Vasodilation Effects of Ketapang (*Terminalia Catappa* Linn) Fruit on Aortic Rings Rat

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Abstract

Hypertension is an important risk factor for cardiovascular disease. Hypertension, or high blood pressure, is a medical condition in which the blood pressure in the arteries is consistently above normal. Hypertension requires long-term treatment to keep blood pressure under control. It is necessary to search for alternative drugs to lower high blood pressure. East Kalimantan is rich in medicinal plant diversity and needs to be explored to discover new drugs for hypertension. Ketapang fruit (*Terminalia catappa* Linn) has the potential to have a vasodilation effect so research is needed to explore vasodilation activity in vitro. The purpose of this research is to determine the vasodilation activity of *Terminalia catappa* fruit using isolated aortic ring bioassay. Twelve Wistar rats were used for bioassays isolated aortic ring with endothelium. The aortic rings were precontracted with phenylephrine. This study used two test groups, Control group and *Terminalia catappa* fruit ethanol extract group (FTC). The FTC and Control concentrations were given cumulatively in chamber of isolated aortic ring. Based on the data, there was quite a significant difference between the Control group and FTC. ($p < 0,002$). The conclusion of this study is FTC causes vasodilation in isolated rat aortic rings with endothelium.

Keywords: *Terminalia catappa*; fruit; vasodilation; aortic ring; bioassay.

INTRODUCTION

Vasodilation is an enlargement of the diameter of a blood vessel that occurs as a result of the process of relaxation smooth muscle in the blood vessel wall. This improves blood circulation and oxygen supply throughout the body[1]. Vasodilation is useful for reducing blood pressure and thus risk health problems especially those related to the cardiovascular system as well will decrease[2]. This vasodilation mechanism is influenced by substrates released by endothelial cells and vascular smooth muscle. These substrate include Nitric Oxide (NO) and prostaglandin I_2 which can relaxes smooth muscles[3].

Drugs that have a vasodilating effect are called vasodilators. Vasodilators commonly used by the public is Angiotensin Converting Enzyme inhibitors (ACEi), Angiotensin Receptor Blockers (ARB), Calcium Channel Blockers (CCB), and beta blockers[4]. Side effects of this medication, if consumed for a long time, causes dry cough, dizziness, headache, nausea, hyperkalemia, hypotension, and bradycardia[5]. To overcome the side effects of conventional drugs, it is necessary exploration to find natural-based medicines that are abundant in Indonesia and can work as a vasodilator. Use of medicinal plants considered practical because it is easy to find, affordable, and does not cause problems dangerous side effects because they are natural[6].

According to Khan & Gilani (2008), one of the plants known to have vasodilating activity is *Terminalia bellerica* fruit. *Terminalia bellerica* fruit extract has been tested in vitro can work as a vasodilator because contains gallic acid. Gallic acid is a gallotannin from the polyphenols group which can increase levels of Nitric Oxide (NO). However, *Terminalia bellerica* does not grow in Indonesia[7]. A plant that grows in Indonesia and has the same genus

as *Terminalia bellerica* is *Terminalia catappa* Linn. Because there are similarities genus between *Terminalia bellerica* and *Terminalia catappa* Linn hence it is possible that *Terminalia catappa* Linn fruit also has vasodilatory activity. This is reinforced because it is also in the fruit of *Terminalia catappa* Linn contains gallic acid which can work as a vasodilator[8].

Terminalia catappa Linn, commonly known as ketapang, is widespread in tropical and subtropical areas. This plant grows a lot in East Kalimantan so it is very easy to find[9]. This plant has been researched that it can be used for good health from leaves, stems, roots, fruit, down to the seeds. However, research related to Ketapang fruit as vasodilators have never been used[10]. Therefore, researchers are interested in testing the vasodilation activity of ketapang fruit ethanol extract in the aorta of white wistar rats. This study used rat aorta because it has a significant similarity to human blood vessels and have a good response to drugs[11].

MATERIALS AND METHODS

This research used a posttest only control group design. This study uses the isolated aorta bioassay in vitro method for testing the aortic tone response activity of white wistar rats. If there is a decrease in aortic tone with a negative value after intervention, that means there is aortic vasodilatory activity. The research was conducted at the Pharmacology Laboratory, Faculty of Medicine Mulawarman University in January 2024.

