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Research Article

The Antihypertension Effect of Fermented Skipjack Tuna (*Katsuwonus pelamis* L.)/Bakasang's Peptide Extract Based on Cardiac's Histopathology and Protease Activity on Hypertensive Rats Induced by Deoxycorticosterone Acetate (DOCA) -Salt

Hilman Nurmahdi[1*], Dyah Kinasih Wuragil[2], Sasangka Prasetyawan[1], Aulanni'am Aulann'am[1,2]

[1]*Biochemistry Laboratory, Department of Chemistry, Faculty of Sciences, Brawijaya University, Malang, Indonesia*
[2]*Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia*

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ABSTRACT

Hypertension is an abnormality of cardiovascular condition, marked by high-blood pressure over the normal condition. One of the factors that causes hypertension is altered equilibrium of Renin-Angiotensin-Aldosteron system (RAAS). This condition, which commonly by genetic could causes excess production of Angiotensin II, which known as vasoconstrictor, carried out by Angiotensin Converting Enzyme (ACE). Angiotensin II could increases the body's blood pressure, and stimulates the production of free radicals, causing hypertension and oxidative stress. One of the common medication for hypertensive patient are using ACE inhibitor pills. Consumption of commercial pills, had a negative effect on the patient, such as skin iritation, cough, allergic reaction, etc. This study was conducted to explore the potential peptide of bakasang extract isolated from fermented skipjack tuna (*Katsuwonus pelamis* L.) for antihypertensive therapy, based on the activity of protease and histopathology of cardiac tissue on hypertensive rats (*Rattus norvegicus*) induced by DOCA-salt. The rats were divided into five groups: (1) negative control group, (2) hypertensive control group, (3) hypertension with *captopril* therapy dose of 5 mg/kg of body weight (BW), (4) and (5) hypertension with bakasang's peptide extract therapy dose of 200 mg/kgBW, and 300 mg/kgBW, respectively. The results showed that bakasang's peptide extract dose of 200 mg/kgBW significantly ($p<0.05$) decrease protease activity and improve histopathology of cardiac tissue on hypertensive rats. Protease activity post-therapy decreased to be 39.27 % from positive hypertension group. It could be concluded that the peptide of bakasang's peptide extract has antihypertension effect (ACE-inhibitors), which was able to improve histopathology and decrease protease activity of the cardiac tissue on hypertensive rats induced by DOCA-salt.

Keywords: Hypertension, Bakasang's peptide extract, Cardiac Histopathology, Protease Activity.

INTRODUCTION

Hypertension is a blood-pressure disorder, which shown by elevation of blood pressure over normal condition. Hypertension is characterized by increased blood pressure in the body that reaches ≥140 mmHg for systolic pressure, and ≥ 90 mmHg for diastolic pressure, with normal blood pressure in human of <120 mmHg for systolic and <80 mmHg for diastolic[1].

Based on data from the WHO in 2009, the number of patients with hypertension, were grew up to 600 million peoples in the world, with the number of people in Indonesia was 27.5% of the total population diagnosed with hypertension[2]. Essential hypertension is the most common type of hypertension that occurs, nearly 95% of the total cases of hypertension[1]. Disruption of the balance system of the Renin-Angiotensin-Aldosterone (RAAS) in the body is one of the causes of essential hypertension[3]. Angiotensin II which is the main product of the RAAS system and is a potent vasoconstrictor, will lead to increase sodium and water retention in kidney that resulted in elevation of body's blood pressure[3]. In addition, angiotensin II stimulates the secretion of aldosterone which causes the activation of NADPH oxidase (NOX)[3,4]. NOX activation will increase the production of free radicals or ROS (Radical Oxygen Species) which leads to oxidative stress, caused by an imbalance concentration between the number of free radicals and antioxidants in the body's defense system, that could lead to a vascular dysfunction[4].

