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The Effect of Ethyl Acetate Fraction of Paku Resam Leaves (*Gleichenia Linearis* (Burm.) Clarke) on Endothelial Cell Dysfunction of White Male Mice

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ABSTRACT

The effect of ethyl acetate fraction of paku resam leaves (*Gleichenia linearis* (Burm. Clarke) on endothelial cell dysfunction of white male mice has been done. Dysfunction of endothelial cells was induced by sodium chloride (NaCl) at a dose of 28 mg/kg BW. Ethyl acetate fraction *G linearis* administered orally at the dose of 35, 70 and 140 mg/kg BW together with NaCl for 14 days. The endothelial cell dysfunction was observed by nitrous oxide (NO) serum levels. The results showed an increasing level of NO in mice serum given the paku resam extract with a dose of 35, 70 and 140 mg / kg BW with increasing percentage of NO levels at 85.66; 62.23 and 48%. After being analysed by one-way ANOVA only the dose of 35 mg / kg BW of the positive control showed the levels of NO significantly different ($p < 0.05$). It can be concluded that ethyl acetate fraction of *G linearis* at a dose of 35 mg / kg BW has the best effect to prevents the endothelial cell dysfunction in mice.

KEY WORDS: *Gleichenia linearis*, endothelial cell dysfunction, Nitric Oxide.

1. INTRODUCTION

Endothelial dysfunction plays an important role in the pathogenesis, development and prognosis of cardiovascular disease. Risk factors associated with endothelial cell dysfunction include: hypertension, smoking, diabetes, age, obesity, displipidemia, and sedentary life style. Many systemic processes that induce endothelial dysfunction is the activation of intracellular oxidative signaling (Simionescu, 2007).

If the endothelial cells impaired by a variety of things such as hemodynamic shear stress, hence its function as a regulator becomes abnormal and is called endothelial dysfunction. Endothelial cell dysfunction is often interpreted as decreasing vasodilation potency of blood vessels, due to a decline in production and bioactivity of local vasodilatory factors, in particular nitric oxide (NO) or other name is derivate of endothelium Relaxing Factor (EDRF) (Xi, 2007). In pathological conditions such as hypertension, stimulation of the endothelium leads to increased production of superoxide anion though NADPH and siklooxigenase (COX). Superoxide can damage the bioavailability of NO. In addition, COX also produces endoperoksida and trombaksan A 2, causing vasoconstriction (Versari, 2009).

With salt-induced hypertension may occur due to disruption of enzyme NO syntetase activity an endothelial cells that play a role in the reaction of L-argininn overhaul to NO and lead down the levels of NO (Hayakawa,1997). Decreased levels of NO resulted vasoconstrictor effect resulting in increased vascular peripheral resistance which leads to hypertension and vascular hypertrophy (Savard, 2011). Hypertension can increase the production of ROS. Reactive oxygen species (ROS) which formed will lead to increasing level of endothelial cell dysfunction that impact to enhancing the reduction of NO so the levels of NO will decrease. Decrease of NO due to the presence of NO synthesis inhibitor of NOS (Nitric Oxide Synthase Inhibitors) such as L-NMMA (NG-monomethyl-L-arginine), or injury of vascular endothelium will improve vascular tone and average arterial pressure (Lewanczuk, 2005). Decreased levels of NO resulted vasoconstrictor effect resulting in increased vascular peripheral resistance which leads to hypertension and vascular hypertrophy (Savard, 2011).

The antioxidants suplement will reduce the free radical so the oxidative stress does not produced and will increase the bioavailibility of NO. Flavonoids as antioxidants can increase NO levels and improve the endothelial function. Flavonoids contain in the plant if regularly consumed could protect the body from cardiovascular disease and some other chronic diseases through its antioxidant effect (Lewanczuk, 2005). Various studies of flavonoids effect on NO levels has been done, several other flavonoids that also have the same activity is rutin (Bondonno, 2012) and anthocyanin (Ugusman, 2014).

