The Effect of Pumpkin Seed Flour (Cucurbita Moschata) on Blood Pressure of Hypertensive Rats (Rattus novergicus L)

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Hypertension is a medical condition where blood pressure increases over the normal limit. One alternative food with the potential for anti-hypertension is pumpkin seeds (Cucurbita Moschata). This study aims to determine pumpkin seed flour's effect on blood pressure changes in hypertensive rats. This type of study is experimental with a pretest-posttest controlled group design. This study's samples were rats induced by Sodium chloride to be hypertensive. Blood pressure measurement using Non-Invasive Blood Pressure CODA (Tail Cuff) method. The rat was divided into a control group, pumpkin seed flour group 4.5 mg, and pumpkin seed flour group 5.4 mg; the intervention was given for 28 days. The analysis in this study used the one-way ANOVA test. The results showed a significant reduction in blood pressure in the rats found in pumpkin seed flour (p<0.05). There was a significant difference in blood pressure before and after treatment between pumpkin seed flour and the control group with a value (p<0.001).

Pumpkin seed flour is effective in reducing the blood pressure of hypertensive rats. This study has the potential to be applied to the human who has hypertension.

Keywords: Blood Pressure; Hypertension; Pumpkin Seed Flour; Rats; Sodium chloride.

The global prevalence of hypertension in people aged 18 years and over with increased blood pressure (systolic blood pressure 140 mmHg or diastolic blood pressure 90 mmHg) is 44.5% based on the male sex 24.0% while based on the female sex 20.5%.1 The 2018 Baseline Health Research data shows that the prevalence of hypertension in people aged 18 years is 34.1%. The highest was in South Kalimantan (44.1%), and the lowest was in Papua (22.2%), and South Sulawesi (32%).2

The use of herbal medicine or plant as part of hypertension treatment has increased in the last decade. This is due to several factors, especially the price of traditional medicines, which are considered cheaper with fewer side effects.3 The plant that can be used is giving pumpkin seeds4, where pumpkin seeds are one of the natural ingredients that have the potential as an anti-hypertensive.5

Pumpkin (Cucurbita Moschata) is a functional food that contains many important nutrients the human body needs.6 The seeds contain many important protein sources, phytosterols, polyunsaturated fatty acids, phytochemicals, sterols, antioxidant vitamins such as carotenoids.
and tocopherols, and trace elements such as zinc and selenium. The nutritional content of pumpkin seeds includes phytosterols, vitamins (vitamins C, E, and beta carotene), and minerals (magnesium, selenium, and zinc) which can reduce hypertension.

The results of a study by Andari et al. showed that rats that had been induced by hypercholesterolemia for 14 days significantly reduced total cholesterol in hypercholesterolemic Wistar rats (p<0.05). A study by Wong et al. showed that supplementation with pumpkin seed oil could help reduce brachial and central blood pressure in postmenopausal women with high blood pressure. It shows that long-term regular consumption of pumpkin seed oil can reduce the risk of heart disease. Before being applied to humans or other primates, a series of experiments using animal models must be carried out first (preclinical research). The Wistar rat is one of the most widely used rats as a model in biomedical research.

In order to increase the use of existing local resources for hypertension treatment, the development and utilization of agricultural waste, namely pumpkin seeds, is carried out into functional food in the form of flour which is expected to overcome nutritional problems, one of which is hypertension.

So far, no previous study has tested the effect of pumpkin seeds on hypertensive rats. Therefore, this study aims to see the effect of giving pumpkin seed flour on the blood pressure of hypertensive rats (Rattus norvegicus L).

**Study Design**

This study is a Quasi-Experimental study with a Non-equivalent control group design.

**Location and Time of Research**

The study was carried out in March-May 2022. Pumpkin seed flour was made at the Food and Nutrition Laboratory of the Research Activities Center (PKP), Hasanuddin University, Makassar. Experimental animal treatment studies were conducted at the Pharmacy Preclinical Testing Laboratory of Indonesian Muslim University (UMI), Makassar.

**Population and Sample**

The sample in this study were male Wistar rats (Rattus norvegicus L) from the Biopharmacy and Biopharmaceutical Laboratory of Pharmacy Faculty at Hasanuddin University, which had been certified. The number of rats in this study was selected through purposive sampling for 24 male Wistar rats, which were divided into three groups, and each group consisted of 8 Wistar rats, as follows:

- **K1**: Normal-fed rats as a control group.
- **P1**: Pumpkin seeds dose of 4.5 mg: Samples induced by pumpkin seed flour as a treatment dose of 1
- **P2**: Pumpkin seeds dose of 5.4 mg: Samples induced by pumpkin seed flour as a treatment dose of 2

In this study, the pumpkin seed doses were 250 mg/kilogram and 300 mg/kilogram of body weight. Previous researchers have used this dose to determine flavonoid levels and toxicity tests of yellow pumpkin seed flour in male Wistar strain rats. After converting to rat weight, the doses used were 4.5 mg and 5.4 mg.

