

ANTIHYPERTENSIVE ACTIVITY ETHANOLIC EXTRACT OF BULB *ELEUTHERINE AMERICANA* MERR ON FRUCTOSE-INDUCED HYPERTENSION RATS

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ABSTRACT

Objective: This study aimed to evaluate ethanolic extract of bulb of *Eleutherine americana* Merr as antihypertensive.

Methods: This study examined the effect of ethanol extract of bulb of *Eleutherine americana* Merr on urine volume, blood pressure, and electrocardiogram (ECG) profile. Diuretic activity was evaluated by the Lipschitz method. Furosemide (3.6 mg/kg) used as standard drug. Animal rats model of hypertension were induced by fructose 66% in the diet for 21 d. Captopril (2.5 mg/kg) used as standard antihypertension. Parameters measured were urine volume for 24 h, systolic and diastolic blood pressure, and electrocardiogram profile.

Results: High fructose diet for 21 d reduced R-R, P-R interval of the electrocardiogram, increased systolic and diastolic blood pressure and heart rate compared to normal group statistically significant ($p < 0.05$). Animals received an ethanolic extract of the bulb of *Eleutherine americana* Merr doses of 25, 50, 100 mg/kg showed lowered systolic and lowered diastolic blood pressure (except for a dose of 100 mg/kg). Dose of 25, 50 mg/kg lowered systolic blood pressure was comparable to captopril 2.5 mg/kg ($p > 0.05$). The group received a dose of 100 mg/kg showed diuretic activity comparable to furosemide 3.6 mg/kg ($p > 0.05$). The group received a dose of 25, 50, 100 mg/kg, showed improved on R-R, P-R interval of the electrocardiogram, and heart rate.

Conclusion: It can be concluded that ethanolic extract of bulb *Eleutherine americana* Merr play important role in regulating blood pressure, increased urine volume and improved electrocardiogram profile.

Keywords: *Eleutherine americana* Merr, Blood pressure, Diuretic, Fructose, Electrocardiogram profile

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INTRODUCTION

Hypertension is an elevation of systolic blood pressure (SBP) to 140 mm Hg or more, and/or an elevation of diastolic blood pressure (DBP) to 90 mm Hg or more. Hypertension classified into different types of hypertension, such as systolic-diastolic hypertension, isolated systolic hypertension or isolated diastolic hypertension [1]. Isolated systolic hypertension (ISH) is an increase in systolic blood pressure (SBP) without an increase in diastolic blood pressure (DBP). It is more common in older adults than systolic-diastolic hypertension (SDH), but both contribute similarly to cardiovascular events [2].

Blood pressure determined by cardiac output and vascular resistance. An increase in cardiac output and/or vascular resistance may result in hypertension. Chronic hypertension can cause changes in the physiology of the heart. This can be measured using electrocardiogram.

Hypertension is a common disease and a major risk factor for stroke, myocardial infarction, vascular disease, and chronic kidney disease [3]. Modern lifestyle might be one of the prime causes of hypertension. High fructose diet in our lifestyle may induce hypertension [4]. Animal and human study has shown that a high fructose diet increased blood pressure [5-7]. As we are aware that hypertension is a silent killer if left untreated may result in a cardiovascular and cerebrovascular complication. In the general population, a tight control of blood pressure can prevent the cardiovascular complications of hypertension. Antihypertension agents are one of the choices for control of high blood pressure. The drugs used in hypertension have a variety of mechanisms of action including diuresis, vasodilation, and antagonism of the renin-angiotensin-aldosterone system. Antihypertension agents should be carefully provided to prevent the evidence of adverse events, because adverse events may induce non-adherence to medication.

