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ANTIHYPERTENSIVE ACTIVITY OF SEPAT LEAF ETHANOL EXTRACT (*MITRAGYNA SPECIOSA* KORTH.) ON WHITE RAT

Elis Susilawati[1*], Muhamad Reza Pahlevi[1], I Ketut Adnyana[2]

[1]Pharmacology Research Group, Faculty of Pharmacy, Bhakti Kencana University, Bandung, West Java, 40161, Indonesia.
[2]Pharmacy School, Bandung Institute of Technology, Jalan Ganesha 10 Bandung, West Java, 40132, Indonesia.

*Corresponding author E-mail: **elis.susilawati@stfb.ac.id**

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ABSTRACT

Empirically, sepat leaf is used toreduce blood pressure. Therefore research will be scientifically tested by in vivo method.The purpose of this research is to prove that sepat leaf ethanol extract has an effect to reduce blood pressure. In this research using 2 methods namely diuretic method and blood pressure measurement using CODA®. In the diuretic method using 25 rats grouped into 5 groups, each group was given 0.9% NaCl and each of them were given Na CMC 0.5%, furosemide 3.6 mg/KgBW, sepat leaf extract doses of 25, 50 and 100 mg/KgBW. Parameters which were seen was urine volume for 8 hours and 24 hours. In the CODA® method, 30 rats were grouped into 6 groups which was given concurrent between therapy and 25% fructose induction in drinks for 21 days. The therapy was given captopril 2.5 mg/KgBW, sepat leaf ethanol extract with doses of 25, 50 and 100 mg/KgBW.The parameters those measured were systolic and diastolic blood pressure. The results of the sepat leaf extract study dose of 50 mg/KgBW can increase urine volume and can reduce blood pressure by the CODA® method. The conclusion from this research is that sepat leaf ethanol extract has antihypertensive activity.

INTRODUCTION

Hypertension is a disease known by almost all people and become a heart disease that can cause death (Michael *et al*., 2014). More appreshensive, hypertension is now called"*silent killer*", because it wasas a deadly diseases without early symptoms, and if the symptoms are felt as intended, it is often considered a normal disorder, this certainly causes sufferers to be too late to realize the presence of hypertension. Hypertension has a high prevalence rate in the world, which is around 1.3 billion suffer from hypertension and that number is increasing every year. It is predicted, thatin 2025 there will be 1.5 billion people affected by hypertension (WHO, 2015).The highest prevalence of hypertension in Indonesia are in Bangka Belitung (30.9%), South Kalimantan (30.8%), East Kalimantan (29.6%) and West Java (29.4%) (Riskesdas, 2013).The high rate of hypertension in Indonesia has been a trigger for researchers to find new breakthroughs on antihypertensive drugs by utilizing natural resources in Indonesia. One of the islands in Indonesia that rich in natural ingredients is in the Borneo island.One of the plants that popularly used by the surrounding community is sepat or kratom which has the Latin name *Mitragyna speciosa* Korth..The plant grows a lot in Kalimantan and it is believed by the surrounding community to

overcome several diseases such as diabetes mellitus, hypertension and wound medicine. In addition, leaf sepat is also used as a drug in several other regions and countries used as medicine for muscle fatigue, diarrhea, coughing, muscle pain, diabetes and hypertension (Srichana *et al*., 2015).Sepat plants contain alkaloids, glycosides, terpenoids, flavonoids and saponins (Rinaldi *et al*., 2017). Sepat plant activities have been widely studied, but until now there has been no scientific research that proves to be antihypertensive.The community used these plants by chewing the leaves of sepat 3-10 sheets 1 day. The use of leaf sepat with more than 10 pieces can cause mild addiction (Pozialeck, 2012).

