KnE Life Sciences

The 3rd IMOPH & the 1st YSSOPH
The 3rd International Meeting of Public Health and
The 1st Young Scholar Symposium on Public Health
Volume 2019

Knowledge E
Engaging minds

Conference Paper

Effect of *Secang* Drink (*Caesalpinia Sappan* L.) on Plasma Nitric Oxide Level and Blood Pressure in Prehypertension Peoples

Ayu Januariyatun[1], Mae Sri Hartati Wahyuningsih[2], and Susetyowati[3]

[1]School of Public Health, Graduate Programme of Medical Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Farmacology and Therapy Department of Medical Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3]Health and Nutrition Programme of Medical Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract

The is experimental with pre-post test control group design. This study aimed to know the effect of *secon*d drink on the plasma NO (Nitric Oxide) level and blood pressure in an officer with prehypertension. The subjects were 36 officers at Balaikota Yogyakarta Indonesia aged 25-45 years that divided into two groups: the intervention group who was given *secon*d drink (3.8-gram *secon*d in 200 mL of water) for four weeks and a control group who not given *secon*d drink. Plasma NO level was measured using the Griess method and blood pressure were measured using a mercury sphygmomanometer. There were no significant differences between intervention and control group ($p>0.05$) of plasma NO level increase (2.69 (-10.69-25.29) µmol/L versus 2.32(-7.65-18.48) µmol/L), systolic blood pressure decrease (4.20±7.27 mmHg versus 3.95±7.08 mmHg) and diastolic blood pressure decrease (4.99±9.12 mmHg versus 4.46±8.62 mmHg). In summary, *secang* drink could increase plasma NO level and decrease blood pressure but not significant compared to the control group. Further research is needed to prove this result.

Keywords: prehypertension, secang drink, Caesalpinia sappan L., nitric oxide, blood pressure

Corresponding Author:
Ayu Januariyatun
ayujanuariyatun@gmail.com

Received: 21 December 2018
Accepted: 23 January 2019
Published: 28 February 2019

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the 3rd IMOPH & the 1st YSSOPH Conference Committee.

1. Introduction

Hypertension is a public health problem in Indonesia by the data of the Health Research (Riskesdas) in 2013 which showed that 25.8% of adults had hypertension [1]. Based on data from SIRS (Hospital Information System) Special Region of Yogyakarta in 2011, the disease is among the ten largest disease cause of death in hospitals. The happens due to hypertension initiate degenerative and cardiovascular diseases. This disease can progress to stroke, heart disease, heart failure, and kidney failure [2].

This disease prevention should have started from prehypertension state. Prehypertension diagnosed to people with 120-139 mmHg for systolic or 80-89 mmHg for diastolic

blood pressure. People with prehypertension had a cardiovascular risk two times greater than normal blood pressure [3].

Secang drink is a purplish red traditional drink that commonly consumed in Indonesia. The benefits of *secang* known as an antioxidant, antibacterial, anti-inflammatory, hypoglycemic activity, vasorelaxant, hepatoprotective, and others. The active polyphenols compound of *secang* (xanthones, coumarins, chalcones, flavonoids, homoisoflavonoid, and brazilin) can also provide blood pressure lowering effects [4]. *Secang* can increase the synthesis of endothelial NO (nitric oxide) to activate guanylate cyclase and increase cGMP (cyclic guanosine 3', 5' monophosphate) which serves to give vasorelaxation effect of human vascular smooth muscle [5].

In this study, *secang* drink give to Balaikota Yogyakarta officers with prehypertension based on data which showed that 32.53% of officers at Balaikota had prehypertension. This study aims to know the effect of *secang* drink to blood plasma NO level, and blood pressure then compared that effect to the control group not give *secang* drink.

