Indexed by : DOAJ DIRECTORY OF OPEN ACCESS JOURNALS Google Scholar Crossref

J.Biomed.Transl. Res
ISSN: 2503-2178

JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

The Effect of *Eurycoma longifolia Jack* on sICAM-1 and eNOS in Rats with High Fat Diet

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Article Info
History:
Received: 17 May 2018
Accepted: 26 Jun 2018
Available: 31 July 2018

Abstract

Background: High fat diets are known to cause a positive fat balance and consequently to the accumulation of adipose mass. The molecular mechanisms underlying the anti-inflammatory activity β-carboline alkaloids *E. longifolia*, which may be useful to prevent or treat diseases of inflammation. Recent studies showed that *E. longifolia Jack* protected HFD animal model from atherosclerosis based on the reducing of atherosclerotic plaque size and formation HFD-rats treated with *E. longifolia Jack*.

Objective: To prove that *Eurycoma longifolia* has anti inflammatory effect on endothelial cell blood vessels of *Sprague Dawley* rat with high fat diet.

Method: Study design was experimental study, by used Randomized Post Test only Control Group Design with Kruskal-Wallis test to analyzed the differences among groups and followed by a Mann Whitney test. Treatment is ethanol or water extract of *Eurycoma longifolia* Jack, and outcome are sICAM-1 and eNOS levels. Thirty *Sprague Dawley* (SD) Rat, were divided into 6 groups. C(-) was SD group, C(+) was group with HFD, X_1 (SD treated with EL dosage 10 mg/kg), X_2 (SD treated with EL dosage 15 mg/kg), X_3 (HFD treated with EL dosage 10 mg/kg), X_4 (HFD treated with EL dosage 15 mg/kg).

Result: No significant difference in sICAM-1 that was found among the studied groups. sICAM-1 levels of X1 and X2 groups were not different than those of negative control group. No different was observed between sICAM-1 level of X3 group than positive control group. There is significant difference in eNOS among six group studied. The control negative group was not significance different than control positive, X2, X3, and X4. X1 showed significant different with negative control, X3 and X4. The positive control showed that no significant different than X1, X2, X3, and X4.

Conclusion: *Eurycoma longifolia* has anti-inflammatory effect especially on eNOS of *Sprague Dawley* rat with high fat diet.

Keywords: *Eurycoma longifolia* Jack; sICAM; eNOS; HFD.

INTRODUCTION

High fat diets are known to cause a positive fat balance and consequently to the accumulation of adipose mass, this diet does not seem to stimulate fat oxidation in the same way in obese and lean subjects[1].

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The HFD increases the level of circulating bad cholesterol which boosts the cardiovascular event risk. HFD also increases the risks of other diseases include diabetes, stroke, and some types of cancers. The oxidative stress occurs due to HFD, this is then results in hypertension and metabolic disorder[2,3].

HFD also causes obesity which became a worldwide epidemic and a major risk factor of several diseases including metabolic syndrome.[4] Globally, about 39% of adults aged 18 years and over were overweight in

2014 and 13% of them are clinically obese.[5] Therefore, prevention and treatment of obesity is an important factor for a healthy condition. The reduction of nutrient digestion and absorption to develop inhibitors of the enzyme without altering the main mechanisms in the digestive system into the most important strategies in the treatment of obesity. [6,7]

HFD was an inducing factor for intercellular adhesion molecule-1 (ICAM-1) expression in the aorta of Wistar rats. HFD effect on ICAM-1 seems to be time dependent. ICAM-1 is one of the first events in the formation of atherosclerotic lesions. Additionally, soluble ICAM-1 measurement of the concentration in plasma is a reliable index of the expression of the molecules in the Wistar rat aorta.[8] Previous studies have found that HFD up-regulated Cav-1 and down regulated of eNOS activity in rat thoracic aorta; switch to a normal diet is restored levels of Cav-1 and eNOS; no change in Cav-1 or eNOS expression was observed in DR rats ate a high-fat diet; high-fat diet increased eNOS (Ser1177) and Akt (Ser473) phosphorylation in diet-induced obese mice.[9]

