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COMPARISON BAY LEAF (SYZYGIUM POLYANTHUM (WIGHT) WALP) EXTRACT WITH 400 MG AND 600MG DOSE ON NITRIT OXIDE CONCENTRATION IN DYSLIPIDEMIC PATIENTS

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Abstract
Introduction: Dyslipidemia is a risk factor for cardiovascular disease. Dyslipidemia is known to trigger damage to endothelial blood vessels so that the disruption of nitric oxide production. Nitric oxide in blood vessels acts as an antiaterosclerotic agent, vasodilator, and prevents platelet aggression. Bay leaf extract is reported to be efficacious in reducing cholesterol, triglyceride levels and can increase levels of nitric oxide. This study aims to compare the effects of bay leaf extract at a dose of 400 mg and 600 mg on nitric oxide levels in dyslipidemic patients.

Method: Clinical trial research with a prospective design. The first group (n = 15) was given therapy with dose of 400mg and the second group (n = 15) was given 600mg which was chosen randomly selected randomly in double disguised. Before and after 30 days of treatment, lipid profile and nitric oxide were examined. Data were analyzed by T-dependent statistical test and Wilcoxon test using SPSS. Significant difference when $p < 0.05$.

Result: Nitric oxide levels before drug administration compared with after treatment. The results of the study found a decrease in group I (41.71 vs 46.62) mmol / L; $p = 0.233$) and group II ((39.89 vs 52.62) mmol / L; $p = 0.006$), statistically significant in group II. Increased levels of nitric oxide in group II were greater than group I ($\Delta 4.91$ vs $\Delta 12.73$), and statistically significant; $p = 0.001$).

Conclusion: The used of bay leaf extract (Syzygium polyanthum) 600 mg for 30 days increases nitric oxide levels greater than 400 mg, and is statistically significant.

Introduction
Dyslipidemia is one of the main risk factors for coronary heart disease (CHD) and stroke in addition to hypertension, smoking, blood glucose abnormalities, and physical inactivity. World Health Organization (WHO) states that coronary heart disease (CHD) and stroke ranks number one and two as the cause of death in the world. Both caused 14.1 million deaths worldwide in 2012. The Indonesian Ministry of Health declared coronary heart disease as the main cause of death in Indonesia. [1]

Dyslipidemia is a major factor in the occurrence of vascular endothelial dysfunction. Dyslipidemia triggers disruption of endothelial nitric oxide (eNOS) production. [2] Nitric oxide (NO) in blood vessel endothelium acts as an anti-atherosclerotic agent, vasodilator, and prevents platelet adhesion and aggregation. Damage to blood vessel endothelium causes interference with the performance of nitric oxide which results in blood vessel intima which triggers thrombus formation in the process of atherosclerosis. [3]

Dyslipidemia treatment by using synthetic drugs has a high risk because it is consumed long term so that it can cause side effects of drugs that cannot be ignored, such as dyspepsia, headache, fatigue, muscle and joint pain, increased transaminases, myopathy, rabdomiolysis, and triggers diabetes mellitus . Therefore, people are starting to reuse drugs from natural materials that are believed to be safer and have relatively few side effects on long-term use. [4]

Bay leaf is often consumed as a fresh salad while old bay leaf is often added as a flavor enhancer to Malay cuisine. In addition, the leaves and stems are also used as traditional medicine in diarrheal diseases, diabetes, skin infections, cataracts, rheumatism, and hypercholesterolemia. The results of previous studies in the mouse model

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of dyslipidemia showed that bay leaf infusion concentrations of 5%, 10%, 20% for 2 weeks significantly decreased total cholesterol levels. [4]

Previous studies of bay leaf extract compared with green tea extract on NO levels in the immune system, NO on macrophages, showed that there was an increase in NO levels in macrophages after bay leaf extract was given, although the increase in NO levels was higher in the group given tea extracts green. [5] In the subchronic toxicity test the safety level of bay leaf extract, a dose of 2 g / kg body weight did not show any disturbance, at a dose of 3 g / kg body weight the new showed an increase in plasma urea and creatinine levels and an increase in liver enzymes. Previous studies in patients with hypercholesterolemia or in mice reported that bay leaf extract (Syzygium polyanthum) reduced cholesterol levels. [6] This study aims to analyze the comparison of bay leaf extract with different doses of nitric oxide levels in patients with dyslipidemia.