2. Methods

The was an experimental study with a pre-post test with control group design. Subjects were chosen based on inclusion criteria and exclusion criteria. The inclusion criteria were prehypertension diagnosed by a doctor (120-139 mmHg for systolic blood pressure or 80-89 mmHg for diastolic blood pressure), 25-45 years old, BMI was between 18.5 to 27 kg/m^2 and willing to sign an informed consent, while the exclusion criteria were subjects with diagnosed of kidney disease, heart disease, adrenal tumor, coarctation aorta, diabetes mellitus, antihypertensive drugs, and corticosteroids consumption, alcohol consumption, smoking, and pregnancy or breastfeeding. Meanwhile, the criteria drop out were not drinking *secang*>1 week and the use of vitamin supplements, minerals, herbs or herbal tea more than two times/glasses a day during the study. A total of 36 subjects were divided into two groups by a simple random allocation using a computer program.

The intervention group was given a *secang* drink once a day for four weeks while the control group does not provide a *secang* drink. *Secang* that given for one package as much as 3.8 g per 200 mL was calculated based on the study [6, 7] and added Diabetasol sugar as non-caloric sugar that did not influence total caloric and phenolic intake. *Secang* that used was taken from the Regional Forest Mangunan Bantul Yogyakarta then processed and packaged by CV Dewie Makmur in Yogyakarta that has production permit. The subjects in the intervention group were asked to make a *secang* drink every morning in water with a temperature of 70-90^{0}C for 20 minutes to get the most optimal active compounds [7].

Characteristic data of respondents, nutrient intake, physical activity, and stress level measured by interview using a validated questionnaire. Nutritional intake was measured three times before the study and three times during the investigation that includes two days of work and one day off. Subjects were asked to fast for 10-12 hours at night before the day of blood sampling to measure the plasma NO level. Blood specimens were taken from the cubital vein as much as 2 ml in the morning and carried out by a health analyst who has license practice. Measurement of plasma NO level used the

Griess method in LPPT UGM unit I. Blood pressure was measured using a calibrated mercury sphygmomanometer at 2:00 p.m. to 16:00 pm. Blood pressure was measured three times by a nurse with ±5 minutes interval and then averaged.

The data was processed using EXCEL program and analyzed using STATA 12 program. The normality of data was tested using the Shapiro-Wilk test then univariate analyzed. Comparisons between groups were tested using an independent t-test for standard data and Mann-Whitney for abnormal data. The Chi-Square analysis used for categorical data. The data presented in mean and deviation standard for average and median and a minimum-maximum value for anomalous data. The effect of *secang* drink to plasma NO level analyzed by Wilcoxon Signed-Rank test and outcome of *secang* drink to blood pressure was examined using repeated ANOVA The significance of the difference are presented by the value of $p < 0.05$.

3. Results

The subjects in this study were officers of the Balaikota Yogyakarta. At least 300 screened into 60 people who met the criteria for inclusion and exclusion, but only 42 peoples who were willing to become respondents. During the pre-test, three respondents were drop out. There were 39 respondents, grouped into two of 20 respondents in the control group and 19 in the intervention group. During for four weeks, there was monitoring of food intake, physical activity, general health, and blood pressure. There was no significant health problem due to *secang* drink that given. Three respondents met the exclusion criteria (smoking and taking supplements that affect blood pressure) and one subject whose lisis blood.

