

Effect of Bamboo Shoot (*Gigantochloa Nigrociliata*) Extract on Decreased Blood Pressure and Tnf-A in Male Wistar Rats (*Rattus Norvegicus*)

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ABSTRACT

Background: Hypertension is a non-communicable disease based on increased pathologies such as RAS, inflammation, and oxidative stress. Bamboo shoot extract contains BSP, phenol, and flavonoids that can reduce inflammatory status, oxidative stress, and ACE. The study aims to determine the effect of bamboo shoot extract on decreased blood pressure and TNF- α in male Wistar Rats made hypertensive.

Subjects and Method: This study used a True Experimental Design with the Posttest Only Control Group Design. The study was located at the Integrated Biomedical Laboratory of the Faculty of Medicine, Sultan Agung Islamic University, Semarang in April-July 2021. The study sample of 24 male Wistar rats was randomly divided into 4 groups. The dependent variables of the study were Blood pressure and TNF- α . The independent variable was bamboo shoot extract. Blood pressure was measured by Pressure Recording Tail Cuff, and TNF- α was measured by using the ELISA method with CATALOG Number KET 9007-48 well. The data were analyzed by using the One Way Anova Test.

Results: This study showed no significant difference from systolic or diastolic blood pressure results of male Wistar rats in the K1, K2, and K3 groups after the intervention. Meanwhile, the average levels of TNF- α decreased significantly in the K3 group (Mean = 1.99; SD= 1.13) compared with the control group Ko (Mean= 16.70; SD= 7.45) and it was statistically significant $p < 0.001$.

Conclusion: There is no significant effect on blood pressure in male Wistar rats that obtained bamboo shoot extract, but there is a significant effect on TNF- α levels in male Wistar rats that obtained bamboo shoot extract.

Keywords: bamboo shoots, hypertension, blood pressure, TNF- α , male wistar rat.

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BACKGROUND

Non-communicable diseases (NCDs) are defined as diseases that are not caused by microorganisms (Budijanto, 2018). Hypertension is a non-communicable disease cha-

racterized by a persistent increase in blood pressure above 140/90 mmHg. Underlying pathologies of hypertension such as the Renin-Angiotensin System, inflammation, oxi-

ductive stress, and vascular disorders that will inflict advanced NCDs such as heart, stroke, and kidney failure (Airaodion et al. 2020), (Budijanto, 2018). Increased oxidative stress on the kidney organs and renal blood vessels that contributes to hypertension. It is proven that the afferent arteriole is the target of ROS, which increases O₂ in the afferent which decreases Nitric Oxide (NO), subsequently increases afferent arteriole vasoconstriction and reduces glomerular filtration rate (Loperena, et al. 2018). Blood pressure is also regulated through the activity of Angiotensin Converting Enzymes (ACEs) and the sympathetic nervous system which interacts with pro-inflammatory cytokines especially TNF- α (Akasha, et al. 2015; Airaodion et al. 2020).

WHO reports that 7 of the 10 leading causes of death in 2019 were NCDs, contributing to 44% of all death-causing diseases in the world (WHO, 2020). The results of Basic Health Research in 2018 showed an increase in the prevalence of NCD hypertension (Budijanto, 2018). NCD Hypertension in Central Java Province ranks first at 68.6% (Prabhakara, 2010). It is reported that hypertension was the highest risk factor for infection in 1,099 patients with COVID-19 (Kulkarni, 2020). A study has reported that the administration of bamboo shoots lower blood pressure, improves oxidant stress status and increases NO levels in serum and Nitric Oxide Synthase activity in the kidneys (Liu, et al. 2012). Another study discovers that the administration of bamboo shoot extract can reduce pro-inflammatory cytokines and leukocytes in the process of atherosclerosis (Soesanto et al. 2019).

Bamboo shoots are known as traditional medicine, and it is used for their antioxidants. Bamboo shoots can also be processed into filling for spring roll snacks, which is a specialty icon of Semarang, Central Java Province (Rohadi et al. 2020). An-

tioxidant activity in bamboo shoots plays a role in suppressing ACE synthesis by normalizing high blood pressure rate, that is blocking lipid peroxidation so as to change oxidative status. Extracts of bamboo shoots water were further extracted with ethyl acetate and n-butanol to produce Bamboo Shoots Peptide which inhibits ACE production. Phenolics and flavonoids in bamboo shoots when combined with ACE inhibitors provide a synergistic effect. Its antioxidant activity increases NO levels in the serum and increases antioxidant activity by donating hydrogen atoms from its phenolic hydroxyl group (Pamba 2015). Flavonoids also affect inflammatory status by inhibiting the production of proinflammatory cytokines (Soesanto et al. 2019).

