Antihypertension Effect from Bakasang’s Peptide Extract Based on MDA Levels in Sera and iNOS Expression in Cardiac Tissue of Rats Hypertensive Model

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ABSTRACT
Hypertension is an abnormality of cardiovascular condition, marked by high-blood pressure over normal condition. One of the factors that causes hypertension is altered equilibrium of Renin-Angiotensin-Aldosteron system, which commonly caused by genetic disorder, could produces excess of Angiotensin II which known as vasoconstrictor. Angiotensin II elevates body’s blood pressure and stimulates production of free radicals causes hypertension and oxidative stress. This research conducted to explore potential antihypertension effect from bakasang’s peptide extract made from fermented skipjack tuna (Katsuwonus pelamis L) based on MDA levels in sera and iNOS expression in cardiac tissue from hypertension rats (Rattus norvegicus) induced by DOCA-salt. Rats were divided into five groups, they were: (1) negative control group, (2) hypertensive control group, (3) hypertension with captopril therapy with dose of 5 mg/kg of body weight (BW), (4,5) bakasang’s peptide extract therapy with dose of 200 mg/kgBW, and 300 mg/kgBW respectively. The results showed that bakasang’s peptide extract with dose of 200 mg/kgBW significantly decrease (p<0.05) MDA level in sera and iNOS expression in cardiac tissue. MDA level decreased up to 32.77%, and iNOS level of expression decreased up to 34.46 % after therapy using bakasang’s peptide extract on hypertension rats. It can be concluded that bakasang’s peptide extract has antihypertension effect (ACE-inhibitor), and capable to decrease MDA levels in sera and iNOS expression in cardiac tissue on hypertension rats induced by DOCA-salt.

Keywords: Bakasang, Hypertension, iNOS, MDA.

INTRODUCTION
Hypertension is a cardiovascular disorder which indicated by the elevation of blood pressure in the body, reaches ≥140 mmHg for systolic pressure and ≥ 90 mmHg for diastolic pressure, compared with normal blood pressure in human is <120 mmHg for systolic and <80 mmHg for diastolic1. The number of patients with hypertension in Indonesia is almost 27,5% of the total population and increase significantly through the years, based on 2004 survey2. With the most common type of cases is essential hypertension, nearly 95% of total cases3. Essential hypertension, which caused by genetical disorder, plays significant role in the development of hypertension. One of the factors that leads to hypertension, is disruption of the balance system of Renin-Angiotensin-Aldosteron System (RAAS). RAAS which normally plays a role in regulating blood pressure and fluid balance in the body, will produce excess volume of Angiotensin II, a potent vasoconstrictor, which induces sodium and water retention in kidney leads to elevation of body’s blood pressure4. In addition, Angiotensin II also stimulates the secretion of Aldosterone which causes activation of NADPH oxidase (NOX)5,6,7. Activation of NOX will increase the production of free radicals (Radical Oxygen Spesies / ROS) leads to oxidative stress after imbalance concentration between free radicals and antioxidants in the body’s8. Deoxycorticosterone Acetate (DOCA)-salt, is a synthetic mineralcorticoid which commonly used in the production of animal model of hypertension, leads to an endocrine hypertension model. Induction of DOCA-salt could form a severe hypertension model by quickly, followed by increasing of oxidative stress. This endocrine type of hypertension allows to be treated using bioactivate peptides as a therapy9.

It has been known that dietary protein is a rich-source of bioactive component which has beneficial effect for health accordance to the sequence of it’s amino acid10. In this research, Bakasang (traditional food from maluku and north sulawesi) which made from fermented skipjack tuna was used as therapeutic agent. Research shown that used of dried skipjack tuna’s meat (Bonito), had potential of antihypertensive effect as an ACE (Angiotensin Converting Enzyme)-inhibitor6,7.
This study is conducted to prove the potential of antihypertension effect from bakasang’s peptide extract, based on MDA (Malondialdehyde) level in sera and iNOS expression in cardiac tissue of hypertensive rat (*Rattus norvegicus*) induced by DOCA-salt.

**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 milliliters 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°C for 5 min. Bakasang mixture’s then centrifuged (4°C) 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°C.