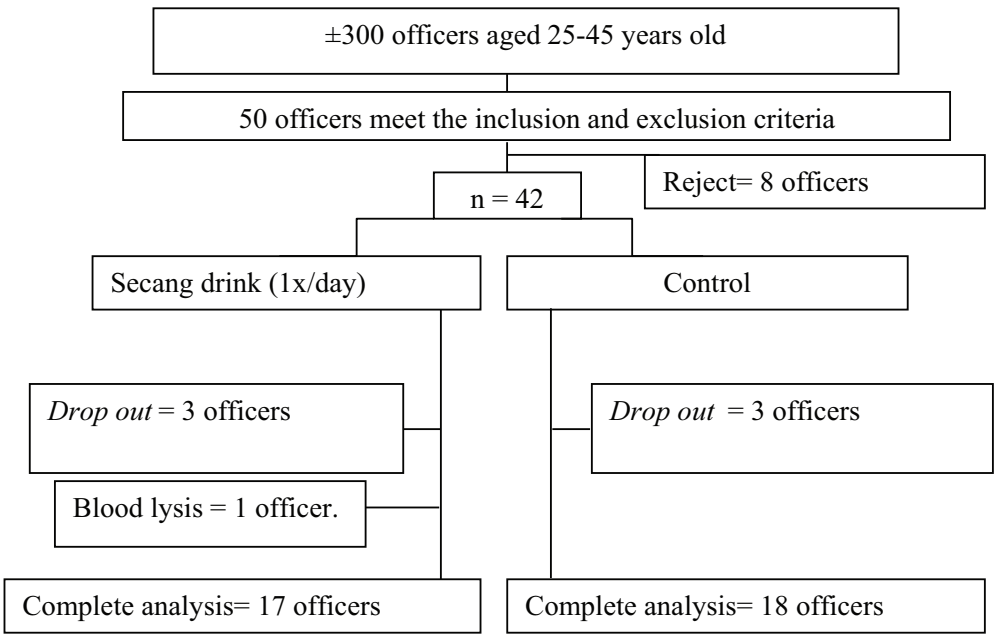

Figure 1: Consort Diagram Flow.

Based on the results, the intervention group did not differ significantly compared with the control group. The evidence by the characteristics of the subjects in the two groups did not change at baseline and during the intervention.

In this study, subjects were asked not to change their usual consumption. The compliance of *Secang* drink consumption in this study monitor by food intake and collected the former "*secang* celup" each week. Average consumption of *secang* drinks by subjects in the intervention group for 28 days was 91% or 25 packages. It was still relatively good because of more than 80%.

Table 1: Characteristics of Subjects.

Variable	Group		Sig (p)
	Intervension (n=18)	Control (n=18)	
Age	37.94 ± 4.11	35.89 ± 5.04	0.188[a]
Gender			
Male	9 (50%)	9 (50%)	1.000[a]
Female	9 (50%)	9 (50%)	
Education			
High School	2 (11%)	7 (39%)	0.126[b]
Diploma	2 (11%)	4 (22%)	
Bachelor	11 (61%)	5 (28%)	
Postgraduate	3 (17%)	2 (11%)	
Systolic blood pressure (mmHg)	117.28 ± 9.19	122.56 ± 7.88	0.070[a]
Diastolic blood pressure (mmHg)	79.83 ± 6.17	78.83 ± 5.09	0.599[a]
Body weight (kg)	58.72 ± 9.54	62.77 ± 7.83	0.172[a]
Height (m)	1.58 ± 0.09	1.62 ± 0.06	0.110[a]
Body Mass Index (BMI)			
Overweight (≥25 kg/m^2)	7 (39%)	6 (33%)	0,729[b]
Normal (18.5-24.99 kg/m^2)	11 (61%)	12 (67%)	
Exercise (x/week)	1.28 ± 0.57	1.28 ± 0.57	1.000[a]
Hypertension family history			
Yes	11 (61%)	10 (56 %)	0.735[c]
No	7 (39%)	8 (44 %)	

Note: Data presented in mean ± SD for standard data and the median (Min-Max) for abnormal data, the significant value of p <0.05. An Independent t-test, [b]Fisher's exact test, and [c]hi-square test.

The data analysis showed there increased plasma NO levels not only in the intervention group but also the control group. There were no significant differences plasma NO level between the intervention and control group (p>0.05). Meanwhile, the effect of *secang* drink had been found to lower systolic and diastolic blood pressure in the intervention group significantly. Based on the results, there was a decline when compared between groups was not significant (p> 0.05). There were 11 of 18 (61%) who initially had prehypertension become normal blood pressure after being given a cup of drinks for

Table 2: Stress Level, Activity Level and Nutritional Intake Before the Study.

