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Research Article
Prevention of Hypertension and Arterial Stiffness by Combination of *Centella asiatica* and *Curcuma longa* in Rats

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Abstract

Background and Objectives: Hypertension is characterized by an increase in systolic and or diastolic blood pressure. Arterial stiffness is an independent cardiovascular risk factor in hypertensive patients. Increasing arterial stiffness correlates with hypertension progression. This study aimed to determine anti-hypertensive activity and reducing arterial stiffness, combination of *Centella asiatica* and *Curcuma longa*, in 25% fructose-induced hypertensive animal models in drinking water and high-fat diet. **Materials and Methods:** This study was conducted on male Wistar rats with 3 methods: Diuretic test, anti-hypertensive test and arterial stiffness test by non-invasive method. Two doses combination of *Centella asiatica* and *Curcuma longa* were at 3.6 and 1.8 g kg^{-1} in juice preparation, compared to furosemide (diuretic test), captopril (anti-hypertensive test). Parameters measured were urine volume, systolic and diastolic blood pressure and Pulse Wave Velocity (PWV). **Results:** Combination of *Centella asiatica* and *Curcuma longa* in juice preparation, increased urine output (diuretic), decreased systolic and diastolic blood pressure and improved arterial stiffness ($p<0.05$) compared to the control group. **Conclusion:** Combination of *Centella asiatica* and *Curcuma longa* in juice preparation has anti-hypertensive activity and improved arterial stiffness.

Key words: Anti-hypertensive, arterial stiffness, *Centella asiatica*, *Curcuma longa*, diuretic, hypertension, pulse wave velocity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hypertension is one of the main risk factors for cardiovascular disease characterized by an increase in blood pressure of more[1] than 140/90 mmHg. The global prevalence of high blood pressure in adults aged 18 years and over was around 24.1% for men and 20.1% in women in 2015. The number of adults with high blood pressure increased from 594 million in 1975 to 1.13 billion in 2015, with an increase especially in low and middle-income countries[2]. According to Indonesia's basic health research report in 2013, the prevalence of hypertension in Indonesia is still quite high at around 25.8%. A number of two-thirds of the prevalence of hypertension are undiagnosed and not treated[3].

There are 4 classes of anti-hypertensive drugs recommended as initial therapy including thiazide diuretics, angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARBs) and calcium channel blockers (CCB). According to JNC 8, it is recommending the target of blood pressure to be achieved by people with hypertension is <150/90 mmHg. However, the measurement of the risk of cardiovascular events is very rarely done in hypertensive patients. In fact, there are reports that most treated hypertension has high arterial stiffness. Arterial stiffness contributes to the pathogenesis of hypertension and cardiovascular disease[4]. This is a finding that supports the use of arterial stiffness measurements in the hypertensive population[5] and helps risk stratification in the management of hypertension[6]. Pulse Wave Velocity (PWV) is one of the parameters currently thought to predict the risk of cardiovascular events. The PWV is a non-invasive method that measures aortic stiffness. Higher aortic stiffness assessed by PWV is associated with an increased risk of cardiovascular events. Aortic PWV is considered a biomarker of clinically important risk of cardiovascular event[7].

Turmeric rhizomes have been reported to have diverse pharmacological activities including antihypertension[8,9]. In addition, *Centella asiatica* leaves containing flavonoids as their active compounds have the potential to reduce blood pressure[10]. However, the benefits of *Centella asiatica* and *Curcuma longa* in reducing blood pressure have no known effect on improving arterial stiffness that usually accompanies hypertension. This study aimed to determine the effectiveness of the combination of *Centella asiatica* and *Curcuma longa* in juice preparations as anti-hypertension and their effects on improving arterial stiffness in male Wistar rats induced by fructose and high-fat feed.

MATERIALS AND METHODS

This study was conducted in the pharmacology laboratory of Bandung School of Pharmacy from February-April, 2018.