**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 study (necropsy).

**Preparation of Bakasang’s Peptide Extract as a Therapy**

The therapeutical dose of bakasang’s peptide 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 therapeutical 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).

**Measurement of MDA Level in Sera**

After all the process has completed, necropsy will be performed and followed by analysis to determine MDA level in sera and observation of iNOS expression on cardiac tissue using immunohistochemistry technique. For the measurement of MDA level in sera, each sera from all groups was pipetted 100 μL and added with 550 μL aquaest. The mixture was homogenized and then added with 250 μL of Tri-Chloroacetic Acid (TCA 10%), 250 μL of HCl 1N and 100 μL of Na-thiobarbituric Acid solution, respectively with homogenized after each addition. The mixture then centrifuged at 500 rpm for 10 minutes and supernatant was taken. Supernatant was heated at 100°C in waterbath for 30 minutes, then cooled at room temperature before measured using visible-spectrophotometer at specific wavelength.

**Analysis of iNOS expression in Cardiac Tissue using Immunohistochemistry Method**

Cardiac tissue were stored in PFA (paraformaldehyde) 10% and then sectioned and attached on object glass (slides) before de-parrafinized and re-hydrated. Slides later was washed by PBS (Phosphate Buffer Saline) solution then added using Hydrogen Peroxide ($H_2O_2$) and incubated for 20 minutes before washed by addition of PBS. Blocking was performed using addition of BSA (Bovine Serum Albumin 5% (w/v)) in PBS solution and incubated 30 minutes at room temperature. Slides was washed using PBS solution and then added by iNOS antibody as primary antibody. Slides later incubated with addition of secondary antibody-biotin labelled and then added using SA-HRP (Strep-Avidin Horse Radish Peroxidase) and DAB (diamino benzidine) solution respectively. Counterstaining was performed by addition of Mayer Hematoxylin before being closed by coverglass. Slides was observed under the microscope and later was analyzed by ImmunoRatio software.

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**Table 1: MDA Levels From Sera.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average Levels (μg/mL)</th>
<th>MDA Negatve Group (%)</th>
<th>Levels Against Hypertension</th>
<th>Decreased Positive Group (%)</th>
<th>Levels Against Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Hypertension</td>
<td>1.012±0.244a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive Hypertension</td>
<td>2.137±0.138c</td>
<td>111.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Captopril mg/kgBW Therapy</td>
<td>1.901±0.223c</td>
<td>-</td>
<td>11.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bakasang mg/kgBW Therapy</td>
<td>1.437±0.198ab</td>
<td>-</td>
<td>32.77</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bakasang mg/kgBW Therapy</td>
<td>1.712 ±0.204bc</td>
<td>-</td>
<td>19.89</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Different superscripts notations indicate significantly different effect (p<0.05).
Statistical Analysis

Statistical analysis was performed using SPSS application for Mac (Version 20) with ANOVA (Analysis of Variance) method and Tukey’s post hoc test (p<0.05).

RESULTS

Based on the results of MDA level in sera (Table 1), it was found that the positive group of hypertension, MDA level had significantly increased compared to negative hypertension group, which had MDA level by 1.012 ± 0.244 μg/mL or increased 111.21%. In captopril group of therapy, MDA level was 1.901 ± 0.223 μg/mL or decreased 11.04% from positive hypertension group. For bakasang group of therapy, MDA levels were 1.437 ± 0.198 μg/mL or decreased 32.77% from positive hypertension group and 1.712 ± 0.204 μg/mL or decreased 19.89% from positive hypertension group, with dose of 200 and 300 mg/kgBW respectively. Table 1 showed that bakasang’s peptide extract therapy significantly decrease MDA level (p<0.05) with dose of 200 mg/kg BW as effective dose for decreasing MDA level in sera.