Variable	Group		Sig (p)
	Intervension (n=18)	Control (n=18)	
Stress Level			
Stress	1 (6%)	2 (11%)	0.500[c]
No Stress	17 (94%)	16 (89 %)	
Activity level			
High activity	7 (39%)	10 (56%)	0.317[d]
Low activity	11 (61%)	8 (44%)	
Nutrition intake			
Energy (kkal)	1454.27 ± 378.70	1452.56 ± 441.53	0.990[a]
Fat (gram)	44.59 ± 20.91	56.92 ± 27.55	0.139[a]
Protein (gram)	51.14 ± 24.25	52.36 ± 15.87	0.859[a]
Karbohidrat (gram)	209.44 ± 70.91	198.76 ± 62.92	0.636[a]
Vitamin A (ug)	306.35 (97.57-1069.97)	385.28 (126.87-3067)	0.506[b]
Vitamin C (mg)	25.88 (8.23-199.17)	36.78 (13-135.97)	0.429[b]
Natrium (mg)	391.4 (34-1271.33)	442.68 ± 251.68	0.506[b]
Tea consumption (x/hari)	1.11 ± 0.51	0.88 ± 0.55	0.201[a]

Note: Data presented in mean ± SD for standard data and the median (Min-Max) for abnormal data, the significant value of p <0.05. An Independent t-test, [b]Mann-Whitney test, [c]chi-square, and[d] Fisher's exact test.

Table 3: Stress Level, Activity Level and Nutrition Intake During The Study.

Variable	Group		Sig (p)
	Intervension (n=18)	Control (n=18)	
Stress Level			
Stress	2 (11%)	0 (0%)	0.486[c]
Not Stress	16 (89%)	18 (100%)	
Activity level			
High activity	10 (56%)	10 (56%)	1.000[d]
Low activity	8 (44%)	8 (44%)	
Nutrition intake			
Energy (kkal)	1450.05 ± 409.69	1512.13 ± 479.59	0.678[a]
Fat (gram)	51.12 ± 16.52	59.39 ± 21.22	0.200[a]
Protein (gram)	50.93 ± 15.87	55.00 ± 20.54	0.509[a]
Karbohidrat (gram)	202.85 ± 72.63	195.93 ± 60.55	0.758[a]
Vitamin A (ug)	489.92 (84.23-1828.1)	285.2 (12-1286.93)	0.055[b]
Vitamin C (mg)	51.68 (18.2-155.83)	30.53 (5-118.07)	0.376[b]
Natrium (mg)	538.11 ± 236.33	552.4 ± 304.05	0.875[b]
Tea consumption (x/hari)	1 ± 0.65	1.09 ± 0.67	0.676[a]

Note: Data presented in mean ± SD for normal data and the median (Min-Max) for abnormal data, the significant value of p <0.05. An Independent test t-test, [b]Mann-Whitney test, [c] Fisher's exact test, and [d] chi-square test.

Table 4: Changes of plasma NO levels after study

Variable	Group		Sig (p)[b]
	Intervension (n=17)	Control (n=18)	
NO level (µmol/L)			
Before	30.44 (20.95-52.21)	30.44 (22.96-53.32)	0.855
After	33.14 (19.57-56.89)	34.81(25.35-47.14)	0.923
Δ	2.69 (-10.69-25.29)	2.32 (-7.65-18.48)	0.775
Sig (p)[a]	0.047	0.044	

Note: Data presented in median (Min-Max), the significance value of p <0.05. [A]Wilcoxon test and [b]Mann-Whitney.