Herbal medicine empirically used by Indonesian people to cure many diseases including high blood pressure. One of promising herbal medicine as antihypertension is the bulb of *Eleutherine americana* Merr. The bulb of *Eleutherine americana* Merr which belongs to the family Iridaceae, a plant originated from West Kalimantan, is known as a Dayak onion. This plant has been used by the Dayak community for generations as a remedy for some types of diseases including breast cancer, gastrointestinal cancer, diabetes, cholesterol, and high blood pressure [8].

Dayak onion contained naphthoquinone compounds and its derivatives such as elecanacine, eleutherine, eleutherol, eleuthermone. Naphthoquinones compounds are known as antimicrobial, antifungal, and antiparasitic. Moreover, naphthoquinones showed antioxidant activity, that protecting the body against free radicals that trigger many diseases, including cardiovascular diseases [7, 8].

This study aimed to determine the role of the ethanolic extract of bulb *Eleutherine americana* Merr in regulating blood pressure as well as its influence on the electrocardiogram profile in fructose-induced hypertensive rats.

MATERIALS AND METHODS

Plants materials

This study was conducted in the Pharmacology Laboratory, Bandung School of Pharmacy, Indonesia. Bulb of *Eleutherine americana* Merr (EA) was collected from Manoko, Lembang, Bandung, West Java, Indonesia and botanically identified at Biology Faculty, Padjadjaran University, Indonesia. The bulb were cut into small pieces, dried, and extracted with ethanol 96% (analytical grade, purchased from Brataco, Indonesia) for 3 d. The extracts were filtered and concentrated by rotary evaporator. Phytochemical analysis was carried out on the dry extract. Doses of *Eleutherine americana* Merr bulb extracts were 25, 50, 100 mg/kg.

Animals

Male Wistar rats of 2 mo age and weighed about 200-250 gram were used in this study. Before treatment, animals were adapted to a cage room temperature (25 °C) and were given access to food and drink for a week. This study was conducted in the Laboratory of Pharmacology, Bandung School of Pharmacy, Bandung, West Java, Indonesia. All experiment procedure was approved by the local animal ethics committee (no. 315/UN6. C1.3.2/KEPK/PN/2016).

Diuretic study

Eighteen male Wistar rats were divided randomly into 6 groups of 3 animals in each group. Group 1 and 2 received vehicle of the drug, group 3 received Furosemide (generic drug purchased from a local pharmacy) dose of 3.6 mg/kg, group 4-6 received an ethanolic extract of bulb *Eleutherine americana* Merr doses of 25, 50, 100 mg/kg respectively. Thirty minutes after a given test drug, a group of 2-6 received 5 ml drinking waters orally. Rats were placed in metabolic cages to separate urine and faeces. The

volume of urine collected was measured at the end of 24 h. During this period, no food was made available for all animals. Diuretic activity determined by urine volume compares to control group.

Animal model hypertension

Eighteen male Wistar rats were divided randomly into 6 groups of 3 animals in each group. Hypertension was induced by fructose 66% in the diet for 21 d. Test drug was given for 21 d along with a high fructose diet. Group 1 and 2 received vehicle of the drug, group 3 received captopril (generic drug purchased from a local pharmacy) dose of 2.5 mg/kg, group 4-6 received *Eleutherine americana* Merr extract doses of 25, 50, 100 mg/kg respectively. Group 1 received standard diet while group 2-6 received 66% fructose (purchased from Brataco, Indonesia) diet for 21 d. Systolic and diastolic blood pressure were measured at 0 and 21 d by noninvasive blood pressure instrument for rodents (CODA® Kent Scientific, USA).