Table 2: Levels of iNOS Expression From Each Group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average iNOS Expression (%)</th>
<th>Increased Levels Against Negative Group (%)</th>
<th>Decreased Levels Against Positive Group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Hypertension (A)</td>
<td>5.975 ± 2.075^e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive Hypertension (B)</td>
<td>49.62 ± 3.633^d</td>
<td>730.46</td>
<td>-</td>
</tr>
<tr>
<td>Captopril Therapy 5 mg/kgBW (C)</td>
<td>43.06 ± 2.157^cd</td>
<td>-</td>
<td>13.22</td>
</tr>
<tr>
<td>Bakasang Therapy 200 mg/kgBW (D)</td>
<td>32.52 ± 4.219^h</td>
<td>-</td>
<td>34.46</td>
</tr>
<tr>
<td>Bakasang Therapy 300 mg/kgBW (E)</td>
<td>35.88 ± 2.039^ic</td>
<td>-</td>
<td>27.69</td>
</tr>
</tbody>
</table>

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

DISCUSSION

The bakasang’s peptide extract, which obtained from extraction of bakasang (fermented skipjack tuna (Katsuwonus pelamis L.)), has potential as an alternative antihypertensive therapy (ACE-inhibitor) by in vitro study and had good ACE-inhibitory activity with average of 68.80%. Bakasang’s peptide extract has better ACE-inhibitory activity compared with another ACE-inhibitor sources, such as douchi (traditional Chinese fermented food from soybean) which has ACE-inhibitory activity of...
Figure 2: iNOS Expression in Cardiac Tissue After Immunohistochemistry Method.  
Note: (A) Negative hypertension, (B) Positive Hypertension, (C) Captopril therapy 5 mg/kgBW, (D) Bakasang therapy 200 mg/kgBW, (E) Bakasang therapy 300 mg/kgBW. (→) Location of iNOS expression.

![Figure 2: iNOS Expression in Cardiac Tissue After Immunohistochemistry Method.](image)

Figure 3: Levels of iNOS Expression From Each Group.

![Figure 3: Levels of iNOS Expression From Each Group.](image)
56.8 – 76.3% and bekasam (fermented fish product, Indonesia) which has ACE-inhibitory activity of 55.17%. This ACE-inhibitory activity from bakasang’s peptide extract, obtained due to the fermentation process of skipjack tuna’s meat by lactic acid bacteria (LAB) such as, Lactobacillus casei, Lactobacillus farciminis dan Lactobacillus plantarum. LAB have the ability to break down the long-chained protein into short-chained fragments (peptides) that could provide positive benefit for the body, one of them as an ACE-inhibitor.

Bakasang’s peptide extract has the ability to decrease MDA level in sera because of its ACE-inhibitory activity. ACE-inhibitor is able to inhibit ACE activity to convert angiotensin I into angiotensin II, which is a potent vasoconstrictor, so that the blood pressure is not get elevated and beside that, production of ROS also become declined. Because, apart as a potent vasoconstrictor, angiotensin II also contributes to stimulate the production of superoxide (O2−) in large quantities. The decline in production of free radicals causes reduced level of oxidative stress, thus the endogenous antioxidants could provide therapeutic effect to the body. This is accordance with the research of Khora (2013) and Wennno et al. (2016), which state that the protein hydrolisate from skipjack tuna’s, even from bonito and bakasang, have potential as an antihypertensive therapy (ACE-inhibitor)13. Captopril therapy is also give a decreasing in MDA level in sera, although not giving significantly different with the positive inhibitory activity from bakasang’s peptide extract, obtained due to the formation of MDA spontaneously or by catalitic reaction with superoxide dismutase (SOD). It has been known that the presence of peroxynitrite radicals and hydrogen peroxide has implication to tissue damage and organ dysfunction, including the heart13. According to the previous research of Sun et al. (2005), although it was known that the inhibition of iNOS did not have a directly effect on lowering blood pressure and left ventricular hypertyrophy, however, the inhibition and lowered expression of iNOS was able to gave therapeutic effect on left ventricular contractile tissue caused by decreased level of oxidative stress, as well known that angiotensin II are able to increase the iNOS expression and oxidative stress which causes reducing performance and function of the heart14.

We conclude that the bakasang’s peptide extract has potential to be an alternative therapy for antihypertension indicated by reduced MDA level in sera and iNOS expression from cardiac tissue in animal’s model of hypertension induced by DOCA-salt.

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REFERENCES