Deoxycorticosterone Acetate (DOCA)-salt is a synthetic mineral corticoid which commonly used in form animal models of hypertension. DOCA-salt induces a form of endocrine hypertension model, which quickly causes severe hypertension followed by increasing of oxidative stress. In addition, this endocrine hypertension model could be treated using bioactive peptide which obtained from a dietary protein[4].

Nowadays, it has been known that dietary protein is a rich source of bioactive components that are beneficial to health in accordance with the sequence of its amino acid[5].

**Author for Correspondence: hilman.nurmahdi@gmail.com*

While in this research, bakasang (Tradiotional fermented food from Maluku and North Sulawesi) was used as therapeutic agent, which known that bakasang were made from fermented Skipjack Tuna, had potential as antihypertensive agent (ACE-inhibitor). Research shown that dried tuna meat (Bonito) have potential as an ACE-inhibitor[6,7]. The aim of this study was to prove the potential of antihypertension from bakasang's peptide based on histopathology and protease activity of the cardiac tissue on hypertensive rats (*Rattus* norvegicus).

MATERIALS AND METHODS

Isolation of Bioactive Peptides from Bakasang

Bakasang samples was obtained from the island of Banda Naira, Maluku, and weighed 200 grams before dissolved in 1000 mililiters of distilled water (Solvent ratios of 1:5 (w/v)). Sample was homogenized using a magnetic stirrer until homogenous (about 15 minutes) and then deactivated in 90^0C for 5 min. Bakasang mixture's then centrifuged (4^0C) for 15 min at 6000 rpm and the supernatant was taken and added with ethanol absolute (1:1 (v/v)), then mixed it before stored overnight at -20^0C. Sample then centrifuged at 10000 rpm (4^0C) and the pellet's was obtained and dried. Then it was added with Tris-HCl buffer (pH 6.8) (1:1 (v/v)) and stored at -20^0C.

Preparation of Hypertensive Rats induced DOCA-salt

The use of animal model in this research was approved by Ethical Clearance Committee of Brawijaya University (KEP-440-UB/2016). Animals which used in this research was Rat (*Rattus norvegicus*) and divided into five groups, there are: (1) Normotensive control group, (2) Hypertensive control group, (3) *Captopril* treated group (5 mg/kg of body weight (BW)), (4) Bakasang's peptide extract (200 mg/kgBW), (5) Bakasang's peptide extract (300 mg/kgBW). Rats were adapted for one week before used. Rats were given standard feed AD II (containing water 12%, proteins's crude 15%, lipid's crude 3-7%, fiber 6%, maximum ash 7%, calcium 0.9-1.1% and phosphorous 0.6-0.9%).

Induction period was 2 times a week for 5 weeks (10 injections), which first five injections contained 20 mg/kgBW and then the next five injections contained 10 mg/kgBW. DOCA-salt was dissolved in corn oil and injections were carried out at subcutan area of cervical section. Rats were given drinking 2% of NaCl (w/v) during induced by DOCA. Blood pressure was measured using the tail-cuff method (CODA tail-cuff Blood Pressure System, Kent Scientific) once a week, until the end of the studt (necropsy).

Preparation of Bakasang's Peptide Extract as a Therapy

The theraupetic dose of bakasang's peptde extract were 200 mg/kgBW and 300 mg/kgBW. The extract was dissolved in distilled water and was carried out by oral administration once daily for four weeks (28 days).

Preparation of Captopril as a Therapy

The theraupetic dose of bakasang's peptide extract were 5 mg/kgBW. Captopril was dissolved in distilled water and was carried out by oral administration once daily for four weeks (28 days).

Histopathological Analysis of Cardiac Tissue

Histopathological analysis was done by Hematoxylin-Eosin staining. Cardiac tissue was stored in PFA (paraformaldehyde) 10% before de-parrafinized, trimmed and then stained using Hematoxylin-Eosin methods.