**Instrument and Procedure**

**Ingredients**

- Pumpkin seed flour, Aquades, ketamine, 70% alcohol, Sodium chloride (Sodium chloride).

**Instrument**

Instruments in this study included record sheets of blood pressure and blood pressure measurements with the Non-Invasive Blood Pressure CODA (Tail Cuff) device. Data on normal blood pressure (normotension) in Wistar rats (Rattus norvegicus) are systolic blood pressure of 129 mmHg, diastolic of 90 mmHg, and heart rate of 350-450 beats per minute.

**Procedures**

**Pumpkin Seed Flour Processing**

Pumpkin seeds that had been washed by clean water were dried in the sun for 7 hours, after that the pumpkin seeds were put in the oven at 70-75 °C for 3 hours then blended until the pumpkin seeds were smooth and sieved using a 70-mesh sieve. Pumpkin seeds were then weighed based on each dose using analytical balance with an accuracy of 0.1 gram. Pumpkin seeds in the form of flour then dissolved with water and filtered until it reaches a volume of 2 ml.

**Adaptation**

In this study, the sample was divided into 3 groups and each group consisted of 8 rats which were then divided into 2 cages where each cage was filled with 4 rats. Prior to the intervention, the
rats were kept in cage at room temperature adapted for 7 days with standard food and water with ad libitum.

**Induction**

After adaptation, blood pressure was measured to the rats. Then all rats were induced by 8% Sodium chloride, namely Sodium chloride was weighed as much as 8 grams and dissolved in 100 ml of distilled water and given orally to the rats as much as 2 ml. After 7 days of giving Sodium chloride, the rats’s blood pressure were measured again, if blood pressure was > 129/90 mmHg that means the rats were in a state of hypertension.14

**Blood Pressure Measurement**

Blood pressure measurement was carried out on the 8th day after the adaptation period to be used as first blood pressure data of rats and on that day 8% Sodium chloride was started to inducted. Then, on the 16th day after Sodium chloride induction, measurement was repeated as hypertension data. At the intervention stage, it was carried out for 28 days. After 28 days the last blood pressure measurement was taken as the last data. There is no lose during this experiment.

**Data Analysis**

The data were statistically analyzed using One Way Anova test to see the differences between all groups. All data were analyzed with SPSS V. 21.0 for windows with a significance level of p<0.05.

**Ethical Consideration**

This study has received approval from the health research ethics commission (KEPK) Faculty of Public Health, Hasanuddin University with number: 3294/UN4.14.1/TP.01.02/2022, all actions taken on the rats are in accordance with ethic on the rats namely replacement, reduction, and refinement.

### Table 1. Systolic Blood Pressure (mmHg) of rats Before and After Intervention

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>Control (C)</th>
<th>Group Dose 1 Treatment (P1)</th>
<th>Dose 2 Treatment (P2)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure after Sodium chloride Induction (mmHg)</td>
<td>8</td>
<td>229.6±22.6</td>
<td>240.0±47.6</td>
<td>252.6±32.4</td>
<td>0.450</td>
</tr>
<tr>
<td>Central Blood Pressure</td>
<td>8</td>
<td>196.6±8.9</td>
<td>126.2±10.9</td>
<td>127.2±7.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intervention for 2 weeks (mmHg)</td>
<td>8</td>
<td>190.3±6.3</td>
<td>114.7±12.5</td>
<td>119.6±9.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood Pressure after Intervention for 4 weeks (mmHg)</td>
<td>8</td>
<td>-39.2±22.3</td>
<td>-125.2±41.0</td>
<td>-133.0±33.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

![Graph 1. Systolic Blood Pressure of Control Group, Dose 1 Treatment Group and Dose 2 Treatment Group](image-url)
**RESULTS AND DISCUSSION**

Table 1 and graph 1 showed that there was a significant decrease in the average systolic blood pressure in the control group (C) after the treatment. On the other hand, there was significant decrease in the average systolic blood pressure of rats in the treatment group dose 1 (P1) and treatment dose 2 (P2) after the treatment and there was a significant difference in systolic blood pressure between group before and after the intervention (p<0.001).

Table 2 and graph 2 showed that there was a significant decrease in the average diastolic blood pressure in the control group (K) after the treatment as well as in the treatment group, dose 1 (P1) and treatment dose 2 (P2). There was a significant decrease in the average diastolic blood pressure in rats after treatment, and there was a significant difference in rats’ systolic blood pressure between groups before and after the intervention (p=0.056).

According to Kusumorini & Yuska, treatment using medicinal plants is more recommended considering that medicinal plant has relatively low side effect compared to synthetic medicine. A lot of understanding and treatments of hypertension have been made and done, but hypertension and its complications still increase uncontrollably. In addition to synthetic medicine, treating the medicinal plant is also beneficial for this disease. Jawi said that giving antioxidants is very useful, especially from plants such as anthocyanins, one of the flavonoids.