Plant materials: The *Centella asiatica* leaves and *Curcuma longa* rhizomes were obtained from Plantation Manoko, Lembang, Bandung, west Java, Indonesia. Plants have been determined in the Laboratory of Biology, Faculty of Science, Padjadjaran University, Bandung, West Java, Indonesia (No. 453/HB/01/2017).

Juice preparation: Fresh leaves of *Centella asiatica* and fresh rhizomes of *Curcuma longa* were made in juice preparations and freeze dried at the Faculty of Pharmacy, Padjadjaran University. A total of 375 g of *Centella asiatica* leaves freshly were washed thoroughly and crushed using a blender (Miyako® blender) with 50 mL of distilled water addition and then squeezed until 340 mL of juice was obtained. The *Centella asiatica* juice was freeze-dried to obtain a dry juice of 20.4 g (2.8%). The same was done to make 752 g of fresh *Curcuma longa* rhizome juice with the addition of 20 mL of distilled water to obtain 390 mL of turmeric juice. The results of freeze dry turmeric rhizome juice were 15.6 g (2.1%).

Two doses juices preparations consisted a combination of *Centella asiatica* and *Curcuma longa* was made with a ratio of at 1.8 and 3.6 g kg^{-1}.

High-fat diet composition: The composition of 40% high-fat diet in accordance with previous research, with slight modification[11]. The 40% high-fat diet contained 60% of the standard diet (Pokphan C551® rat-food purchased from a local store) and 40% of fat (consist of duck eggs, quail eggs, margarine and beef fat). All ingredients were mixed until homogeneous and then dried in a 37°C oven.

Animals: This study conducted on Wistar male rats 2 months old weighing 200-250 g were obtained from D'Wistar (provider of test animals), Majalaya, Bandung, west Java, Indonesia. Animals were acclimatized for 14 days in a cage with standard feed and drinking water *ad libitum* and 12 h of dark and light cycle was maintained. All procedures were conducted on animals during the study, approved by the Ethics Committee of the Faculty of Medicine, Padjadjaran University, Bandung, west Java, Indonesia (No.143/UN6.KEP/EC/2018).

Diuretic study: The diuretic effect was carried out by the Lipschitz method[12]. A total of 20 male Wistar rats were grouped randomly into 4 groups consisting of group 1 (received drug carriers), group 2 (received 3.6 mg kg^{-1} furosemide), group 3 and 4 (received combination juice of *Centella asiatica* and *Curcuma longa* (dose of at 1.8 and 3.6 g kg^{-1}). All groups received 5 mL of 0.9% NaCl after administration of the test drug. Urine volume of each group was collected every hour for 5 h. The diuretic effect was marked by an increase in urine volume for 5 h of observation.

Anti-hypertension design study: The anti-hypertensive activity test was carried out *in vivo* by using non-invasive measurements. Measurement of systolic and diastolic blood pressure was carried out using a CODA® device. A total of 25 male Wistar rats were grouped randomly into 5 groups consisting of group 1 (received drug carriers), group 2 (received drug carriers), group 3 (received captopril 2.5 mg kg^{-1}), groups 4 and 5 receiving a combination of *Centella asiatica* and *Curcuma longa* with 2 different doses (at 1.8 and 3.6 g kg^{-1}). All groups except group 1 received a high-fat diet and 25% fructose in drinking water for 21 days. Measurements of blood pressure were performed on day 0, 14 and 21.

Measurement of arterial stiffness: Arterial stiffness was measured non-invasively using the PWV method (pulse wave velocity). Measurements are performed with tools and methods in accordance with previous research[13]. In summary, this non-invasive PWV measurement method uses an electrocardiogram (ECG) and photoplethysmogram (PPG) sensors. The ECG measures the potential difference between the left and right arms using the right foot as a general reference. The potential difference is due to the contraction of the heart muscles. The peak of the ECG signal (R-wave) is used as the first reference point when the ventricle contracts to pump blood out of the heart. The PPG sensor is located at the base of the tail to measure changes in blood volume. The PPG foot signals are used as the second reference point at the time of arrival of blood pumped from the heart. The increase in PWV values shows the progression of arterial stiffness. Measurement of arterial stiffness was carried out on day 0, 14 and 21 after treatment.