Table 1: Number group of treatment for antihypertension and ECG study

No	Diet	Drug
1	Normal	0.5% CMC (carboxy methyl cellulose) 5 ml/kg
2	High fructose diet (66% fructose)	0.5% CMC (carboxy methyl cellulose) 5 ml/kg
3	High fructose diet (66% fructose)	Captopril 2.5 mg/kg
4	High fructose diet (66% fructose)	Ethanolic extract of bulb <i>Eleutherine americana</i> Merr (25 mg/kg)
5	High fructose diet (66% fructose)	Ethanolic extract of bulb <i>Eleutherine americana</i> Merr (50 mg/kg)
6	High fructose diet (66% fructose)	Ethanolic extract of bulb <i>Eleutherine americana</i> Merr (100 mg/kg)

Electrocardiogram measurement

In this research, electrocardiogram (ECG) measured the change in ECG profile after high fructose diet for 21 d. An animal ECG device (developed by the Department of Biomedical, Institute of Technology Bandung, ITB) connected directly to a laptop. Three electrodes were put on the right foot and two hands of the rat. The rats were either restrained to a platform or hold by hand. The ECG device was developed based on a single-lead ECG analog front end AD8232 chip. The chip was configured with a 0.5 Hz two-pole high-pass filters and followed by a two-pole 40 Hz low-pass filter. The op amp stage was set for a total system gain of 1100. The ECG analog signal was then converted into digital by using 14 bits resolution ADC (Advantech USB 4704) with a sampling rate of 1000 samples/second. The ECG signal was recorded on the PC for 10 seconds.

Data analysis

Data were collected for statistical analysis. The value is expressed as mean±standard deviation mean from three rats for each group. The

results were statistically analysis by one-way ANOVA. The significant difference was confirmed by p-value<0.05.

RESULTS

Percentage of yield extract

Percentage of yield extract of bulb *Eleutherine americana* Merr was found to be 5.7% w/w.

Phytochemical screening

The result of phytochemical analysis of an ethanolic extract of bulb *Eleutherine americana* Merr revealed the presence of flavonoids, saponin, and phenolic compounds.

Effect on urine volume

Group 3-6 showed increased in urine output statistically significant compared to normal and positive groups (p<0.05). The diuretic effect of group 4 and 5 showed significant different compared to group 3, while a group 6 showed comparable to group 3.

Table 2: Effect of furosemide and ethanolic extract of bulb *Eleutherine americana* Merr on urine volume for 24 h of rats

Group of treatment	Urine volume±SD (ml)
Normal control	0.4±0.1
Water	2.8±0.2
Water+furosemide (3.6 mg/kg)	5.3±0.2*
Water+bulb <i>Eleutherine americana</i> Merr (25 mg/kg)	4.2±0.2*
Water+bulb <i>Eleutherine americana</i> Merr (50 mg/kg)	3.5±0.1*
Water+bulb <i>Eleutherine americana</i> Merr (100 mg/kg)	5.2±0.2*

Values are expressed as mean±SD from three rats. Significant at *p<0.05, when compared to high fructose group

Systolic and diastolic blood pressure

Fructose diet for 21 d in the animal study showed increased in systolic and diastolic blood pressure (group 2). Administration of bulb *Eleutherine americana* Merr extract for 21 d revealed a reduction in

systolic, diastolic blood pressure and heart rate (group 4-6) that statistically significant compared to group 2. Group 4 and 5 that received extract dose of 25, 50 mg/kg showed a comparable effect in systolic blood pressure reduction but significantly different in diastolic blood pressure compared to group 3 (received captopril 2.5 mg/kg) (table 3).

Table 3: Effect of captopril and ethanolic extract of bulb *Eleutherine americana* Merr on systolic blood pressure of rats

Group of treatment	Systolic blood pressure±SD (mmHg)
Normal control	111.67±0.6
High fructose	148.67±1.53
High fructose+captopril (2.5 mg/kg)	101.33±2.52 *
High fructose+bulb <i>Eleutherine americana</i> Merr (25 mg/kg)	104.33±2.52 *
High fructose+bulb <i>Eleutherine americana</i> Merr (50 mg/kg)	104.67±2.52 *
High fructose+bulb <i>Eleutherine americana</i> Merr (100 mg/kg)	129.67±2.08 *

Values are expressed as mean±SD from three rats. Significant at *p<0.05, when compared to high fructose group

