Effect Soursop (Annona muricata L.) Leaf Aqueous Extract (SLAE) on Remodeling of Ventricle Heart Tissue in Obesity Rats Model

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ABSTRACT

A high-fat and high-fructose diet can lead to obesity which contributes to an increased risk of heart failure complications. Soursop leaves are known to have the potential of antioxidant, but research about the effects of soursop leaf on complications of heart failure has never been studied. This study evaluated whether soursop leaf aqueous extract can inhibit the total of cardiomyocyte necrosis, cardiomyocyte diameter, and density of cardiac collagen in obese rats. The heart organs of obese rats that have been paraffin, which randomly divided into normal group, obesity group, and soursop leaf aqueous extract (SLAE) group with dose I (100 mg/kgbw), II (200 mg/kgbw), and III (400 mg/kgbw) (n=6 rats). The rats were induced by high-fat high-fructose and SLI diets for 10 weeks. The number of necrosis and diameter cardiomyocytes were measured by using Hematoxylin Eosin staining, while the density of cardiac collagen was measured by Masson’s Trichrome staining and then observed with a trinocular and dot side microscope at 200x and 400x magnification. Statistical analysis using One Way ANOVA and continued by LSD test. We found that group SLAE with doses I, II, and III significantly reduced the number of cardiomyocyte necrosis and cardiac collagen density compared to the obesity group. The SLI group with doses II and III significantly reduced the diameter of cardiomyocytes compared to the obesity group, while the SLAE group with the dose I was unable to reduce the diameter of cardiomyocytes. In conclusion, Soursop leaf aqueous extract can inhibit the increase of cardiac collagen density, number necrosis and diameter cardiomyocyte.

Keywords: Soursop leaf, TLTF diet, obesity, necrosis, cardiomyocyte diameter, collagen density

Introduction

Obesity can lead to hyperlipidemia which contributes to an increased risk of heart failure complications (Powell-Wiley et al., 2021). Heart failure is defined as a chronic progressive condition in which the heart muscle is unable to pump enough blood to meet the body’s need for oxygen. In addition, the prevalence of obesity is increasing every year in the world, around 39% of the world’s population and 33.5% of the Indonesian population (Kementerian Kesehatan RI, 2018; Afshin et al., 2017). According to the Institute for Health Metrics and Evaluation (IHME) Global Burden of Disease, around 4 million deaths from obesity, more than two-thirds of which are caused by cardiovascular disease (Powell-Wiley et al., 2021; Afshin et al., 2017).

Pathophysiologically, hyperlipidemia can cause adipose tissue dysfunction and fat deposits in the heart muscle which will induce lipotoxicity and insulin resistance so that oxidative stress in
heart muscle increases (Powell-Wiley et al., 2021). This causes cardiomyocyte necrosis and the formation of fibrosis in the myocardium. In addition, hyperlipidemia can cause atherosclerosis in the arteries so that it will cause narrowing of the arteries. This will increase peripheral resistance so that the pumping power of the heart will increase, causing an increase in blood pressure (Powell-Wiley et al., 2021). If this happens chronically, it will cause hypertrophy, dilatation and fibrosis of the myocardium. Cardiomyocyte necrosis, fatty deposits in the heart muscle, and fibrosis will cause a decrease in the elasticity and contractility of the heart muscle, resulting in heart failure (Lorell & Carabello, 2000).

Although many drugs are currently used for weight loss, orlistat (lipase inhibitor) is one of the most effective and safe drugs for the treatment of obesity and cardiovascular diseases such as heart failure (Powell-Wiley et al., 2021). However, several studies have stated that orlistat has side effects of cholelithiasis and acute pancreatitis (Filippatos et al., 2008). These side effects encourage to search for alternative medicines from natural ingredients that are cheaper with minimal side effects (Coria-Téllez, 2018). Recently, herb-based traditional medicine has been widely used and is rapidly growing among many countries, and World Health Organization has encouraged the utilization of natural medicine as a potential therapeutic strategy. Soursop leaf (Annona muricata L.) is a plant that has medicinal properties. In vivo studies have proven that soursop leaf extract can lower blood glucose by preventing damage to pancreatic beta cells that are damaged by oxidative stress (Suastuti et al., 2015; Asmonie, 2014; Esmawati, 2015). Another study showed that soursop leaf extract has saponin compounds that can reduce lipid profile levels by inhibiting cholesterol absorption in the intestine (Wurdianing et al., 2015). Until now, research on the effect of soursop leaf aqueous extract on complications of heart failure in obese patients has not been carried out.