MATERIAL AND METHODS

Material: The materials used in this study were leaf sepat (*Mitragyna speciosa* Korth.), fructose 25%, captopril, furosemide, aquadest, reagent mayer, dragendroff, ethanol 96%, HCl P, Mg powder, ethanol, $FeCl_3$ 0.3%, NaOH 1 N, chloroform, H_2SO_4 P, Na CMC, physiological NaCl 0.9%, HCl 2 N, toluene, ammonia 25%, HCl 10%, amyl alcohol, gelatin 2%, ether and anhydrous acetic acid.

Extraction: The sepat leaves that we used were obtained from Muara Baruh Village, North Amuntai District, North Hulu Sungai Regency, South Kalimantan Province. To ascertain the truth of these plants, determination was made at the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru.The results of the determination with the number 164d/LB.LABDASAR/XII/2018 indicate that the plants used in this study are true leaf sepat with the Latin name *Mitragyna speciosa* Korth..A total of 700 grams of dried leaf simplicia powder were macerated with ethanol 96% solvent at room temperature for 3 days with solvent replacement every 1 x 24 hours, 5000 mL in the first day and 3600 mL for the second and third days.The maserate was then concentrated with an evaporator at 60^{o}C, followed by a water bath until thick extract was obtained.

Animal: The experimental animals that we used were 30 male wistar rats with a weight of 200-300 grams at the age of 2 months (Hasimun *et al*., 2017). Test animals were obtained from D'Wistar, Majalaya, Bandung, West Java, Indonesia.All animals are placed and treated according to ethical guidelines that have been approved by the research ethics commission of Padjadjaran University Bandung with letter number 28 / UN6.KEP / EC / 2019.Before the experiment, mice were adapted 14 days and fed standardly and drank water ad libitum.

Diuretic test: Test animals were divided into 5 groups, each group divided into 5 animals.All rats in the experimental group were given physiological NaCl 0.9% as much as 20 mL/KgBW orally (Yulinah, 2015). Group 1 was given Na CMC 0.5% 10 mL/KgBW, Group 2 was given a suspension of furosemide 3.6 mg/KgBW, Group 3 was given 25 mg/KgBW sepat leaf extract, group 4 was given 50 mg/KgBW sepat leaf extract, group 5 given sepat leaf extract 100 mg/KgBW.Urine volume was observed from 30, 60, 120, 180, 240, 300, 360, 420, 480 and 1440 (Wardani & Andrianta, 2016).

Antihypertensive: Antihypertensive tested in vivo with non-invasive methods. The measurement of systolic and diastolic blood pressure used a CODA® device. Test animals were divided into 6 groups, each group divided into 5 animals.Five groups of test animals were given 25% fructose simultaneously in drinks with captopril or sepat leaf extract for 21 days according to their respective groups, except in group 1 only 0.5% Na CMC was given. Measurements were made on days 0, 7, 14 and 21.

Data analysis: The obtained data were analyzed using the SPSS application with the One Way Anova method. To see the differences between groups using the LSD test with a confidence level of 95%.

RESULT AND DISCUSSION

Extraction: The thick extract obtained as much as 200 grams with% yield is 28.57%. In the Novindriana (2014) study, thick extracts were obtained with% yield, which was 31.14%.

Calculation of rendemen:

$$\frac{The\ weight\ of\ the\ thick\ extract\ obtained}{heavy\ simplicia} x\ 100\%$$

$$= \frac{200\ gram}{700\ gram}\ x\ 100\% = 28.57\%$$

Simplicia Characterization: The results of the simplicia characterization of sepat leaf plants (*Mitragyna speciosa* Korth.) Can be seen in Table 1.

Phytochemical Screening: The results of phytochemical screening of sepat leaf plants (*Mitragyna speciosa* Korth.) Can be seen in Table 2.

