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Original Article

In vivo study of the antihypertensive effect of bidara leaf (*Ziziphus spina-christi*) during pregnancy

Tasya Nurlaila Dilla [1]✉, Suharyo Hadisaputro [1], Aris Santjaka [1]

[1] *Postgraduate Program in Applied Health, Poltekkes Kemenkes Semarang, Central Java, Indonesia*

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CORRESPONDENCE

Phone: +6282346330691

E-mail: tasyanurlaila12@gmail.com

A B S T R A C T

Background: Bidara is drought tolerant and very easy to grow in tropical climates such as Indonesia. Bidara contains a combination of calcium, potassium, and magnesium, and active flavonoid compounds, and antioxidant activity that play a role in inhibiting free radical damage, improving endothelial function so that it can potentially lower blood pressure. Previous studies explained that a dose variant of no more than 300mg/kg BW is beneficial while minimizing pathological changes. However, there has been no research related to the effect of bidara leaf in lowering blood pressure, so it is necessary to do related research.

Objective: Analyze the effect of bidara leaf extract at a 200 mg/kg BW dose and 300 mg/kg BW on systolic and diastolic blood pressure.

Methods: 24 pregnant female Wistar rats induced hypertension, aged 6-8 weeks with a weight of 130-230 grams. The rats were randomized so that they consisted of 2 control groups and two experimental groups, which were given various doses of bidara leaf for nine days. Blood pressure was measured using non-invasive CODA.

Results: The blood pressure of rats in the bidara leaf extract group at doses of 200mg/kg BW and 300mg/kg BW decreased systolic and diastolic compared to the control group ($p<0.05$). The 200mg/kg BW dose group experienced a decrease in blood pressure of 12.3% for systolic and 16.32% for diastolic; the 300mg/kg BW dose group experienced a decrease in blood pressure of 19.99% for systolic and 27.73% for diastolic.

Conclusion: Bidara leaf extract can reduce the blood pressure of pregnant rats with hypertension.

INTRODUCTION

Hypertension significantly contributes to the high maternal mortality rate. World Health Organization estimates that the maternal mortality rate in the world due to hypertension is 14%.[1] The pathophysiology of hypertension in pregnant women is associated with the spiral artery vascular remodeling process's failure to trigger vasoconstriction that inhibits uteroplacental blood flow, resulting in placental hypoxia and ischemia. Placental hypoxia and ischemia can cause an increase in the formation of free radicals that trigger oxidative stress resulting in endothelial dysfunction that can interfere with nitric oxide production and become a clinical manifestation of preeclampsia.[2]

The combination of macrominerals such as calcium, magnesium, and potassium and the presence of flavonoids are considered to lower blood pressure by increasing serum nitric oxide in endothelial cells and reducing contractility in smooth muscle so that it can inhibit vasoconstriction.[3–6] Medicinal plants that have previously been shown to lower blood pressure are bay leaf and celery leaf, which contain macrominerals that influence blood pressure. The macromineral content in bay leaf on 100grams is 834mg calcium, 120mg magnesium, and 529mg potassium, while celery leaf contains 40mg calcium, 11mg magnesium, and 260mg potassium.[7,8] Bay leaf water decoction can reduce blood pressure by 6.77% for systolic and 3% for diastolic [4], while the ethanol extract of celery can reduce systolic blood pressure by 5.7% and diastolic by 5.95%.[3] Although it can lower blood pressure, previous preclinical studies

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have stated that celery leaf cannot be recommended as an antihypertensive during pregnancy because it is thought to have the exact mechanism as captopril, so there is a risk of causing fetal weight loss.[9]

Bidara leaf contains more potassium (673mg%), calcium (1270mg%), magnesium (169mg%) than celery and bay leaf,[10] in addition, there are active flavonoid compounds (9,6±1,2mg QE/g DW)[11] and potent antioxidant activity with IC50 90.9584 ppm.[12] Previous studies have examined the toxicity test on bidara leaf extract and found that a dose of 300mg/kg BW was beneficial because it could prevent and minimize the pathological changes caused by mercury in the kidneys.[11] Bidara leaf extract at a dose of 600mg/kg BW had a toxic risk to the liver, characterized by changes in the histopathological structure of liver cells.[13] Bidara leaf has various benefits when used with the correct dose, so that in its application as a non-pharmacological treatment. People in Lorestan Province have used bidara leaf as a medicinal plant to control high blood pressure.[14]

