

Effect of *Averrhoa bilimbi* Fruit Extract on Blood Pressure and Mean Arterial Pressure of NaCl Induced Hypertensive Rats

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ABSTRACT

Objective: *Averrhoa bilimbi* fruit contains potassium and flavonoid expected to play a role in lowering blood pressure. This study aim to examine the effect of *Averrhoa bilimbi* fruit extract on blood pressure and mean arterial pressure in NaCl-induced hypertensive rats.

Materials and methods: This research employs pre- and post-test randomized control group design and uses 15 rats which are divided into 3 groups. On day 6, all groups are pretreated with NaCl 8% solution to induce hypertension for 14 days. On day 20, the control, furosemide and bilimbi groups are respectively treated with distilled water, 0.72 mg/200 g BW furosemide, and 150 mg/200 g BW *Averrhoa bilimbi* fruit extract for 7 days. The (systolic and diastolic) blood pressure and mean arterial pressure of each group are evaluated on day 20

and 27. The results are analyzed with Repeated Anova. **Results:** The mean systolic and diastolic blood pressure of the control, furosemide and bilimbi group before treatment are (128.4±11.8/88.0±7.8 mmHg); (142.8±13.6/85.4±16.7 mmHg); and (144.8±15.1/98.0±14.9 mmHg) respectively. The mean systolic and diastolic blood pressure of the control, furosemide and bilimbi group after being treated are (88.0±11.8/60.8±7.8 mmHg); (98.0±13.6/71.0±16.7 mmHg); and (85.4±15.1/65.0±14.9 mmHg) respectively. The mean arterial pressure of the control, furosemide and bilimbi groups are (69.4±8.2 mmHg); (79.6±14.8 mmHg); (71.4±15.0 mmHg) respectively. There is a statistically significant difference in the blood pressure and the mean arterial pressure among the groups (p=0.000). *Averrhoa bilimbi* fruit extract has an effect on the blood pressure and the mean arterial pressure of NaCl-induced hypertensive rats.

Keywords: *Averrhoa bilimbi* fruit extract, blood pressure, mean arterial pressure.

Introduction

High blood pressure is an elevation of arterial systolic or diastolic pressure which may be affected by an increase of cardiac output or total peripheral resistance or both.¹ High blood pressure, or commonly known as hypertension, is a modifiable risk factor of cardiovascular disease. Many therapies can be used to lower blood pressure studied, but hypertension incident rate remains high.² People, especially in elderly, tend to change to herbal medicine to help control their chronic disorder³ such as blood pressure. *Averrhoa bilimbi* fruit contains potassium and flavonoid expected to lower blood pressure⁴⁻⁶, but there are only few researches of the antihypertensive effect of this fruit.

Hypertension is also a risk factor of more serious cardiovascular diseases, like coronary artery disease, congestive cardiac failure, ischemic and hemorrhagic stroke, and

kidney failure.⁷ Hypertension incident rate in Indonesia is relatively high, in which Riskesdas data show that 25.8% of >18 years old people have hypertension.⁸ Hypertension incidents in Indonesia occur more in women with a percentage of 52.3%. Based on 2010 census data, 33 million >40 years old Indonesians have hypertension. 12 million out of the 33 million population are aware of their hypertension condition, and 8.5 million people have therapy.⁹ The increasing number of hypertension patients requires serious handling. Antihypertensive medicine has commonly been used, but people tend to switch to herbal medicine that it is seemed to have minimum side effect. One of the efforts is to consume *Averrhoa bilimbi* fruit.

The research conducted by Bipat (2008) finds that consuming *Averrhoa bilimbi* leaf extract may lower cardiac output, and thus, may lower blood pressure.¹⁰ Another research also shows that *Averrhoa bilimbi* fruit juice is able to lower laboratory rodent's systolic blood pressure at dose 2ml/200gBW.¹¹ *Averrhoa bilimbi* fruit and its juice has a very sour flavor that is unpleasant for some people ¹², therefore *Averrhoa bilimbi* extract is an alternative to gather the benefit of *Averrhoa bilimbi* without leaving sourness in palate.