Statistical analysis: The data obtained were statistically analyzed by one-way ANOVA using SPPS software. Significant differences between the test group and the control group showed the effect of the test drug. The p-values <0.05 were considered statistically significant.

RESULTS

Screening of secondary metabolite: The results of the examination of secondary metabolites contained in the leaves of *Centella asiatica* and rhizome of turmeric in fresh juice and freeze-dried results are shown in Table 1.

Diuretic activity: The results of urine accumulation for 5 h are shown in Fig. 1. There was an increase in urine volume in the group receiving the test drug. It showed a diuretic effect.

Percentage of increase in body weight: All treatment groups showed an increase in body weight after receiving a high-fat diet and fructose 25% in drinking water for 21 days. The increase in body weight calculated in percentages (compared to the normal group) for all treatment groups is shown in Fig. 2. The group that received the test drug showed a smaller increase in body weight than the control group.

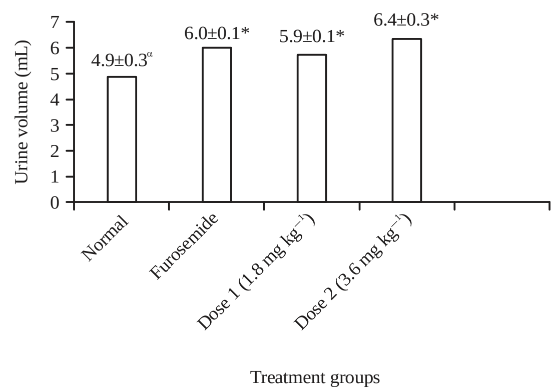

Fig. 1: Results of urine accumulation for all treatment groups for 5 h

*There were significant differences compared to normal groups (p<0.05), α: There were significant differences compared to group received furosemide (p<0.05)

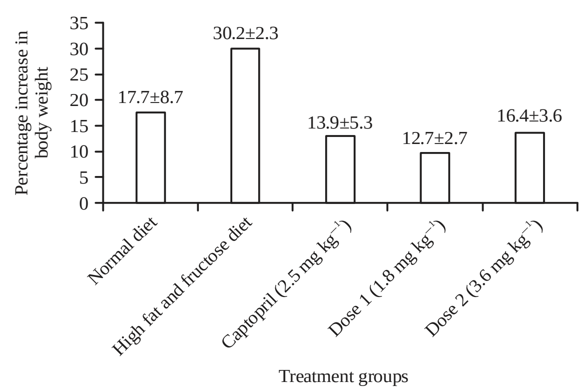

Fig. 2: Increase in body weight (%) of rats, after receiving a diet high in fat and fructose 25% in drinking water, for 21 days for all treatment groups

Table 1: Secondary metabolite content of *Centella asiatica* leaves and *Curcuma longa* rhizomes in fresh juice and dried juice

	Fresh juice		Dried juice	
Screening	*Centella asiatica*	*Curcuma longa*	*Centella asiatica*	*Curcuma longa*
Alkaloid	-	-	+	+
Flavonoid	+	+	+	+
Saponin	+	+	-	-
Tannin	+	+	+	+
Quinone	-	+	-	+
Steroids	+	-	+	-
Triterpenoids	+	+	+	+

+: Identified, -: Not identified

Table 2: Average systolic blood pressure measurement results on day 0, 14, 21 after receiving a high-fat diet and 25% fructose in drinking water for all treatment groups

	Systolic blood pressure (mmHg) ±SD measurement after treatment (days)		
Groups	0	14	21
Normal	116.8±2.4	118.0±2.6*	116.3±2.2
High-fat and fructose diet	116.3±1.0	158.0±3.6$^{\alpha}$	191.5±2.7
Captopril 2.5 mg kg^{-1}	116.3±2.1	120.5±1.3*	99.8±1.7$^{*\alpha}$
Dose 1 (1.8 g kg^{-1})	116.5±1.3	117.5±0.1*	107.0±2.2$^{*\alpha}$
Dose 2 (3.6 g kg^{-1})	115.8±2.1	96.8±2.2$^{*\alpha\beta}$	96.0±1.6$^{*\alpha}$