However, strong evidence still needs to state that bidara leaf functions as a safe and effective antihypertensive for consumption. The accuracy of the research results was assessed through monitoring for side effects and toxicity. The community at large has used non-pharmacological treatments that utilize herbs. However, animal testing needs a solid preclinical basis for dosage and quality enforcement before conducting large-scale clinical trials in humans.[14,15] Rats were used as research subjects because they have several advantages, like being easy to handle.[16] This study aims to prove the effectiveness of various doses of bidara leaf extract on lowering blood pressure in pregnant rats with hypertension.

METHOD

Study Design

This is an experimental study using animals with a randomized pretest-posttest control group design.

Study Site

This research was carried out in three different university laboratories in Semarang, namely the manufacture of extracts at the Food Technology Laboratory of Diponegoro University, the manufacture of a pregnant rat model as well as hypertension induction at the Biology Laboratory of the State University of Semarang, and the administration of extracts as well as blood pressure measurements were carried out in the pharmacology laboratory of Wahid Hasyim University.

Materials

The surgical instruments used in this study were: medical gloves, surgical scissors, anatomical tweezers, Petri dish glass, stainless-bottom wax surgical board, closed container, caliper, and millimeter block paper. The other tools include a rat cage, non-invasive CODA® instrument set, trays, scissors, labels, markers, syringes, measuring flask tubes, oral probes, and digital scales. The main ingredients used in this research are bidara leaf extract (*Ziziphus spina-christi*), aqua dest, 96% ethanol, NaCl, and dexamethasone for hypertension induction. Materials used in the operation of pregnant rats, namely: NaCl and chloroform.

Plant Extraction

The bidara leaf is mashed first using a blender so that they become dry bidara leaf powder. The dried bidara leaf powder was macerated with 96% ethanol solvent for ±3x24 hours, accompanied by occasional stirring. When the process has been completed, it continues with filtering. However, the residue is macerated again with 5L ethanol so that the maceration is carried out two times within ±2x24 hours, and proceed with the filtration process. The last stage is evaporation to remove the solvent so that only the compound or leaf extract is left by using a rotary evaporator at a temperature of 45-50ºC to produce extracts in the form of thick or paste dosage forms.[13]

In Vivo Procedure

Animal Preparation

The experimental animal used in this study was a white female rat Wistar strain. Rats were acclimatized for seven days, equipped with cages with wood husks, with a room temperature of 25-26ºC and 76-87% humidity.

Making Pregnant Rats

The process of making pregnant rats is carried out when female rats are in estrus. Initial management is to equalize the estrus period of female rats and use the pheromone method for three days by placing female rats in a typical cage with smell or urine previously occupied by male rats. On the third day of implementing the pheromone method, mating was carried out in the afternoon until the next day, sperm cells were found in the vaginal smear on microscopic examination. That day was declared as the first day of pregnancy.[17]

Making Rats with Hypertension Model

The research sample that had been determined was divided into an intervention group and a control group without intervention. The control group without intervention was a group of pregnant rats without being induced to have hypertension. In contrast, the intervention group was a group of hypertensive rats induced by dexamethasone 0.018mg given three times a day and 3ml of 8% NaCl a day given orally for seven days starting from day one until the 7th day of pregnancy to experience an increase in blood pressure to exceed normal blood pressure in rats.[18,19]

Experimental Procedure

Twenty-four rats that were declared pregnant based on microscopic examination were divided into three groups with induced hypertension using dexamethasone and NaCl, while 1 group without induced hypertension, namely six rats for the bidara leaf extract group at a dose of 200mg/kg BW, six rats for the bidara leaf extract group at a dose of 300mg/kg BW, six rats in the positive control group that only induced hypertension, and six rats in the negative control group without hypertension-induced and extract administration. Both experimental groups were given bidara leaf extract once a day for nine days with different doses of 200mg/kg BW and 300mg/kg BW.