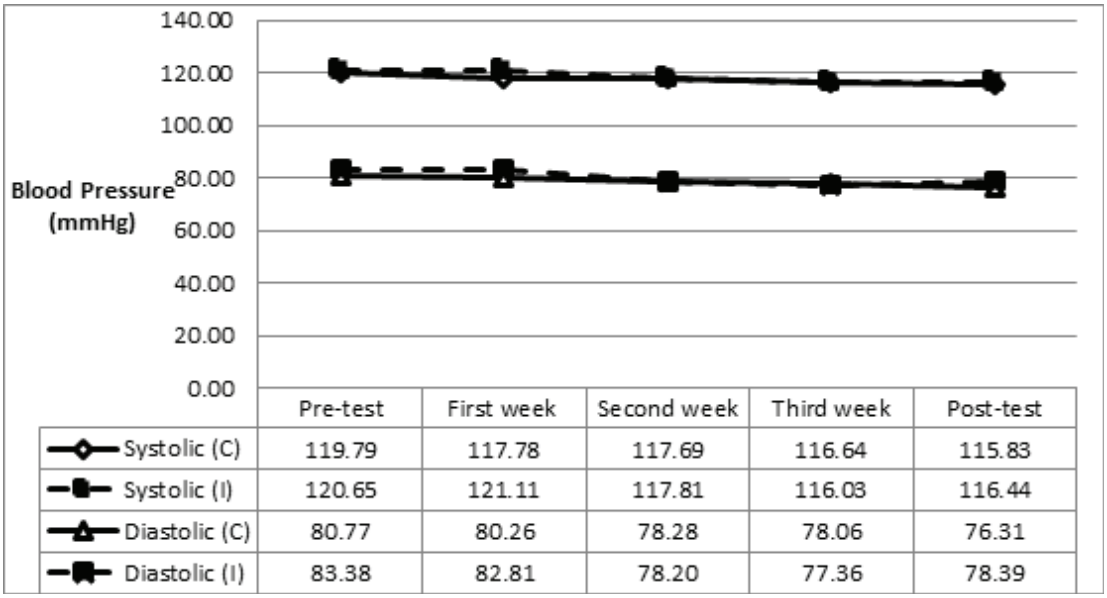

Figure 2: Changes in Blood Pressure during the study (*Note: (I) = Intervention and (C) = Control*).

Table 5: Changes of blood pressure after the study.

Variable		Group		Sig (p)
		Intervention (n=18)	Control (n=18)	
Systolic blood pressure (mmHg)	Before	120.65 ± 5.78	119.78 ± 7.58	0.704[b]
	After	116.44 ± 7.78	115.833 ± 7.89	0.816[b]
	Δ	-4.20 ± 7.27	-3.95 ± 7.08	0.639[a]
	Sig (p)	0.016[a]	0.310[a]	
Diastolic blood pressure (mmHg)	Before	83.38 ± 7.35	80.76 ± 8.57	0.333[b]
	After	78.38 ± 4.25	76,31 ± 7,47	0.311[b]
	Δ	-4.99 ±9,12	-4.46 ± 8.62	0.478[a]
	Sig (p)	0.000[a]	0.084[a]	

Note: Data presented in mean ± SD, significance value of p<0.05. [a] Repeated ANOVA Test and [b] Independent t-test

Table 6: Classification of blood pressure after study.

Blood pressure classification	Intervention		Control		Sig (p)
	n	%	n	%	
Prehypertension	7	38.89	11	61.11	0.182[a]
Normal	11	61.11	7	38.89	
Total	18	100	18	100	
Note: the significance value of p<0,05 [a]chi-square test					

Table 7: The Relationship of the Plasma NO Level and Blood Pressure Changes.

Variable	Plasma NO Level	
	R	*p-value*
Systolic blood pressure	-0.0562	0.748[a]
Diastolic blood pressure	0.0619	0.724[a]
Note: the signifikansi value p<0,05. [a]n Independent t-test		

four weeks. This proportion was more than the healthy subjects without being given any treatment (7 out of 18 people or 39%) in the control group.

4. Conclusions

The increasing plasma NO level in the group given *secang* drink described in studies on isolated rat aorta cells and human umbilical endothelial vein. *Secang* extract can give vasorelaxation effects by induces the increasing formation of NO, NOS activity and influx of extracellular Ca^{2+} in cells [6] and inhibiting the activity of arginase II of human umbilical endothelial vein [9]. Meanwhile, plasma NO level of the control group was not given *secang* drink also increased. It relates to the type of NO in the blood consist of three kinds of isoforms (iNOS, nNOS, and eNOS) that cannot measure using Griess Method. The NO level in subcutaneous adipose tissue of human also influenced by gene expression [10], age, sex, smoking behavior, BMI, cholesterol, and triglyceride levels [11]. Gene expression, cholesterol, and triglyceride level not measured in this study. The results are different from the study of chocolate given for 15 days increased the NO level significantly [12]. It is likely due to differences in active substances. The results as same as with spinach juice not given any effect on the NO level in serum of men with prehypertension [13]. High physical activity has a more powerful influence on the NO level [14]. The also may occur because of the proportion of subjects with high activity more than subjects with low action.

