e-ISSN : 2621-9468

Type of the Paper (Research Article)

Study in vitro and in silico on effectiveness noni fruit extract (*Morinda Citrifolia*) to reducing hypertension

La Ode Abdul Haris Hijriansyah[1*], Hermilasari [2], Hardyanty Subair[3], Irianto[4], Andi Alief Utama Armyn[5], Supartina Hakim[6]

[1] Undergraduate Student, Program Study of Physical Therapy, Faculty of Nursing, Hasanuddin University, Indonesia
[2] Fresh graduate Student, Program Study of Physical Therapy, Faculty of Nursing, Hasanuddin University, Indonesia
[3] Fresh graduate Student, Program Study of Nutrition, Faculty of Public Health, Hasanuddin University, Indonesia
[4] Lecturer, Program Study Physical Therapy, Faculty of Nursing, Hasanuddin University, Indonesia
[5] Lecturer, Program Study of Medical Doctor, Faculty of Medicine, Hasanuddin University, Indonesia
[6] Master Student, Faculty of Physical Therapy, Mahidol University, Thailand

Abstract

Hypertension is one of the major causes of stroke. Stroke can be prevented by controlling hypertension. Noni fruit proved to have antihypertensive effect. Noni Fruit contains scopoletin and xeronin compounds that play a role in antihypertensives. This study aims to determine the effectiveness of noni fruit extract to controlling hypertension. The research method used is pre-test and post-test matched control group. The 6 Wistar rats were divided into 3 groups consisting of 1 treatment group and 2 control groups. Group P1 was induced by using ketamine 0,05 ml + epinephrine 0,2 ml + 6 ml noni fruit extract, group K (-) induced by ketamine 0,05 ml + epinephrine 0,2 ml without extract, and group K (+) induced by using ketamine 0,05 ml + epinephrine 0,2 ml + captopril 2,5 mg. The results showed that epinephrine can be used as a hypertensive inducer. Noni fruit extract as much as 6ml can provide antihypertensive effects. In Vitro, test result showed that noni fruit extract can reduce blood pressure by an average decrease in blood pressure of 58,5 mmHg While captopril 25,5 mmHg. in addition to the in vitro test, the results of the in-silico test showed that the noni fruit extract can significantly reduce blood pressure compared to anti-hypertensive drugs (captopril). the value of scopoletin in noni fruit is -7,6. and captopril only -5,7.

Article History
Received June 06, 2020
Accepted December 12, 2020

Keyword
Hypertension,
Noni fruit extract,
In Silico,
In Vitro,

1. Introduction

WHO (World Health Organization) suggests that hypertension occurs when a person's condition has systolic blood pressure equal to or above 160 mmHg and/or a diastolic blood pressure equal to or above 90 mmHg consistently over time(1). WHO reported in 2011 that about 972 million people or 26,4% of the world's population lives suffered from hypertension and it will reach to 29,2% in 2025 (2). Indonesia Basic Health Research (Riskesdas) in 2013 revealed that the prevalence of hypertension among people aged more than 18 years was 25,8%. Based on the Riskesdas in 2013, the percentage of hypertension in South Sulawesi was 28,1%. Riskesdas found that the prevalence of stroke in Indonesia from 8,3 % in 2007 increased to 12,1% in 2013. While, South Sulawesi was the highest region in the prevalence of stroke with 17,9% in 2013.

* Correspondence : **La Ode Abdul Haris Hijriansyah** harishijriansyah@gmail.com

ISSN 2621-9468 online, DOI 10.20956/canrea.v3i2.329

Indonesian Stroke Foundation recorded that among countries in Asia, Indonesia has the highest number of prevalence stroke and is still increasing. It was also reported that the prevalence of stroke becomes the second rank case with people age above 60 years and fifth rank with the age of 15-59 years(3). Hypertension is a major cause of stroke. Based on the previous study, people with hypertension have 2 times more at risk of stroke. Sorganvi et al in 2014 also reported that hypertension increases the risk of stroke in 3,8 times (4) .

As mentioned above, hypertension requires more attention in treatment because it is one of the most important factors cause of stroke. Therefore, to minimize the increased prevalence of stroke can be done by controlling hypertension. Generally, most people use chemical drugs in reducing hypertension.

Chemical drugs have several disadvantages that impact negative side effects for the body. Many studies already conducted to search chemical drugs with better effectiveness with minimal side effects through traditional medicine, for example, used noni and cucumber fruits. Previous studies showed that noni juice can reduce hypertension (5). The results found that a significant decrease in blood pressure used noni juice. However, the limitation of previous studies can trigger a vomiting response from respondents due to the digestive system reaction with the taste and sting smell of noni fruit. In addition, it is not a known appropriate dosage of noni fruit than can decrease blood pressure. Therefore, this study aims to compare the effectiveness of noni extract in reducing hypertension through In Vitro and In Silico studies.

