Impact of Black Garlic on Biomarkers of Arterial Stiffness and Frontal QRS-T Angle on Hypertensive Animal Model

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ABSTRACT
Background: Hypertension is a silent killer disease that continues to increase every year. Non-optimal treatment of hypertension may heighten the risk of dangerous cardiovascular problems. Black garlic (BG) has been known as an antihypertensive; however, its effect on biomarkers of arterial stiffness and QRS-T angle direction is still not yet understood. Objectives: This study to identify the role of BG as an antihypertensive agent on biomarkers of arterial stiffness and QRS-T angle direction. Materials and Methods: The study was conducted on 30 male Wistar rats, which were randomly grouped into six, consisting of groups 1 and 2 (receiving drug carrier), group 3 (receiving captopril at 2.5 mg/kg), groups 4, 5 and 6 (receiving BG suspension at doses of 50, 100 and 200 mg/kg, respectively). All groups, except group 1, were given a high-fat diet and 25% fructose in drinking water for 28 days. Test drugs were given from day 15 to day 28 (for 14 days). The parameters measured were blood pressure, nitric oxide (NO) level in the serum, pulse wave velocity (PWV), heart rate and the QRS-T angle direction. Results: the BG doses of 50, 100 and 200 mg/kg lowered blood pressure, increase NO serum level; ameliorate PWV and wide QRS-T angle (p<0.05). Conclusion: BG has anti-hypertensive activity and its antihypertensive effect of BG through the improvement of arterial stiffness and QRS-T angle direction. Key words: Black Garlic, Hypertension, Nitric oxide, Arterial Stiffness, QRS-T angle.

INTRODUCTION
There is a close relationship between hypertension and arterial stiffness and both as predictors of cardiovascular events and mortality. These suggest that antihypertensive drugs should be able to reduce blood pressure and improve arterial stiffness. Uncontrolled hypertension triggers left ventricular hypertrophy. It is characterized by widening the QRS-T angle direction derived from electrocardiogram (ECG). Therefore, arterial stiffness and QRS-T angle direction of the heart both become important biomarkers in assessing the antihypertensive agents. A large artery or Aortic stiffness which continues to raise can cause an increase in pressure and produces an abnormal pattern of pressure on the left ventricle. In this case, the stiffness of the aorta which causes a rise in afterload of the left ventricle may induce left ventricular hypertrophy, thus disrupting the diastolic and systolic functions in the left ventricle. The findings of the clinical studies showed that the QRS-T angle of more than 90° was associated with an increase in the risk of cardiac sudden deaths. The method of measurement of the angle of spatial QRS-T is a reliable, reproducible, non-invasive method with a relatively small level of error. Garlic has been recognized by people in various countries, including in Indonesia and it is also commonly used as the treatment for various kinds of diseases, including hypertension. Thus, the development of black (fermented) garlic products as food supplements is highly likely since garlic has been considered a relatively safe food ingredient.

Black garlic is produced from fresh garlic (Allium sativum L.), from the Liliaceae family, which has been heated for a specified time duration, at a specified high temperature and high humidity level. The process of making black garlic starts from turning the cloves of garlic into a dark-color, to have a sweet taste and soft texture with a chewy consistency. The duration of fermentation varies depending on the culture, factory and purpose. Fresh garlic that is processed into black garlic at a temperature of 70-80°C produces better quality and taste, as well as the total phenol content which increases significantly so that it increases its antioxidant capacity. This leads to the growth of pharmacological activities of black garlic including anti-obesity, anti-hyperlipidemia, anti-diabetic, anti-oxidant and anti-inflammatory. The heating process with varying temperatures, ranging from 60°C to 90°C, greatly affects the contents of phytochemicals generated. It was found that the temperature ideal to produce black garlic is at 70°C, in addition to the humidity and the process of fermentation.

Black garlic has been reported to have antihypertensive effects because it contains stable nitrates converted to nitric oxide (NO) in the body, making it able to reduce blood pressure. However, the black garlic effects on arterial stiffness and spatial QRS-T angle are yet to study. This present study aims to investigate the effects of black garlic on biomarkers PWV and QRS-Tangle in hypertensive animals induced by a high-fat diet and 25% fructose in drinking water.