Paku resam (*Gleichenia liearis* (Burm) Clarke) is one of the plants that contain flavonoids. Paku resam leaves contains flavonoid sulfate form of kaempferol 3-O-glukopiranosil 7-O-NaSO4 and kaempferol 3-O glycosides. Kaempferol isolated from paku resam leaves can increase the activity of superoxide dismutase enzyme (SOD) at the dose of 5 mg / 200 kg BW (Wang, 2002). The ethyl acetate fraction of *G linearis* is able to reduce the levels of malondialdehyde significantly (Pauziah, 2012).

This study aimed to see whether endothelial cell dysfunction can be induced by NaCl administration. It is expected that this research can provide the knowledge to people that the ethyl acetate of fraction *G linearis* is one of the medicinal plants that can be used as an alternative treatment of endothelial dysfunction as well as provide

scientific information on the effect of the ethyl acetate fraction of *G linearis* (Burm.) Clarke against the levels of NO in order to be used as supporting data for the next research.

2. MATERIALS AND METHODS

A total of 15 kg of *G linearis* leaves boiled in 20 liters of water for 90 minutes and then filtered while hot, boiled water allowed to stand for 24 hours in the refrigerator. Water boiled and then filtered with filter paper to separate the precipitate and the supernatant. The supernatant was fractionated with ethyl acetate (3x1500 ml), then the solvent evaporated with a rotary evaporator at 60°C.

Characterization of ethyl acetate fraction of *G linearis* leaves involved testing of ion sufat, thin-layer chromatography (Ibnu, 2005). The calculation of total flavonoids levels (making of standard solution of kaempferol, manufacturing test solutions, the measurement wavelength of maximum absorption spectrophotometry UV-VIS (Andersen, 2006).

A total of 25 mice were divided into 5 groups of normal animals (KN) the group was given saline 28 mg/ kg (KP) and the group of ethyl acetate fraction of *G linearis* dose of 35 mg / kg BW, 70 mg / kg BW and 140 mg / kg BW given concurrently by NaCl a dose of 28 mg / kg. for 14 days. All the animals were sacrificed and the were serum taken for determination of the NO levels.

The serum levels of NO were obtained determined by using the ELISA methode Assay™ Total Nitric Oxide Assay microplate spectrophotometer and production of Bio-Rad. Preparation Colomeric Nitric Oxide Assay Kit.

3. RESULTS AND DISCUSSION

The obtained results of ethyl acetate fraction of *G linearis* yield is 0.13% against fresh sample, dry powder yellow-brown color, bitter taste, and typical smell with total flavonoid content is equivalent to 7.2067% pure kaempferol with elution of ethyl acetate-methanol-water (50: 3: 10) (Simionescu, 2007) stains seen with sitroborat with 366 nm wave length, positive for sulfate ions as shown in figure.1 and table.1 below.

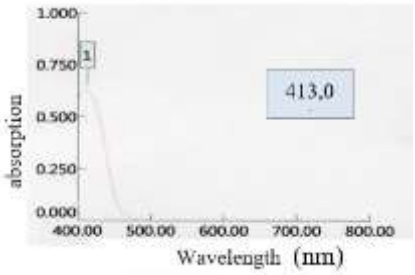

Figure.1. Measurement of maximum absorption wavelength of kaempferol

Table.1. Absorbance of standard solution of kaempferol

Content (µg/ml)	Absorbance
40	0.263
60	0.375
80	0.501
100	0.631
120	0.754

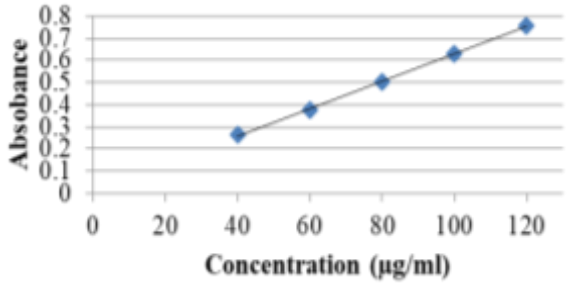

Figure.2. The regression curve of kaempferol standard solution

Information: $y = 0.006x + 0.0096$; $R^2 = 0.9994$

Table.2. Results of absorbance measurements of samples

No.	Concentration (mg/ml)	Absorbance	Average
1.	1	0.451	0.442
2.	1	0.429	
3.	1	0.446	

