	1.25 ± 0.50^{ab}
P1 (385 mg/kg BW)	1.25 ± 0.50^{ab}
P2 (770 mg/kg BW)	1.00 ± 0.00^{a}
P3 (1540 mg/kg BW)	2.00 ± 0.00^{bc}

Note: Data were analyzed using ANOVA and Tukey test with 95% confidence level. Different letters in one column show a real difference ($p<0.05$).

Results of histologic cardiac observations showed that streptozotocin-induced rat had higher numbers of necrotic cells (234,50 ± 6.56) than streptozotocin-non induced rat (172.00 ± 7.62). Normal cells in diabetics will experience a decrease in the amount caused by damage to cells. This is in accordance with the results of research conducted by Sari (2015), that the induction of streptozotocin causes heart organ to permanent cell damage in the form of myocardium cell cytoplasm vacuolization.

Necrosis is the death of tissue cells due to injury when individual is alive. Microscopically, the core changes are the loss of chromatin image, the core wrinkle, not vesicular anymore, the core appears denser, the color is dark black (pyknosis), the core is divided into fragments, torn (caryokinesis), and no longer to take much color because it is pale not real (caryolisis) (Suhita et al. 2013).

Tukey test results showed that rat treated with jengkol fruit peel extract at each dose were significantly different from those given streptozotocin. This means that the administration of jengkol fruit peel extract is able to repair cell damage in the heart of diabetics by reducing the number of necrosis cells. In rat treated with jengkol fruit peel extract at dose of 385 mg/kg BW, it had no differ significantly to the control group rats without streptozotocin induced induction. This means that the extract of jengkol fruit peel at dose of 385 mg/kg BW had a better effect on reducing the number of necrosis cells

compared to the comparison (glibenclamide drug), so that this effect was close to the normal condition of the streptozotocin-non induced rat. The rat treated with jengkol fruit peel extract at dose of 385 mg/kg BW had fewer necrotic cells (174.25 ± 6.34) than glibenclamine-treated rat (185.50±6.76).

The peel of jengkol fruit is known to contain tannin and flavonoids, which apparently prevent and repair cell damage by increasing the number of normal cells. Karodi et al. (2009) states that tannin performs wound healing activity by increasing regeneration and organization of new tissues, whereas according to Harisaranraj et al. (2009), flavonoids are water-soluble antioxidants, which can clean up free radicals, so that oxidative cell damage can be prevented and they also have strong anticancer and anti-inflammatory activity.The results of microscopic histological observation show that the changes of rat heart happen due to the administration of streptozotocin, glibenclamide drug or treatment given in three doses (385 mg/kg BW, 770 mg/kg BW, and 1540 mg/kg BW), which could be observed from the degeneration of fat, degeneration of hydropic, and inflammatory cell infiltration.

Scoring Damaged CellThe result of ANOVA test for cell damage score showed that F_{count} (15,533)> F_{table} (2,77), It means there is a significant difference at least one group of treatments showed that fruit jengkol skin extract had an effect on histological structure of female rat (*R. novergicus*) Wistar heart. It also means that the administration of jengkol fruit peel extract had a significant effect on the degree of mast cell damage in female rat (*R. novergicus*) Wistar heart. The data analysis was continued with Tukey test to find out the most significant different treatment.

Tukey test results showed that in mice treated with jengkol fruit peel extract at dose of 385 mg/kg BW and 770 mg/kg BW did not differ significantly to negative control group that not induced by streptozotocin (Table 2). This means that the jengkol fruit peel extract of doses of 385 mg/kg BW and 770 mg/kg BW has a better effect on the reduction of cell damage caused by fat degeneration, hydropic degeneration, and inflammatory cell infiltration when they were compared with comparison (glibenclamine drug) so that their effects were near the normal condition of rats with no streptozotocin induction. In rat treated with jengkol fruit peel extract at dose of 1540 mg/kg BW was not significantly different from that of the positive control group induced by streptozotocin. This shows the extract of ethanol skin jengkol dose 1540 mg/kg bb had not been able to repair the damage of heart cells in people with diabetes mellitus.

Based on the observation, jengkol fruit peel extract is known to reduce necrosis in the cell. Fat degeneration is a metabolic disorder of cells will cause cell damage and initiate the occurrence of necrosis. According to Aisyah et al. (2014), the damage of heart cells is characterized by the presence of vacuoles that accumulate on the walls. The vacuoles are fatty deposits, which are known as foam cells. If it is sedentary, the foam cell hardens and may clog the blood vessels, which is known as atherosclerosis. Fat degeneration is an abnormal fat deposits in cells that lie between connective tissue or degenerative changes leading to cellular necrosis. This occurs in conditions of diabetes mellitus, malnutrition, ischemic and severe anemia.