MATERIALS AND METHODS
Garlic fermentation
The cloves of garlic (Allium sativum L.) were collected from the local farm Ciwidey, Bandung. Plant determination was carried out at the Laboratory of the School of Life Science and Technology, Bandung.
Institute of Technology (1233 / 11.CO2.2 / PL / 2019). The process of garlic fermentation as black garlic (BG) began by cleaning garlic cloves. The garlic cloves were then wrapped in the aluminum foils and put into the oven at the temperatures of 65°-70°C for 15 days. After 15 days of fermentation, the BG was put into the sterilized glass containers with impermeable lids. A total of 1630 g of fresh garlic processed at 70°C for 15 days can produce 605 g of black garlic. The percentage of yield obtained can be calculated as 37.12% (605 g of 1630 g of fresh garlic).

**Animals**

This study performed on the male Wistar rats with the weights of 200-250 g and the ages of 2-3 months. All of the rats were acclimatized at cages with good ventilation and were fed a normal diet (Pokphan C551) and given ad libitum water before testing to assure the normal growth and behavior. Animal cages were maintained at the temperature of around 22°C and 50% humidity with 12 hr of light and dark cycles. All series of procedures had followed the regulations and had been approved by the Research Ethics Commission of Padjadjaran University Bandung as listed in the Ethics Approval letter with the registration number 287/UN6.KEP/EC/2019. The study was performed in vivo in the rats which were randomly grouped into six groups. These six groups consisted of normal control and positive control (receiving drug carrier) groups, standard drug (receiving captopril 2.5 mg/kg) group and black garlic (receiving BG of 50, 100 and 200 mg/kg) groups.

**High-Fat Food Composition**

A total of 1000 g of standard feed was mixed with 400 g of fat. The fat contained 155 g of butter, 90 g of eggs and 155 g of tallow. The composition of the 90 g of eggs was made of a mixture of 1 duck egg and 4 quail eggs.9

**Evaluation of the antihypertensive effect of fermented garlic**

The systolic and diastolic blood pressure was measured by the CODA Rat Tail-Cuff Blood Pressure System (CODA® Kent Scientific Corporation). The working principle of the tool was by installing the tail-cuff in the form of rubber on the tails of the rats, in which the cuff had a Volume Pressure Recorder (VPR), a special sensor that could measure the changes in the volume of blood and the cuff was placed at the base of the rat tail. The rat was detained in a restrainer and heated it artificially to maintain the normal blood pressure. The cuff automatically inflated to give pressure on the rat tail and then the pulse of flowing blood could be detected. The blood pressure was measured on days 0, 7, 14, 21, 28 after treatment. The antihypertensive effect of black garlic was expressed if there were significant differences in the treatment groups compared to the control group (p<0.05).

**Evaluation of the effect of black garlic on arterial stiffness, heart rate and spatial QRS-T angle**

The arterial stiffness and heart rate after having black garlic treatment were measured by a non-invasive method, using the ECG and PPG sensors following the methods published previously. The measurement of the QRS-T angle also used the same tool, by adding two ECG channels to the device to allow the obtainment of frontal ECG leads. Heart rate was determined by measuring the distance of R-R in PQRST waves of the ECG results.

**Evaluation of the levels of nitric oxide in the serum**

On day 28, the measurement of NO levels in the serum of test rats was done by using the spectrophotometry on a wavelength of 300-600 nm using the Griess assay which can detect the presence of nitrile in the serum. The principle of the Griess assay is the diazotization between nitric acid (from nitrites in acidic conditions) and primary aromatic amines (sulfanilic acid). Diazonium salts produced from this diazotization are then reacted (coupled) with alpha-naphthylamine to form red compounds.10

**Data Analysis**

The obtained data were analyzed statistically, by the SPSS software 14.0 version, to identify the effect of black garlic on the treatment groups compared to the control group. Significant differences in effect between treatment groups were indicated by p-value (p<0.05).

**RESULTS**

The difference in physicochemical properties between BG and fresh garlic showed in Table 1. The garlic fermentation process by heating at 70°C for 15 days increases the ash content, acid content, water-soluble components and ethanol-soluble components. Meanwhile, the loss on drying of fresh garlic was greater than BG.