Averrhoa bilimbi fruit contains potassium, calcium, vitamin B1 (thiamine), vitamin A, and ascorbic acid ions.⁵ *Averrhoa bilimbi* fruit extract also has antioxidant effect serving to capture free radicals and contains tannins, saponins, phenols, and flavonoids.¹³ Potassium is expected to lower blood pressure with Na⁺K⁺ATPase pumping stimulation in non-striated muscles of blood vessel, which causes vasodilation¹⁴, while flavonoid is expected to stimulate nitric oxide release, which also cause vasodilation.¹⁵ The other potassium's blood pressure lowering mechanism is to increase sodium excretion related to diuretic effect to lower cardiac output.^{16,17} An increase of blood volume and cardiac output may be caused by NaCl¹⁸ induction which causes an increase of blood sodium level.^{18,19} Therefore, the effect of *Averrhoa bilimbi* fruit extract on lowering blood pressure in male albino Wistar strain rat.

Materials and methods

Blood Pressure Measurement

The systolic and diastolic blood pressure measurement (mmHg), and the mean arterial pressure (mmHg) of Wistar rat recorded in the measuring instrument monitor, after NaCl 8% solution induction, and after treatment given in the morning of days 20 and 27 of the research using non-invasive blood pressure measuring instrument of tail cuff method of Kent Scientific brand.

Hypertension Induction

Rats were induced with NaCl 8% solution daily for 14 days.

Preparation of *Averrhoa bilimbi* extract

The *Averrhoa bilimbi* fruit extract is *Averrhoa bilimbi* fruit slices which are dried and made into extract with maceration technique using ethanol 70% solvent and given at dose 150 mg/200g BW mixed with ad 2 ml distilled water per oral once daily in afternoon using feeding tube to the laboratory rodents for 7 days.

Study design and animals

This experimental research employs a pre- and post-test randomized control group design. The 3 months old male Wistar strain rats with ± 250 g body weight kept at the Laboratory of Biology, Faculty of Medicine, Sultan Agung Islamic University, Semarang. 15 rats are taken, which are then randomly divided into 3 groups of treatment. After adaptation, the three groups are fed with standard feed and induced with 3 ml NaCl 8% solution daily for

14 days. The three groups are measured for systolic and diastolic blood pressure, and mean arterial pressure after NaCl 8% solution induction on day 20. The control group is given with standard feed and 2 ml distilled water without any treatment, the furosemide group is given with standard feed and furosemide treatment at dose 0.72 mg/200 g BW and the *Averrhoa bilimbi* group is given with standard feed and *Averrhoa bilimbi* fruit extract at dose 150 mg/200 g BW for 7 days. After treatment, on day 27, the systolic and diastolic blood pressure and mean arterial pressure after treatment are measured on day 27. The rats are terminated using chloroform inhalation at the end of research. The rats' blood pressure is measured at the Laboratory of Physiology, Faculty of Medicine, Sultan Agung Islamic University, Semarang.

Statistical analysis

The results are analyzed using IBM SPSS Statistics 24 program for Windows. The data analyzed are in the form of mean systolic and diastolic blood pressure and mean arterial pressure of the control group, furosemide group and *Averrhoa bilimbi* group. The data are descriptively tested for mean value and standard deviation. The mean three groups' data of systolic and diastolic blood pressure and mean arterial pressure pre- and post- treatment are tested for normality using Shapiro-Wilk test and for homogeneity using data variance test (Levene test), resulting in all normally distributed data and homogenous variance of all data. The parametric requirements are satisfied, thus it employs a Repeated Anova test, followed with a Post Hoc Bonferroni test. This study was performed after being approved by Health/Medical Research Bioethic Commission, Medical Faculty of UNISSULA (No. 367/XI/2017/Bioethic Commission).