Method

Study Design

This study is a clinical trial with a prospective design. The target population is all dyslipidemic patients in Medan who meet the inclusion and exclusion criteria from August 2019 to November 2019. Research subjects who meet the criteria are given an explanation and asked to give written consent (informed consent) to participate in the study. Examination data was consist of height (m), body weight (kg), body mass index, waist size (cm) and subjects were fasted for 10-12 hours then blood samples were taken in the cubital fossa area and examined at the laboratory. Nitric oxide levels, lipid profile, fasting blood glucose levels on the 1st day and 31st day. Then all subjects were randomized and disguised into two groups, where there was group I who received bay leaf extract therapy 2x200 mg and group II who received 2x300 mg bay leaves extract.

Statistical Analysis

Univariate analysis was performed to determine the frequency distribution based on sociodemographic research subjects before the treatment was started. Bivariate analysis used the independent T test / Man Whitney U and the dependent T test / Wilcoxon is used to compare changes in values of laboratory, blood pressure and BMI in both groups after administration of bay leaf extract, and considered significant when $p < 0.05$.

Result

The research subjects were 30 people divided into two groups. The basic characteristics of the research subjects are detailed in table 1.

Table 1. Characteristics of dyslipidemic patients in Medan

Characteristic	Dose		*p*
	Group I (2x200 mg)	Group II (2x300mg)	
Gender:			
Male	0 (0,0%)	1 (6,7%)	
Female	15 (100,0%)	14 (93,3%)	
Age (Years)	51 (35-60)	49 (37-55)	0,478
Body Weight (Kg)	65 (50-87)	65 (60-81)	0,806
Height (m)	1,55±0,03	1,55±0,04	0,932
BMI (kg/m^2)	27,5±3,2	27,4±0,9	0,871
Waist Size (cm)	93 (81-97)	94 (90-96)	0,217
SBP (mmHg)	120 (110-130)	120 (110-130)	0,935
DBP (mmHg)	80 (70-80)	80 (70-80	0,539
NO (mmoL/L)	41,7(14,1-314,4)	39,9(14,4-130,4)	0,917
TC (mg/dL)	229,1±14,9	271,7±52,16	0,005*
HDL (mg/dL)	51,1±7,7	49,3±8,5	0,550
LDL (mg/dL)	155±22,5	175,7±35,4	0,066
TG (mg/dL)	149,9±70,5	202,8±114,5	0,139
FGL (mg/dL)	94,2±15,0	91,4±18,3	0,658

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Abbreviations: BMI: Body Mass Index, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein TG: Triglyceride, FGL: Fasting Glucose Level, TC: Total Cholesterol, NO: Nitrit Oxide, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure. *: $p<0.05$.

Subjects in group I were all women (100%). In the second group II women were 14 people (93.3%) and men were 1 person (6.7%). There were no statistically significant differences in the baseline characteristics between the two groups except value of TC was higher in group II.

Table 2. Comparison of nitrit oxide and lipid profiles levels between group I and group II.

	Group I				Group II				
	H_0	H_{30}	Δ	*p*	H_0	H_{30}	Δ	*p*	Δ*p*
NO (mmoL/L)	41.7 (14.1-314.4)	46.6 (12.7-377.3)	4.9	0.233	39.8 (14.4-130.4)	52.6 (14.1-156.5)	12.7	0.006 *	0,001 *
TC (mg/dL)	229 ±14.9	217.5 ±23	11.6	0.012 *	271.7±52.1	225.9±30.8	45.8	0.002 *	0,785
HDL (mg/dL)	51 ±7.7	50.06 ±7.5	1.07	0.318	49.3 ±8.5	47.7 ±5.8	1.6	0.344	0,573
LDL (mg/dL)	155 ±22.5	146.6 ±29.3	8.34	0.035 *	175.7±35.4	145.2±33.1	30.4	0,001 *	0,019
TG (mg/dL)	149.9 ±70.5	112 ±37.9	37.8	0.009 *	202.8±114.5	138.6±49.7	64.2	0,016 *	0,050