The Variable, Instrument, and Measurement

The dependent variable in this study was systolic and diastolic blood pressure which was measured using a non-invasive CODA instrument. Measurements were carried out without anesthesia and only using the tail-cuff auto-pickup method, which was placed in the rat's tail to monitor blood pressure equipped with a Volume Pressure Recorder sensor. When administering blood pressure measurements, it should be noted that during the examination, the rats are placed on a warmer or heating board so that blood pressure can be measured accurately as well as its constituents by the VPR system.[20]

Statistical Analysis

Data analyzed with Manova test with a significant level at $p < 0.05$.

Ethical Consideration

This research has been registered with the Research Bioethics Commission of Sultan Agung Islamic University Semarang with the Ethical Clearance number 8/1/2021/Bioethics Commission.

RESULTS

Experiment dose 200mg/kg BW experienced a 12.3% decrease in systolic and 16.15% diastolic, while the Experiment dose 300mg/kg BW decreased systolic by 20% and diastolic by 27.73%. The decrease in systolic blood pressure experienced by the two experimental and control groups at the time of measurement is shown in Figure 1, and diastolic blood pressure is shown in Figure 2. Both experimental groups experienced a significant decrease in blood pressure on the 6th day for the experiment 300mg/kg BW dose group and on the 9th day for the experiment 200mg/kg BW dose group ($p<0.05$).

The effect of bidara leaf extract on reducing blood pressure in pregnant rats with hypertension was indicated by a decrease in systolic and diastolic in the experimental group and the difference in the value of the difference in decline between the experimental group and the control group. After rats were induced with hypertension using NaCl and dexamethasone, the systolic and diastolic values were higher than average blood pressure values. The mean value of the difference in blood pressure reduction between groups is shown in Table 1, which significantly differs between the two experimental groups and the two control groups ($p<0.05$).

Table 1. The Difference in The Mean Delta Value of Blood Pressure Reduction Between Groups

	Group		*Δpretest-posttest 3*		*Mean difference*	*p-value*	*R-squared*
Systolic	Experiment dose 200mg/kg BW	Experiment dose 300mg/kg BW	16.67	29.17	-12.500	0.136	0.603
		Positive control	16.67	-3.8	20.467	0.025	
		Negative control	16.67	-15.17	31.833	0.001	
	Experiment dose 300mg/kg BW	Positive control	29.17	-3.8	32.967	0.001	
		Negative control	29.17	-15.17	44.333	0.00001	
	Positive control	Negative control	-3.8	-15.17	11.367	0.193	
	Negative control	Experiment dose 200mg/kg BW	-15.17	16.67	-31.833	0.001	
		Experiment dose 300mg/kg BW	-15.17	29.17	-44.333	0.00001	
		Positive control	-15.17	-3.8	-11.367	0.193	
Diastolic	Experiment dose 200mg/kg BW	Experiment 300mg/kg BW	15.83	30	-14.167	0.160	0.457
		Positive control	15.83	-10.6	26.433	0.017	
		Negative control	15.83	-5.17	21.000	0.043	
	Experiment dose 300mg/kg BW	Positive control	30	-10.6	40.600	0.001	
		Negative control	30	-5.17	35.167	0.002	
	Positive control	Negative control	-10.6	-5.17	-5.433	0.599	
	Negative control	Experiment 200mg/kg BW	-5.17	15.83	-21.000	0.043	
		Experiment 300mg/kg BW	-5.17	30	-35.167	0.002	
		Positive control	-5.17	-10.6	5.433	0.599	