2. Materials and Methods

2.1 Tools

Tools and materials used scales, 1 ml spoit, blood pressure analyzer, blood beaker, test animal cages, animal scales, stirring rods, aluminum foil, filter paper selectron BA 85 type, rotary evaporator, spatula lab, simplicial oven, cup porcelain, glass funnel, jar simplicial,

2.2 Materials

Noni fruit, epinephrine medicine, water pro injection, captopril, aquades, and ketamine.

2.3 Procedure

2.3.1 Making Extract

Noni fruit was dried used a simplicial oven with temperature 55^0 C for 2-3 hours. After drying, simplicial was mashed. After simplicial was mashed then maceration that used 70 % ethanol about 1000 ml (1 liter) for 5 days. The ratio of ethanol and simplicial is 1 : 1 (1 liter for 1 kg of simplicial). After that, simplicial was filtered used filter paper 2 times. The filter paper used is selectron BA 85 type with a pore diameter of 0,45 μm. Then, the solvent contained active substance with a used rotary evaporator at a pressure of 20 Psi and 120 rpm with temperature of 50^0 C, so that a thick extract was produced as much 10 gram. Then, the thick extract was dissolved using aquades 50 ml.

2.3.2 Selection and preparation of animal testing

Animal testing used wistar strain rats aged 2-3 months with body weight >150 grams. 6 rats were used and divided into 3 treatment groups. One control group (-) and (+) has 2

rats in each and treatment group consisted of 2 rats. Control group (-) only hypertension will be made and will not be treated with extracts or captopril. While control group (+) will be treated with captopril 2,5 mg.

2.3.3 Treatment of animal testing

Has been approved by the Ethics Commission section of Hasanuddin University Faculty of Medicine for treatment of tested animals. Before treatment, animal testing was given standard food consumption and drank water for 4 weeks to maximize their body weight >150 grams. Then, all animal testing was grouped and induced by ketamine about 0.05 ml and epinephrine 0,2 ml for a day. in one day the treatment was carried out 2 times injection (1 injection of ketamine and 1 time injection of epinephrine).

Treatments for animal testing have been divided into 3 groups as follow;

P1 : Induced with ketamine 0,05 ml + epinephrine 0,2 ml + 6 ml noni extract.
K (-) : Induced with ketamine 0,05 ml + epinephrine 0,2 ml without extract.
K (+) : Induced with ketamine 0,05 ml + epinephrine 0,2 ml + captopril 2,5 mg

Giving ketamine and epinephrine aims to increase hypertensive in wistar rats during anesthetized state that will facilitate measurement of blood pressure. After induced ketamine and epinephrine, 20 minutes later blood pressure was measured by a blood pressure analyzer. Then, wistar rats was induced by noni extract and 20 minutes later, the blood pressure was measured again.

2.3.4 Measurement of rat blood pressure

Measurement of rat blood pressure was used blood pressure analyzer with tail-cuff method. This tool can show the results of blood pressure measurements through a monitor device.

2.3.5 In Silico Test of Active Compounds

In silico test can be used to find out interaction between a compound and the target cell protein as a receptor. Interaction of compounds with receptors can be visualized by computational methods with PyRx and PyMol that can be used to determine the pharmacophore of a compound(6). The virtual screening process was used to find compounds that were most potential to become drugs, in case the compounds contained noni extracts. The target in the in silico test were scopoletin compounds in noni fruit and captopril drugs against protein NOS3 Which play a role regulating blood pressure in blood vessels.

3. Results and Discussion

3.1 Group K- (without extract)

Rats negative control group (K-) was made hypertension during anesthetized state without extract. Rats initial blood pressure in K- group was 101/71 mmHg and 115/100 mmHg. After induced with 0,05 ml ketamine and 0,2 ml epinephrine, the blood pressure reaches 178/121 mmHg and 193/140 mmHg. After 20 minutes, final blood pressure was measured. The results showed that rats in group K- (without extract) increase blood pressure consistently. Last blood pressure was 179/119 and 192/138 mmHg.