The subject of the plant used in the research is ketapang fruit (*Terminalia catappa* Linn) which is green, whole, fresh, and not rotten. *Terminalia catappa* fruit was taken in Samarinda, East Kalimantan. Species identification was assisted by taxonomists from the Ecology Laboratory and Tropical Forest Biodiversity Conservation, Faculty of Forestry, Mulawarman University No. 278/UN17.4.08/LL/2023. The subject of the isolated organ bioassays used in research is the aortic ring of wistar white rats (Rattus novergicus) with a length of 3 mm with endothel. The criteria for test animals are male rat, healthy, with white fur clean, actively moving, weight 200-300 g, and 2-4 months old[12]. The size of the Isolated Aorta Bioassay sample used in this study is 6 rats to get 6 aortic rings with endothel for each group. Ethical approval was obtained from the Faculty of Medicine's Research Ethics Commission Mulawarman University on January 15th 2024 No. 12/KEPK-FK/I/2024.

The material used in this research is *Terminalia catappa* fruit. After washing with running water and draining at room temperature, the fruit cut crosswise with a size of 3 mm. Then, the fruit is dried in an oven at 50°C for seven days. The fruit is turned upside down every day until it is dry and easy to break. After that, the fruit is coarsely ground and put it in a closed, airtight box labeled and dated, and stored in a dry place away from sunlight before being used for research. In this research, chemicals used for extraction, 96% pharmaceutical grade ethanol[13]. To carry out the *Terminalia catappa* fruit extraction procedure used equipment in the form of digital scales, ovens, filters, paper filter, rotary evaporator, vacuum pump, bottle for maceration, beaker glass, measuring cup, and a jar to hold the extract. Research materials used in testing vasodilatory activity aorta are ketamine, Wistar rat aorta, DMSO, absolute ethanol, methacholine, phenylephrine, HCl, Kreb's Henseleit solution, and carbogen gas (a mixture of 95% O2 + 5% CO2). To perform isolated aorta bioassay, the equipment used are digital scales, pH-meters, digital thermometers, 5 and 20 ml syringes, tissue scissors, tweezers, petri dishes, micropipette, isolated organ bath, isometric force transducer, octal bridge amplifier, PowerLab, Chart reader ver. 8 for Windows, SigmaPlot.

The first step taken was to give the rat anesthesia using ketamine. Rat were euthanized by cervical discollation after deep asleep (has no response when clamped). After that, the abdominal rat was opened with scissors from the abdomen to the thorax and up to the neck. Removal of the entire aorta starting from the inferior part of the diaphragm to the aortic root, carried out carefully so as not to damage the endothelium. The aorta has been taken and placed in a petri dish filled with solution Kreb's-Henseleit. Then the aortic connective tissue is cleaned and the aorta is cut transversely with a size of around 3 mm[12]. The aortic ring that has been cut is attached to the tissue holder on the wrong side one side of the aorta. On the opposite side, it is attached to the tissue holder

connected to the isometric force transducer which has been connected to octal bridge amplifier and computer with LabChart installed. Furthermore, the aorta was inserted into a chamber filled with 10 mL of 37°C Kreb's Henseleit solution and supplied with carbogen gas. The Kreb's-Henseleit solution was changed every 15 minutes and the aorta is allowed to equilibrate for 90 minutes until tone is established stable. After that, test the aortic contraction response with 10^{-6} M phenylephrine solution and endothelial integrity test with 10^{-5} M methacholine solution[14]. If after being precontracted with phenylephrine solution the aortic tone increases until it reaches a peak on the computer graph, meaning the aortic contraction has a good response. Then, after administering methacholine, there is a response to a decrease in tone aorta, meaning that the aorta used has an endothel. Then the aorta is flushed with Kreb's-Henseleit solution several times every 10 minutes. After returning to the basal tone, the aorta is ready to be tested for its vasodilation response[14].

This study used two test groups, namely the control group (extract solvent consisting of DMSO and distilled water) and the FTC group (ethanol extract of *Terminalia catappa* Linn fruit). Each group will 6 concentration variations were made, namely log concentration -2.0, -1.5, -1, -0.5, 0.0, 0.5 mg/ml. This concentration will be entered into the chamber containing the aorta cumulatively. After the aorta was prepared, it was tested for tone response to FTC by providing a log of the cumulative FTC concentration in that chamber contains the aorta. This FTC concentration log starts at -2, -1.5, -1, -0.5, 0, and 0.5. For the control group, the concentration of extract solvent used was the same with the FTC group, and repetition was carried out six times. The results are expressed in percent aortic tone. If the percent aortic tone is negative, that means there is aortic vasodilation activity[14]. Tonus percent value aorta can be calculated by:

$$\% \text{ Aortic tone} = \frac{Final\ tone\ FTC - Maximum\ tone\ PE}{Maximum\ tone\ PE} \times 100\%$$

Data were tabulated in mean ± SEM. Data analysis using SigmaStat ver. 13. One way anova test to compare the Control and Extract groups. The t-test was used to compare two concentration groups for normally distributed data. The statistics are significantly different with $p<0.05$.