Diuretic Activity Test: A diuretic test was conducted to determine whether sepat leaf extract has a diuresis effect, because the diuresis process affects blood pressure by removing ions that play a role in increasing / decreasing blood pressure. This test uses urine volume parameters observed for 8 hours and 24 hours.Observations for 8 hours due to the duration of work of furosemide is 6-8 hours and observations for 24 hours aimed at finding out the effect of diuresis on extracts had a longer duration than the comparison group namely furosemide. The results of urine volume obtained during the test can be seen in Figure 1. From these results it can be seen that the comparison group and extract group had more urine volume than the normal group for 24 hours.The first test of sepat leaf extract dose 25 mg/KgBW had a diuresis effect, but it was not as good as the comparison of furosemide dose of 3.6 mg/KgBW. Test II, namely sepat leaf extract dose of 50 mg/KgBW also had a better diuresis effect compared to sepat leaf extract dosage of 25 mg/KgBW, but it was not as good as the comparison of 3.6 mg/KgBW furosemide dose.Test III, namely sepat leaf extract dosage of 100 mg/KgBW had a better diuresis effect than leaf extract sepat dose of 25 mg/KgBW and 50 mg/KgBW and had the strength of diuresis effect which was similar to the comparison of furosemide dose 3.6 mg/KgBW.To make it more convincing about the relationship between leaf sepat extract as a diuretic effect, an analysis using SPSS One Way Anova and Post Hoc LSD Test was conducted. In ANOVA testing, the data must be distributed evenly with a significant value (P>0.05).The data used in ANOVA has a significant value of 0.157, meaning that the value is more than 0.05. This shows that the data is evenly distributed so that One Way Anova can be tested.The ANOVA test results using the F test, obtained F count value 39.430 with a significant value of 0.000. Decision making is based on the ratio of F count and F table, if F count is smaller than F table (F count <Ftable) then H_0 is accepted and if F count is greater than F table (F count> F table) then H_1 is accepted.From the ANOVA, the calculated F value is greater than F table (39.430> 2.87) then H_1 is accepted and H_0 is rejected. This shows that there is a correlation between leaf ethanol extract and diuretic effect.

Testing systolic blood pressure with CODA® method: Testing of antihypertensive activity using the CODA® tool was carried out for 21 days. This test uses experimental animals namely male white wistar rats which were weigh more than 200 g of 25% fructose induced in drinks.Blood pressure measurements were carried out 4 times namely 0, 7, 14 and 21 days. CODA® devices use a Volume Pressure Recording (VPR) sensor. VPR uses a volumetric method so that it can measure blood volume and blood flow in the tail non-invasively.VPR can measure 6 parameters simultaneously, namely systolic blood pressure, diastolic blood pressure, average blood pressure, heart rate, tail blood volume and tail blood flow (Malkoff, 2015). This test is preventive, where the administration between induction and therapy is carried out simultaneously. This test is divided into 6 groups, where each group has 5 test animals. The results of systolic blood pressure can be seen in Table 3. The results of the test data

were analyzed using SPSS One Way Anova based on the homogeneity test with a value ($p>0.05$). The statistical results show the value of homogeneity on days 0, 7, 14 and 21 values ($p>0.05$), meaning that the data is distributed homogeneously and can be continued with One Way Anova testing. Based on the One Way Anova analysis at T_0, the significant value was more than 0.05 ($p> 0.05$), indicating that there was no effect of a decrease in systolic blood pressure that occurred because T_0 had not been treated. At T_7, T_{14} and T_{21} the significant values obtained were below 0.05 ($p<0.05$), indicating that there was an effect of decreasing systolic blood pressure on days 7, 14 and 21.