Table 4: Effect of captopril and ethanolic extract of bulb *Eleutherine americana* Merr on diastolic blood pressure of rats

Group of treatment	Diastolic blood pressure±SD (mm Hg)
Normal control	80.33±1.53
High fructose	102.67±2.52
High fructose+captopril (2.5 mg/kg)	78.00±2.00 *
High fructose+bulb <i>Eleutherine americana</i> Merr (25 mg/kg)	85.67±2.08 *
High fructose+bulb <i>Eleutherine americana</i> Merr (50 mg/kg)	86.67±2.52 *
High fructose+bulb <i>Eleutherine americana</i> Merr (100 mg/kg)	113.33±2.52 *

Values are expressed as mean±SD from three rats. Significant at *p<0.05, when compared to high fructose group High fructose diet for 21 d showed a reduction in R-R, P-R waves and increased in heart rate. Administration of captopril and bulb *Eleutherine americana* Merr improved of ECG profile and heart rate (table 5 and fig. 1).

Table 5: Effect of captopril and ethanolic extract of bulb *Eleutherine americana* Merr on electrocardiogram (ECG) profile of rats

Group of treatment	Mean of interval wave±SD (minutes)			
	R-R	P-R	QRS	S-T
Normal control	0.11±0.006*	0.03±0.006*	0.03±0.008	0.03±0.01
High fructose	0.10±0.002	0.02±0.003	0.04±0.008	0.03±0.004
High fructose+captopril (2.5 mg/kg)	0.12±0.006*	0.03±0.006*	0.03±0.003	0.03±0.006
High fructose+bulb <i>Eleutherine americana</i> Merr (25 mg/kg)	0.12±0.004*	0.03±0.004*	0.03±0.004	0.03±0.009
High fructose+bulb <i>Eleutherine americana</i> Merr (50 mg/kg)	0.11±0.005*	0.03±0.002*	0.03±0.006	0.04±0.01
High fructose+bulb <i>Eleutherine americana</i> Merr (100 mg/kg)	0.12±0.004*	0.03±0.004*	0.04±0.003	0.03±0.006

Values are expressed as mean±SD from three rats. Significant at *p<0.05, when compared to high fructose group

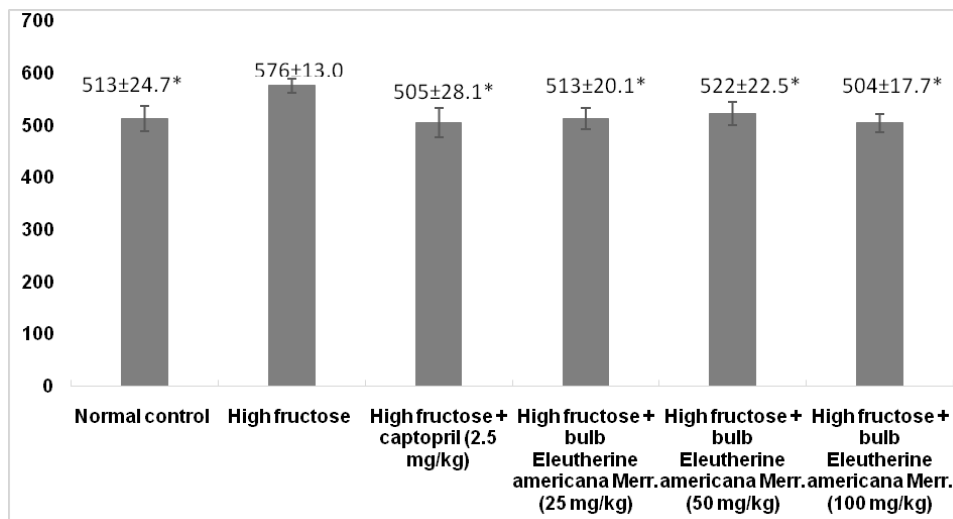


Fig. 1: Effect of captopril and ethanolic extract of bulb *Eleutherine americana* Merr on the heart rate of rats. Values are expressed as mean±SD from three rats. Significant at *p<0.05, when compared to high fructose group