RESULTS

The isolated aorta bioassay test uses the endothelium of Wistar rat aorta. Before the isolated aorta test is carried out bioassay, testing the integrity of rat aortic contractility with
using phenylephrine 10^{-6} M is given at the 100 second and the results are obtained increase in aortic tone, reaching peak contraction at the 500 second and continuing flat until the 1500 second. This shows the administration of phenylephrine can cause vasoconstriction. After that, 10^{-4}M methacholine was given 1500 seconds and found a decrease in aortic tone until 1580 seconds, then wash out at the 2000 second. This proves the aorta ring rat that used has endothel.

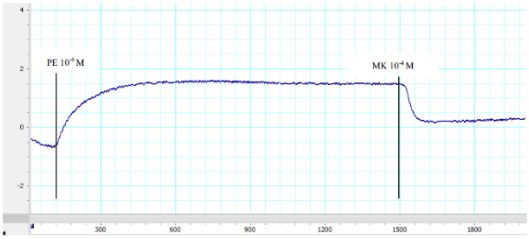

Fig 1. The aorta was precontracted with phenylephrine and tested for endothelial integrity with methacholine.

In the Control group testing, 10^{-6} M phenylephrine was given first at the 100 second then continue giving 6 Controls doses, at the 700, 800, 900, 1000, 1100, and 1200 seconds.

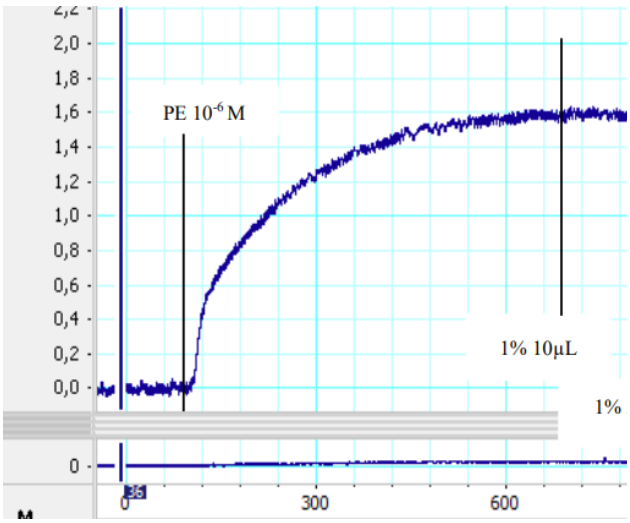

Fig 2. The aorta was precontracted with phenylephrine and tested with a control group.

For testing the extract group (FTC), 10^{-6} M phenylephrine was given first at the 100 second, then continue with the administration of ethanol extract *Terminalia catappa* Linn fruit in 6 doses, at the 700, 800, 900, 1000, 1100, and 1200.

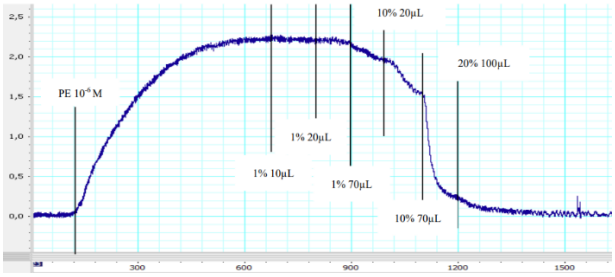

Fig 3. The aorta was precontracted with phenylephrine and tested with an extract group.

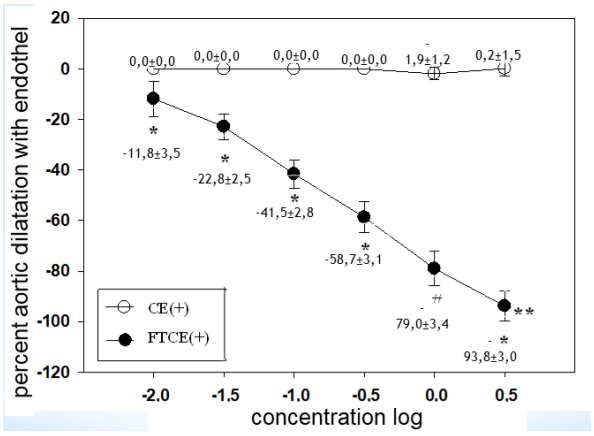

Fig 4. Percentage curve of aortic dilatation against log concentration of Control and Extract in rat aortic ring bioassay with endothel. n=6 rats. Precontraction with PE 10^{-6}M. CE(+) = control, aortic ring with endothel. FTC(+) = ethanol extract of *Terminalia catappa* fruit. Statistical tests significantly with $p < 0.05$.

Based on the data, there was quite a significant difference between the Control group and FTC. In Figure 4, it can be seen that there is no significant change in aortic tone after administration of Control with low or high concentrations. Meanwhile, on to giving FTC cumulatively there is a significant decrease in aortic tone. The more higher the concentration of the extract given, the greater the percent reduction aortic tone. The larger the number produced, the effect generated will be stronger. This proves that there is strong pharmacological activity in the aorta rat after administration of FTC.

