

# Antihypertension Activity of *Averrhoa bilimbi* Fruit Juice on Sodium Chloride and Prednisone-Induced Rats

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**ABSTRACT:** Hypertension is a condition where systolic and diastolic blood pressure continuously increases, causing heart disease, kidney disease, hardening of the arteries, eye damage, and stroke (brain damage). Bilimbi (*Averrhoa bilimbi* L.) is traditionally used to lowering high blood pressure. The research objective was evaluating the activity of *A. bilimbi* as an antihypertensive. The research was conducted in vivo using rats and the CODA blood pressure analysis instrument. The doses used for *A. bilimbi* juice were 150, 300, and 600 mg/kg BW, respectively. *A. bilimbi* juice at a dose of 600 mg/kg BW/day for 14 days can reduce systolic and diastolic blood pressure in male Wistar rats induced by 2.5% NaCl and 1.5 mg/kg BW prednisone. The results of the study showed that *A. bilimbi* juice at a dose of 600 mg/kg BW had antihypertensive activity.

**Keywords:** antihypertensive; bilimbi; NaCl; prednisone



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## 1. Introduction

Hypertension can be defined as a persistent increase in blood pressure. The prevalence of hypertension continues to increase every year and contributes to the emergence of various types of diseases such as cardiovascular, stroke, and kidney disease [1-3].

Treatment of hypertensive patients can be used in two ways, non-pharmacology and pharmacology. Non-pharmacology treatment of hypertension can be done by improving daily lifestyles such as losing excess weight, doing regular exercise, and reducing foods that contain high levels of fat and salt [4]. Pharmacological treatment of hypertensive patients is carried out by administering synthetic antihypertensive drugs [5,6]. However, long-term use of antihypertensive drugs often causes unwanted side effects [7]. It is necessary to consider alternative therapies for treating hypertension using natural products [8]. One of the natural products that have the potential to be used for treating hypertension is bilimbi [9].

Bilimbi (*Averrhoa bilimbi* L.) belongs to the *Oxalidaceae* family. This fruit is commonly found in tropical and subtropical regions such as Indonesia, Malaysia, Thailand, Vietnam, Bangladesh, Sri Lanka, and India. In Indonesia, bilimbi was known as "Belimbing Wuluh". Traditionally bilimbi has been used to treat several diseases [9,10]. Research related to the use of water stew from bilimbi to reduce hypertension has been reported before [11]. Therefore, this study contributes the exact dose of antihypertensive activity of bilimbi fruit juice on sodium chloride and prednisone-induced rats.

## 2. Materials and methods

### 2.1. Materials

*A. Bilimbi* fruit was obtained from the plantation in Bandung. Sodium chloride (NaCl), aquadest, and sodium carboxymethyl cellulose (NaCMC) were obtained from Sakura Medical Ban-

dung (all chemicals are pharmaceutical grade), and medicines such as prednisone and captopril were obtained from an Islamic hospital in the city of Bandung, Indonesia.

### 2.2. Plant determination

Determination of *A. bilimbi* fruit was carried out at the Center for Plant Conservation at the Bogor Botanical Gardens of the Indonesian Institute of Sciences, Indonesia.

### 2.3. Sample preparation

The picked *A. bilimbi* fruit was washed clean and dried on the surface by wiping it with a tissue and weighed. After weighing, the fruit was cutted into small pieces and put in a blender. The obtained juice was stored in a brown bottle at a controlled temperature.

### 2.4. Phytochemical screening

Phytochemical screening was carried out to detect compounds in the juice such as alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids. The screening method was carried out using chemical reagents, then observing the color changes that occur, sediment and foam appear.

### 2.5. Experimental animals

The experimental animals used were healthy Wistar strain male rats aged 3 months weighing 200-210 grams. Research procedures on experimental animals have been accepted by the Ethical Committee of Padjajaran University (No. 196/UN6.KEP/EC/2018).