Protein Isolation of Cardiac Tissue

Cardiac tissues were weighed 0.3 grams and then homogenized using cold mortar. Homogenous later were added with PBST-PMSF buffer up to 1.5 mL (1:5 (w/v)) and then mixed. Then, the samples were put into ultrasonic-sonicator about 10 minutes and then centrifuged at 6000 rpm about 15 minutes. Superantants later were added by ethanol absolute (1:1 (w/v)) and mixed before stored in -20^0C overnight. The samples then centrifuged at 10000 rpm about 15 minutes (4^{0-}C) and the pellets were obtained and dried. Added Tris-HCl buffer (pH 6.8) (1:1 (v/v)) and stored at -20^0C.

Measuring Protease's Activity from Isolated Protein

Isolated protein from cardiac tissues were taken about 100 µL and then added with casein solution (500 ppm) about 200 µL and mixed. Then, added with phophate buffer solution (0.1 M) pH 7 about 300 µL and mixed. Incubated for 1 h at 37^0C. Cooling the mixture at room temperature and later were added with TCA solution (4% (w/v)) 400 µL. Centrifugated at 4000 rpm for 10 min, and supernatant were taken about 300 µL, and dilluted until 5 times of volume using phosphat buffer solutios (0.1 M) pH 7. Protease's activity was measured using UV Spectrophotometry at 275 nm. Same procedure were done without adding of isolated protein from cardiac tissue as a blank standart. Tyrosine standard was use for standard curve.

RESULT AND DISCUSSION

Histopathology Analysis of Cardiac Tissue

Induction of DOCA-salt showed tendencies toward of hypertension in animal models (Rats). It was characterized by gradual elevation of systolic blood pressure, elevation of MDA levels in kidney and abdominal aorta and histopatology alteration in kidney and abdominal aorta rather than normotennsive group[4]. The used of bakasang's peptide extract, as an alternative antihypertension therapy, showed repairing effect based on histopathology of cardiac tissue.

Wenno *et al.,*[8] stated that Bakasang, which made from fermented Skipjack Tuna (*Katsuwonus pelamis* L.), had potential to be an ACE-inhibitor (an antihypertensive therapy) after being tested *in vitro*. This report showed that ACE-inhibitory activity from bakasang's peptide extract was about 68.80 %. This was better, when compared with another ACE-inhibitor sources, such as Douchi (Chinese traditional fermented soybeans) which have 56.8 – 76.3 % of ACE-inhibitory activity and Bekasam (fermented fish product, Indonesia) which having 55.17 % of ACE-inhibitory activity.

normotensive group (Figure 1. A), showed normal signs of cardiac tissue, including normal nucleus, which did not indicate a change that marks the occurrence of necrosis and none of visible signs of hypertrophy. In the positive control group (Figure 1. B), it showed that hypertrophy in the cardiomyocyte tissue indicated by the enlargement of the