*There were significant differences compared to normal groups (p<0.05), α: There were significant differences compared to the group received a high-fat and fructose diet (p<0.05), β: There are significant differences compared to captopril group (p<0.05)

Table 3: Average diastolic blood pressure measurement results on day 0, 14, 21 after receiving a high-fat diet and 25% fructose in drinking water for all treatment groups

	Diastolic blood pressure (mmHg) ±SD measurement after treatment (days)		
Groups	0	14	21
Normal	78.0±1.8	78.0±1.6*	76.8±1.2
High-fat and fructose diet	77.5±2.4	111.0±10.8$^{\alpha}$	131.5±21.0
Captopril 2.5 mg kg^{-1}	76.8±2.2	81.0±0.82*	64.5±1.3*
Dose 1 (1.8 g kg^{-1})	77.8±1.8	76.3±1.71*	73.3±3.3*
Dose 2 (3.6 g kg^{-1})	78.5±1.3	66.8±1.71$^{*\alpha\beta}$	59.3±1.7$^{*\alpha}$

*There were significant differences compared to the group received a high-fat and fructose diet (p<0.05), α: There were significant differences compared to normal groups (p<0.05), β: There are significant differences compared to captopril group (p<0.05)

Results of systolic blood pressure measurement: The group that received high-fat diet and 25% fructose in drinking water for 21 days caused an increase in systolic blood pressure by 64.7% (increased from 116.3-191.5 mmHg). The results of systolic blood pressure measurements for all treatment groups are shown in Table 2. The group that received the combination of *Centella asiatica* and *Curcuma longa* showed a statistically significant decrease in systolic blood pressure (p<0.05) compared to the control group. Systolic blood pressure decreases with increasing doses.

Results of diastolic blood pressure measurement: The group that received foods containing high fat and 25% fructose in drinking water for 21 days showed an increase in diastolic blood pressure by 41.6% (increased from 77.5±2.4-131.5±21.0 mmHg). The results of diastolic blood pressure measurements for all treatment groups are shown in Table 3. The group that received the combination of *Centella asiatica* and *Curcuma longa* showed a statistically significant decrease in diastolic blood pressure (p<0.05) compared to the control group. Diastolic blood pressure decreases with increasing doses.

Results of PWV measurement: The group that received high-fat diet and 25% fructose in drinking water showed an increase in arterial stiffness which was marked by an increase in the value of the pulse wave velocity (PWV). The PWV measurement results for all treatment groups are shown in Table 4. The group that received the combination of *Centella asiatica* and *Curcuma longa* showed improved arterial stiffness significantly different (p<0.05) compared to the control group. The effect of improving arterial stiffness increases with increasing doses.

Table 4: PWV values after 14 and 21 days of treatment for all treatment groups

Groups	Average of PWV (cm sec^{-1})±SD measurement after treatment (days) 0	14	21
Normal	386±12	381±13*	386±16
High-fat and fructose diet	383±7	653±10$^{\alpha}$	746±12
Captopril 2.5 mg kg^{-1}	383±7	389±9*	390±15*
Dose 1 (1.8 g kg^{-1})	387±9	388±9*	374±11*
Dose 2 (3.6 g kg^{-1})	382±15	370±8$^{*\alpha\beta}$	269±14$^{*\alpha\beta}$

*There were significant differences compared to group received a high-fat and fructose diet (p<0.05), α: There were significant differences compared to normal groups (p<0.05), β: There are significant differences compared to captopril group (p<0.05)

DISCUSSION

Fructose consumption has increased since the last decade and is considered to be the cause of the increasing prevalence of hypertension and metabolic disorders. This occurs through the mechanism that fructose activates vasoconstrictors, inactivates vasodilators and stimulates the sympathetic nervous system excessively[14]. The results proved that consumption of fructose and high-fat foods for 21 days increases blood pressure and arterial stiffness in an animal model.