The decreasing systolic and diastolic blood pressure in the intervention group describe in previous studies [5]. *Secang* extract lowered α1-receptor agonist phenylephrine-precontracted aortic ring significantly through increased endogenous NO and cGMP formation. *Secang* also can reduce blood pressure by inhibits vasoconstriction through

norepinephrine, blocking voltage and Ca^{2+} receptor that independent from the endothelium [15]. The effect of *secang* drink on lowering blood pressure also can be through the antioxidant activity of its polyphenols that reduce oxidative stress [16]. The decrease in oxidative stress can affect blood pressure by lowering peroxides nitrite (NOX) from NO [17]. Meanwhile, there was a decrease in blood pressure in the control group that not statistically different compared to the intervention group. The blinding method not used in this study can affect the result.

Secang drink was not strong enough to give a significant effect on the decrease of blood pressure compared with the control group. These results have similarities with the study [18] that consumption of tea for four weeks did not affect blood pressure reduction. Meanwhile, this result is different from the study of chocolate that decreased lower systolic blood pressure significantly [12]. Polyphenols of tea drink can be affected by heating then decrease the antioxidant activity [19]. It may occur that using hot water in the making of drink influenced the polyphenols activity.

The control group had a blood pressure decrease were not statistically different in the intervention group. The decline can be affected by factors that not examined in this study. According to [20], the blood pressure control by hemodynamic hypertension, renin-angiotensin system, autonomic nervous system, endothelial dysfunction, insulin sensitivity, genetic and kidney function. The control mechanism influenced by age, gender, genetics, BMI, stress levels, physical activity, salt intake, and blood lipid levels. In this study, several factors that influence blood pressure has minimized through the inclusion and exclusion criteria. These factors are analyzed using statistical and showed no difference between groups. Meanwhile, lipids and blood cholesterol not studied because of limitation. It may influence the results.

Plasma NO was associated with blood pressure can be caused by the low bioavailability of NO in their function as vasorelaxant [21]. Bioavailability of NO produced by eNOS is influenced by many factors: the expression of protein / mRNA eNOS, the availability of L-arginine as a substrate which is constrained by ADMA, the availability of NO cofactors including BH4 catalyzed by GTP cyclohydrolase, protein interactions such as Caveolin, HSP90, modification posttranslational and the reaction of NO with SOD against peroxynitrite anion.

There was plasma NO level increase and blood pressure decreased significantly in the group that given *secang* drink, but there was no difference compared to the control group. For further study, it is expected to study the influence of *secang* drink to blood pressure with the blinded method.

Acknowledgment

The authors would like to thank LPDP (Lembaga Pengelola Dana Pendidikan) for financial support of the study.

Ethical Approval

The study was conducted in June-August 2016 in the Balaikota Yogyakarta and has received permission from the ethics committee of the medical faculty of UGM (KE / FK / 464 / EC / 2016).

Competing Interest

The authors declare they have no conflict of interest. The authors alone are responsible for the content and writing of this article.

References

[1] Widyakarya Nasional Pangan dan Gizi: Angka Kecukupan Gizi Indonesia tahun 2013. Jakarta. Departemen Kesehatan Republik Indonesia. 2013.

[2] Sundari F, Amalia L, Ekawidyani KR: Minuman Cincau Hijau (Premna oblongifolia Merr.) dapat Menurunkan Tekanan Darah pada Wanita Dewasa Penderita Hipertensi Ringan dan Sedang. Jurnal Gizi Pangan. November 2014, 9(3): 203 – 210.

[3] Izzo JL: Prehypertension: Demographics, Pathophysiology, And Treatment. Current Hypertension Report. 2007;9:264-8.

[4] Nirmal, Nilesh P, Rajput, Mithun S, Prasad, Rangabhatla GSV, Ahmad, Mehraj: Brazilin from Caesalpinia Sappan Hearthwood and Its Pharmacological Activities: A Review. Asian Pacific Journal of Tropical Medicine. 2015; 8(6): 421 – 430.