3.2 Group K + (2.5 mg captopril)

Before group K+ was made hypertensive, Rats initial blood pressure was 124/78 mmHg and 130/85 mmHg. After induced with 0,05 ml ketamine and 0,2 ml epinephrine, rat blood pressure increased to 195/118 mmHg and 188/120 mmHg. 20 minutes later, rats with hypertension were given captopril. Then after 20 minutes, blood pressure was measured. Rats blood pressure dropped to 171/102 and 161/100 mmHg. However, rats blood pressure still showed hypertension, but captopril can still reduce blood pressure. It can be seen that blood pressure significantly decreases when epinephrine was induced and after given 25,5 mmHg captopril.

3.3 Group P1 (with Noni Extract)

Before rats in group p1 were made hypertension, rats initial blood pressure was 127/93 mmHg and 104/81 mmHg. After induced with 0,05 ml ketamine and 0,2 ml epinephrine, rats blood pressure raised to 190/143 mmHg and 181/131 mmHg. After induced with epinephrine, 20 minutes later rats blood pressure was measured. Rats with hypertension were given a solution of 6 ml of noni extract orally. Blood pressure was measured after 20 minutes. The results showed that rats blood pressure decreased to 130/80 mmHg and 124/68 mmHg. This condition showed that noni extract can significantly decrease systole blood pressure as well as reduce hypertension. The average decrease in systole blood pressure after given noni extract was 58,5 mmHg.

