DENTINO
JURNAL KEDOKTERAN GIGI
Vol VIII. No 2. September 2023

TOXICITY TEST OF *CHANNA MICROPELTES* EXTRACT BETWEEN NORMAL WISTAR RATS AND DIABETES MODEL BASED ON CARDIAC HISTOPATHOLOGY

Annisa Noviany[1)], Amy Nindia Carabelly[2)], Juliyatin Putri Utami [3)]
[1)] Undergraduate Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin
[2)] Departement of Oral Pathology and Maxillofacial, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin
[3)] Departement of Biomedicine, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

ABSTRACT
Background: *DM is metabolic disease can prolong wound healing phase and cause oxidative stress that can affect cardiac damage such as degeneration, hypertrophy, and necrosis. They can be inhibited by the effect of 16 ml/kg BW CM containing palmitic acid (18.30%), albumin (3.6147g/dl), and amino acids are leucine (1.58%) and valine (0.97%). It's proven to accelerate wound healing in normal Wistar rats and DM. However, CM extract on the cardiac is unknown, so an acute toxicity test was carried out for 14 days.* ***Purpose****: To analyze the effect of oral administration of CM extract at an effective dose of 16 ml/kg BW on the histopathological exam of degeneration, hypertrophy, and cardiac necrosis of normal Wistar rats and diabetic models.* ***Method****: This study is true experimental study with a post-test only control group design which is divided into 4 groups are 2 groups of Wistar rats normal and diabetic models who were given CM extract at an effective dose of 16 ml/kg BW, 2 groups consisted of normal Wistar rats and diabetic models which were only given BR2 feed.* ***Results****: T-Independent test and the Mann-Whitney test showed that there was a significant difference between degeneration and hypertrophy ($p<0.05$) and necrosis. There was no significant difference ($p>0.05$).* ***Conclusion****: The effective dose of CM extract 16 ml/kg BW had an effect on the cardiac of normal Wistar rats and diabetic models in the form of a decrease in the level of damage to degeneration, hypertrophy, and cardiac necrosis.*

Keywords: Cardiac, *Channa micropeltes*, Degeneration, Hypertrophy, Necrosis.
Correspondence: Annisa Noviany, Faculty of Dentistry, Lambung Mangkurat University, Jl. Veteran No 128B Banjarmasin; E-mail corresponding author: annisanov54@gmail.com

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by blood sugar levels (hyperglycemic) as a result of impaired insulin production, insulin secretion, or insulin resistance. The prevalence of DM in Indonesia based on data from the Ministry of Health of the Republic of Indonesia (2019) is 10,7 million people, while the province of South Kalimantan is 1.3 million people.[1] One of the complications of DM is cardiac disease and the field of dentistry is oral diabetes as a chronic inflammation due to the prolongation of the wound healing phase due to hyperglycemia conditions which then causes oxidative stress.[1] Stress Oxidative oxidation can be inhibited by antioxidants that neutralize free radicals produced by Reactive Oxygen Species (ROS). One of these antioxidants is albumin which contains content that is used in traditional medicine.[2]

Traditional medicine is one part of alternative medicine for health maintenance, treatment, and disease prevention which is considered safer when compared to modern medicine because it has relatively lower side effects.[3] Toman fish or *Channa micropeltes* (CM) is a specific fish with specific the highest albumin content from the Channadae group was 5.35%.[4] CM had to go through a toxicity test to qualify as traditional medicine.

The use of traditional medicine should not have adverse side effects so it requires knowledge of drug doses and the dangers of a substance through toxicity tests. Toxicity tests can then be seen from changes in the structure or function of the cardiac organ.[5] In DM conditions, oxidative stress occurs and then causes endothelial dysfunction. This condition is an indicator of degeneration, namely air accumulation, hypertrophy, which is an increase in

cell size and cell nucleus, and necrosis, where cells undergo lysis.[6] This then triggers atherosclerosis which can trigger cardiac disease.[7,8]

In the studies of Nicodemus et al (2014) and Apriasari et al (2020), it was found that the effective dose of CM extracts for wound healing in normal Wistar rats and diabetic models was a dose of 16 ml/kg BW. This dose was effective in closing the wound and wound contraction completely in normal Wistar rats on day 7 and diabetic rat model on day14. Based on this, it is necessary to conduct further research on the toxicity test of CM extract at an effective dose of 16 ml/kg BW on histopathological features of degeneration, hypertrophy, and cardiac necrosis of Wistar rats.[9,10] analysis of toxic effects occurred within 14 days after presenting the preparation orally in a single dose or repeated doses to test animals for 14 days.