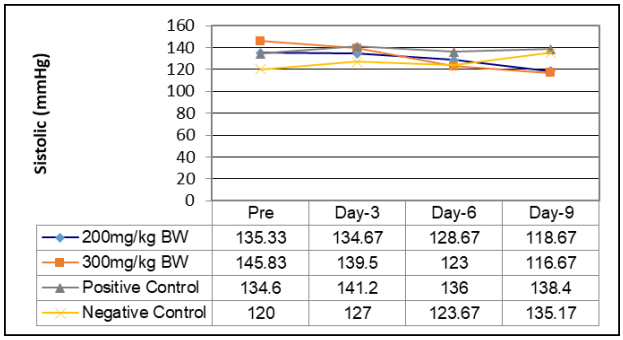

Figure 1. *Changes in Mean Systolic Blood Pressure*

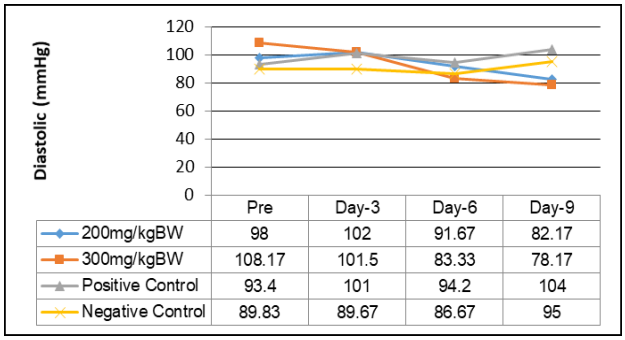

Figure 2. *Changes in Mean Diastolic Blood Pressure*

DISCUSSION

This study found the more significant effect of bidara leaf extracts on systolic blood pressure than diastolic, due to the significant macro minerals such as potassium and magnesium. The content of potassium in bidara leaf plays a role in controlling cardiac output, which is closely related to the cause of changes in systolic, as well as the presence of magnesium in bidara leaf, which plays a role in maintaining heart rhythm so that it remains in normal condition, improving blood flow to the heart while providing a sedative effect. However, to reduce diastolic, there is only the role of flavonoid active compounds that can affect the work of Angiotensin-Converting Enzyme. It triggers vasodilation, which will reduce peripheral decrease diastolic resistance.[21]

The magnitude of the effect of giving bidara leaf extract was also assessed through the finding of a significant interaction between group associations and the period of giving the extract with a p-value of 0.0001 for systolic and 0.006 for diastolic so that it can be interpreted that bidara leaf extract was given in various doses with the time of administration. The various extracts, namely the 3rd day, the sixth day, and the ninth day simultaneously (together), contributed to the decrease in blood pressure.

Hypertension consists of various classifications. A systolic blood pressure of 130-139mmHg or a diastolic of 80-89mmHg has been included as the first hypertension stage. The blood pressure has increased with a systolic value of 140mmHg, or a diastolic 90mmHg has been included as a second stage hypertension.[22] Hypertension is caused by many factors and is complex due to neurohormonal factors. Oxidative stress plays an essential role in the pathophysiology of hypertension. It causes endothelial dysfunction that affects reduced nitric oxide levels, thereby inhibiting the endothelial relaxation process and resulting in hypertension.[2–5] Oxidative stress is defined as an imbalance between the production of free radicals and the ineffective use of antioxidants in the body, thus triggering cell damage that results in diseases, including hypertension. The severity of endothelial damage due to excessive oxidative stress will affect lower nitric oxide production so that it interferes with vasodilation which results in severe hypertension.[23]

In this study, the experimental group given bidara leaf extract also experienced a decrease in blood pressure. Experiment 200mg/kg BW had a decrease in systolic blood pressure of 12.3% (16.67mmHg) and a decrease in diastolic blood pressure of 16.32% (15.83mmHg). Experiment 300mg/kg BW had a percentage decrease in systolic blood pressure reaching 19.99% (29.17mmHg), and 27.73% (30mmHg) for diastolic. Other than that, the experimental group did not have a difference in blood pressure reduction on the sixth and ninth days during the consumption period of bidara leaf extract. However, the treatment was continued until the ninth day to decrease blood pressure until it reached the normal classification. The experimental group which was included as the hypertension induction group experienced a decrease in blood pressure because bidara leaf with varying doses, namely experiment 200mg/kg BW and experiment 300mg/kg BW, had more macromineral content such as potassium (673mg%), calcium (1270mg%), and magnesium (169 mg%) as well as flavonoid bioactive compounds (9.6±1.2mgQE/g DW) which play an important role in controlling blood pressure. Bidara leaf also has antioxidant activity with an IC50 value of 23.4 ppm, categorized as having robust activity.[10–12]