DISCUSSION

Ketapang fruit that has been dried and mashed is made into shapes extract. Extracts are concentrated preparations resulting from the extraction process. Extraction is carried out to separate the chemical components needed using solvent. One of the commonly used extract solvents is ethanol. Ethanol is able to dissolve polar and nonpolar compounds. Ethanol is capable dissolves flavonoid compounds and phenolic compounds which are the ingredients chemistry of this ketapang fruit. In addition, when compared with other solvents, ethanol is relatively non-toxic, easy to obtain, and safe for the extract to be extracted used as medicine and food[15].

Therefore, this research uses the ethanol solvent method extraction in the form of maceration. This method is widely used because it is quite affordable and easy to do. However, this method is quite time consuming and requires a lot of solvent. Maceration is done by soaking the simplicia for 1-7 days at room temperature and shaking every day. Process This soaking and shaking will trigger the solvent to penetrate as well breaks down the cell walls of the extracted plant so that the active substance components will dissolved[16]. After maceration,

concentration will be carried out extract with a rotary evaporator at a temperature of 50°C to evaporate the solvent to obtain a concentrated preparation. After that, the concentrated extract will be stored in an airtight jar[13].

The vasodilation activity test in this study used the isolated aorta bioassay in vitro. This means that this research was not influenced by absorption, distribution, metabolism and excretion systems or pharmacokinetic effects. Apart from that, in vitro research is also fast, easy and affordable[17]. In this study, Wistar rat aortas were used. The aorta is the largest artery in the body and has receptors which is the same as other blood vessels[18]. Research using rat aortas is quite accurate because rat aortas are similar with human blood vessels and has a response when given drug intervention. Another advantage of using rat aortas is the blood vessels large so it is easy to prepare and has stable contractility[11].

In this study, phenylephrine was used as a vasoconstrictor for increase aortic tone before administering the extract. Phenylephrine is an agonist α1 adrenergic receptor. Phenylephrine after binding to its receptor can stimulates the release of intracellular calcium and activates the myosin light chain resulting in vasoconstriction[19]. In the endothelial integrity test, methacholine was used. Methacholine is NO vasodilator. Methacholine acts directly on muscle muscarinic receptors plain. Methacholine will stimulate the release of NO produced by cells endothelium. NO will diffuse from the endothelium to the smooth muscle and will activate it intracellular guanylyn cyclase to form cyclic guanosine monophosphate. It will trigger a decrease in intracellular calcium concentration vasodilation[20].

The results of testing the rat aorta after preparation showed that the aorta was still alive and has endothelium. This can be seen from testing with phenylephrine 10^{-6}M and methacholine 10^{-5}M. When the aorta was given 10^{-6}M phenylephrine at the 100 second it appeared aortic tone increases to plateau at 700 seconds and continues with administration of 10^{-5}M methacholine at 1500 seconds showed decreased aortic tone until the 2000 second. In the isolated aorta bioassay test with precontracted endothelium with PE then given a cumulative concentration of fruit ethanol extract *Terminalia catappa* Linn (FTC) causes a decrease in aortic tone in accordance with given concentration. This shows that the FTC extract has vasodilatory activity. Vasodilation activity of rat aorta after FTC administration this is because *Terminalia catappa* Linn fruit contains gallic acid, elagic acid, and cyanidin-3-glucoside which have been studied to have vasodilatory activity[8,21–23].

Gallic acid acts on the endothelium by activating the NOS enzyme so NO production will increase. Apart from that, gallic acid can also downregulates angiotensin, including its receptors and enzymes[8]. The effect of activating the NOS enzyme also occurs due to the presence of metabolites another secondary ingredient found in *Terminalia catappa* fruit is elagic acid. Elagic acid also acts on the endothel to increase NO production[24]. These three secondary metabolites is a group of phenolic acids. On *Terminalia catappa* fruit also contains flavonoids, namely cyanidin-3-glucoside. This compound can reduces oxidative stress in endothelial cells and increases NO production[25].

This study only proves the vasodilation activity of FTC extract on endothelial rat aorta. This is because of the various compounds contained in it Ketapang fruit has been studied to have the function of increasing NO production produced by endothelial cells. Therefore, further research is needed regarding whether FTC extract can also have an effect on rat aorta without endothelium which means it can also work on the smooth muscles of blood vessels. If extract also works on rat aortas without endothelium, meaning the extract also has internal effects the process of MLC dephosphorylation and inhibition of calcium channels in smooth muscle.

CONCLUSION

Ethanol extract of *Terminalia catappa* fruit causes aortic ring rat vasodilation with endothel.

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