The systolic blood pressure in all treatment groups for 28 days showed in Table 2. The group that received a high-fat and 25% fructose diet showed a significant increase in systolic blood pressure compared to the normal control group on days 7 and 14. Meanwhile, the group receiving black garlic at doses of 50, 100 and 200 mg/kg showed a decrease in systolic blood pressure. There was a decrease in systolic blood pressure in the administration of BG at doses of 50, 100 and 20 mg/kg which were comparable to the captopril group (p<0.05). Table 3 describes the differences in diastolic blood pressure for 28 days of treatment for all groups.

The diastolic blood pressure on day 0 showed no significant differences in all groups of treatment. On day 7-28, the diastolic pressure continued to rise and there were significant differences from the normal control group. The results showed that a high-fat diet and 25% fructose for 28 days significantly increased (p<0.05) the diastolic blood pressure. Table 4 shows the effect of BG on PVW values during 14 days of therapy. BG at doses of 50, 100 and 200 mg/kg showed comparable decreases in PVW values (p<0.01). The PVW value of the positive control group increased significantly compared to the normal control group (p<0.05). Whereas, the BG group decreased significantly against the positive control group (p<0.05). There was no difference in the BG group at doses of 50, 100 and 200 mg/kg.

Table 5 describes the effect of the treatment on heart rate, as measured for 28 days. The control positive group shows a significant increase in heart rate compared to the normal group. While the BG group showed a significant decrease in heart rate compared to the positive control group. There was no difference in the BG group at doses of 50, 100 and 200 mg/kg.

Table 6 describes the effects of BG at doses of 50, 100 and 200 mg/kg on the direction of the QRS-T angle. BG narrows the wide-angle QRS-T, which differed significantly compared to the positive control group.

<table>
<thead>
<tr>
<th>No</th>
<th>Physicochemical Test</th>
<th>Black Garlic (%)</th>
<th>Fresh Garlic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Ash Content</td>
<td>2.5</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>Levels of Acid-insoluble Ash</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol Soluble Content</td>
<td>34.5</td>
<td>9.6</td>
</tr>
<tr>
<td>4</td>
<td>Water Soluble Content</td>
<td>56.3</td>
<td>23.2</td>
</tr>
<tr>
<td>5</td>
<td>Loss on Drying</td>
<td>0.1</td>
<td>61.7</td>
</tr>
</tbody>
</table>

Garlic fermentation carried out at a temperature of 70°C for 15 days.
Figure 1 shows the effect of BG on vasodilator NO levels in the blood. The group receiving BG at a dose of 200 mg/kg showed higher NO levels compared to a dose of 50 and 100 mg/kg.

**DISCUSSION**

The fructose diet has been known to increase blood pressure due to the formation of aldehyde compounds as a result of fructose metabolism. Aldehydes will bind to the protein membranes of sulfhydryl groups, causing calcium channel in the membrane of vascular smooth muscle. As a consequence, free calcium levels in the vascular smooth muscle cytosol will elevate, which triggers vascular hyperactivity, vasoconstriction and peripheral resistance; thus, leading to an increase in blood pressure. Moreover, the consumption of fructose and high-fat diet increased the distribution of fat in the body; therefore, induced obesity. The increased

### Table 2: Effects of BG on systolic blood pressure (mmHg) on day 0, 7, 14, 21 and 28 treatment.

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment Group</th>
<th>Average Systolic Blood Pressure ± SD (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>T0: 102.8±1.9; T7: 104.6±2.1; T14: 114.8±2.6; T21: 108.0±1.8; T28: 108.8±2.1</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>T0: 114.8±2.6; T7: 144.0±2.1; T14: 176.0±2.3; T21: 193.4±2.3; T28: 213.2±2.3</td>
</tr>
<tr>
<td>3</td>
<td>Captopril 2.5</td>
<td>T0: 104.0±2.5; T7: 145.0±1.6; T14: 177.0±1.5; T21: 151.2±0.8; T28: 132.4±1.8</td>
</tr>
<tr>
<td>4</td>
<td>BG 50</td>
<td>T0: 103.8±1.3; T7: 143.8±2.2; T14: 178.0±1.8; T21: 151.8±1.9; T28: 133.6±3.5</td>
</tr>
<tr>
<td>5</td>
<td>BG 100</td>
<td>T0: 102.0±2.5; T7: 143.0±1.0; T14: 177.8±1.9; T21: 150.8±0.8; T28: 131.0±1.9</td>
</tr>
<tr>
<td>6</td>
<td>BG 200</td>
<td>T0: 103.2±2.2; T7: 143.2±2.2; T14: 178.0±2.0; T21: 150.4±1.5; T28: 130.2±1.3</td>
</tr>
</tbody>
</table>

BG: black garlic doses of 50, 100, 200 mg/kg

(*) significantly different from the normal/negative control group (p < 0.05).