Results

Figure 1, Figure 2, and Figure 3 show the results of mean systolic and diastolic blood pressure and mean arterial pressure. All of the three groups' data are normally distributed and homogenous ($p > 0.05$). The Repeated Anova test with Greenhouse-Geisser correction results in significant difference in systolic ($F(1.000; 14.000) = 63.424; p = 0.000$), diastolic ($F(1.000; 14.000) = 20.371; p = 0.000$) blood pressure and mean arterial pressure ($F(1.000; 14.000) = 33,144; p = 0.000$). The mean systolic blood pressure value after NaCl 8% solution induction and after treatment declines by 48.20 mmHg ($p = 0.000$). The mean diastolic blood pressure value after NaCl 8% solution induction and after treatment declines by 32.40 mmHg ($p = 0.000$). The mean arterial pressure value after NaCl 8% solution induction and after treatment declines by 37.67 mmHg ($p = 0.000$).

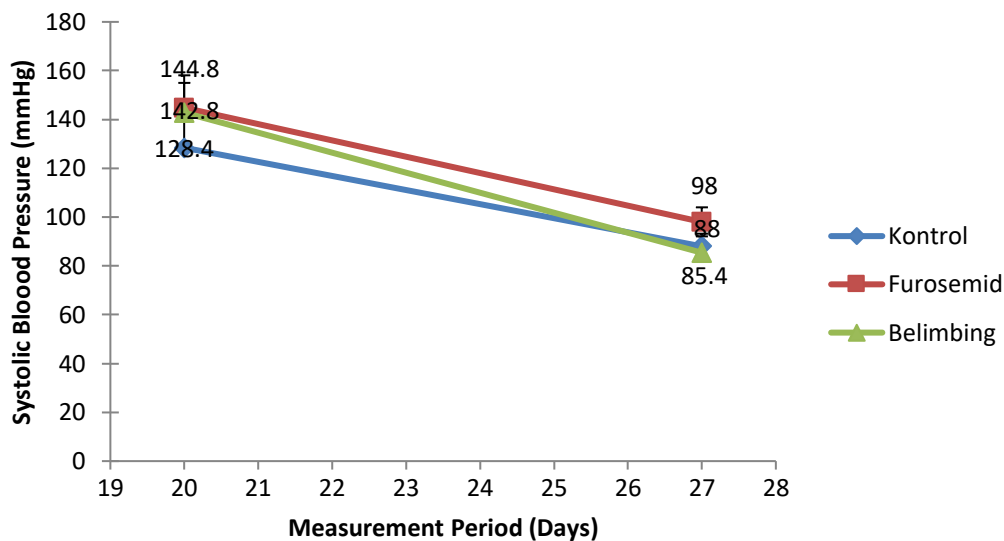


Figure 1. Result of systolic blood pressure after NaCl 8% solution induction (day 20) and after treatment (day 27).