Material and Methods

Research design

The type of research was True Laboratory research with post-test group control only design to determine the histological changes of heart muscle tissue, in male Wistar rats induced by HFHF diet and SLAE at a dose of 100 mg/kgbw, 200mg/kgbw and 400 mg/kgbw. Diet is given orally for 10 weeks which was carried out in the study of Dini Sri Damayanti et al., 2020.

Research Time and Place of Research

This research was conducted at the histology laboratory of the Faculty of Medicine, Islamic University of Malang, and the Laboratory of Anatomical Pathology, Faculty of Medicine, Universitas Brawijaya, in June-September 2021.

Tools and Materials

The tools are Dot Slide Virtual Microscope (Olympus), Trinocular Microscope, hematoxylin-eosin staining set, and Masson trichrome staining set. The materials used in this research are rats' hearts induced by a high-fat high-fructose diet and soursop leaf aqueous extract.

Experimental Procedure

White Wistar rats (Rattus novergicus) were reared and acclimatized in the UB Medical Faculty laboratory for 2 weeks. After acclimatization, experimental rats were induced with a high-fat high-fructose (HFHF) diet and soursop leaf aqueous extract (SLAE) simultaneously for 70 days or 10 weeks, with a total of 30 rats divided into 5 groups (Damayanti, 2020). The five groups were normal control group (NG) namely the group without HFHF and SLAE diet induction (n=6), obesity group (OG), the group with HFHF diet induction but without SLAE induction (n=6), and then treatment group 1 (TG1) the group with diet induction HFHF and SLAE at a dose of 100 mg/kgbw (n=6), treatment group 2 (TG2) the group with diet induction HFHF and SLAE at a dose of 200 mg/kgbw (n=6), and treatment group 3 (TG3) is the group with diet induction HFHF and
SLAE at a dose of 400 mg/kgbw (n=6). At the end of the treatment (day 70) the experimental rats were sacrificed and surgery was performed to take the cardiac organs. Furthermore, the rat heart organ was blocked by paraffin. Each rat’s heart was taken so that 30 samples of rat heart would be prepared for histology using hematoxylin-eosin and mason trichome staining. The experimental rats were male, 3 months old, weighing 150-250 grams, healthy rats characterized by good activity and good appetite.

**Preparation of Cardiac Histology Slide with Hematoxylin Eosin (HE) Staining**

Heart block paraffin was cut to 4 cm thickness using a microtome and then placed on the slide. Then the slides were put in xylol solution for ± 3 minutes for deparaffinization. After that, slides were put into 80%, 90%, and 100% alcohol solutions for about 2 minutes. Then washed with water flow for ± 3 minutes. After that, the slides were added to the hematoxylin solution for 7 minutes. After that, the slide was added to 0.6% HCL solution. Then the slides were put in a 0.5% lithium carbonate solution. After that, the slides were added to the solution. Furthermore, the slides were put into 80%, 90%, and 100% alcohol. Last, slides were given entellan to glue the slide material with the glass object (Ariyadi & Suryono, 2017).

**Preparation of Cardiac Histology Slide with Masson's Trichrome (MT) Staining**

Heart block paraffin was cut to 4 cm thickness using a microtome and then placed on the slide. Then the slides were put in xylol solution for ± 3 minutes for deparaffinization. Furthermore, the slides were put into 70%, 95%, and 100% alcohol solutions for about 2 minutes. Then the slides were rinsed with water flow for ± 3 minutes. The slides were then immersed in Bouin's solution for 1 hour. After that, wash under running water for 5-10 minutes. Next, the slides were stained with Weigert’s Iron Hematoxylin solution for 10 minutes. Rinse with warm water for 10 minutes, then wash again with normal water. Then soaked in a solution of Biebrich Scarlet-Acid Fuchsin for 10-15 and wash with water. Furthermore, the slide was soaked with a solution of Phosphomolybdic-Phosphotungstic Acid for 10-15 minutes. Move directly (without rinsing) to the Aniline Blue solution for 5-10 minutes. The slides were briefly rinsed with water and then soaked in 1% Acetic Acid for 2-5 minutes and washed in distilled water. Then the slides were dehydrated with 95% Ethyl Alcohol and then cleaned with Xylene (Medical Center U,2020).