The content contained in the bidara leaf has its respective role in lowering blood pressure. The macro-mineral content is thought to lower blood pressure through vasodilation which causes a decrease in total peripheral retention and increases cardiac output. Adequate calcium intake can affect the concentration of extracellular calcium, which plays a vital role in endothelium synthesis to repair physiological damage caused by oxidative stress. In addition to calcium, magnesium also increases nitric oxide and improves endothelial function.[24,25] Potassium which is in charge of changing the activity of renin-angiotensin and together with calcium, can affect the level of smooth muscle contraction so that vasodilation occurs and results in a decrease in blood pressure; magnesium and flavonoids, which act as ACE inhibitors and antioxidants which are tasked with suppressing oxidative stress thereby reducing continuous damage to the endothelium as well resulting in

increased nitric oxide synthesis which acts as a potent vasodilator in relaxing blood vessels and lowering blood pressure, besides that the fulfillment of potassium, calcium, and magnesium can also suppress sodium concentrations in overcoming mineral imbalances so that it can affect arterial pressure.

The experimental and positive control groups were pregnant rats induced by hypertension using dexamethasone to decrease nitric oxide production while disrupting endothelial cell function and causing an increase in blood pressure while inhibiting intrauterine fetal growth. Compared with the positive control group, the experimental group was known to have significant differences because the experimental group was given treatment using bidara leaf extract, in contrast to the positive control group, which after induced hypertension but was not given treatment so that they had unstable blood pressure. This underlies why there is no significant difference in blood pressure values between experiment 200mg/kg BW and experiment 300mg/kg BW. Both doses could reduce blood pressure until it reached the standard blood pressure classification. Researchers reviewed in terms of the time of administration, and it was found that the bidara leaf extract at a dose of 300mg/kg BW could significantly reduce blood pressure on day 6, in contrast, to experiment 200mg/kg BW which experienced a significant decrease in blood pressure on day 9.

The results of this study are in line with research conducted that assessed that the active flavonoid content in the leaf of the *Ziziphus mauritiana* given was considered to prevent an increase in blood pressure and improve glomerulosclerosis that occurs due to hypertension.[26] This study is also in line with the other research that showed that *Ziziphus spina-christi* extract has an anxiolytic activity that can treat anxiety disorders associated with oxidative stress modulation. It can also affect serum malondialdehyde levels.[27] The difference between this study and previous research related to bidara leaf is that the research subject is devoted to being pregnant, affecting the duration of hypertension induction and extract administration. In contrast, previous studies were male rats with 28 days of extract administration. No research on bidara (*Ziziphus spina-christi*) explicitly discusses its effect on systolic and diastolic blood pressure.

Negative control group rats in this study that were not induced by hypertension and were not given extracts tended to be more challenging to adapt to the CODA space so that even though they had been given time to adapt, they still had unstable blood pressure due to modulation of oxidative stress during pregnancy, in contrast to rat in the group experiments that tend to be calmer and do not fight, which is thought to be due to the bidara leaf extract that is given besides affecting blood pressure also has an anxiolytic activity that can treat other variables such as anxiety disorders experienced by rats.

This study did not examine teratogenicity by assessing the number of fetuses and fetal weight. At the time of surgery, the rats found that not all 24 rats had developing fetuses, and many of them had stunted growth and were suspected of having implantation failure. Previous studies have stated that there is a 70% chance of embryo failure to implant or implantation failure, which clinically even though the results of a pregnancy test through the examination of human chorionic gonadotropin (HCG) in the urine are positive, but the process can fail before the formation of the intrauterine gestational sac.[28] Different studies also assessed that implantation success in rats occurred in a short period starting from day 4 to day 6 of pregnancy, marked by points suspected as implantation sites.[29]

One of the causes of disruption of the pregnancy process and fetal growth inhibition in this study was the induction of hypertension through the administration of dexamethasone. Dexamethasone causes an increase in nitric oxide synthase trafficking inducer (NOSTRIN) protein to weaken nitric oxide production, which is found in the placenta of women with preeclampsia and gestational hypertension. Thus, affecting endothelial cell function and causing intrauterine growth restriction.[18]

CONCLUSIONS AND RECOMMENDATION

Bidara leaf extract can reduce blood pressure in various doses, especially the dose of 300mg/kg BW, which has decreased significantly on the sixth day. Researchers suggest that this study can further research, such as histopathological tests on the uterus.

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