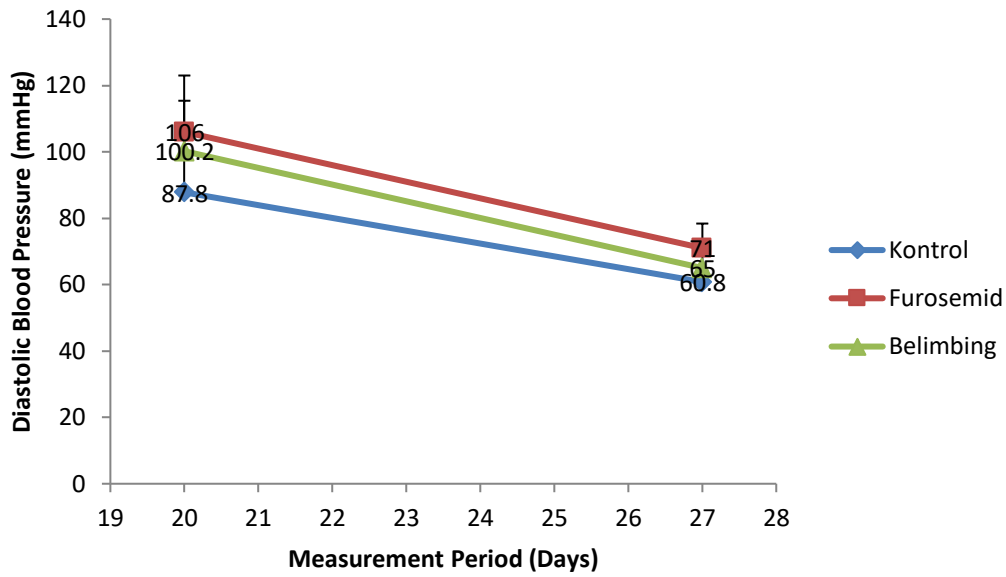


Figure 2. Results of diastolic blood pressure after NaCl 8% solution induction (day 20) and after treatment (day 27).

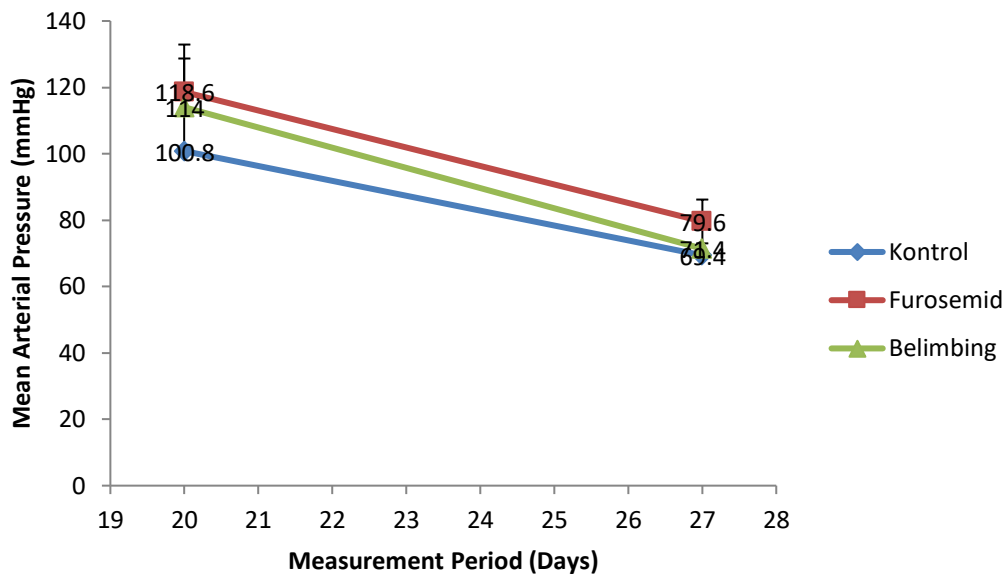


Figure 3. Result of mean arterial pressure (MAP) after NaCl 8% solution induction (day 20) and after treatment (day 27).

Discussion

The elevation of mean systolic and diastolic blood pressure after NaCl 8% solution induction in this research conforms to the research conducted by Riyadi (2016) that using NaCl 8% solution for 14 days may elevate rat's systolic and diastolic blood pressure.²⁰ Blood pressure elevation in furosemide and *Averrhoa bilimbi* groups may be stated as hypertension since it satisfies hypertension criteria, which are systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg.^{21,22} The systolic and diastolic blood pressure elevation with NaCl 8% solution induction in this research is also expectedly caused by an increase of sodium level in plasma and cerebrospinal fluid which stimulates sympathetic nerve in heart and blood vessel, which may increase peripheral resistance through vasoconstriction and increase heart contractility, and thus increases stroke volume, cardiac output and blood pressure.^{1,18,19,23} The other mechanism of blood pressure elevation in this research is expectedly through an increase of circulating blood volume because of an increase of blood sodium level which causes an increase of water reabsorption in renal tubule.^{1,18,24}

The control group that receives distilled water has their blood pressure declined, although the blood pressure decline with distilled water intake is not as high as that of furosemide and *Averrhoa bilimbi* fruit extract intake. This does not conform to the research conducted by Cunha *et al.* (2016) that also uses distilled water as a negative control in the research, but the distilled water does not show any blood pressure decline.²⁵ Physiologically, body will perform compensation mechanism by stimulating baroreceptor reflex in aortic arch and carotid sinus through sympathetic nervous inhibition to lower heart rate and reduce peripheral resistance.^{1,18,26} This baroreceptor reflex stimulation mechanism is also expected to lower the rats' systolic and diastolic blood pressure of control group with distilled water intake.