**Count the Number of Cardiomyocyte Necrosis**

Cardiac histology slides that had been stained with HE were then observed for the number of cardiomyocytes observed using a trinocular microscope with 400x magnification in 10 fields of view for each sample preparation. Counting the number of cardiomyocyte necrosis cells manually with Image J application and observed by three people, then the results of 10 fields of view by 3 different observers were averaged to get the total number of results. Cell necrosis is characterized by pyknotic cell nuclei / shrinking cell nuclei, karyorrhexis/cell nucleus fragmentation, and karyolysis/melting of nucleus chromatin (Nurasidah et al., 2020). Counting of cardiomyocyte necrosis using the proportion formula (%) = a/b x 100%.

Note:

a: Number of necrotic cells
b: Total number of cells

**Count the number of cardiomyocyte diameter**

A cardiac histology slide that had been stained with HE was then observed for the diameter of the cardiomyocytes which were observed using a trinocular microscope with 400x magnification in 10 fields of view for each sample preparation. The cardiomyocyte diameter was measured Image J application and observed by three people, then the results of 10 fields of view by 3 different observers were averaged to get the total results. Cardiomyocytes were measured by calculating the diameter of the cardiomyocytes from the cell membrane boundary with units.
of micrometers (µm) in transverse and longitudinal sections. The calculation of the cardiomyocyte diameter is by using the length of the vertical diameter (x) with x/y 1.2, if x/y > 1.2 then the length of the cardiomyocyte diameter was excluded (Sulistyowati et al., 2017). This helped eliminate cells sectioned tangentially (Tracy & Sander, 2011).

Note:
- a: vertical axis cardiomyocyte diameter
- b: horizontal axis cardiomyocyte diameter

**Calculation of collagen connective tissue density in cardiac tissue**

Cardiac histology slides that had been stained with MT were then observed for the density of cardiac collagen connective tissue which was observed using a Dot slide microscope with 200x magnification in 10 fields of view for each sample preparation. The calculation of the density of collagen connective tissue was carried out with Image J using the Image RGB stack menu and adjusting the threshold, then using the measure menu the percentage of collagen density was obtained (Li et al., 2017). Observations were made by three people, then the results of 10 fields of view by 3 different observers were averaged to get the total number of results. The result is a percentage of the blue area that indicates the presence of collagen deposits (Loganathan et al., 2006).

**Data analysis techniques**

The data are expressed in the form of mean ± SD. Furthermore, the data was carried out by the Saphiro Wilk test to determine the homogeneity and normality of the data. After that, the One-way ANOVA statistical test was performed for differences between groups with a significant degree of p<0.05. If there is significance, the analysis is continued with the least significant difference (LSD) test to determine the comparison of each treatment group. Data analysis was carried out using SPSS statistical software (Statistical Product and Service Solution) version 25.

**Results and Discussion**

**Effects of Soursop Leaf Aqueous Extract (SLAE) on the number of cardiomyocyte necrosis of the heart of rats in obesity model**

Obesity induction with HFHF diet significantly increased the number of cardiomyocyte necrosis about 2 times compared to normal controls (p<0.05). Giving SLAE doses of 100 mg/kgbw (TG1), 200 mg/kgbw (TG2), and 400 mg/kgbw (TG3) significantly reduced the number of cardiomyocytes necrosis with a percentage of about 20% compared to the obesity group (p<0.05). The administration of SLAE was not significantly different between doses in reducing the number of cardiomyocyte necrosis with the strongest effect at a dose of 400 mg/kgbw (p>0.05). The number of cardiomyocyte necrosis in the SLAE group was 60% higher than in the normal group (p<0.05). The effect of differences in the number of cardiomyocyte necrosis.

The HFHF diet significantly increased the amount of necrosis in OG compared to NG (p<0.05). A high-fat diet can induce the formation of ROS and proinflammatory cytokines through adipose tissue dysfunction mechanism that triggers macrophage activation. In addition, the accumulation of excess fat in heart tissue can also cause lipotoxicity so that it will form proinflammatory cytokines (Powell-Wiley et al., 2021). An increase in ROS and proinflammatory cytokines will cause damage and death of cardiomyocytes. Increased levels of free fatty acids, inflammatory cytokines, and lipids in non-adipose tissue can lead to impaired insulin receptor signaling resulting in insulin resistance (Heymsfield & Wadden, 2017). The occurrence of insulin resistance will lead to failure of glucose uptake, mitochondrial dysfunction, activation of alternative pathways of glucose metabolism, and increased production of ROS, causing cardiomyocyte necrosis (Powell-Wiley et al., 2021; Heymsfield & Wadden, 2017).