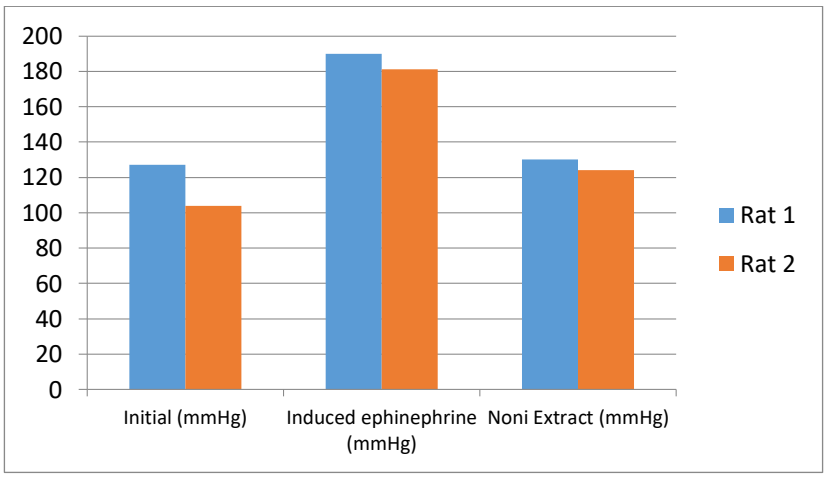

Figure 1. Comparison rats systole blood pressure in group K-

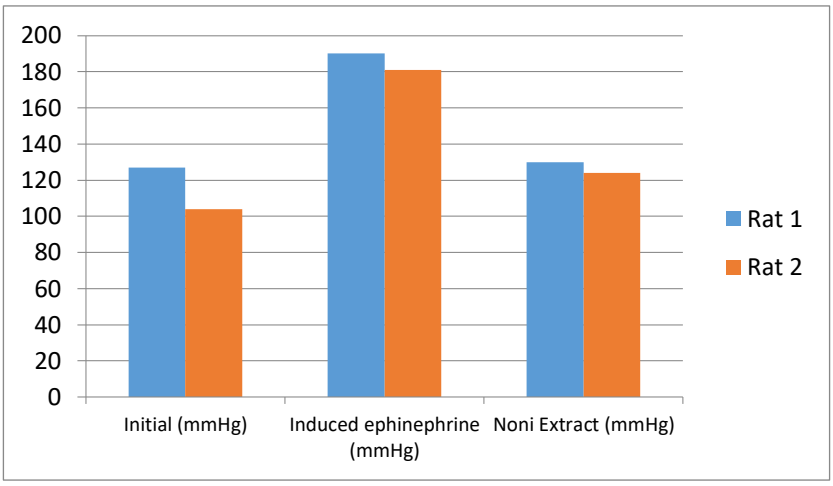

Figure 2. Comparison rats systole blood pressure in group K+

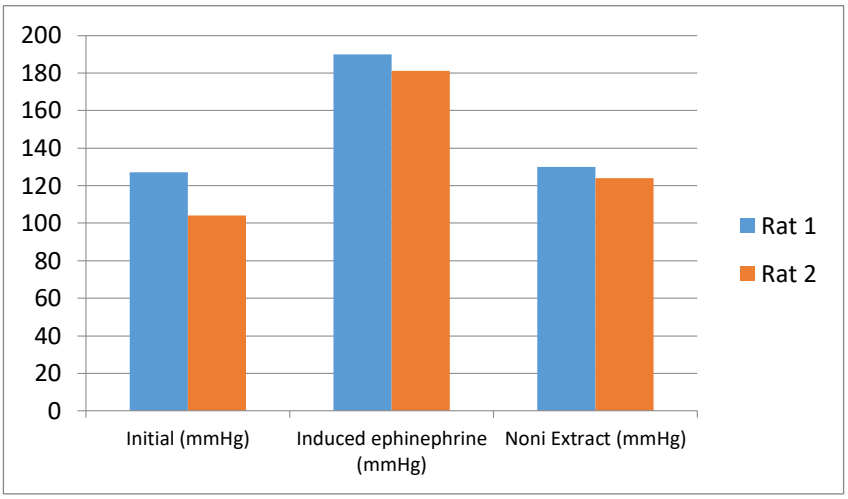

Figure 3. Comparison rats systole blood pressure in group P1

Noni is an annual plant (perennial) in the form of shrubs that can live in various places such as fields, beaches, and forests(7). Noni fruit has many benefits, reduce hypertension is one of benefits of noni (8)

Noni fruit contains scopoletin and xeronine that have potential to reduce hypertension. Scopoletin is one of the substances in noni that can bind serotonin which is one of the important chemicals in the human body. Noni fruit contains a type of phytonutrients, namely scopoletin. Scopoletin has a function to widen narrowed blood vessels and also can increase MDA levels in the body with increasing SOD activity in the body(8) . Not only scopoletin, xeronine also was in noni fruit. Consumption of noni juice will help supply xeronine hormone(9). Xeronine can increase blood vessel permeability to potassium ions. Experts from Standford University, Hawai University, University of California (UCLA), Union College of London, University of meets in France found that noni is a plant that plays a role in lowering blood pressure. Noni fruit can cure various diseases such as hypertension that contains prexeronin that can work on endothelial vasoactive, therefore can reduce blood pressure. Active ingredients scopeletin noni has function to normalize pressure blood in the presence of a spasmolytic effect. The spasmolytic effect is characterized by there is dilation of blood vessels (vasodilation) due to muscle relaxation innocently, the effect is similar in manner antihypertensive drug action. Effect as

antihipotent indicated by inhibits inducible nitric oxide synthase (iNOS), which will inhibits the formation of nitric oxide (NO) because NO has an effect vasodilation (10).

To strengthen the results of in vitro tests, in silico tests were also carried out through computerized modeling methods to see the activity of active compounds contained in noni extracts that had been made.

Tabel 1. Comparison Value of Binding Affinity Active Compound (Scopoletin) and Captopril To NOS3 Protein

Ligand	Binding Affinity
Captopril_NOS3	-5.7
Scopoletin_NOS3	-7.6

*The result of the analysis with PyRx Autodock Vina

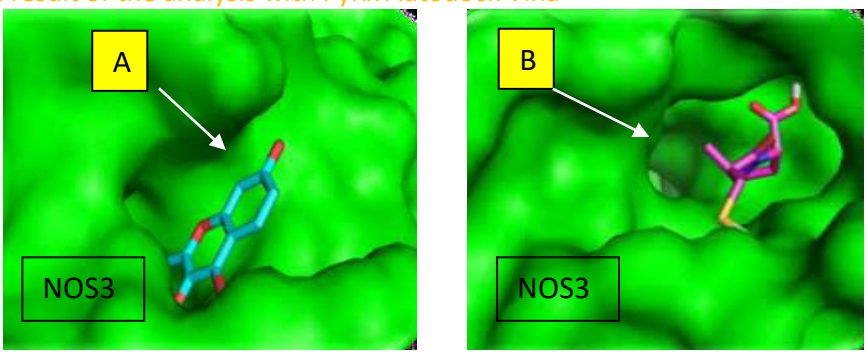

Figure 4. Visualization Active compound of Scopoletin on noni extract (A) and Anti-Hypertension drug (Captopril) (B) with cell receptor Target was NOS3 *(Nitric Oxide Synthase)*