Abbreviations: TC: Total Cholesterol, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, TG: Trygliceride, H_0: before being given extract, H_{30}: after being given extract for 30 days, delta: the difference between the results of the examination before being given the extract is reduced after being given the extract for 30 days, *: $p<0.05$.

There was an increase in mean nitric oxide before and after treatment in group I [(41.7 (14.1-314.4) vs 46.6 (12.7-377.3)] mmol / L; p value = 0.233) but not statistically significant, whereas in group II there was a statistically significant increase [39.8 (14.4-130.4) vs 52.6 (14.1-156.5)] mmol / L; p value = 0.006 and the difference in value of increasing nitric oxide in both groups was statistically significant and (27.81 ± 33.79 vs 25.65 ± 33.23) ng / dL; value of p = 0.013)]. The mean increase in nitric oxide in group I compared with group II was statistically significant (4.9 vs 12.7) mg / dL; Δp value = 0.001).

Both groups showed a statistically significant reduction in total cholesterol, TG and LDL before and after treatment, but the mean increase of LDL was only statistically significant (Δp= 0.019)

In both group we found a decrease in mean HDL cholesterol before treatment compared with after treatment, but not statistically significant [(51.1 + 7.7 vs 50.06 + 7.5) mg / dL; p value = 0.318) and (49.3 + 8.5 vs 47.7 + 5.8) mg / dL; value of p = 0.344)].

Table 3. Comparison of BMI and blood pressure between group I and group II.

	Group I				Group II				
	H_0	H_{30}	Δ	*p*	H_0	H_{30}	Δ	*p*	Δ*p*
BMI (kg/m^2)	27,5 ±3,2	27,2 ±3,2	0,32	0,129	27,4 ±0,97	27,1 ±1,0	0,3	0,65	0,076
SBP (mmHg)	120 (110-130)	120 (110-130)	0	0,713	120 (110-130)	120 (110-120)	0	0,15	0,098
DBP (mmHg)	80 (70-80)	75 (65-90)	5	0,25	80 (70-80)	80 (60-80)	0	0,51	0,085

Abbreviations: BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, H_0: before being given extract, H_{30}: after being given extract for 30 days, *: $p<0.05$

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Comparison of BMI and blood pressure levels beetween group I and group II detailed in table 3. There was a non-significant decrease in BMI in both groups, nd the comparison of decreases for BMI in both groups (0.32 vs 0.33) was not statistically significant (**Δ**p = 0.076). There was no changes in systolic blood pressure values in group I (120 (110-130) mmHg vs 120 (110-130) mmHg; p = 0.713) and in group II (120 (110-130) mmHg vs 120 (110-120) mmHg; p = 0.152). In the measurement of diastolic blood pressure there was a decrease in group I (80 (70-80) vs 75 (65-90)) but it was not statistically significant (p = 0.25), whereas in group II there was no change in diastolic blood pressure. Comparison of the difference in diastolic blood pressure reduction in the two groups was also not statistically significant (**Δ**p = 0.085).

Discussion

In this study, we found a better decrease in lipid profile in group II by giving 2x300 mg bay leaf extract for 1 month compared to group I by giving 2x200 mg bay leaf extract for 1 month. This finding has similarities with previous studies that have conducted research on 25 male Wistar rats given bay leaves with concentrations of 5%, 10% and 20% have the effect of reducing total cholesterol levels and its potential is equivalent to simvastatin. [4] This study showed a significant decrease in total cholesterol levels in bay leaf extract in group I and group II for 30 days where the decrease in cholesterol levels was greater in group II, but this comparison was not statistically significant (Δp = 0.785). In addition to total cholesterol there was also a significant decrease in LDL values in both groups, where the decrease in LDL in group II is greater than group I and statistically significant (**Δ**p=0.019) The results of this study also showed a significant decrease in TG in in group I and in group II., where the decrease in TG in group II was greater than group I, but this comparison was not statistically significant (Δp = 0.050). There was also a decrease in HDL values in both groups but it was not significant, where the decrease in LDL in group II is greater that group I and it was not statisticaly significant (Δp= 0.573).