RESEARCH METHODS

This research is a true experimental study with posttest-only control design with ethical eligibility No. 069 / KEPKG-FKGULM / EC / VI / 2022 by the Faculty of Dentistry Lambung Mangkurat University. The study population was healthy Wistar rats weighing 200-300 grams and 2-3 months old. Wistar rats did not die, were disabled, and lost >10% body weight after adaptation. Samples were taken using the technique of simple random sampling in four groups. The groups are (K1) a group of normal Wistar rats fed BR2 orally for 14 days, (K2) a group of normal Wistar rats fed BR2 and CM extract with an effective dose of 16 ml/kg BW orally for 14 days, (K3) a group of diabetic Wistar rats fed BR2 orally for 14 days, and (K4) a group of diabetic Wistar rats fed BR2 and CM extract at an effective dose of 16 ml/kg BW orally for 14 days.

CM Extract Manufacture

CM used in this study had total weight 11 kg. The CM used in this study had a total weight of 11 kg. CM meat that has been cleaned should be steamed in a pan for ±30 minutes at a temperature of 70-80^{o}C. The meat is then wrapped in flannel cloth and whatman paper and then put into a hydraulic press for the pressing process. The resulting extract was put into a 7.5 ml test tube and centrifuged at 6000 rpm speed for 15 minutes. The results of centrifugation obtained 700 ml (oil and water phases) and 50 ml precipitate which was then separated. The CM extract was stored in a refrigerator at 4^{o}C in a clean dark glass bottle to prevent oxidation and contamination by covered with aluminum foil.

Induction of Diabetes Mellitus

The Wistar rats model used was a type 1 diabetes mellitus model by injecting streptozotocin (STZ) at a dose of 40 mg/ kg BW. Blood sugar levels were checked with a glucometer before and 3 days after STZ induction. The blood sugar level of diabetic rats is ≥126 mg/dL.

Experimental Animal Treatment

Wistar rats were given CM extract 16 mL/kg BW orally using a gastric probe for 14 days. On 15th day, Wistar rats were euthanized by being given intraperitoneal anesthesia with a mixture of *ketamine-xylazine* 1:1 ratio *ketamine* a dose of 91 ml/kg BW and *xylazine* 9.1 ml/kg BW and injected as much as 10 ml/kg BW for each experimental animal, wait a few minutes until they are unconscious dan the cardiac organ is taken.

Handling of Wistar Rat Carcasses

Wistar rats had their cardiac organs removed were cleaned first with water and wrapped in white cloth and then buried to a depth of 75 cm.

The Making of Histopathological Preparations

Histopathological preparations were made by fixing the cardiac organ. Cardiac immersed in solution Buffer Neutral Formalin (BNF) 10% for 24 hours and washed with water. In the processing stage, the cardiac is inserted into 12 different reagents. The embedding stage, the cardiac is taken with tweezers and blocked using a paraffin block. The hardened paraffin blocks were cut with a thickness of 4-7 µm using a microtome. Samples are inserted into a floating bath that contains water with a temperature of 49^{o}C which is to stretch the cut and put it on the object glass. The object glass is put into the incubator for ± 2-3 hours until the sample dries.

In the staining process, the tissue slices located on the object glass are inserted into the reagent and washed with water. Tissues soaked in Haematoxylin (10 minutes) and washed with water (10 minutes). The sample is immersed in Eosin for 3-5 minutes, then soaked in ethanol. Dehydrated ethanol 70%, 80%, and 90% (2 times). Then done clearing with Xylen (3-4 times). Canada balsam is applied to an object glass that has been colored and then covered with cover glass. Histopathological were observed using microscope OLYMPUS U-CTR-2 with 400x magnification in 5 fields of view.