The use of natural products in health problem now become noticeable.[10] *Eurycoma longifolia Jack* (Simaroubaceae family) is one of the most popular medicinal plants in Southeast Asia, including Myanmar, Thailand, Laos, Indonesia, Cambodia, and Malaysia.[11] Local *E. longifolia Jack* has many local name, for example: Tongkat Ali (in Malaysia), Bidara Bitter (in Indonesia) and Cay babinh (in Thailand) meaning as a tree that can cure hundreds of diseases.[12] This plant contains quassinoids, squalene derivatives, neolignans biphenyl, tricullane-type triterpene, canthine-6-one and β-carboline alkaloids. Quassinoid is a bitter substance found in Simaroubaceae family that has a lot of biological activity as anticancer, anti-microbial, anti-inflammatory and many other protective effect.[13] Recent studies showed that *E. longifolia Jack* protected HFD animal model from atherosclerosis based on the reduce atherosclerotic plaque size and formation HFD-rats treated with *E. longifolia Jack.*[14] Similar animal model also showed that *E. longifolia Jack* reduced levels of triglyceride and blood pressure of HFD-rats.[15] Antioxidant and anti-inflammatory effect of *E. longifolia Jack* had been proven in *in vitro* study.[16] However, it remains unknown whether any anti-inflammatory effect of *E. longifolia Jack* involves in the protection of HFD-rats.

β-carboline alkaloid 7-MCPA (7-methoxy- (9H-β-carbolin-1-yl) - (E) -1-propenoat acid) isolated from *E. longifolia* hairy root culture Nrf2 is activated via a ROS dependent p38 MAPK and 7-MCPA anti-inflammatory effects associated with activation of 7-MCPA-induced of Nrf2 / HO-1 pathway. This study clarified the molecular mechanisms underlying the anti-inflammatory activity of β-carboline alkaloids *E. longifolia*, which may be useful to prevent or treat diseases of inflammation.[17] Eurycomalactone, 14,15β-dihydroklaieanone, and 13.21-dehydroeurycomanone identified as potent inhibitors of NF-kB with IC50 values of <1 pM.[18] For this study, the mice will be treated and given a high-fat diet EL extract from the roots as much as 5 mg and 10 mg / kg body weight for 14 days to determine the levels of sICAM-1 and eNOS.

This research aimed to prove that *Eurycoma longifolia* has anti-inflammatory effect on endothelial cell blood vessels of *Sprague Dawley* rat with high fat diet.

MATERIALS AND METHOD

This study design was experimental study, by used randomized post test only control group design. Kruskal-Wallis test was used to analyze the differences among groups and followed by a Mann Whitney test. Population of this study was *Sprague Dawley* (SD) Rat, with inclusion criteria were: male; weight 150-200 gram; 8 - 9 weeks of age; and healthy indicated by active movement and anatomically normal. Thirty *Sprague Dawley* (SD) Rat, were divided into 6 groups. C(-) was SD group, C(+) was group with HFD, X_1 (SD treated with EL dosage 10 mg/kg), X_2 (SD treated with EL dosage 15 mg/kg), X_3 (HFD treated with EL dosage 10 mg/kg), X_4 (HFD treated with EL dosage 15 mg/kg).

Treatment was ethanolic or water extract of *Eurycoma longifolia* Jack, and outcome were sICAM-1 and eNOS levels. High fat diet fed composition were additional cholesterol 2%, cholic acid 0.2%, and pig oil 5%. This the highest fat diet compotition confeed PARS 200 gram, yolk of quail egg, cholesterol 8 gram, cholic acid 0.8 gram, pig oil 40 ml, water 51.2 ml. Total of high fat diet was 40 gram/rat. Before treatment, rats have been adapted for 7 days, observed for healthy condition and was weighted, and then randomly were alocated into 6 groups. Body weight was evaluated every week. Each rat was treated in the separate plastic cage. Every day, researcher scaled the rat's feed to know the intake of rat/ day. During treatment, rat was given high fat diet feed and extract *Eurycoma longifolia* Jack dosage 10 mg/kg BW and 15 mg/kg BW until 60days treatments by force feeding (oral).