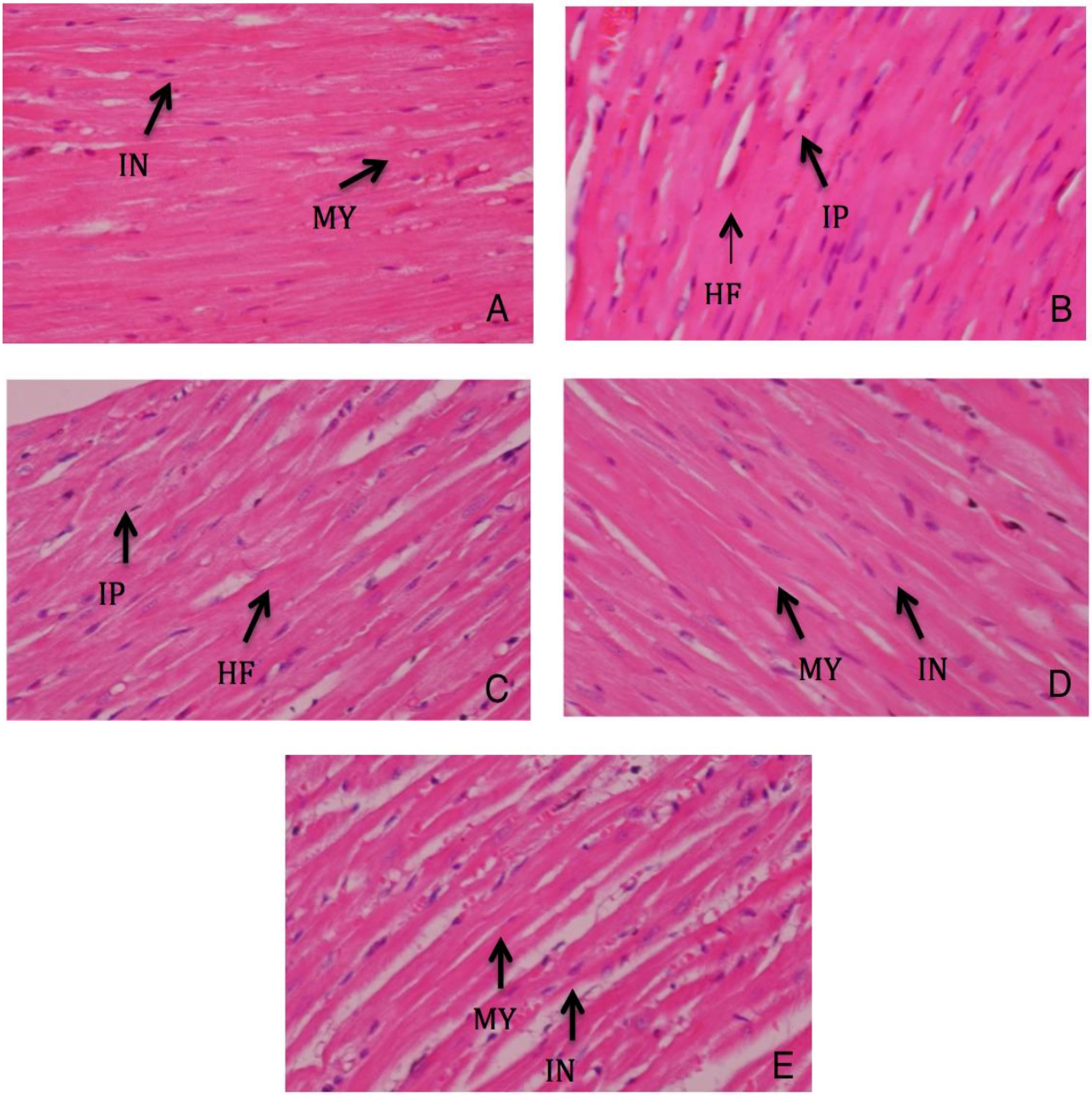

Figure 1: Histological features of cardiac tissue (Rats) with a magnification of 400x. Description: (A) Negative control (normotensive), (B) Positive control (hypertension), (C) Captopril's therapy (5 mg/kgBW), (D) Bakasang's peptide extract therapy (200 mg/kgBW), (E) Bakasang's peptide extract therapy (300 mg/kgBW). (IN) Normal nucleus, (MY) Cardiomyocyte normal cells, (IP) Nucleus undergo picnotic phase, (HF) Hypertrophy.

cardiac muscle size as an adaptation of the increased work of the heart to pump blood due to hypertension. Figure 1. B also shown alteration of cell nucleus, which indicates damage, due to necrosis or cell death process. The process of necrosis can be observed with a change in the cell nucleus into a pictnotic phase, which is characterized by shrinkage of cells or picnotic core area.

Captopril's theraphy (Figure 1. C), shown that hypertrophy was still occur and still had a picnotic area on nucleus of cardiac mucles cells. In fact, as has been known that captopril is one type of commercial hypertension therapy and is a source of antioxidants, this was because a small dose of captopril used, at 5 mg/kgBW. Research from Pechanova[9], stated that the therapeutic use of captopril 50 mg/kgBW has an antioxidant effects in mice. Widyanti[10], also stated that the recommended dose of captopril to prevent hypertrophy is 100 mg/kgBW.