(a): significantly different from the induction/positive control group (p < 0.05).

(β): significantly different from the comparison group/captopril 2.5 mg/kg (p < 0.05).

### Table 3: Effects of BG on diastolic blood pressure (mmHg) on day 0, 7, 14, 21 and 28 after treatment.

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment Group</th>
<th>Average of Diastolic Blood Pressure ± SD (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>T0: 70.4±1.5; T7: 72.4±2.2; T14: 75.4±2.1; T21: 75.6±2.6; T28: 77.0±1.9</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>T0: 70.0±3.5; T7: 105.4±2.1; T14: 122.4±3.4; T21: 129.6±1.8; T28: 173.6±2.7</td>
</tr>
<tr>
<td>3</td>
<td>Captopril 2.5</td>
<td>T0: 69.6±2.1; T7: 106.4±2.7; T14: 123.4±3.7; T21: 113.6±2.6; T28: 86.4±2.7</td>
</tr>
<tr>
<td>4</td>
<td>BG 50</td>
<td>T0: 70.2±5.9; T7: 106.4±5.1; T14: 123.4±2.6; T21: 115.4±2.3; T28: 88.2±2.7</td>
</tr>
<tr>
<td>5</td>
<td>BG 100</td>
<td>T0: 70.0±2.2; T7: 106.0±4.3; T14: 123.6±2.9; T21: 113.6±2.4; T28: 86.2±3.5</td>
</tr>
<tr>
<td>6</td>
<td>BG 200</td>
<td>T0: 71.0±2.8; T7: 106.8±2.8; T14: 124.4±3.2; T21: 111.4±1.1; T28: 83.6±2.1</td>
</tr>
</tbody>
</table>

BG: black garlic doses of 50, 100, 200 mg/kg

(*) significantly different from the normal/negative control group (p < 0.05).

(a): significantly different from the induction/positive control group (p < 0.05).

(β): significantly different from the comparison group/captopril 2.5 mg/kg (p < 0.05).

### Table 4: Results of Pulse Wave Velocity (PWV) in cm/s ± SD on day 0, 7, 14, 21 and 28 after treatment.

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment Group</th>
<th>Average of PWV (cm/s) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>T0: 346±4.3; T7: 348±3.4; T14: 351.8±1.5; T21: 353.8±1.3; T28: 356.2±1.3</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>T0: 347.6±3.4; T7: 442.8±2.9; T14: 551.6±1.1; T21: 541.4±0.5; T28: 676±2.3</td>
</tr>
<tr>
<td>3</td>
<td>Captopril 2.5</td>
<td>T0: 344.4±3.4; T7: 444.6±4.9; T14: 553.6±2.3; T21: 559.6±3.4; T28: 520±2.3</td>
</tr>
<tr>
<td>4</td>
<td>BG 50</td>
<td>T0: 347±1.9; T7: 446.4±1.7; T14: 551.8±1.1; T21: 537.6±1.1; T28: 516.6±1.4</td>
</tr>
<tr>
<td>5</td>
<td>BG 100</td>
<td>T0: 346.6±1.5; T7: 446.6±1.1; T14: 551.8±1.5; T21: 536±0.7; T28: 513.2±1.9</td>
</tr>
<tr>
<td>6</td>
<td>BG 200</td>
<td>T0: 346±3.1; T7: 446.6±1.1; T14: 551±1.7; T21: 534.2±0.8; T28: 511±1.3</td>
</tr>
</tbody>
</table>

BG: black garlic doses of 50, 100, 200 mg/kg

(*) significantly different from the normal/negative control group (p < 0.05).

(a): significantly different from the induction/positive control group (p < 0.05).