RESULTS

ICAM-1

The highest level of ICAM-1 in the group SD was found in X2 group and followed by negative control and X1 group. The highest level of sICAM-1 in the HFD groups was the positive control group followed by X3 and X4 groups (Graph 1.). Normality test showed that the distribution level of sICAM-1 in each group was normally distributed including negative control (p = 0.200), X2 group (p = 0.091), positive control (p = 0.200), and X4 group (p = 0.144) and not normally distributed in the X1 and X3 group (p = 0.003 and p = 0.001), respectively. The homogeneity test with Kruskall Wallis test showed that the group was not homogen (p = 0.023). The test showed that no significant difference among groups (p = 0.087).

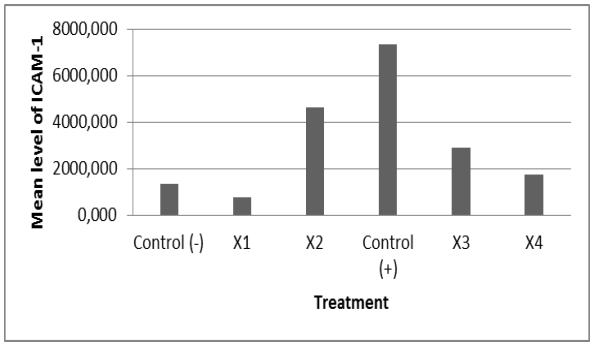

Figure 1. Level of sICAM-1
(Control (-): SD, X1: SD treated with EL dosage 10 mg/kg ; X2: SD treated with EL dosage 15 mg/kg; Control (+): HFD; X3: HFD treated with EL dosage 10 mg/kg; X4: HFD treated with EL dosage 15 mg/kg)

Table 1. Mann Whitney U test Level of sICAM-1

Group	Median (min – Max) pg/ml	*p* value				
		Pos	X1	X2	X3	X4
Neg	(1689.40 – 15109.48)	0.056	0.548	0.548	0.548	0.841
Pos	(-491.36 – 2863.66)		0.016*	0.310	0.095	0.056
X1	(11.89 – 3199.16)			0.056	0.421	0.421
X2	(347.39 – 12928.72)				0.151	0.310
X3	(-155.86 – 14606.23)					0.310
X4	(179.64 – 6050.93)					

sICAM-1 levels of X1 and X2 groups were not different than those of negative control group (p = 0.548 and p = 0.548). By comparing of positive and negative control, it was found sICAM-1 level of positive control was nearly significantly lower than negative control control (p = 0.056). There was no different result observed between sICAM-1 level of X3 group than positive control group (p = 0.095), however sICAM-1 levels of X4 group was nearly significantly lower than positive control (p = 0.056). Additionally sICAM-1 levels of both X3 and X4 groups were comparable to that of negative control (p = 0.548 and p = 0.841) (Table 1).

eNOS

Circulating eNOS level of X1 and X2 was higher than negative control group. Interestingly, eNOS level on positive control group was higherthan negative control group. Those e NOS level on X3 and X4 were below detectable level i.e. lower than either positive or control (Graph 2.)

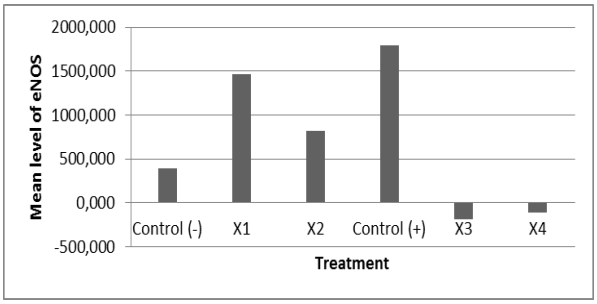

Figure 2. Mean level of eNOS
(Control (-): SD, X1: SD treated with EL dosage 10 mg/kg ; X2: SD treated with EL dosage 15 mg/kg; Control (+): HFD; X3: HFD treated with EL dosage 10 mg/kg; X4: HFD treated with EL dosage 15 mg/kg)