In the group with bakasang's therapy of 200 mg/kgBW, (Figure 1. D), it can be seen that the most significant improvements in the histological picture of the cardiac tissue has occurred. Which hypertrophy in cardiomyocytes had reduced and approached normal state, which also supported by improvements myofibril (muscle fibers) as well as many emerged nucleus of normal cells. Whereas, for the group of rats with bakasang's peptide extract therapy 300 mg/kgBW (Figure 1. E), it can be seen that

Table 1: Protease activity from isolated cardiac tissue.

Group	Average of Protease Activity (U)	Increasing of Protease Activity Against Normotonsive (%)	Decreasing of Protease Activity Against Hypertension (%)
Negative	0.0466 ± 0.0065^{a}		
Positive	0.1672 ± 0.0119^{c}	258.11	
Catopril Therapy	0.1129 ± 0.0072^{b}		32.48
Bakasang Therapy 200 mg/kgBW	0.1015 ± 0.0172^{b}		39.27
Bakasang Theraphy 300 mg/kgBW	0.1022 ± 0.0082^{b}		38.82

Note: Different notations indicate significantly different effect ($p<0.05$).

had been improvements on cardiomyocyte, from its original state (hypertrophy) toward normal (negative control), as well as normal nucleus, but better result was obtained on Bakasang's therapy of 200 mg/kgBW.

Hypertensive rats therapy using bakasang's peptide extract 200 mg/kgBW provides the most improvement effect of repairing histology of cardiac tissue from hypertensive rat, rather than captopril and bakasang's peptide extract 300 mg/kgBW. This can be occurs because their potential as ACE-inhibitor contained in the bakasang's peptide extract. ACE-inhibitor would inhibit the work of the ACE enzyme (Angiotensin Converting Enzyme) which converts angiotensin I, which is inactive to angiotensin II which is a vasoconstrictor. It would be no vasoconstriction occurs and reducing the work of the heart to pump blood. In addition, because of the nature of angiotensin II, which also acts as stimulator of formation of ROS, then by the inhibiton of the ACE enzyme, convertion of angiotensin I into angiotensin II did not occur, causing the production of ROS was decreased, thereby reducing the level of oxidative stress, and natural antioxidants in the body would to work properly.

Protease Activity of Isolated Cardiac's Protein

Protease activty was measured based on tyrosine formed from the hydrolysis of casein[11]. The results showed that bakasang's peptide extract could reduce protease activity ($p<0.05$). Normotensive group as shown, had the lowest protease activity of 0.0466±0.0065 U. Induction of DOCA-salt was able to produce animal model of hypertension, which also increasing the protease activity up to 0.1672 ± 0.0119 U or increaseed 258.11 % from normotensive group. At captopril therapy group, protease activity had reduced up to 0.1129 ± 0.0072 U or decreased 32.48 % from hypertensive group. From bakasang's peptide extract therapy 200 mg/kgBW, protease activity had reduced up to 0.1015 ± 0.0172 U or decreased 39.27 % from hypertensive group, and for bakasang's peptide extract therapy 300 mg/kgBW, protease activity had reduced up to 0.1022 ± 0.0082 U or decreased 38.82 % from hypertensive group. It shown that bakasang's peptide extract therapy 200 mg/kgBW had the lowest decreasing of protease activity against hypertensive group, although as not significant against other therapeutic groups ($p<0.05$).

At normal condition, the presence of some proteases, like matrix metalloproteinase (MMP), Calpain, Cathepsin, Chymase and Caspase are helping to maintain the integrity and function of cells in heart. But, the alteration of activation from these enzymes have suggested to contribute in progression of cardiac failure. There are two major group of proteases, which have play a role in cardiac dysfunction because of hypertension, Extracellular protease (including MMP-2, MMP-9 and Cathepsins) and intracellular protease (Including Cathepsins and Chymase)[12].

MMP family has known as main causes of extracellular damage in heart disease. Which degrades many of structural proteins including collagen, fibronectin, elastin and proteoglycan in extracellular matrix (ECM). While activation of MMP-2 has caused proteolytic damage in extracellular and intracellular sector of cardiomyocytes, especially in sarcomers[12].