(β): significantly different from the comparison group/captopril 2.5 mg/kg (p < 0.05).
masses of fat cells result in a growth in the production of angiotensinogen which subsequently triggers the systemic activation of the renin-angiotensin-aldosterone system (RAAS); and thus, vasoconstriction and increases in blood pressure.\textsuperscript{12}

The present study revealed that BG has an anti-hypertensive effect. This study is in line with those reported by Miao (2014) which found that the effect of black garlic in lowering blood pressure is thought to occur through angiotensin-converting enzyme (ACE) or inhibit the formation of angiotensin II.\textsuperscript{13} It was reported that black garlic heated for 45 days at a temperature of 40-85°C contained polyphenols, ajoene, S-allyl-L-cysteine and flavonoids that showed more potent antioxidant activity.

High blood pressure is also accompanied by arterial stiffness. This conformed to the previous study which found that fructose diets cause endothelial dysfunction, which plays an important role in producing nitric oxide. This disrupts the production of NO as a vasodilator. Nitric oxide plays an important role in the homeostasis regulation of blood pressure. A decrease in nitric oxide levels is one of the causes of arterial stiffness which results in persistent high blood pressure.\textsuperscript{14}

Arterial stiffness can be measured non-invasively by measuring pulse wave velocity (PWV). It is related to cardiovascular conditions. This confirms the results of clinical studies that found antihypertensive drugs, apart from being able to lower blood pressure, are also able to reduce arterial stiffness.\textsuperscript{15} The present study indicates that black garlic improves arterial stiffness as evidenced by decreasing PWV and heart rate.

There is plenty of evidence that an increase in heart rate is associated with a greater risk of developing hypertension and atherosclerosis.

Increased heart rate is a strong predictor of cardiovascular morbidity and mortality. Moreover, experimental research in animals indicated that hemodynamic disruption was associated with a higher heart rate and had a direct impact on the walls of the arteries leading to the occurrence of atherosclerotic plaques.\textsuperscript{16}

The changes in blood pressure in the arteries will affect the movement of artery walls. There is a positive correlation between blood pressure and heart rate with arterial stiffness.\textsuperscript{17}

It has been reported that BG contains higher ajoene compounds compared to fresh garlic\textsuperscript{13} which inhibits the entry of calcium ions into the cell resulting in a decrease in intracellular calcium levels. This affects hyperpolarization and relaxation of smooth muscle which results in vasodilation resulting in a decrease in blood pressure.\textsuperscript{18}

A decrease in serum NO levels indicates a disturbance in endothelial function. The group that received BG showed an increase in serum NO levels (Figure 1). This is likely to occur due to the disruption of endothelial function due to diet.\textsuperscript{19} Nitric oxide plays a central role in maintaining blood pressure homeostasis. Any interference in NO bioactivity can cause vasoconstriction and arterial stiffness, which heighten the risk of hypertension. Serum nitric oxide levels are inversely correlated with heart rate.

Increased heart rate with arterial stiffness.
exhibits antioxidant activity by regulating NO production. SAC inhibits iNOS expression in macrophages and increases NO in endothelial cells. This has an anti-inflammatory effect and prevents atherosclerosis.\textsuperscript{23}

The diet contained high-fat and 25% fructose could lead to heart dysfunction. Shifts and changes in the angle of QRS-T describe the abnormalities in the heart. It has been reported previously that when the QRS-T angle reached 120\degree or above, there would be abnormalities in the left ventricle generally identified as ischemia.\textsuperscript{24}

The BG showed an improvement in the QRS-T wave and showed left angle shifts or returned to a normal angle. These results corroborated those in previous studies which suggested that black garlic administration was believed to be able to correct abnormalities in the left ventricle of the heart, thereby reducing the risk of ischemic heart disease.\textsuperscript{25}

**CONCLUSION**

Black garlic obtained by fermentation at temperature 70\degree C for 15 days has potential as an antihypertensive, which improves arterial stiffness, heart rate and QRS-T angle direction. Increased serum nitric oxide levels contribute to this effect. Further research is needed.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ABBREVIATIONS**

**BG:** Black garlic; **ECG:** Electrocardiogram; **PPG:** Photoplethysmogram; **PWV:** Pulse wave velocity; **NO:** Nitric oxide; **RAAS:** Renin-angiotensin-aldosterone system; **Frontal QRS-T angle:** A spatial angle between QRS vector and T vector; **SAC:** S-allyl cysteine.

**REFERENCES**