Total flavonoid levels in the sample is = 72.0667 µg KE/ml (72.0667 µg kaempferol equi./ml)

The observation of ethyl acetate fraction of *G linearis* administration in endothelial cell dysfunction induced by NaCl in mice showed an elevated levels of NO. Average levels of NO in negative control group was 3.547 nmol/mL; positive control was 1.719 nmol/mL; treatment dose of 35 mg/kg BW were 3.192 nmol/mL; 70 mg/kg BW 2.789; and 140 mg/kg BW of 2.545 nmol/ml, as seen in Table.3.

Tabel.3. The NO serum level of mice induced by NaCl after the administration of ethyl acetate fraction of *G. linearis*

Mice	NO level (nmol/µl)				
	KN	KP	D1	D2	D3
1	2.892	1.340	3.089	1.779	2.094
2	2.744	1.783	2.419	3.611	2.389
3	2.596	1.749	2.872	4.457	2.862
4	5.954	2.005	4.388	2.005	2.833
Average± SD	3.547 ± 1.609	1.719 ± 0.277	3.192 ± 0.844	2.789 ± 1.287	2.545 ± 0.370

KN = Negative control; KP = Positive control; D1, D2 & D3 = doses 35 mg/KgBW; 70 mg/KgBW and 140 mg/KgBW.

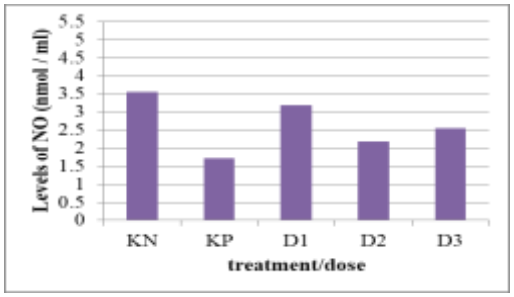

Figure.2. Histogram of NO levels in serum of each group of dose

KN = Negative control; KP = Positive control; D1, D2 & D3 = Doses 35 mg/KgBW; 70 mg/KgBW and 140 mg/KgBW

From Figure.2, and the average levels of NO serum in positive control was 1.719 lower than the negative control (normal animals), the value was 3.547 nmol / ml. Low levels of serum NO indicates that NaCl indeed can reduce levels of NO serum. The average serum levels of NO in treatment groups given the ethyl acetate fraction *G. linearis* a dose of 35 mg/KgBW, 70 mg/Kg BW, and 140 mg/KgBW respectively was 3.192; 2.789 and 2.545 nmol/ml. When compared with the positive control, the NO serum levels was increase. Percentage of the elevation in treatment groups a dose of 35 mg/KgBW was 85.66%; in 70 mg/KgBW was 62.23%; and in 140 mg/Kg BW was 48% (Table.4). Based on the table.4, we can see a dose of 35 mg/KgBW had the highest levels of NO. We can concluded that a dose of 35 mg/KgBW provided the highest protective effects of endothelial cells compared with a dose of 70 mg/KgBW and 140 mg/KgBW.

Table.4.The Percentage of increasing levels of NO in treatments groups compared to positive control group

Group	**Doses of Treatment Groups**	**The Percentage of increasing levels of NO in treatments groups compared to positive control group**
D1	35 mg/KgBW	85.662 %
D2	70 mg/KgBW	62.234 %
D3	140 mg/KgBW	48.001 %

D1, D2 & D3 = Doses 35 mg/KgBW; 70 mg/KgBW and 140 mg/KgBW

Based on statistical analysis by one-way ANOVA as shown in table.5, the p value = 0.026 then followed by Bonferroni analysis as shown in table.6, we can see that there is a significant difference between the positive control group compared to the treatment group given the ethyl acetate fraction of *G. linearis* a dose of 35 mg/KgBW ($p < 0.05$). While there is no significant difference between the positive control group treated with a dose of 70 mg/ KgBW and 140 mg/KgBW ($p > 0.05$). Therefore, the ethyl acetate fraction of paku resam dose of 35 mg/KgBW administration have a significant impact on NO serum levels in experimental animals induced by NaCl.