Activation of MMP family is affected by elevated formation of free radicals and oxidative stress, which produced as result of the increasing levels of NADPH oxidase by angiotensin II enzyme[12]. The potential as an ACE-inhibitor which contained in bakasang's peptide extract will inhibit the work of ACE enzyme (Angiotensin Converting Enzyme) which converts angiotensin I to angiotensin II, resulting no increased concentration of NADPH oxidase which leading to reduced of free radicals formation.

CONCLUSION

The conclusion of this study is that the peptides from bakasang's extract has an antihypertension effect (ACE-inhibitor) and able to repair histopathology and decrease protease activity of the cardiac tissue on hypertensive rats induced by DOCA-salt.

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REFERENCE

1. Sedayu B., S. Azmi, dan Rahmatini, 2015, *Karakteristik Pasien Hipertensi di Bangsal Rawat Inap SMF Penyakit Dalam RSUP DR. M. Jamil Padang Tahun 2013*, Jurnal Kesehatan Andalas, Vol :4 (1).
2. Rahajeng E. dan S. Tuminah, 2009, *Prevalensi Hipertensi dan Determinannya di Indonesia*, Artikel

Penelitian, Majalah Kedokteran Indonesia, Vol: 59, No:12.

3. Nafrialdi, 2007, *Farmakologi dan Terapi Edisi 5*, Balai Penerbit FKUI, Jakarta.
4. Padaga M.C., A. Aulanni'am, H. Sujuti, Widodo, 2015, *Blood Pressure Lowering Effect and Antioxidant Activity of Casein Derived from Goat Milk Yogurt in DOCA-Salt Hypertensive Rats*, International Journal of PharmTech Research, Vol: 8, No: 6: p :322-330.
5. Korhonen H, and A. Pihlanto, 2006, *Bioactive Peptides: Production and Functionality*, Journal of International Dairy Journal, Vol: 16, p: 945 –960.
6. Khora S.S., 2013, *Marine Fish-Derived Bioactive Peptides and Proteins for Human Therapeutics*, International Journal of Pharmacy and Pharmaceutical Sciences, Vol: 5.
7. Brown N.J., and D.E. Vaughan, 1998, *Angiotensin-Converting Enzyme Inhibitors*, Journal of Circulation, Vol: 97, Hal: 1411-1420.
8. Wenno M.R., E. Suprayitno, A. Aulanni'am, Hardoko, 2016, *The Physicochemical Characteristics and Angiotensin Converting Enzyme (ACE) Inhibitory Activity of Skipjack Tuna (Katsuwonus pelamis) "Bakasang"*, Jurnal Teknologi (Science & Engineering), 78:4-2.
9. Pechanova A., 2007, *Contribution of Captopril Thiol Group to the Prevention of Spontaneous Hypertension*, Journal of Physiological Research, Vol: 56.
10. Widyanti I.K., M.C. Padaga, D.K. Wuragil, 2014, *Pengaruh Terapi Water Soluble Extract (WSE) Yoghurt Susu Kambing Terhadap Kadar Malondialdehyde (MDA) dan Gambaran Histopatologi Jantung Tikus (Rattus norvegicus) Model Hipertensi Induksi Deoxycorticosterone Acetate (DOCA)-Salt*, Student Journal Vetschool PKH-UB, No: 4, Vol: 1.
11. Shofia, V., C. Waranmasalembun, S. Prasetyawan, A. Aulanni'am, 2015, *Methanol Extracts Potential of Mas Ngur Shells (Atactodea striata) Against Protease Profile and Description of Histopathology of Jejunum Rats Exposed by Indometacin*, Journal of Pure and Applied Chemistry Research, Vol: 4 (2), p: 77-81.
12. Muller, A.L., D. Freed, L. Hryshko, N. S. Dhalla, 2012, *Implication of Protease Activation in Cardiac Dysfunction and Development of Genetic Cardiomyopathy in Hamsters*, Canadian Journal of Physiology and Pharmacology, Vol: 90, p : 995-1004.