Analysis of Histopathological Preparations

Histopathological analysis in this study used 3 indicators is degeneration, hypertrophy, and necrosis. With criteria, are:

- Degeneration: there are changes in the cytoplasm of cells in the form of swelling, and accumulation of water or fat.
- Hypertrophy: there is an increase in the size of the cell and the nucleus of the cell.
- Necrosis: there are changes in the cell nucleus in the form of clumping or lysis.

The method of assessment is in the form of scoring, are:[6]

- Score 0: Normal (0% damage).
- Score 1: Minor damage (>0%-25%).
- Score 2: Moderate damage (>25%-50%).
- Score 3: Heavy damage (>50%-75% or more).

Data Analysis and Statistical Evaluation

The research data obtained were analyzed using the test *T-Independent* test and *Mann-Whitney* test to compare and see the significant difference between 2 different groups. The data in the study were processed using a computer program SPSS.

RESULT

Microscopic Examination in Normal Rats
Degeneration

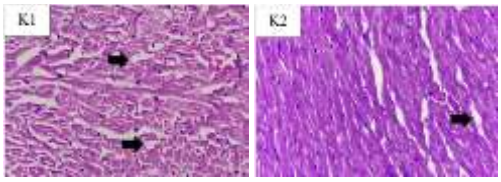

Figure 1: Degeneration. (K1) A group of normal Wistar rats fed BR2 diet was found to have moderate damage covering 25-50% of the total field of view. (K2) A group of normal Wistar rats with CM extract found mild damage of <25% of the total visual field. Arrows indicate degeneration.

Hypertrophy

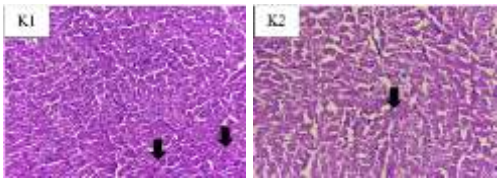

Figure 2: Hypertrophy. (K1) A group of normal Wistar rats fed BR2 diet was found to have moderate damage covering 25-50% of the total field of view. (K2) A group of normal Wistar rats with CM extract found mild damage of <25% of the total visual field. Arrows indicate hypertrophy.

Necrosis

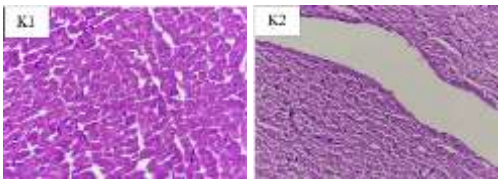

Figure 3: Necrosis. (K1) A group of normal Wistar rats fed BR2 feed. (K2) Wistar rat group normal with CM extract. There was no histopathological picture of necrosis.

Microscopic Examination in Diabetic Rats Model
Degeneration

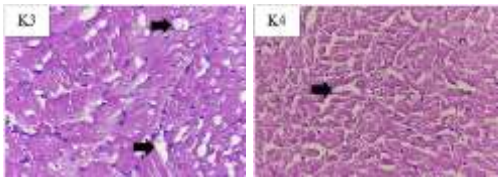

Figure 4: Degeneration. (K3) The group of diabetic Wistar rats that were given BR2 diet was found to have moderate damage covering 25-50% of the total field of view. (K4) A group of diabetic Wistar rats with CM extract was found to have mild damage of <25% of the total visual field. Arrows indicate degeneration.

Hypertrophy

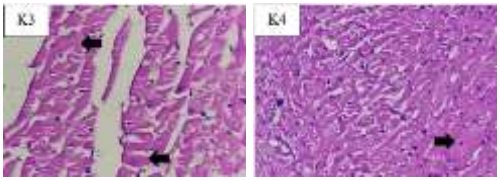

Figure 5: Hypertrophy. (K3) The group of diabetic Wistar rats that were given BR2 diet was found to have moderate damage covering 25-50% of the total field of view. (K4) The group of diabetic Wistar rats with CM extract was found to have mild damage of <25% of the total visual field. Arrows indicate hypertrophy.