Administration of soursop leaf aqueous extract significantly (p<0.05) increased the number of cardiomyocyte necrosis in rats induced by HFHF diet. This is due to the presence of compounds
in soursop leaves that have antioxidant activity (Coria-Téllez et al., 2018; Bhardwaj et al., 2019). The antioxidant effect on soursop leaves is an indirect effect on reducing the number of cardiomyocyte necrosis played by phenolic compounds and flavonoids by neutralizing free radicals through oxidation (Makuasa & Ningsih, 2020). Free radical neutralizing activity will reduce the inflammatory reaction which will prevent the increase in proinflammatory cytokines such as IL-6 and TNF-α thereby preventing an increase in the number of cardiomyocyte necrosis (Makuasa & Ningsih, 2020; Balderrama-Carmona et al., 2020). In addition, flavonoid compounds can also prevent the formation of ROS in the mitochondria of cardiomyocytes to reduce the occurrence of cell necrosis (Balderrama-Carmona et al., 2020).

In addition to the antioxidant effect, soursop leaves also have an anti-inflammatory effect (Coria-Téllez et al., 2018; Ishola et al., 2014). The anti-inflammatory effect of soursop leaves is direct in preventing an increase in the number of cardiomyocyte necrosis through flavonoid compounds. Flavonoid compounds can prevent the formation of proinflammatory cytokines such as IL-6 and TNF-α (Moghadamtousi et al., 2015; Ellulu et al., 2017). In addition, soursop leaves also contain quercetin compounds (flavonoid group) which can increase the binding of GLP-1 with GLP-1R in fat tissue so that it will cause an increase in cAMP production in fat tissue. An increase in cAMP will reduce the expression of the NF-kB gene so that the production of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α will decrease (Coria-Téllez et al., 2018; Moghadamtousi et al., 2015; Al-Dwairi et al., 2018). There are also flavonoid compounds of the flavone group such as naringenin and hesperetin which play a role in reducing the formation of cardiac TNF-α to prevent an increase in the number of cardiomyocyte necrosis (Moghadamtousi et al., 2015). In another study, it was stated that flavonoid compounds have an antinoseptic mechanism by inhibiting cyclooxygenase (COX-1 and COX-2) and lipoxygenase (LOX) which inhibit inflammation (Poma et al., 2011). Molecularly, the decrease in the formation of TNF-α can reduce the number of cardiomyocyte necrosis due to the small amount of TNF-α so that it does not bind to TNFR1 so that it will not induce cardiomyocyte necrosis. Inhibition of proinflammatory cytokine expression gives the possibility that the flavonoid compounds contained in soursop leaves have a cardioprotective mechanism in humans (Poma et al., 2011; Fioranelli et al., 2021). Damayanti et al. (2020) research stated that soursop leaf infusion also has the potential to inhibit DPP4. A decrease in DPP4 can cause a decrease in the binding between DPP4 and caveolin protein in macrophage cells located in adipocytes so it causes a decrease in the synthesis of proinflammatory cytokines (Zhang et al., 2013).

Soursop leaves also contain alkaloids and flavonoid compounds that can reduce lipid profiles through the mechanism of inhibiting HMG CoA-reductase (Hafidz et al., 2017; Rochima et al., 2021). In obese patients, decreased lipid profile in the body will reduce lipid accumulation in non-adipose tissues such as heart muscle (Powell-Wiley et al., 2021; Heymsfield & Wadden, 2017). Therefore, the increase in lipotoxicity also decreases, thereby preventing the increase in cardiomyocyte necrosis by decreasing pro-inflammatory cytokines and ROS produced (Hafidz et al., 2017; Savini et al., 2016).

In this study, there were no significant differences (p>0.05) between SLAE doses in experimental rats in inhibiting the increase in the number of cardiomyocyte necrosis in obese rats. However, the decrease in the amount of necrosis was directly proportional to the increase in the dose of soursop leaf aqueous extract given to rats so that it had a dose-dependent effect, namely an increase in dose accompanied by an increase in effect although the difference was not significant with the strongest effect at a dose of 400 mg/kgbw. Damage in the form of necrosis in cardiomyocytes is caused by the activity of proinflammatory cytokines such as IL-6 and TNF-α and the activity of ROS (Coria-Téllez et al., 2018).
Effect of Soursop Leaf Aqueous Extract (SLAE) on cardiomyocyte diameter in obesity rat heart model