Discussion

Fat degeneration is an abnormal fat accumulation in the cytoplasm, vacuoles and urgent nuclei to the edges. Fat degeneration describes the abnormal accumulation of triglycerides in parenchymal cells. The etiology of fatty degeneration is toxin, protein malnutrition, diabetes mellitus, obesity and anoxia. The consequences of changes of fatty depend on the amount of fat deposits. If there is not too much fat deposits, the cell function is not disrupted, but if there is excessive fat deposits, it will cause changes in fatty cells and can cause necrosis (Suhita et al. 2013).

Cell cytoplasm vacuolization is a feature of hydropic degeneration, which is the accumulation of further water in the cells due to mitochondrial damage, the cessation of ATP production and the failure of the sodium pump causing an increase in osmotic pressure in the cell. Severe hydropic degeneration results in necrosis of the cells (Salim and Balqis 2017). Hydropic degeneration occurs when a vacuole containing water in a cytoplasm containing no fat or glycogen is present, the cytoplasm becomes pale and swollen with fluid retention. This change is generally a result of metabolic disorders such as hypoxia or chemical toxicity. This change is reversible, although it can also be irreversible if the cause of the injury persists. If a rupture of the plasma membrane occurs and changes in the nucleus, then the cell becomes irreversible and the cell dies (Kasno 2005).

Hydrophic degeneration is characterized by cellular swelling, presence of empty spaces (vacuoles), enlarged and docked cells. Hydropic degeneration is a reversible cell lesions with more severe intracellular accumulation when it is accompanied by albumin. Its etiology is similar to cell swelling, only the intensity of pathological stimuli is more severe and the duration of exposure to pathologic stimulation is longer. Hydropic degeneration is common in epithelial cells (Suhita et al. 2013).

According to Braun and Anderson (2010), dead cells are chemically altered, adjacent living tissues respond to the change and cause an inflammatory reaction. Cell inflammation is a vascular reaction which results in the delivery of fluids, dissolved substances and cells from the blood circulation to the interstitial tissues of the necrosis region.

Inflammation or an inflammatory reaction is an important mechanism that the body needs to defend itself from a variety of dangers that disrupt the balance, as well as improve the structure and disruption of tissue function caused by the hazard. Inflammation is characterized by plasma protein fluid transfer and leukocytes from the blood circulation into the tissues in response to hazards. Inflammation can be characterized by redness, heat, swelling, pain and disruption of body functions. Histopathologically, inflammation is characterized by infiltration of inflammatory cells (Baratawidjaja 2002).

The results showed that the treatment using jengkol fruit peel extract (*A. pauciflorum*) can affect normal cell

count, necrosis cell count, fat degeneration, hydropic degeneration, and inflammation of inflammatory cell, which is almost the same with negative control, without streptozotocin induction. Fruit ethanol extract of jengkol fruit having good effect on each parameter was at dose 770 mg/kg BW, which could increase normal cell count, decrease cell necrosis, and repair cell damage based on fat degeneration, hydropic degeneration, and inflammatory cell infiltration. This suggests that jengkol fruit peel extract (*A. pauciflorum*) has the potential to improve the histologic damage of rat (*R. novergicus*) heart that induced by streptozotocin.

Biochemical compounds in the peel extract of jengkol fruit (*A. pauciflorum*) such as tannins, flavonoids, alkaloids, quinones, steroids/triterpenoids, saponins, polyphenols can reduce cellular necrosis and increase regeneration of new tissues in cardiac myocardium. These compounds also act as antioxidants that help repair tissue damage to the heart. Jengkol fruit peel also contains protein, vitamin A, B vitamins, phosphorus, and calcium that plays a role for the development of the body and can repair back the damaged cells.

Based on the results of this study, it can be concluded that the provision of jengkol fruit peel extract (*A. pauciflorum*) can improve the damage of the histological structure of the heart, which could be observed from the number of necrosis cells and score damage picture caused by fat degeneration, hydropic degeneration, and inflammatory cell inflammation on Wistar rat (*R. norvegicus*) induced by streptozotocin. The dose of ethanol extract from jengkol fruit peel (*A. pauciflorum*), which effectively repair the cell damage in female rat (*R. norvegicus*) wistar heart induced by streptozotocin was 385 mg/kg BW. However, toxicity test of ethanol extract of jengkol fruit peel for 28 days is required to know the toxic level of jengkol fruit peel to other body organs, in order to prevent other negative effects that can be inflicted on people with diabetes mellitus. In addition, further research on the effect of duration of jengkol fruit peel extract on histological and morphological structure of Wistar female rat is required, so that the optimum duration of time in the extract of jengkol fruit peel can be obtained.

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