Table.5. Results of one-way ANOVA statistical analysis of NO levels in mice induced by NaCl (SPSS 17.00) after the ethyl acetate fraction of *G. linearis* administration

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.269	3	0.090	4.408	0.026
Within Groups	0.244	12	0.020		
Total	0.512	15			

Table.6. The Bonferroni Advanced Test

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	KP	D1	-0.34330*	0.10079	0.031	-0.6611	-0.0255
		D2	-0.26688	0.10079	0.128	-0.5846	0.0509
		D3	-0.25742	0.10079	0.152	-0.5752	0.0603
	D1	KP	0.34330*	0.10079	0.031	0.0255	0.6611
		D2	0.07642	0.10079	1.000	-0.2413	0.3942
		D3	0.08588	0.10079	1.000	-0.2319	0.4036
	D2	KP	0.26688	0.10079	0.128	-0.0509	0.5846
		D1	-0.07642	0.10079	1.000	-0.3942	0.2413
		D3	0.00946	0.10079	1.000	-0.3083	0.3272
	D3	KP	0.25742	0.10079	0.152	-0.0603	0.5752
		D1	-0.08588	0.10079	1.000	-0.4036	0.2319
		D2	-0.00946	0.10079	1.000	-0.3272	0.3083

*The mean difference is significant at the 0.05 level

Kaempferol (flavonoids) which contained in ethyl acetate fraction of *G linearis* can increase the levels of NO possibly by increasing the activity of endothelial NOS (eNOS), an enzyme that synthesizes NO in endothelial cells. In addition, increased levels of NO can also occur due to the effect of kaempferol (flavonoids) as natural antioxidants. In rats fed by high salt diet showed the decreasing levels of free radicals or ROS (Reactive Oxygen Species), and the administration of antioxidants can intensify the the SOD and NO levels (Saidu, 2012).

The ethyl acetate fraction of *G linearis* administration with a dose of 35 mg/KgBW had a higher NO levels compared with a dose of 70 mg/KgBW and 140 mg/KgBW. This is probably occured by the reduction antioxidant activity concomitant with the increasing doses of antioxidant activity of flavonoids. At high concentrations, the antioxidant activity of phenolic groups often decrease or disappear, even these antioxidants become prooxidant (Cillard,1980). By the addition of flavonoids, would produce a radical antioxidants (A*) that are more stable. However, at high concentrations, flavonoids will be a tie breaker of free radicals.

From the results, we can concluded that the ethyl acetate fraction of *G. linearis* administration can increase the serum levels of NO in NaCl-induced mice at all doses with the highest increasing levels of NO was seen in treatment group by a dose of 35 mg/KgBW that is 85.66%. A dose of 35 mg/KgBW significantly different compared with positive control group and there were not significantly differences with the treatment groups at a dose of 70 mg/KgBW and the dosage of 140 mg/KgBW compared to the positive control group ($p > 0.05$).

4. CONCLUSION:

The results of the study of the effect of ethyl acetate fraction of paku resam leaves (Gleichenia linearis (Burm. Clarke) on endothelial cell dysfunction of white male induced by sodium chloride (NaCl) at a dose of 28 mg/kg BW showed an increasing level of NO in mice serum given the paku resam extract with a dose of 35, 70 and 140 mg/kg BW with increasing percentage of NO levels at 85.66; 62.23; and 48%. After being analysed by one-way ANOVA only the dose of 35 mg / kg BW of the positive control showed the levels of NO significantly different (prevents the endothelial cell dysfunction in mice).

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