#### 2.5.1. Antihypertension activity test in rats induced by NaCl and prednisone

A total of 30 rats were adapted for 7 days. The mice were given food and drink *ad libitum* during the adaptation phase. Rats were randomly divided into five groups (n=5), which are described in Table 1. This study used a combination of NaCl and prednisone to induce hypertension in rats. The NaCl used in this study had a concentration of 2.5% and the prednisone dose used was

**Table 1.** Grouping of animals for antihypertension activity test (CODA non-invasive)

No	Test group	Description
1	Negative control	Receiving 0.5% Na-CMC.
2	Positive control	Receiving 2.5% NaCl and prednisone 1.5 mg/kg BW
3	Standard group	Receiving captopril at a dose of 2.5 mg/kg BW
4	ABJG-1 group	Receiving bilimbi juice at a dose of 150 mg/kg BW
5	ABJG-2 group	Receiving bilimbi juice at a dose of 300 mg/kg BW
6	ABJG-3 group	Receiving bilimbi juice at a dose of 600 mg/kg BW

ABJG: *A. bilimbi* juice group

**Table 2.** Phytochemical screening results of *A. bilimbi* juice

Secondary metabolites	Result
Alkaloids	-
Flavonoids	+
Saponins	-
Tannins	+
Quinone	+
Steroids/triterpenoids	+

Description:

(+) : Detected

(-) : Not detected

1.5 mg/kg [12]. Induction with NaCl and prednisone was carried out for 14 days. After the induction phase is complete, it was continued with the therapy phase for 14 days. *A. bilimbi* juice was given orally to all groups with doses 150 mg/kg BW, 300 mg/kg BW, and 600 mg/kg BW, respectively. Blood pressure measurements for all test groups were carried out at  $T_0$  (before induction),  $T_{15}$  (after induction), and  $T_{30}$  (after therapy).

### 2.5.2. Blood pressure measurement

The instrument used in measuring rat blood pressure was Kent Scientific's CODA non-invasive blood pressure. This tool simultaneously recorded systolic and diastolic blood pressure through a transducer located in the tail-cuff [13].

### 2.5.3. Statistic analysis

Results are expressed in mean  $\pm$  standard error mean (SEM). The statistical method used was One-Way ANOVA followed by a Bonferroni Post hoc test (using SPSS version 25). Results were considered significant when  $p < 0.05$ .

## 3. Results and discussions

### 3.1. Phytochemical screening results

The results of the phytochemical screening showed that *A. bilimbi* contains flavonoids, quinones, tannins, and steroids/ triterpenoids (Table 2). The chemical content detected in *A. bilimbi* might be responsible for antihypertensive activity [14].

### 3.2. Hypertension activity test in rats induced by NaCl and prednisone

In this study, the animals used were healthy Wistar rats aged 3 months weighing 200-210 grams, which were then carried out in the adaptation stage. The adaptation stage was carried out for 7 days. After acclimatization for 7 days, the blood pressure of the mice was measured (known as  $T_0$ ). The results showed that the rats had normal systolic and diastolic blood pressure values, namely systolic  $<120$  mmHg and diastolic  $<80$  mmHg at  $T_0$ .

After obtaining data on systolic and diastolic blood pressure in rats, enter the induction stage. The induction stage was carried out for 14 days. The induction stage was carried out by administering a combination of 2.5% NaCl and 1.5 mg/kg body weight prednisone. The results showed that at  $T_{15}$  the systolic blood pressure was more than 150 mmHg and the diastolic blood pressure was more than 100 mmHg. This increase were in systole and diastolic occurred in all groups, except negative control. This explains that giving a combination of 2.5% NaCl and 1.5 mg/kg BW prednisone orally for 14 days can cause an increase in blood pressure of the experimental animals. NaCl has a mechanism for causing hypertension by increasing plasma volume and cardiac output [12]. Meanwhile, the mechanism by which prednisone causes hypertension may affect the balance of water, salt, and electrolytes [15,16]. The standard drug used in this study was captopril. Captopril is a drug from the Angiotensin Converting Enzyme Inhibitor (ACEI) class which is often used in hypertensive patients. ACEI works