Administration of HFFH diet in the obesity group significantly increased cardiomyocyte diameter by about 2% compared to normal controls ($p<0.05$). The administration of SLAE at a dose of 100 mg/kgbw (TG1) was not able to reduce the diameter of cardiomyocytes compared to OG, while the administration of SLAE at a dose of 200 mg/kgbw (TG2) and a dose of 400 mg/kgbw (TG3) was significantly different in reducing the diameter of cardiomyocytes by a percentage of about 2% compared to KP. ($p<0.05$). The administration of SLAE was not significantly different between KP2 and KP3 in reducing cardiomyocyte diameter with the strongest effect at a dose of 200 mg/kgbw ($p>0.05$). The effect of differences in cardiomyocyte diameter between groups.

In calculating the diameter of rat cardiomyocytes, a significant increase in the obesity group compared to the normal group was thought to be due to the induced high-fat diet high in fructose which would be converted into triglyceraldehyde which is a raw material for cardiac metabolism (Prahastuti, 2011). Accumulation of lipid accumulation in the heart causes lipotoxicity and increased oxidative stress which overstimulates mitochondria and activates the molecular mechanisms of cardiac remodeling (Horst & Serlie, 2017). Accumulation of free radicals during lipid oxidation causes protein and DNA damage, whereas hypophosphorylation of the cardiac protein titin will relieve the brake on prohypertrophic stimulation which in turn induces cardiomyocyte hypertrophy (Dickhout et al., 2011; Fang et al., 2008).

However, the cardiomyocyte diameter of the obesity group is $23.39 \pm 0.17 \mu m$, which is still low compared to the baseline size of normal rat cardiomyocytes (20-35 $\mu m$). In the study, Zhang et al. (2016) explained that giving a high-fructose diet was able to cause an increase in the size of cardiomyocytes and cardiac hypertrophy at week 20 after treatment. Meanwhile, in the study of Huang et al. (2016), it was stated that the induction of a high-fat, high-fructose diet could cause cardiomyocyte remodeling and hypertrophy at week 12 after treatment. Therefore, it can be concluded that the increase in cardiomyocyte diameter by giving HFFH diet induction for 10 weeks in this study has not been able to induce pathological hypertrophy due to the low time of HFFH diet administration and differences in fat composition in the HFFH diet which may still be too low. In addition, the lack of stimulation that causes a direct increase in the workload of the heart also affects the occurrence of cardiomyocyte hypertrophy (Sulistyowati et al., 2017).

Giving soursop leaf aqueous extract on TG2 and TG3 was able to significantly reduce the diameter of cardiomyocytes ($p<0.05$) by 2% compared to OG. Soursop leaves have antihyperlipidemic activity which is played by alkaloids and flavonoids through inhibition of the HMG CoA-reductase enzyme as previously described. In another study, it was stated that soursop leaves also contain saponins and alkaloids that can inhibit the activity of pancreatic lipase enzymes. It was also stated in the study of Maulana et al. (2019) that soursop leaf aqueous extract with doses 200 mg/kgbw and 400 mg/kgbw had the effect of reducing serum LDL levels and increasing serum HDL levels in rats induced by a high-fat high-fructose diet for 10 weeks. Therefore, with this antihyperlipidemic effect, less fat is formed and the pathophysiological cascade of hyperlipidemia in obesity leads to constriction of blood vessels thereby increasing blood pressure and finally causing cardiomyocyte hypertrophy can stop. In addition, soursop leaves also contain flavonoids which have antioxidant effects that will neutralize and reduce the formation of oxidized LDL and increase SOD levels to prevent atherosclerosis, narrow blood vessels, and prevent protein and DNA damage that induces cardiomyocyte hypertrophy (Makuasa & Ningsih, 2020; Thrisnadia et al., 2019). This explains how the ability of TG2 and TG3 in reducing the increase in cardiomyocyte diameter compared to the obesity group.

Meanwhile, TG1 was not able to reduce the increase in the diameter of rat cardiomyocytes with HFFH diet induction. This is probably due to the low dose of TG1 causing the content of active compounds that have antihyperlipidemic and antioxidant mechanisms to remain low so that they are unable to inhibit the increase in cardiomyocyte diameter (Trindade et al., 2011). This explains why TG1 is not able to reduce the increase in cardiomyocyte diameter compared to OG.
**Effect of Soursop Leaf Aqueous Extract (SLAE) on the density of collagen connective tissue heart in obesity model rats**

Obesity-induced by HFHF diet significantly increased the collagen density of rat heart tissue about 2.5 times compared to normal controls (p<0.05). The administration of SLAE doses of 100 mg/kgbw (TG1), 200 mg/kgbw (TG2) and 400 mg/kgbw (TG3) significantly reduced the amount of cardiac collagen density by 35% respectively compared to the obesity group (OG) (p<0.05). The administration of SLAE was not significantly different between doses in reducing the amount of collagen density in the heart of rats with the strongest effect at a dose of 100 mg/kgbw (p>0.05). The amount of collagen density in the SLAE group was 65% higher than the normal group (p<0.05).