Necrosis

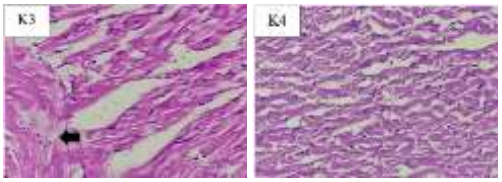

Figure 6: Necrosis. (K3) The group of diabetic Wistar rats that were given BR2 diet was found to have minor damage of <25% of the total visual field. (K4) The group of diabetic Wistar rats with CM extract showed no signs of necrosis. The arrow indicates the presence of necrosis.

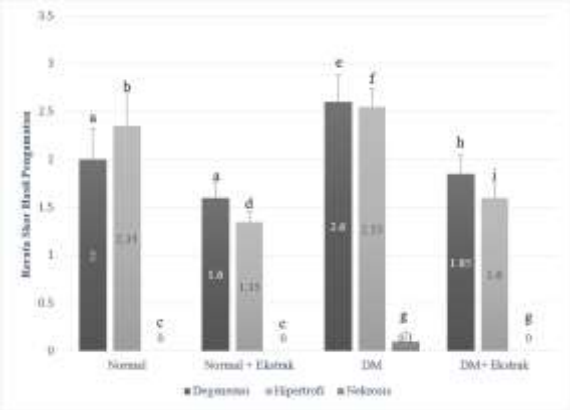

Figure 7: The histopathological mean and standard deviation of degeneration, hypertrophy, and necrosis of normal Wistar rats and diabetic models with significant differences by code (letters).

DISCUSSION

The results showed that there was degeneration in the normal K1 and K2 groups of rats. The K1 group found degeneration with moderate damage compared to the K2 group, which had mild degeneration. Cell degeneration is a cell disorder that occurs as a result of mild injury with increased cell damage due to oxidative stress which increases the inflammatory response as an immune response. This can eliminate the cause of the injury and a healing process occurs. Increased injury due to oxidative stress leads to cell degeneration. The injury is reversible so that it can return to normal if the injury is removed.[11,12]

The results showed that there was hypertrophy in the normal K1 and K2 groups of rats. The K1 group found hypertrophic images with a moderate degree of damage compared to the K2 group which had hypertrophic images with mild damage. Hypertrophy can occur due to the body's reaction to psychosocial stressors such as mental stress or the burden of life in the form of physiological, behavioral, and subjective responses to stress. At the cellular level, there will be hypertrophy with increasing thickness and length of cardiomyocytes as a form of physiological adaptation.[13] This occurs when the compressive load has been exceeded thereby reducing the contractile ability of the cardiac. Psychosocial stress adds to the role in increasing the formation of free radicals that will release the hormones cortisol and norepinephrine.[14]

Psychosocial stress causes an increase in gluconeogenesis and glycogenolysis so that blood glucose increases. Blood glucose that increases acutely or chronically will cause oxidative stress. Hyperglycemia causes cell damage due to the accumulation of intracellular ROS, through non-enzymatic glycation mechanisms on proteins producing AGEs, glucose auto-oxidation increasing superoxide radicals and SOD enzyme damage, formation of sorbitol through the polyol-sorbitol pathway (aldose reductase), and activation of Protein Kinase C.[14] ROS directly impair mitochondrial structure and function and cause decreased energy production and mitochondrial respiratory function.[12]

Normal rat groups K1 and K2 which were given treatment for 14 days did not show any signs of necrosis. The process of necrosis occurs for more than 14 days under normal conditions. Necrosis of the cardiac can occur due to the inability of the cardiac to compensate for changes such as lack of oxygen. Cells will have a reversible injury as a form of adaptation with hypertrophy and degeneration to restore normal homeostasis of the body. When the injury continues, it will cause irreversible muscle death, namely necrosis.[11,12] This can explain the occurrence of damage to degeneration and hypertrophy of cardiac cells, which in the normal group have the same degree as the diabetic group.