Elevated levels of proinflammatory cytokines (TNF- α) have been associated with hypertensive patients (Moneim Abdel, 2020). The effect of increasing ROS and pro-inflammatory cytokines is oxidative stress that triggers damage to DNA, proteins, and lipids in some organs of the brain, and kidneys (Togliatto, et al. 2017). An increase in O₂- in the anterior afferent decreases NO, which in turn increases the vasoconstriction of the afferent arteriole, thereby reducing the glomerular filtration rate. ACE is involved in a blood pressure regulation mechanism that converts decapeptides in inactive Angiotensin I (Ang I) into Ang II (Liu et al. 2012). Hypertension involves an increase in the enzyme Ang-II which has an impact on the activation of NADPH oxidase and results in endothelial dysfunction in afferent arterioles. Podocyte injury is characterized by increased proteinuria then the Ang-II enzyme induces excessive ROS production in cell lines (Moneim Abdel, 2018).

Bamboo shoots extract ethyl acetate and n-butanol produce Bamboo Shoots Peptide / BSP compounds that inhibit ACE

synthesis. Natural phenolic compounds with BSP contained in food will experience inhibition of ACE, thus providing a synergistic effect as an antihypertensive. The phenol and flavonoid antioxidants in bamboo shoots provide an increase in NO production which then donates one H atom from the hydroxyl phenolic group (Awol 2015). Flavonoids in bamboo shoots also decrease the number of monocytes, where monocytes are part of the formation of foam cells. Flavonoids also act as anti-inflammatory, namely inhibiting the synthesis of pro-inflammatory cytokines (TNF- α) and reducing thrombocyte aggregation (Soesanto et al. 2019). This study looked at how the effect of bamboo shoot extract on decreased blood pressure and TNF- α in male wistar rats.

The study aims to determine the effect of the administration of bamboo shoot extract 0.8 and 1.2 mg/gram body weight on decreased blood pressure and decreasing TNF- α levels in male Wistar Rats (*Rattus norvegicus*) compared to those that did not obtain bamboo shoot extract.

SUBJECTS AND METHOD

1. Study Design

A true experimental design with The Post-test Only Control Group Design study design. The study location was in the integrated biomedical laboratory of the Faculty of Medicine, Sultan Agung Islamic University, Semarang. The study was conducted in April-July 2021

2. Population and Sample

The population used was Male Wistar Rats (*Rattus norvegicus*) using ethical clearance from the Bioethics Commission for Medical Research of Sultan Agung Islamic University Semarang. Study samples were random and those that met the inclusion criteria (male, healthy, age 2-3 months, body weight 150-200 grams). The exclusion criteria were anatomical defects and dropout (death). The

total study population was 24 male Wistar rats, so the size of samples was 6 per group.

3. Study Variables

The dependent variables of this study were blood pressure and TNF- α . Meanwhile, the independent variable of this study was bamboo shoot extract.

4. Operational definition of variables

Tumor Necrosis Factor Alpha (TNF- α) is an inflammatory cytokine synthesized or secreted by macrophages and synthesized by CD4, lymphocytes, neutrophils, NK cells, and mast cells. Inflammatory cytokine was examined by the ELISA method in units of mg/L. It used a ratio data scale.

Blood pressure is a clinical parameter in assessing blood pressure that primarily determines therapeutic. The determination of arterial blood pressure is determined by the volume released by the heart into the arteries, the elastance of the artery walls, and the rate of blood flow out of the artery. The increase in blood pressure above 140/90 mmHg. It was measured by the Volume Pressure Recording Tail Cuff method in units of mmHg. It used a ratio data scale.

Bamboo shoot extract is a young bamboo plant that is extracted to produce the necessary substances. The administration of bamboo shoot extracts with the addition of 96% ethanol solvent. It used an ordinal data scale.