The HFHF diet significantly increased the density of rat cardiac collagen connective tissue in OG compared to NG (p<0.05). Andersen et al (2019) explained that myocardial fibrosis can be caused by many causes such as conditions of excess pressure on the walls of the heart muscle, neurohormonal activity, cardiomyocyte death, oxidative stress, and inflammation. In general, the mechanism underlying the increase in collagen density is influenced by monocytes of the HFHF diet-induced inflammatory reaction. In addition to secreting proinflammatory cytokines such as TNF-α and IL-6, monocytes also release Connective Tissue Growth Factor (CTGF) and Tumor Growth Factor (TGF)-β which can cause the conversion of fibroblasts to myofibroblasts and stimulate the formation of collagen in the interstitial space by decreasing collagen degradation. And increase the expression of extracellular matrix genes, namely α-smooth muscle actin (α-SMA) (Li et al., 2017; Andersen et al., 2019).

In this study, the administration of soursop leaf aqueous extract at all doses inhibited the increase in cardiac collagen density in rats with HFHF diet induction significantly compared to the obesity group (OG) (p<0.05). These results are following research by Usunomena et al. (2016) which explains that the content of acetogenins, flavonoids, alkaloids, saponins, tannins, and vitamin C in soursop leaves is capable of suppressing the formation of fibrosis in the liver by suppressing the accumulation of extracellular matrix, increasing lysosomal membrane stability and the ability to hepatic synthesis. Meanwhile, in the study of Balderrama et al. (2008), it was stated that the polyphenol content was able to regulate the lipid profile and reduce liver fibrosis.

Soursop leaves also have antioxidant and anti-inflammatory activity as previously described. The antioxidant and anti-inflammatory effects are also played by flavonoid compounds [8, 30]. With the presence of antioxidant and anti-inflammatory mechanisms, it can overcome the factors that trigger the occurrence of fibrotic extracellular matrix in heart tissue, triggering factors such as oxidative stress, inflammation, the presence of profibrotic cytokines, and cardiomyocyte death (Andersen et al., 2019). The mechanism of flavonoids that act as an anti-inflammatory is by inhibiting the activity of nuclear factor-kappa B (NF-kB) which further inhibits monocyte adhesion and further inflammatory processes (Ellulu et al., 2017; Poma et al., 2011).

In this study, there was no significant difference (p>0.05) between doses of soursop leaf aqueous extract given in inhibiting the increase in the density of the rat heart collagen connective tissue with the strongest effect at a dose of 100 mg/kgbw or treatment group 1 (TG1). This is thought to occur due to the presence of excessive flavonoid content in IDS doses of 200 mg/kgbw and 400 mg/kgbw which act as pro-oxidants thereby triggering an increase in free radicals and an increase in levels of inflammatory cytokines such as TNF-α in the heart (Skibola & Smith, 2000). The increase in free radicals and inflammatory cytokines triggers the formation of the fibrotic extracellular matrix in the heart. In this study, the impression was non-dose dependent because there was no increase in the effect that accompanied the increase in the dose given to experimental rats. This is probably because there are complex and multicomponent compounds in soursop leaves that have various mechanisms of action so that increasing the dose is not accompanied by an increase in effect (Coria-Téllez et al., 2018; Moghadamtousi et al., 2015).
Conclusion

From the results of the study, it can be concluded that dietary induction of HFHF increased the number of necrotic cardiomyocytes, cardiomyocyte diameter, and cardiac collagen connective tissue density compared to normal controls. And then, administration of soursop leaf aqueous extract at a dose of 100 mg/kgbw, 200 mg/kgbw and 400 mg/kgbw significantly inhibited the increase in the number of cardiomyocyte necrosis and density of collagen connective tissue. Administration of soursop leaf aqueous extract at a dose of 200 mg/kgbw and 400 mg/kgbw was significantly able to inhibit the increase in cardiomyocyte diameter.

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