Normality test performed in every group studied showed that eNOS level was normally distributed (p> 0.05). These were SD groups negative control (p = 0.053), X1 (p = 0.200) were normally distributed, however X2 group was not normally distributed (p = 0.010). These were also observed in groups with HFD including positive control (p = 0.193), X3 group treated with 10 mg/kg BW/day EL(p = 0.200) and X4 group treated with 15 mg/kg BW/day EL (p = 0.200) normally distributed. Test homogeneity showed that the groups are not homogen (p = 0.000). Kruskall Wallis test showed significant difference among six group studied (p = 0.033). The Mann Whitney U test indicate the different between groups in these study (Table 2).

The control negative group was not significance different than control positive, group II, group III, and group IV (p = 0.841; p = 0.310; p = 0.056; p = 0.095), respectively. The group I showed significant different with negative control, group III and group IV (p = 0.032; p = 0.008; and p = 0.008), respectively. The positive control showed that no significant different than group I, group II, group III, and group IV (p = 0.690; p = 0.310; p = 0.095; and p = 0.151), respectively.

Table 2. Post Hoc Analyses of eNOS

Groups	Median (Min – Max) pg/ml	*p* value				
		Pos	X1	X2	X3	X4
Neg	(-30.84 – 4732.55)	0.841	0.032*	0.310	0.056	0.095
Pos	(-30.84 – 649.65)		0.690	0.310	0.095	0.151
X1	(552.44 – 2205.04)			0.151	0.008*	0.008*
X2	(-614.11 – 48892.76)				0.690	0.690
X3	(-614.11 – 260.80)					0.841
X4	(-516.90 – 552.44)					

DISCUSSION

The aim of study was observing the effect of root extract of *Eurycoma longifolia* in reduces sICAM-1 and eNOS levels of *Sprague Dawley* rat with high fat diet. This study was done to further explore of the

mechanism used by EL in protecting vascular endothelial cells in HFD individual. EL effect on sICAM and eNOS levels were observed in rats receiving a combination of EL and HFD for two months period. Endothelial NOS that is commonly expressed in endothelial cells, has many favorable effects, including maintaining vascular dilatation, influencing blood pressure, vasoprotectitive and anti-atherosclerosis development and progression.[19]

This study showed that the EL can not reduce levels of ICAM-1 of *Sprague Dawley* rat that have been fed by HFD. The serum ICAM-1 levels of negative controls receiving normal diet were not different than the positive controls receiving HFD. The HFD may not be sufficient or may not have been feed for a sufficient duration to induce changes in ICAM-1 concentration. The previous study which used baboon as the model of animal showed that high cholesterol of high fat on ICAM-1 did not significant change during 7 weeks period.[20]

This study showed that the eNOS levels of negative control consuming SD only were lower than those of receiving HFD. The eNOS concentrations were significantly lower by consuming HFD for 7 weeks period.[20] These findings were supported by studies conducted on mice receiving high-fat diets that showed decreased eNOS expression in the liver, heart and medulla kidney.[21] The HFD mice used in this study were confirmed as animal models of atherosclerosis exhibited by the presence of foam cells in the aortic wall.[22] The reduced circulating eNOS levels observed in HFD mice may involve the formation of foam cells because the presence of eNOS is capable of preventing the formation of decay cells.[23] The formation of foam cells, occurs after macrophages take OxLDL.[24] OxLDL has the ability to inhibit eNOS activity.[25] Several other factors may explain the decrease in eNOS production in HFD mice. TNF-α may be one of the contributing factors in eNOS level decline. Studies using similar HFD mice showed an increase in the number of cells expressing TNF-α significantly.[26] TNF-α reduces the rate of eNOS mRNA from endothelial cells of human coronary arteries.[27] The HFD rat model for atherosclerosis shows reduced production of eNOS.