5. Study Instruments

Blood pressure was measured with a Pressure Recording Tail Cuff.

TNF- α was measured by ELISA method CATALOG Number KET9007-48well with EliKine Rat TNF- α ELISA Kit 48 wells (IP) specifications.

Bamboo shoot extract was made using 96% ethanol solvent using a Soxhlet tool.

6. Data analysis

Blood pressure and TNF- α levels were analyzed using Shapiro Wilk as the normality test and Levene Test as the homogeneity

test, normally distributed and homogeneous data were tested by using One Way Anova and Post Hoc LSD.

7. Research Ethics

Ethical clearance was obtained from the Bioethics Commission for Medical Research of Sultan Agung Islamic University Semarang, Central Java, Indonesia, No. 212/VI-/2022/Bioethics Commission.

RESULTS

1. Univariate Analysis

The results of the study in table 1 show systolic blood pressure (Mean= 126.30; SD= 20.81), diastolic blood pressure (Mean= 90.46; SD= 15.96), TNF-α (pg/mL) level (Mean= 7.80; SD= 6.75).

2. Bivariate Analysis

Based on the Table of Average Systolic and Diastolic Blood Pressure, was analyzed using the Shapiro-Wilk normality test and the Levene Test of homogeneity test. Shapiro Wilk normality test and Levene Test of homogeneity test on blood pressure resulted in $p > 0.05$, which means the data on systolic and diastolic blood pressure were distributed normally and homogeneous therefore it used a parametric test One way ANOVA with a follow-up test was Post hoc test to determine the differences across groups.

Table 2 describes the average systolic

blood pressure before the lowest intervention was administered to group K2 (Mean= 98; SD= 8.00) compared with control group Ko (Mean= 144; SD= 18.04) and it was statistically significant $p < 0.001$.

Table 3 describes the average systolic blood pressure after the lowest intervention was administered to the control group Ko (Mean= 110; SD= 22.02) compared with group k3 (Mean= 139; SD= 11.38) and it was statistically insignificant $p = 0.018$ so the result was unreliable.

Table 4 describes the average diastolic blood pressure before the lowest intervention was administered to the K2 group (Mean= 98; SD= 8.00) compared with control group Ko (Mean= 129; SD= 12.75) and it was statistically significant $p = 0.003$.

Table 5 describes the average diastolic blood pressure after the lowest intervention was administered to the Ko and k3 groups (Mean = 77; SD= 16.11) compared with group K2 (Mean= 96; SD= 11.95) and it was statistically significant ($p = 0.042$).

Table 6. describes the average number of TNF-α after the lowest intervention was administered to the K3 group (Mean= 1.99; SD= 1.13) compared with control group Ko (Mean= 16.70; SD= 7.45) and it was statistically significant ($p < 0.001$).

Table.1 Sample Characteristics

Variables	Mean	SD
Systolic Blood Pressure	126.30	20.81
Diastolic Blood Pressure	90.46	15.96
TNF-α (pg/mL) level	7.80	6.75

Table 2. Average Pre-Intervention Systolic Blood Pressure

Groups	N	Mean	SD	p
Ko (Control)	6	144	18.04	<0.001
K1 (Intervention 1)	6	133	18.25	
K2 (Intervention 2)	6	98	8.00	
K3 (Intervention 3)	6	139	11.38	

Table 3. Average Post-Intervention Systolic Blood Pressure

Groups	N	Mean	SD	p
K0 (Control)	6	110	22.02	0.018
K1 (Intervention 1)	6	133	16.54	
K2 (Intervention 2)	6	134	8.00	
K3 (Intervention 3)	6	139	11.38	

Table 4. Average Pre-Intervention Diastolic Blood Pressure

Groups	N	Mean	SD	p
K0 (Control)	6	129	12.75	0.003
K1 (Intervention 1)	6	100	14.21	
K2 (Intervention 2)	6	98	8.00	
K3 (Intervention 3)	6	105	18.31	

Table 5. Average Post-Intervention Diastolic Blood Pressure

Groups	N	Mean	SD	p
K0 (Control)	6	77	16.11	0.042
K1 (Intervention 1)	6	95	13.21	
K2 (Intervention 2)	6	96	11.95	
K3 (Intervention 3)	6	77	16.11	