[5] Hu CM, Kang JJ, Lee CC, Li CH, Liao JW, Cheng YW: Induction of Vasorelaxation Through Activation of Nitric Oxide Synthase in Endothelial Cells by Brazilin. European Journal of Pharmacology 468 (2003) 37-45.

[6] Xie YW, Ming DS, Xu HX, Dong H, But PPHB: Vasorelaxing Effects of Caesalpinia sappan Involvement of Endogenous Nitric Oxide. Journal Life Sciences 67. 2000; 1913 – 1918.

[7] Badami S, Moorkoth S, Rai SR, Kannan E, Bhojraj S. Antioxidant Activity Of Caesalpinia sappan Heartwood. Biol Pharm Bull 26. 2003; 1534–1537;.

[8] Farhana H, Maulana IT, Kodir RA: Perbandingan Pengaruh Suhu dan Waktu Perebusan terhadap Kandungan Brazilin pada Kayu *Secang* (Caesalpinia Sappan L.). Prosiding penelitian SPeSIA Unisba. 2015.

[9] Shin W, Cuong T, Lee JH, Min B, Jeon BH, Lim HK, and Ryoo S: Arginase Inhibition by Ethylacetate Extract of Caesalpinia sappan Lignum Contributes to Activation of Endothelial Nitric Oxide Synthase. Korean J Physiol Pharmacol. 2011; Vol 15: 121-128,.

[10] Cahjono H, Budhiarta AAG: Hubungan Resistensi Insulin dengan kadar Nitric Oxide pada Obesitas Abdominal. Fakultas Kedotekteran Universitas Udayana Denpasar. 2007.

[11] Ghasemi A, Asl SZ, Mehrabi Y, Saadat N, Azizi F: Serum Nitric Oxide Metabolite Levels in General Healthy Population: Relation to Sex and Age. Life Sciences 83. 2008; 326-331.

[12] Sudarma V, Sukmaniah S, Siregar P: Effect of Dark Chocolate on Nitric Oxide Serum Levels and Blood Pressure in Prehypertension Subjects. Acta Med Indones-Indonesia J Intern Med. 2011; Vol 43 No 4.

[13] Sutadarma IWG, Sayogo S, Effendi I: Pengaruh Jus Bayam terhadap Kadar NOX Serum dan Tekanan Darah pada Laki-laki Dewasa Muda. Majalah Kedokteran Indonesia. 2010; Vol. 60; 4, April.

[14] Isral GN, Afriwadi, Sulastri D: Hubungan Aktivitas Fisik dengan Kadar Nitric Oxide (NO) Plasma pada Masyarakat di Kota Padang. Jurnal Kesehatan Andalas. 2014; 3(2).

[15] Yan Y, Chen Y, Lin YH, Guo J, Niu Z, Li L, Wang S, Fang L, Du G: Brazilin Isolated From The Heartwood Of Caesalpinia Sappan L Induces Endothelium-Dependent And -Independent Relaxation Of Rat Aortic Rings. Acta Pharmacologica Sinica.2015; 36: 1318-1326.

[16] Clark JL, Zahradka P, Taylor CG: Nutrition Efficacy Of Flavonoids In The Management Of High Blood Pressure. Reviews Advance Access published October 21, 2015.

[17] Galleano, M., Pechanova, O. & Fraga, Cesar G. Hypertension, Nitric Oxide, Oxidants, and Dietary Plant Polyphenols. Current Pharmaceutical Biotechnology Bentham Science Publishers Ltd; 2010.

[18] Hodgson JM, Puddey IB, Burke V, Watts GF, Beilin LJ: Regular Ingestion Of Black Tea Improves Endothelial Function In Humans. Clinical Science. 2002; 102:195–201.

[19] Routray W, Orsat V: Microwave-assisted extraction of flavonoids: A Review. Food and Bioprocess Technology. 2012; 5, 409-424.
[20] Kaplan, NM, Flynn JT: Kaplan's Clinical Hypertension: Ninth Edition. Philadelphia: Lippincott Williams & Wilkins. 2006.
[21] Huang, Paul L. eNOS, Metabolic Syndrome and Cardiovascular Disease. Trends in Endocrinology and Metabolism. 2009; Vol. 20 No.6