The results of observations of the K3 and K4 groups induced by diabetes were found to have degeneration. The K3 group found degeneration with moderate damage compared to the K4 group, which had mild degeneration. According to Hroob et al (2019), there are changes in the structure and function of the myocardium that occur as a result of hyperglycemia that can cause cardiac cell dysfunction in humans and animals.[15] Hyperglycemia will induce the formation of ROS and AGEs. AGEs are formed when glucose produces stable covalent protein modifications. These proteins directly damage and contribute to the formation of ROS thereby triggering oxidative stress faster than normal conditions. Oxidative stress creates a pathological state in the presence of cardiac cell dysfunction. Injury due to cardiac cell dysfunction will affect normal homeostasis and increase pro-inflammatory cytokines so that it triggers cardiac cell degeneration in structures in cells such as mitochondria and cytoplasm.[16]

The results of the observation of the K3 and K4 groups induced by diabetes were found to be hypertrophic. The K3 group found hypertrophic images with a moderate degree of damage compared to the K4 group which had hypertrophic images with mild damage. According to Wassertrum et al (2018), diabetes mellitus is a key factor in changes at the molecular and cellular levels of myocytes, culminating in structural and functional abnormalities in the cardiac. Diabetes mellitus causes hyperglycemia that induces metabolic and molecular changes in myocardial cells resulting in cellular

injury.[17] These metabolic changes produce ROS from mitochondria, reduced capacity of the main antioxidant enzyme Glutathione Reductase (GR), and the formation of AGEs, thereby triggering oxidative stress faster than normal conditions. [16] Increased oxidative stress will trigger many responses associated with Diabetic Cardiomyopathy (DCM) such as activation of Matrix Metalloproteinase (MMP) which alters extracellular matrix architecture and modulates signal transduction pathways resulting in cardiac cell dysfunction.6 Cardiac cell dysfunction causes injury that disrupts normal homeostasis thereby increasing pro-inflammatory cytokines and triggering the process of cardiac cell hypertrophy.[8]

The results of the observation found that the picture of necrosis in the diabetic group was only given BR2 (K3) feed but there was no picture of necrosis in the K4 group. ROS plays an important role in necrosis.[18] Diabetes mellitus in the K3 and K4 groups can increase ROS. High ROS levels can be detected in necrotic cell death and can induce lipid membrane peroxidation and plasma membrane leakage.[18] Necrotic cells experience a lack of oxygen supply, resulting in a decrease in ATP production. Lack of ATP leads to failure of the sodium pump and the influx of calcium and water resulting in cell swelling and increased oxidative stress. Increased oxidative stress causes mitochondrial damage so that pro-apoptotic proteins exit the mitochondria and cause cell death through the mechanism of necrosis.[18]

Early studies show that diabetes increases necrosis by up to fourfold in cardiomyocytes.[19] According to Chen et al (2020), a mouse model of diabetes induced by STZ exhibits metabolic dysfunction, cardiac structural abnormalities, or functionally similar to human DCM pathology. STZ induction in the diabetic group led to metabolic abnormalities, including high concentrations of serum plasma glucose which then led to inhibition of insulin secretion resulting in the production of ROS by STZ. ROS generated by fatty acid oxidase (NADPH) oxidase induces cell death or necrosis.[19]

In this study, there was a decrease in the mean score of observations in groups K2 and K4 who were given CM extract. The CM extract contains fatty acids such as palmitic acid (18.30%), albumin (3.6147 g/dl), and amino acids, namely leucine (1.58%) and valine (0.97%).[20] Palmitic acid will be broken down to acetyl-CoA through the oxidation process. The acetyl-CoA will enter the Krebs cycle and produce Adenosine Triphosphate (ATP), Flavin Adenine Dinucleotide (FADH2), Nicotinamide Adenine Dinucleotide (NADH), and Carbon Dioxide (CO2). NADH and FADH2 will be converted into ATP so that more ATP is produced.[21] According to Mitra et al (2018), the cardiac requires 70% of ATP from fatty acid oxidation to maintain and optimize the contractile function of the cardiac to pump blood which can inhibit degeneration, hypertrophy, and cardiac necrosis.[22]