Nowadays, molecular biology studies changed from blood pressure were affected by the brain to kidneys to can also be caused by endothelial problems. Vascular endothelium can produce nitric oxide (NO) that has characteristics as vasoactive gas(11). Another mechanism for hypertension is an increase in peripheral resistance caused by vasoconstriction of blood vessels. The process of vasoconstriction and vasodilation of blood vessels was influenced by level NO that can cause vasodilation of blood vessels. Levels NO were impacted by the presence of NOS enzyme that expressed by NOS3 gene(12) . Therefore, NOS3 protein is one of the proteins that can regulate blood pressure. In silico test, the researcher performed to see the binding value of target affinity cell protein (NOS3) to the contained in noni as ligand. Silico tests showed that noni extract can significantly decrease blood pressure compared to antihypertensive drug Captopril. The active compound contains in noni was scopoletin that has -7,6 of binding affinity value. While anty-hypertension drug (captopril) has only -5,7 of binding affinity value. The lower binding value of affinity was the stronger the binding between active compounds and the target cell protein that is NOS3 protein. Binding affinity shows the scale of stregth of the ligand ti bind to the receptor. The lower the binding affinity results in the affinity between the receptor and the ligand the higher and vice versa. Ligands of value binding affinity is the least negative category best ligands (13). Vascular endothelium can produce nitric oxide (NO), a relaxing factor that acts as a vasoactive gas (vasodilator) with the help of Endothelial Nitric Oxide Synthase (eNOS). This enzyme is an expression of the NOS3 gene, which in the event of a mutation can reduce NO production, causing vasoconstriction and increased peripheral resistance of blood vessels and ultimately leading to hypertension. However, if the

scopoletin compound binds to NOS3, the stability of the bonds will be maintained so that NO production as a vasodilator will remain (14).

This proves that noni extract can reduce blood pressure. Active compound was found in noni extract binds to target cell receptor protein (NOS3) that showed in blood vessels.

4. Conclusions

Noni extract with volume of 6 ml proven to reduce high blood pressure. Noni extract can reduce systolic blood pressure with an average reduction in blood pressure of 58,5 mmHg. While, captopril as a positive control with 25,5 mmHg in average drop of blood pressure of 25,5. Not only vitro test, but also silico test proves showed that noni extract can significantly reduce hypertension compared to anty-hypertension drugs (captopril) that contain active compounds (scopoletin) in noni. The affinity scopoletin binding value was -7,6. While, anti-hypertensive drugs (captopril) only have binding affinity values was -5,7. The lower the value of the binding affinity was the stronger the binding of the active compound to the target cell. Therefore, Scopoletin was contained in noni fruit has a greater ability (significantly) in lowering blood pressure compared with anti-hypertensive drugs (captopril).

Acknowledgements

1. Thanks to Kemenristekdikti for providing funds for this research.
2. Thanks to the Physiotherapy department who has provided support throughout the research.
3. Thank you to the Pharmacy department of Hasanudin University and Indonesian Muslim University who have helped provide laboratories.
4. Thanks to the supervisor and teammates who helped complete this research.

References

1. World Health Organization, Islands S. WHO statistical profile. World Health Organisation: Geneva, Switzerland. 2015.
2. World Health Organization. Global status report on noncommunicable diseases 2014. World Health Organization; 2014.
3. Burke TA. The epidemiology of stroke in the East Asian region: a literature- based review. 2006;1(4):208-15.
4. Sorganvi. Review. Risk factors for stroke: a case control study. 2014;6(3):46.
5. Nowak. Effects of Acute Consumption of Noni and Chokeberry Juices vs. Energy Drinks on Blood Pressure, Heart Rate, and Blood Glucose in Young Adults. 2019;
6. Ekins. In Silico Pharmacology for Drug Discovery : Methods for Virtual Ligand Screening and Profiling. Br J Pharmacol. 2007;
7. Wigati. medicine Hypotensive activity of ethanolic extracts of Morinda citrifolia L. leaves and fruit in dexamethasone-induced hypertensive rat. 2017;22(1):107-13.
8. Ali M. Health benefits of Morinda citrifolia (Noni): A review. 2016;8(4)
9. Maheswari. Invigorating Efficacy of cucumis sativas for healt care and radiance. Int J Chem Pharm Sci. 2014;2(3)
10. Yuliana C. Noni fruit (Morinda citrifoliaL.) For Reduce High Blood Pressure. Major J. 2015;4
11. Sulastri. The Role of Antioxidant Intake on eNOS3 Gene Expression in Minangkabau

Ethnic Grup Patient with Hypertension. 2011;60(12)
12. Li J. Association of eNOS gene polymorphisms with essential hypertension in the Han population in southwestern China. 2011;10(3):2202-12.
13. Faqih. Screening of Flavonoid Derivatives as Candidates for nsP2 Protease Inhibitors from Chikungunya Virus Using Molecular Docking. JC-T (Journal Cis-Trans). 2019;3(1)
14. Amelia. Role Of Expression Of Nitrite Oxide Synthage Gen (Nos3) On The Event Of Hypertension Essence. Med J Lampung Univ. 2018;7(2)