Blood pressure in the control group (C) showed a decrease, but the control group (C) was the group that had the smallest decrease among all groups. This is in line with a study conducted by Uthia & Yuliana, which showed that there are metabolic processes and diuresis in the body of the rats so that blood pressure in the body of the rats can change. Stress can cause blood pressure

<table>
<thead>
<tr>
<th>Variables</th>
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<th>Control (K)</th>
<th>Group Dose 1 Treatment (P1)</th>
<th>Dose 2 Treatment (P2)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure after Sodium chloride Induction (mmHg)</td>
<td>8</td>
<td>136.3±23.8</td>
<td>127.3±11.5</td>
<td>120.1±20.2</td>
<td>0.261</td>
</tr>
<tr>
<td>Central Blood Pressure Intervention for 2 weeks (mmHg)</td>
<td>8</td>
<td>129.0±14.7</td>
<td>95.8±5.0</td>
<td>93.3±5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood Pressure after Intervention for 4 weeks (mmHg)</td>
<td>8</td>
<td>119.2±19.7</td>
<td>91.1±3.6</td>
<td>89.6±5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Changes in Blood Pressure</td>
<td>8</td>
<td>-17.1±12.1</td>
<td>-36.2±13.2</td>
<td>-30.5±18.8</td>
<td>0.056</td>
</tr>
</tbody>
</table>

**Graph 2.** Diastolic Blood Pressure of Control Group, Dose 1 Treatment Group and Dose 2 Treatment Group
to rise temporarily. If the stress has passed, blood pressure will usually return to normal.\textsuperscript{19}

Blood pressure in the group given pumpkin seed flour at a dose of 4.5 mg (P1) and pumpkin seed flour at a dose of 5.4 (P2) showed that the average blood pressure decreased after 28 days of intervention. Where the decrease in systolic and diastolic blood pressure for the treatment group dose 1 (P1) ranged from 125.25/36.25 mmHg to 114.75/91.13 mmHg, and the decrease in blood pressure for the treatment group dose 2 (P2) ranged from 133/30, 5 mmHg to 119.63/89.63 mmHg. In this study, the blood pressure of the rats was below $129/90$ mmHg, which indicated that the blood pressure of the rats in groups P1 and P2 had returned to normal. According to Thompson in Puspitaningrum et al, a tested substance is said to have an antihypertensive effect if it can reduce systolic blood pressure $\geq 20$ mmHg.\textsuperscript{19} In this study, if there was a decrease in systolic blood pressure $\geq 20$ mmHg, it can be said that pumpkin seeds have the potential as an antihypertensive.\textsuperscript{20}

This is because pumpkin seeds contain high enough anthocyanin flavonoids so that they can reduce blood pressure in rats given high doses of Sodium chloride; it is possible through the mechanism of an increase of antioxidant that it improves Nitrous oxide (NO) bioavailability so that it can maintain stable endothelial function.\textsuperscript{21} Flavonoids are also useful to lower blood pressure by releasing substances, namely nitric oxide, and balancing some hormones in the body. The mechanism of flavonoids in lowering blood pressure is by retarding the angiotensin-converting enzyme (ACE). It is known that the angiotensin-converting enzyme (ACE) plays an important role in the formation of angiotensin II which is one of hypertension causes. Angiotensin II causes blood vessels to be narrow, which can raise blood pressure. ACE inhibitor causes blood vessels to be wider so that more blood flow to the heart, which causes a decrease in blood pressure.\textsuperscript{22}

Pumpkin seeds also have high potassium content. Potassium intake in pumpkin seeds can overcome excess sodium because potassium functions as a diuretic and retard the release of renin so that blood pressure returns to normal.\textsuperscript{23} A diuretic is a substance that increases the formation of urine. The term diuretic has two meanings; the first indicates an increase in urine volume percentage produced, and the second indicates the amount of expenditure or excretion of water-soluble substances. The main function of diuretics is to mobilize edema fluid which means changing the fluid balance so that the extracellular fluid returns to normal.\textsuperscript{13} Based on Rustina’s study the ethyl acetate extract of pumpkin seeds contains phenol hydroquinone compounds, alkaloids, and triterpenoids.\textsuperscript{24} The three phytochemical compounds have hydroxyl groups to have antioxidant effects. Phenol compounds can muffle free radicals by donating their hydrogen atoms to a free radical, and alkaloid derivatives such as indole compounds, quinolones, and melatonin can provide antioxidant effects in the body.

**CONCLUSION**

Based on the analysis and discussion that the researcher has described, it can be concluded that pumpkin seed flour (dose 4.5 mg and dose 5.4 mg) effectively reduces blood pressure in hypertensive rats. Pumpkin seeds contain flavonoids, potassium, and antioxidants, proven to lower the blood pressure of hypertensive rats. This study has the potential to be applied to human who has hypertension.

**ACKNOWLEDGEMENT**

None.

**Conflict of Interest**

There is no conflict of interest.

**Funding Sources**

There is no funding sources.

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