Testing diastolic blood pressure with CODA® method: In diastolic blood pressure testing is the same as testing systolic blood pressure. Diastolic blood pressure testing using test animals wistar strain white rats with weights of more than 200 g were given simultaneously between therapy (extract / drug) and fructose induction with a concentration of 25% in drinks for 21 days.Blood pressure measurements using CODA® were performed on days 0, 7, 14 and 21. The results of diastolic blood pressure of all treatment groups for 21 days can be seen in Table 4. The results of the test data were analyzed using SPSS One Way Anova based on homogeneity test with a value ($p>0.05$). The statistical results show the value of homogeneity obtained is greater than 0.05 ($p>0.05$).This shows that the data can be received/accepted and continued to One Way Anova. Based on the One Way Anova analysis at T0, the significant value was more than 0.05 ($p>0.05$), indicates that there was no effect of a decrease in diastolic blood pressure that occurred because at T_0, no treatment had been given. At T_7, T_{14} and T_{21}, the significant values were obtained which were below 0.05 ($p<0.05$), indicating that there was an effect of decreasing diastolic blood pressure on days 7, 14 and 21.The application fructose 25% in drinks given to test animals has been shown to increase systolic and diastolic blood pressure. Drinks given to test animals has been shown to increase systolic and diastolic blood pressure.The mechanism of fructose in increasing blood pressure is by increasing plasma uric acid levels so that inflammation occurs and eventually increases blood pressure. In addition, fructose can also stimulate the sympathetic nervous system, affect the renin angiotensin system, reduce NO levels and stimulate oxidative stress which ultimately increases blood pressure (Desmawati, 2017).A leaf sepat that tested commonly can prevent blood pressure from rising at a dose of 25 mg/KgBW, 50 mg/KgBW and 100 mg/KgBW. The class of compounds that are thought to be responsible for lowering blood pressure in leaf sepat are flavonoids, alkaloids and saponins.According to Loizzo *et al* (2007) the mechanism of flavonoid compounds in reducing blood pressure by influencing the work of the ACE enzyme (Angiotensin Converting enzyme) Inhibition of ACE will inhibit changes in angiotensin I to angiotensin II, which causes vasodilation so that resistance to peripheral resistance decreased and can lower blood pressure.According to Tengo *et al* (2013) the mechanism of the alkaloid compound in reducing blood pressure is similar to β-blocker antihypertensive drugs, namely negative and chronotropic negative inotropic so that the decrease in cardiac output, decreased heart rate and lack of contraction strength of the myocardium.According to De Souza *et al* (2004) the mechanism of the group of saponin compounds in reducing blood pressure is the occurrence of a diuresis process. Saponins reduce plasma volume by removing water and electrolytes, especially sodium, so that ultimately cardiac output decreases.

CONCLUSION

Based on the research that has been done, it can be concluded that the leaf ethanol extract has antihypertensive activity. At a dose of 50 mg/KgBW of sepat leaf extract can increase urine volume by a diuretic method and can reduce blood pressure by the CODA® method.