by inhibiting the conversion of angiotensin-I to angiotensin-II. During the induction phase, the blood pressure of the rats in the captopril group, namely for systole and diastole respectively, was 158.67/74.5 mmHg. However, when they entered the treatment phase, the blood pressure experienced a significant decrease. The results showed that captopril efficiently reduced rat's systolic and diastolic blood pressure during the treatment phase (systolic:  $99.17 \pm 6.77$ ; diastolic:  $74.5 \pm 6.16$ ) mmHg.

The final stage was the treatment stage. At this stage, rats with induced hypertension were given *A. bilimbi* juice for 14 days. The effect of giving *A. bilimbi* juice with variations in the dose can be seen in Tables 3 and 4. Tables 3 and 4 show the effects of ABJG on rat blood pressure induced by NaCl and prednisone. As stated above, in this study the doses used were 150 mg/kg BW, 300 mg/kg BW, and 600 mg/kg BW. The results showed that a dose of 600 mg/kg BW effectively reduces systolic and diastolic blood pressure when compared with the other two doses.

**Table 3.** Results of average systolic blood pressure (mmHg)

Group	T <sub>0</sub>	T <sub>15</sub>	T <sub>30</sub>
Negative control	108 ± 8.79	105.83 ± 10.21	102.83 ± 7.88 <sup>#</sup>
Positive control	101 ± 7.67	162.5 ± 14.8 <sup>*</sup>	144.83 ± 18.96 <sup>&amp;</sup>
Captopril 2.5 mg/kg BW	101.67 ± 10.5	158.67 ± 16.43 <sup>*</sup>	99.17 ± 6.77 <sup>#</sup>
ABJG 150 mg/kg BW	98.5 ± 11.95	167.5 ± 9.48 <sup>*</sup>	112.17 ± 7.22 <sup>#</sup>
ABJG 300 mg/kg BW	100.17 ± 5.19	155.17 ± 17.77 <sup>*</sup>	101.5 ± 3.62 <sup>#</sup>
ABJG 600 mg/kg BW	107.17 ± 10.63	161 ± 15.53 <sup>*</sup>	100.83 ± 5.85 <sup>#</sup>

**Table 4.** Results of average diastolic blood pressure (mmHg)

Group	T <sub>0</sub>	T <sub>15</sub>	T <sub>30</sub>
Negative control	78 ± 4.38	77.5 ± 4.51	72.17 ± 4.26 <sup>#</sup>
Positive control	76 ± 7.82	119.33 ± 24.67 <sup>*</sup>	105.83 ± 14.72 <sup>&amp;</sup>
Captopril 2.5 mg/kg BW	70.17 ± 11.02	112.83 ± 21.46 <sup>*</sup>	74.5 ± 6.16 <sup>#</sup>
ABJG 150 mg/kg BW	73.83 ± 7.88	112.5 ± 16.6 <sup>*</sup>	78.33 ± 2.42 <sup>#</sup>
ABJG 300 mg/kg BW	73.33 ± 2.8	114.83 ± 13.41 <sup>*</sup>	75 ± 3.52 <sup>#</sup>
ABJG 600 mg/kg BW	76.17 ± 6.94	121 ± 25.18 <sup>*</sup>	74.67 ± 4.72 <sup>#</sup>

Description :

\* : significantly different from the negative control

# : significantly different from the positive control

& : significantly different from the standard group

#### 4. Conclusion

The research results found that *A. bilimbi* juice had activity as an antihypertensive in rats induced with NaCl and prednisone. The optimum dose of *A. bilimbi* fruit juice that effectively lowers blood pressure (both systolic and diastolic) is at a dose of 600 mg/kg body weight when compared to doses of 150 mg/kg body weight and 300 mg/kg body weight.

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