The regulation of eNOS expression induced by mechanical forces, other stimuli has been shown to modulate the expression of eNOS mRNA in vitro. Most of these stimuli have been implicated in vascular pathophysiology, and they include other mechanical forces, cell growth, cytokines, lipoproteins, growth factors, and oxidative stress. Changes in eNOS mRNA expression and cell ability to produce NO are generally in the order of two to threefold. Although this modulation level may seem simple, it should be remembered that small changes in NO levels may have significant physiological effects. In the case of vascular relaxation, the dose response to NO is quite steep; small increases in NO concentrations resulted in major changes in vascular tone.[28] Recent research has provided a more detailed understanding of the molecular mechanisms involved in eNOS expression modulation.

Although EL treatment has no significant different on ICAM-1, but EL with a dose of 15 mg / kg is lower than EL at a dose of 10 mg / kg. Many bioactive compounds have been isolated from *E. longifolia*, such as quassinoid, canthine-6-one alkaloids, β-carbolin alkaloids, squalene derivatives, triterpenestirucallane, biphenylneolignans, phenolic compounds, and bioactive steroids. Through a bioguided isolation approach, it has recently identified several inhibitors of the NF-κB transcription factor (the kappa-light core factor of activated B cells) at the root of *E. longifolia*.[29] One of the most interesting compounds appears to be Eurycoma lactonequassinoid C-19. The transcription factor NF-κB is a central performer in the inflammatory response that regulates, for example, the expression of endothelial adhesion molecules, such as VCAM-1, ICAM-1, or E-selectin, which is essential in inflammatory initiation since adhesion molecules promote extravasation of leucocytes to the site of injury.[30] NF-κB signaling pathways are activated in response to pro-inflammatory cytokines such as TNFα or other pro-inflammatory stimuli, such as lipopolysaccharide (LPS).[31] NF The family of transcription factors consists of five transcription factor proteins (p65 (RelA), c -Rel, RelB, p50, and p52) which are usually found as homo or heterodimer. In most cell types, the prevalent retained p65 / p50 heterodimer is inactivated in the cytoplasm by closing the sequence of nuclear localization by one of several kB protein inhibitors (IκB), which IκBα is a prototypical member.[31] Pro-inflammatory stimulation induces a signal cascade which causes the phosphorylation of IκB kinase (IKK) in the activation loop (Ser177 and Ser181). The phosphorylated IKK complex in turn phosphorylates IκB to mark degradation through the 26S proteasome.

The results of this study also show that EL does not affect the eNOS level. However, the level of eNOS in group IV is higher than in group III. The *E. longifolia* water extract increases the expression of NOS to form NO and also inhibits PDE3, PDE4 and PDE5. It is known to be involved in a myriad of biochemical processes in the human body including smooth muscle relaxation.[32] Thippeswamy & Marris reported that NO produced by nNOS induced cGMP synthesis. Penile eruption is a proven androgen-dependent NO-mediated process. In penile erection, NO places its target molecule from guanylylcyclase that dissolves on the smooth muscle surface of the corpus cavernosum and causes a conformational change in the enzyme leading to increased production of a 3'-5'-cylic messenger guanosine. monophosphate (cGMP) of guanosine triphosphate (GTP). cGMP induces relaxation of smooth muscle in the corpus cavernosum and allows blood to flow into the penis where it becomes trapped. The degradation and loss of cGMP from smooth muscle tissue causes contraction and normal blood flow in and out of the corpora cavernosa. PDE5 is dominant in the penis, breaking cGMP into GMP by catalyzing the reaction that damages the phosphodiester bond[33]. *E. longifolia* helps maintain high levels of cGMP in the corpora

cavernosa by pressing PDE5 to break down cGMP. Inhibition of PDE5 increases the duration of cGMP remain in smooth muscle tissue thus increasing.[33]

CONCLUSION

No significant difference on level of ICAM-1 was found among groups with or without HFD treatment. Significant difference on the level of eNOS was found among groups with or without HFD treatment.

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