DISCUSSION

High-fructose diets increase the activity of sodium and chloride transporters, resulting in a state of salt overload in an animal study. This condition increases blood pressure. Moreover, Excess fructose can activate vasoconstrictors, inactivate vasodilators, and overstimulate the sympathetic nervous system, these can contribute

to hypertension [10]. In accordance with our study that fructose diet for 21 d showed elevated in systolic and diastolic blood pressure and increased heart rates (table 3 and 4). Fructose diet showed changed in electrocardiogram profile (table 5).

Extract of bulb *Eleutherine americana* Merr dose of 25, 50 and 100 mg/kg showed diuretic activity. The dose of 100 mg/kg was the

highest diuretic effect comparable to furosemide 3.6 mg/kg. Diuretic furosemide increased the urinary output as well as an increase of sodium, potassium and chloride ions concentration in urine. This effect is valuable in the treatment of hypertension and enhancing the effect of others antihypertensive agents [11]. Moreover, increase in urine output result in systolic and diastolic blood pressure reduction, and heart rates reduction. Our study showed that provision of bulb *Eleutherine americana* Merr extract dose of 25,50, and 100 mg/kg for 21 d reduced systolic and diastolic blood pressure, and heart rates compared to control group (table 3 and 4, fig. 1).

The electrocardiogram (ECG) is an important tool for the study of cardiac electrophysiology [12]. The fluid loss during diuretic therapy results in a change of ECG including an increase in the amplitudes of P-waves, QRS complexes, and T waves; durations of P waves, QRS complexes, and QT intervals [13]. The hazard of diuretic-induced ventricular ectopic activity warrants correction of hypokalemia. Furosemide increased the risk of hypokalemia [14] results in increased heart rate.

In the present study showed that high fructose diet decreased of RR and PR intervals, and increased the heart rates. Administration extract of bulb *Eleutherine americana* Merr for 21 d improved ECG profile, RR, and PR intervals, and heart rates comparable to normal group ($p > 0.05$) (table 5, fig. 1).

Heart rate variability has been used as an important tool for the study of cardiac autonomic control [15]. Variability in heart rate can increased risk for ventricular arrhythmia event [16]. Our study suggested the role of bulb *Eleutherine americana* Merr in preventing ventricular arrhythmia by controlling the heart rates.

The Multiple Risk Factor Intervention Trial found that for any level of diastolic and systolic blood pressure was the major determinant of cardiovascular risk in aged over 50 y. The elevation of systolic blood pressure raises left ventricular afterload and myocardial work. Left ventricular hypertrophy is a known risk factor for congestive heart failure and cardiovascular events that associated with arterial stiffness (correlated with atherosclerosis) [17]. The present study showed that administration of bulb *Eleutherine americana* Merr reduced systolic and diastolic blood pressure. It suggested that bulb *Eleutherine americana* Merr has an important role in ameliorating the arterial stiffness. Further study needed.

CONCLUSION

The bulb of *Eleutherine americana* Merr showed antihypertensive activity in fructose-induced hypertension animal model. It has a potential role in regulating systolic, diastolic blood pressure and heart rate. Proposed mechanism of bulb *Eleutherine americana* Merr in lowering blood pressure are stimulating urine output (diuretic) and improvement of heart rate.

AUTHOR CONTRIBUTION

Patonah Hasimun directed the entire series of research and evaluates the overall research results.

Hasballah Zakaria developed a test method and validated the results of electrocardiogram measurements.

Elis Susilawati evaluated the results of the diuretic test and animal handling treatment.

Jeany Dwiylia Wardiono is a technical executive in anti-hypertensive research.

CONFLICT OF INTERESTS

Declared none

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