Previous studies have found that the combination of Sambiloto extract (Andrographis paniculata) and bay leaf (Syzygium polyanthum) for 30 days reduced lipid profile and ferritin levels better than simvastatin, but statistically not significant. [7] Other studies have concluded that the combination of bitter extract (Andrographis paniculata) and bay leaf (Syzygium polyanthum) 2 x @ 150 mg for 30 days in patients with dyslipidemia in addition to reducing lipid profile also significantly reduces Apo-B levels. [8]

The results of this study indicate an increase in levels of nitric oxide in bay leaf extract for 30 days in group I but not statistically significant (p=0.233) and in group II which is statistically significant (*p* value = 0.006), where the increase on NO levels was higher in group II. The comparison of the increase in nitric oxide is also statistically significant (Δp = 0.001). Previous studies have suggested that the effect of giving bay leaf extract on experimental animals at a dose of 56.7 mg for 3 months increases nitric oxide levels in macrophages. [5] But until now there have been no studies conducted on humans. It was concluded that the administration of bay leaf extract capsules at a dose of 2x300 mg had a greater effect on increasing levels of nitric oxide compared to the dose of 2x200 mg. However, because long-term research to see side effects has never been done so further research is needed on administering the right dose to produce optimal effects without causing side effects.

Body mass index in the group given bay leaf extract at a dose of 2x200 mg for 30 days (group I) did not show any significant changes before and after the intervention, nor did the groups given extract with 3x300 mg for 30 days (group II).

This finding is not in accordance with the results of the study which showed that ethyl acetate fraction of bay leaves ethanol extract at a dose of 180, 360 and 720 mg / kgBB was able to lose weight, and there was a linear correlation between increasing the dose of ethyl acetate fraction of bay leaves in weight loss. Sutrisna et al (2018) previously also observed changes in body weight of mice before and 15 days after the intervention of bay skin extract. Further studies with better design are needed to evaluate weight changes before and after the administration of bay leaf extract.

Systolic and diastolic blood pressure did not show significant changes in both groups. The results of this study are different from the results of previous studies. showed aqueous bay leaf extract at a dose of 20-100 mg / kg and residual methanolic extract of bay leaf at a dose of 40-100 mg / kg significantly by reducing mean arterial pressure and systolic and diastolic blood pressure with dose-dependent properties. [10] Bay leaf extract is reported to have a significant vasorelieving effect via the nitric oxide pathway, which may be mediated by autonomic receptors.

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However, the difference between this study and the two studies is that this study involved humans while both studies used mice as research samples [11]

The strength of this study is the first study conducted to assess the effect of giving bay leaf extract with a different dose in humans on increasing levels of nitric oxide in dyslipidemic patients. This research has weaknesses, among others, research subjects have been given education about the recommended diet for dyslipidemia, but there is no uniform diet and physical activity in the research subjects so that it can lead to bias in research results. Research time is too short to detect long-term side effects that may arise due to the combination of bay leaf extract (Syzygium polyanthum).

Conclusion

Bay leaf extract (Syzygium polyanthum) at a dose of 600 mg increases nitric oxide levels greater than the dose of 400 mg, and also treatment of bay leaf extract (Syzygium polyanthum) at a dose of 600 mg decreases the level LDL greater than the dose of 400 mg and it is statistically significant. Based on the results of this study it is recommended that further research be done with more specific subjects, stricter adherence to dietary monitoring, serial blood tests and longer duration of treatment to obtain better results.

Conflict of Interests

The authors declare there is no conflicts of interest regarding the publication of this paper.

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