The results showed that the group received a high-fat and fructose diet increased body weight by more than 20%. This showed that the group was obese. There is an increase in leptin concentration (indicating the occurrence of leptin resistance) as a result of the body's failure to control body weight due to the imbalance between energy intake and expenditure[15]. The state of obesity increases the risk of portal hypertension, metabolic syndrome and type 2 diabetes mellitus[16]. The combination of *Centella asiatica* leaves and *Curcuma longa* rhizomes in a juice preparation, works synergistically as a diuretic, reduced systolic and diastolic blood pressure as well as improved in arterial stiffness. The findings of this study confirm and prove more clearly the combination of *Centella asiatica* leaves and *Curcuma longa* rhizomes in addition to reducing systolic and diastolic blood pressure also able to improve arterial stiffness that occurs in animal models of hypertension.

The results showed that *Centella asiatica* and *Curcuma longa* can improve arterial stiffness. This result is in line with previous studies that asiaticoside as the active component of *Centella asiatica* improves metabolic disorders and hemodynamic disturbances in rat animal models induced by high-fat and high-carbohydrate diets. The effects of these improvements are related to asiaticoside as an antioxidant, anti-inflammatory and able to restore eNOS/iNOS expression[17]. Curcumin as an active compound in turmeric rhizomes is reported to improve carbohydrate and fat metabolism in animal models that receive a high fructose diet. This occurs through a mechanism of modulating stress oxidation and the inflammatory process. Therefore, curcumin can be a therapeutic option to overcome metabolic syndrome[18]. The combination of *Centella asiatica* leaves and *Curcuma longa* rhizomes in a juice preparation works synergistically as a diuretic. The results of this study are consistent with studies that reported the diuretic effect of turmeric rhizomes dose of 200 and 400 mg kg^{-1} in models of nephrotoxic rats induced by gentamicin[19]. The molecular mechanisms of *Centella asiatica* and *Curcuma longa* in reducing blood pressure and improving arterial stiffness are explained by previous studies. Tetrahydrourcumin as the main metabolite of curcumin can reduce blood pressure in cadmium-induced hypertensive animal models. These effects occur through anti-oxidant activity, anti-inflammatory. Tetrahydrourcumin improves vascular dysfunction and arterial stiffness in rats through increased bioavailability NO[20].

Moreover, asiatic acid is a triterpenoid compound in *Centella asiatica* which has the activity of repairing human aortic endothelial cells that experience hyperpermeability due to TNF-α. Hyperpermeability in endothelial cells is the first stage in atherosclerosis which begins with endothelial cell dysfunction. Therefore, asiatic acid plays an important role in preventing atherosclerosis[21]. The need to develop further research on antihypertensive drugs in addition to being able to reduce blood pressure is also able to improve arterial stiffness. This non-invasive method of measuring PWV can be used for studies related to blood vessel elasticity. So that the risk of cardiovascular events can be prevented through earlier measurements especially in high-risk populations.

CONCLUSION

The results of this study can be concluded that the combination of *Centella asiatica* and *Curcuma longa* in juice preparation is effective and works synergistically as antihypertensive in reducing systolic and diastolic blood pressure and is able to improve arterial stiffness (improvement of PWV value). In addition, the diuretic effect of the combination of *Centella asiatica* and *Curcuma longa* contributes to a decrease in blood pressure. These effects can decrease the risk of cardiovascular complications. However, the effect of improving arterial stiffness needs further research to prove its effect on the production of nitric oxide *in vitro*.

SIGNIFICANCE STATEMENT

This study discovered the effect of *Centella asiatica* and *Curcuma longa* in combination on improving vascular elasticity in hypertensive animal models using non-invasive methods. The results of this study prove that hypertension is accompanied by arterial stiffness. This study will help the researchers to uncover the critical areas of arterial stiffness that can occur in hypertension, hyperlipidemia, atherosclerosis and other inflammatory diseases. Thus a new theory on herbal medicine as anti-arterial stiffness may be arrived at.

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