The rats of the furosemide group with furosemide intake at dose 0.72 mg/200 g BW have their mean blood pressure declined more than that of the control group. This result conforms to the research conducted by Mojiminiyi *et al.*, (2012) which shows that furosemide may lower rat's blood pressure induced with NaCl 8%.²⁷ The rats' systolic and diastolic blood pressure decline of the furosemide group occurs through a compensation mechanism with baroreceptor reflex stimulation at carotid sinus and aortic arch.^{1,18,26} The furosemide group's other mechanism underlying the blood pressure decline is with inhibition of transporters Na, K and Cl in ascending loop of Henle of kidney which inhibits water reabsorption process, thus a natriuresis process occurs and reduces cardiac output.^{28,29} Furosemide also stimulates prostaglandin E₂ (PGE₂) production, which causes vasodilation and reduces peripheral resistance.²⁸

The *Averrhoa bilimbi* group has the highest difference in the mean lowering of systolic and diastolic blood pressure among the three groups of the research. This result conforms to the research conducted by Safitri & Candra (2015) that also shows that *Averrhoa bilimbi* fruit juice may lower rat's systolic blood pressure induced with high fructose and high fat feed.¹¹ The rats' systolic and diastolic blood pressure decline of the *Averrhoa bilimbi* group occurs through baroreceptor reflex stimulation at carotid sinus and aortic arch.^{1,18,26} The other mechanism playing a role in lowering the blood pressure of the *Averrhoa bilimbi* group is expected to be mediated by potassium ions which inhibits Na⁺K⁺ATPase pumping and flavonoid content which stimulates nitric oxide (NO) production. Na⁺K⁺ATPase pumping inhibition and NO production cause relaxation of vascular endothelium, causing vasodilation and decreasing peripheral resistance.^{6,14,15,17} Potassium ion in *Averrhoa bilimbi* fruit extract expectedly inhibits water and sodium reabsorption process at renal proximal tubule. Water

and sodium reabsorption inhibition at renal proximal tubule may increase diuresis process and thus lowers blood pressure through reduction of intravascular fluid volume.^{16,17}

Averrhoa bilimbi fruit extract also contains a large number of ascorbic acid or vitamin C.⁵ The vitamin C content in *Averrhoa bilimbi* fruit extract expectedly serves to lower rat's blood pressure through antioxidant effect by reducing oxidative stress and help with correction of vascular endothelial function through nitric oxide (NO) production which causes vascular relaxation and vasodilation and reduces peripheral resistance.^{30,31} These blood pressure lowering mechanisms by *Averrhoa bilimbi* fruit extract expectedly cause higher difference in mean systolic and diastolic blood pressure decline from distilled water and furosemide at dose 0.72 mg/200 g BW intakes.

This research does not measure urine volume, urea creatinine level, glomerular filtration rate (GFR), PGE2 level, blood and urine sodium level, thus it needs further research with longer induction period; systolic and diastolic blood pressure periodic measurement; PGE2 level measurement; rat's urine volume, urea creatinine level, GFR, and blood and urine sodium level measurement after *Averrhoa bilimbi* fruit extract administration.

Conclusion

Averrhoa bilimbi fruit extract lowers blood pressure and mean arterial pressure of male albino Wistar strain rat induced with NaCl 8% solution.

Ethical Approval

This research proposal was accepted by the Health/Medical Research Bioethic Commission, Medical Faculty of UNISSULA (No. 367/XI/2017/Bioethic Commission).

Conflict of Interest

None declared

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Author's Contribution

Idea owner of this study: Rafida M

Study design: Tyagita N

Data gathering: Rafida M

Writing and submitting manuscript: Rafida M, Safitri AH, Tyagita N

Editing and approval of final draft: Safitri AH

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