Table 6. Average Post-Intervention TNF-α levels

Groups	N	Mean	SD	p
K0 (Control)	6	16.70	7.45	<0.001
K1 (Intervention 1)	6	8.65	1.65	
K2 (Intervention 2)	6	4.50	1.39	
K3 (Intervention 3)	6	1.99	1.13	

DISCUSSION

There was no significant effect of blood pressure measurement results on samples from both the control group and the test groups that had been treated with bamboo shoot extract. The rats' normal blood pressure was 129 (systolic) / 90 (diastolic) mmHg. Blood pressure in Wistar rats can be influenced by several factors such as age, sex, race, and heredity (genetics). Sexual dimorphism in blood pressure is commonly found in all types of mammals, i.e. males have higher blood pressure and are more likely to increase than females (Nugroho et al., 2018). In Tables 3 and 5 it can be said that the induced samples already had hypertension. After the intervention process with the administration of bamboo shoot extract with a concentration of 40, 80, and 160 mg, it obtained results that were not significant eno-

ugh. The criteria of substance can be said to have an antihypertensive effect if it can reduce systole pressure ≥ 20 mmHg (Fidrianny et al., 2009).

These results were different from a study conducted by (Makatita et al., 2020) that showed that bamboo shoot extract had a positive effect on decreased blood pressure and cholesterol. The results of this study could also be affected by the effect of ethanol concentration that affects flavonoid levels. It was in line with a study by (Riwanti et al., 2019) on the use of 50%, 70%, and 96% ethanol solvents, that the highest total flavonoid level was in the ethanol solvent 70% at 0.1300% w/w. It is influenced by the polarity of the solvent which can be associated with the highest flavonoid level in the medium solvent of ethanol 70%. The polarity of ethanol increases as its concentration in

water decreases. Ethanol has an OH group (carboxyl group) which can form a hydrogen bond with OH (carboxyl group) of flavonoid compounds so as to cause an increase in the solubility of flavonoid compounds in ethanol.

The duration of a study in the administration of bamboo shoot extract doses in a study (Sunarti, 2020) regarding the anti-hypertensive effects of Bambu Tali's sprout ethanol extract for male white rats Sprague Dawley extracted by using maceration method with 70% ethanol for 21 days was proven to lower blood pressure for male white rats Sparague Dawley. The duration of this study in the administration of bamboo shoot extract doses was 15 days.

There was a significant effect on TNF- α levels from the results of blood pressure measurements of samples from both the control group and test groups that had been treated with bamboo shoots extract. It is supported by (Soesanto et al., 2019), that bamboo shoots are believed to contain flavonoids that inhibit the inflammatory status of TNF- α , IL-1 β , IL-6, and IL-17 and reduce thrombocyte aggregation. Flavonoids also reduce the number of monocytes in the blood so that the formation of foam cells also decreases which is characterized by a decrease in Vascular Cell Adhesion Molecule-1 (VCAM-1) and Monocyte Chemoattractant Protein-1 (MCP-1). The phenol and flavonoid antioxidants in bamboo shoots provide an increase in NO production which then donates one H atom from the hydroxyl phenolic group (Awol, 2015). Flavonoids in bamboo shoots also decrease the number of monocytes, as monocytes are part of the forming of foam cells. Flavonoids also act as anti-inflammatory, namely inhibiting the synthesis of pro-inflammatory cytokines (TNF- α) and reducing thrombocyte aggregation (Soesanto et al., 2019).

For the next study, it is expected to add the dose of bamboo shoot extract and the duration of bamboo shoot extract administration of more than 15 days. Also, the making of bamboo shoot extract by using the maceration method with ethanol solvent concentration of 70% to lower systolic and diastolic blood pressure.

AUTHOR CONTRIBUTION

Surati: The main researcher as the chief researcher was responsible for the study and improvement of the journal

Lilik Setyowatiningsih: Research fellow 1 assisted with research thinking and material assistance

Qurrotu A'yuni Auliya: Research fellow 2 assisted in the implementation of the study and material assistance

SY Didik Widiyanto: Research fellow 3 contributed to material assistance

EM Sutrisna: Research fellow 4 contributed to material assistance

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CONFLICT OF INTEREST

There is no conflict of Interest in this study.

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