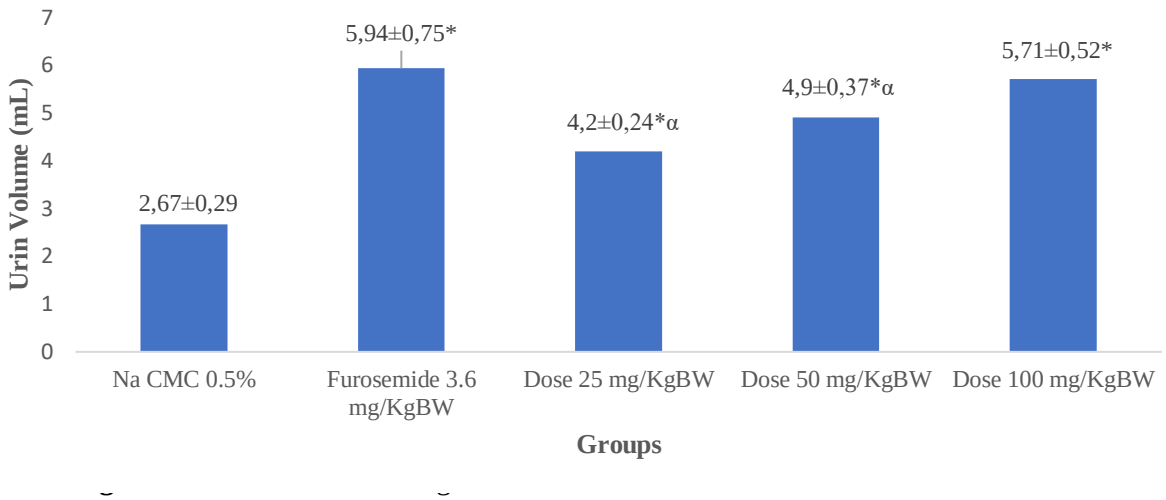

Fig 1: Result of urine accumulation for all treatments groups for 24 h

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

Table 1: Results of Characterization of Sepat Leaf Simplicia

Characterization	Grade (%)	Lestari (2018) (%)
Total Powder Ash	5	-
Water Rate	8	10.47
Drying Shrinkage	15.11	10.35
Water Soluble Extract Rate	10	1.1
Ethanol Soluble Extract Rate	16	1.7

Class of Compounds	Simplicia	Extract	Novindriana (2014)
Alkaloid	(+)	(+)	(+)
Flavonoid	(+)	(+)	(+)
Quinone	(+)	(+)	(-)
Phenol	(+)	(+)	(+)
Saponin	(+)	(+)	(+)
Triterpenoids	(+)	(+)	(+)

Table 2: Results of Phytochemical Screening of Sepat Leaf

(-) : Not Identfied (+) : Identfied

Table 3: Result ofAverage Systolic Blood Pressure (mmHg)±SD on Day 0, 7, 14 dan 21

Groups	Average Systolic Blood Pressure (mmHg) ± SD			
	T_0	T_7	T_{14}	T_{21}
Na CMC 0.5%	108.80±1.30	109.60±1.14*β	111.00±1.41*	111.00±1.22*β
Fructose 25%	108.80±1.92	127.00±1.87αβ	146.20±2.49αβ	170.00±2.55αβ
Captorpil 2.5 mg/KgBW	109.00±3.00	116.00±2.74*α	113.20±1.30*	110.60±1.14*α
Dose 25 mg/KgBW	109.20±2.39	116.20±2.77*α	122.20±2.59*αβ	118.40±1.82*β
Dose 50 mg/KgBW	108.80±2.39	112.40±1.82*β	115.60±1.52*α	110.60±1.52*α
Dose 100 mg/KgBW	109.00±2.24	110.40±2.51*β	106.20±3.11*αβ	103.00±2.91*αβ

α : There were significant differences compared to normal groups ($p<0.05$), * : There were significant differences compared to positive control groups ($p<0.05$), β : There were significant differences compared to captopril groups ($p<0.05$).

Groups	Average Diastolic Blood Pressure (mmHg) ± SD			
	T_0	T_7	T_{14}	T_{21}
Na CMC 0.5%	77.40±2.19	79.60±2.07*	80.20±2.17*	80.40±1.52*β
Fructose 25%	76.00±3.39	87.40±1.95α	94.00±2.74αβ	101.20±1.92αβ
Captorpil 2.5 mg/KgBW	78.00±1.87	80.60±2.30*	79.00±2.74*	76.60±1.67*α
Dose 25 mg/KgBW	77.20±2.17	82.60±2.61*α	86.00±1.58*αβ	82.40±1.82*β
Dose 50 mg/KgBW	77.80±1.92	80.20±1.92*	82.40±1.82*β	76.60±1.67*α
Dose 100 mg/KgBW	77.40±2.07	78.60±2.30*	74.60±1.67*αβ	70.00±2.00*αβ

Table 4: Result Average Diastolic Blood Pressure (mmHg)±SD on Day0, 7, 14 dan 21

α : There were significant differences compared to normal groups ($p<0.05$), * : There were significant differences compared to positive control groups ($p<0.05$), β : There were significant differences compared to captopril groups ($p<0.05$).

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