CM extract contains albumin which is an antioxidant that can capture (Reactive Oxygen Species) ROS and activate (Nuclear factor-erythroid-2 related factor 2) NrF2 in fighting oxidative stress. NrF2 will form (Antioxidants Response Element) ARE which will stimulate the activity of the SOD enzyme. SOD will neutralize ROS components so that oxidative stress is inhibited. Inhibition of oxidative stress and decreased cardiac dysfunction will reduce inflammatory cytokines so that they can maintain and reduce cardiac cell damage.[1,2]

The content of amino acids such as leucine and valine in the CM extract plays a role in lipid metabolism so that it increases HDL (High-Density Lipoprotein), lowers LDL (Low-Density Lipoprotein) levels, and triglyceride levels.20 This will protect cells and vascular endothelium from damage and reduce cell dysfunction. and endothelium by inhibition of pro-inflammatory cytokines. If oxidative stress is inhibited and dysfunction decreases, it will be able to maintain and reduce cardiac cell damage such as degeneration, hypertrophy, and cardiac necrosis.[7,23]

Based on the research, there was significant differences in the picture of hypertrophy in the normal groups. There was a significant difference in the degeneration and hypertrophy features in the diabetic groups. Necrosis has been found in the diabetic group treated with BR2. It can be concluded that *Channa micropeltes* have antioxidant effect that inhibits cardiac damage to normal and diabetic Wistar rats. The research can be used as an alternative medicine to accelerate diabetic wound healing and wounds that occur on the oral mucosa or skin.

REFERENCES

1. Carabelly AN, Utami JP, Aspriyanto D, Reksi MH, Puspitasari D, Rachmadi P. Effect of Channa Micropeltes in The Granulation, Fibrosis and Necrosis of Diabetic Wound Healing. Dentino (Jurnal Kedokteran Gigi). 2021; 4(2): 171-176.
2. Apriasari ML, Ainah Y, Febrianty E, Carabelly AN. Antioxidant Effect of Channa Micropeltes in Diabetic Wound of Oral Mucosa. International Journal Pharmacol. 2018; 15(1): 137-143.
3. Sumayyah S, Salsabila N. Obat Tradisional : Antara Khasiat dan Efek Sampingnya. Farmasetika. 2017; 2(5): 1-4.
4. Firlianty, Suprayitno E, Nursyam H, Handoko, Mustafa A. Chemical Composition and Amino Acid Profile of Channidae Collected From Central Kalimantan, Indonesia. IEESE International Journal Science Technology (IJSTE). 2013; 2(4): 25-31.

5. Hilma N, Nuri N, Puspitasari E, Ningsih IY. Gambaran Histopatologi Organ Jantung Tikus Putih Jantan Galur Wistar (Rattus norvegicus) dalam Uji Toksisitas Akut Kombinasi Ekstrak Daun Jati Belanda (Guazuma ulmifolia Lmk.) dan Kelopak Bunga Rosella (Hibiscus sabdariffa L.). Pustaka Kesehatan. 2018; 6(2): 240-244.
6. Izazi F, Prayogo B, Hestianah EP, Zulkarnain E. Histopatologi Jantung Tikus Pada Toksisitas Sub Kronis Ekstrak Etanol 70% Kulit Buah Jeruk Citrus nobilis Lour. Journal of Herbal, Clinical and Pharmaceutical Science. 2020; 1(2): 1-5.
7. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology 10th ed. Singapore: Elsevier; 2018: p.397-400.
8. Lilly LS. Pathophysiology of Cardiac Disease A Collaborative Project of Medical Students and Aculty 6th ed. Boston: Wolters Kluwer; 2016: p.144-166.
9. Nicodemus, Andrie M, Luliana S. Uji Efek Penyembuhan Luka Sayat Ekstrak Ikan Toman (Channa micropellets) secara Oral pada Tikus Putih Jantan Wistar. Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN. 2014; 1(1): 1–14.
10. Apriasari ML, Syahadati MA, Carabelly AN. Clinical Analysis of Channa micropeltes for Treating Wound of Diabetes Mellitus. Berkala Kedokteran. 2020; 16(1): 1-10.
11. Nazarudin Z, Muhimmah I, Fidianingsih I. Segmentasi Citra untuk Menentukan Skor Kerusakan Hati secara Histologi. Seminar Nasional Informasi Medis VIII. Yogyakarta: Proceeding Book; 2017: hal 15-21.
12. Pangestiningsih TW, Aninditya DP, Puspitandaru GG, Sofana I, Pratiwi R. Degenerasi Dan Nekrosis Pada Neuron Penyusun Sistem Saraf Enterik Di Usus Halus Dan Usus Besar Tikus Yang Diinjeksi Paraquat Dichloride. Livestock and Animal Research. 2021; 19(2): 188-196.
13. Yandriani R, Karani Y. Patogenesis Hipertrofi Ventrikel Kiri. Jurnal Kesehatan Andalas. 2018; 7(2): 159-167.
14. Srivastava KK, Kumar R. Stress, Oxidative Injury and Disease. Indian Journal Clinical of Biochemistry. 2015; 30(1): 3–10.
15. Al Hroob AM, Abukhalil MH, Hussein OE, Mahmoud AM. Pathophysiological Mechanisms Of Diabetic Cardiomyopathy And The Therapeutic Potential Of Epigallocatechin-3-Gallate. Biomed Pharmacother. 2019; 109(1): 2155–2172.
16. Brunvand L, Heier M, Brunborg C, Hanssen KF, Fugelseth D, Stensaeth KH, Jorgensen KD. Advanced Glycation End Products In Children With Type 1 Diabetes And Early Reduced Diastolic Cardiac Function. BMC Cardiovascular Disorders. 2017; 17(1): 1–6.
17. Barman C, Pandey R, Singh J, Sodhi K. Molecular Insights Into Diabetic Cardiomyopathy. International Journal of Research in Medical Sciences. 2015; 3(7): 1564–1570.
18. Adnyana IMO. Stroke Iskemik dari Patofisiologi Sampai Kematian Sel (Apoptosis Dan Nekrosis) Dan Astrosit Sebagai Target Neuroprotektor. Denpasar: Intisari Sains Media; 2020: hal 52-55.
19. Chen Y, Hua Y, Li X, Arslan IM, Zhang W, Meng G. Distinct Types Of Cell Death And The Implication In Diabetic Cardiomyopathy. Front Pharmacol. 2020; 11(42):1–9.
20. Fitriyani E, Nuraenah N, Deviarni IM. Perbandingan Komposisi Kimia, Asam Lemak, Asam Amino Ikan Toman (Channa micropeltes) dan Ikan Gabus (Channa striata) dari Perairan Kalimantan Barat. Manfish Jurnal. 2020; 1(2): 71-82.
21. Murray RK, Granner DK, Rodwell VW. Biokimia Harper edisi 29. Jakarta: Buku Kedokteran EGC; 2014: hal 52–153.
22. Luptak I, Sverdlov AL, Panagia M, Qin F, Pimentel DR, Croteau D, Siwik DA, Ingwall JS, Bachschmid MM, Balschi JA, Colucci WS. Decreased ATP Production And Myocardial Contractile Reserve In Metabolic Cardiac Disease. Journal of Molecular and Cellular Cardiology. 2018; 116(1): 106-144.
23. Cojocaru E, Leon-Constantin MM, Ungureanu C, Trandafirescu MF, Maștaleru A, Trandafir LM, Petrariu FD, Bădelescu OU, Filip N. Hypolipemiant Actions And Possible Cardioprotective Effects Of Valine And Leucine: An Experimental Study. Medicina (Lithuania). 2021; 57(3): 1-10.