Effects of Herbal Ingredients (*Allium sativum, Punica granatum, Curcuma longa, Curcuma xanthorrhiza*) on FATP3 Gene Expression in Aorta of High Fat Diet-fed Rats: A Preliminary Study

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Abstract : FATP (Fatty Acid Transport Protein) is a protein that facilitates uptake of LCFA (Long Chain Fatty Acid) by activating it into CoA-thioester and trapping them in the cell. FATP3 is critical for LCFA uptake in endothelial cells. Herbal ingredients are well known as anti-hyperlipidemic and anti-atherosclerotic agents, but the molecular mechanism for these effects are still unclear. Twenty-eight male Wistar rats used in this study were divided into negative control, positive control (HFD), and treatments (175 mg/kg BW *Allium sativum, Punica granatum, Curcuma longa, Curcuma xanthorrhiza*, and 1.8 mg/kg BW Rosuvastatin), each group consisted of 4 rats. The rats were given vitamin D 700.000 mg/kg BW single dose to all groups except for negative control, continued with HFD combined with herbal ingredients for twelve weeks. After treatments, the rats were sacrificed, RNA was extracted from the aorta to perform semi-quantitative PCR (FATP3 and GAPDH). We found no significant differences in FATP3 gene expression between all groups. In summary, herbal ingredients (*Allium sativum, Punica granatum, Curcuma longa, Curcuma xanthorrhiza*) do not influence FATP3 gene expression in the aorta of high fat diet-fed rats.

1 INTRODUCTION

Population around the world has been through a modern transition, where the trends of a sedentary lifestyle and over calories become more prominent than under-nutrition (Shao et al., 2017). This transition might lead to obesity that served as a risk factor for developing metabolic syndrome, thus

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increasing the risk for atherosclerosis (Aboonabi, Meyer, & Singh, 2019). Recent research has shown that metabolic syndrome promotes atherosclerotic lesions, and endothelium might mediate some of the effects (Aboonabi et al., 2019; Goldberg & Bornfeldt, 2013). The main risk factor for developing obesity that might contribute to the incidence of atherosclerosis is dietary fat intake (Csige et al., 2018). The previous study has shown that dietary intake of Long Chain Fatty Acid (FATP) in large amounts might induce lesions of atherosclerosis (Blair, Sepulveda, & Papachristou, 2016).

LCFA, mostly found in our dietary lipid intake, require active transport into the blood flow (Dallinga-Thie et al., 2010). FATP (Fatty Acid Transport Protein), a family of transmembrane proteins, has been proven to improve LCFA cellular uptake (C. Hagberg, Mehlem, Falkevall, Muhl, & Eriksson, 2013; Stahl, Gimeno, Tartaglia, & Lodish, 2001). FATP has 6 members, ranging from FATP1 until FATP6, that could be found in many organs utilizing fatty acid (Stahl et al., 2001). FATP3 is expressed in endothelial cells including aorta and works synergically with FATP4 to induce LCFA uptake (C. E. Hagberg et al., 2010). Abnormal LCFA influx into skeletal muscle, heart, the liver might lead to insulin resistance, oxidative stress, and eventually apoptosis, therefore it is important to study the mechanism of LCFA uptake by FATP to identify potential therapy for a metabolic disease that might lead to atherosclerosis (Aboonabi et al., 2019; Anderson & Stahl, 2013; Kazantzis & Stahl, 2012).

Herbal ingredients, such as *Allium sativum, Punica granatum, and Curcumin* has been known for having the effect of anti-atherosclerosis (Koscielny et al., 1999; Majeed, Ghafil, Fatima, Hadi, & Mahdi, 2021; Supekar & Kale, 2015). Low-dose curcumin reduce atherogenesis in a mouse model of human atherosclerosis through the suppression of CD36 (a FATP) in macrophages (Hasan et al., 2014). *Allium sativum* inhibited the thickening of neointimal in rabbits given high cholesterol diet, and in cell culture treated with atherosclerosis patient's serum, reduced atherogenic potential was shown (Sobenin et al., 2016; Sobenin, Myasoedova, Iltchuk, Zhang, & Orekhov, 2019). *Punica granatum* reduced the progression of atherosclerosis in hypercholesterolemic mice (de Nigris et al., 2007). A clinical trial of *Curcuma longa* in patients at risk of CVD showed evidence of *Curcuma xanthorrhiza* beneficial effects on serum TG and LDL-C levels (Qin et al., 2017). The study of *Curcuma xanthorrhiza* has proven that *C. xanthorrhiza* decreased LDL-Cholesterol level and Total-Cholesterol level, and increased HDL-Cholesterol level in dyslipidemic Sprague Dawley rats (Budiarto et al., 2017). Although many kinds of research have proven the anti-hyperlipidemic and anti-atherosclerotic effect of these herbal ingredients, little is known about the detailed molecular mechanism involving FATP3 in the aorta.

In the present study, we want to elaborate on the effect of herbal ingredients in the aorta of high-fat diet-fed rats. According to previous study, atherosclerosis could be induced by vitamin D3 single dose and three months of high lipid diets in rats (Pang et al., 2010). Therefore, in this study, we aim to know whether a high-fat diet would influence FATP3 in the aorta of Wistar rats after supplementation of herbal ingredients (*Allium sativum, Punica granatum, Curcuma longa,* and *Curcuma xanthorrhiza*).

2 METHODS (AND MATERIALS)

2.1 Animals

Twenty-eight male Wistar rats, aged 8 weeks, weight 200-220 grams, were divided into seven groups (negative control, positive control, *A. sativum, P. granatum, C. longa, C.xanthorrhiza*, and Rosuvastatin). The rats were put in a cage per group and given a high-fat diet. The temperature was maintained between 22-24°C each day and light-dark cycle every 12 hours. The rats were environmentally habituated for 1 week, continued with vitamin D3 700.000 mg/kg BW orally single dose, then high-fat diet for 12 consecutive weeks, except the negative control group that was given standard chow diet (Pang et al., 2010). The treatment was given for 12 weeks and there was 175 mg/kg BW of *A. sativum, P. granatum, C. longa, C. xanthorrhiza* ethanol extract, and 1.8 mg/kgB Rosuvastatin. On the final day of treatments, rats were terminated, and aorta was taken, then stored in a -80°C refrigerator until further use for RNA extraction and PCR.

All procedures were conducted according to the use and care of laboratory animal guidelines (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, 2011). Ethical approval was obtained from the Faculty of Medicine's Research Ethics Committee in Universitas Kristen Maranatha-Rumah Sakit Immanuel Bandung with the number 160/KEP/XI/2020.

2.2 RNA Extraction and Semi-quantitative PCR

We conducted extraction of RNA from stored aorta using Trisure reagent, with the proportion of 200 ul Trisure per 10-20 mg sample (BIO-38033, Bioline, London). After measuring the purity and concentration of the extracted RNA using 260/280 nm absorbance spectrophotometry (51119300, Multiskan Go Microplate Spectrophotometer, Thermo, Netherland), we conducted semi-quantitative PCR using One-Step RT PCR Kit (BIO-65409, Bioline, London). We used GAPDH as the housekeeping gene. After PCR, we continued with electrophoresis, then visualization of the gels using Bluepad, and image quantification using Image J. Primer sequences used in this study were as follows: for FATP3:
Fwd 5'- CTGGGACGAGCTAGAGGAAG -3',
Rev 5'- GCTGAGGCCAGAGGTCTAAC -3'
(Lee et al., 2017)
GAPDH:
Fwd 5'- GTTACCAGGGCTGCCTTCTC-3',
Rev 5'- GATGGTGATGGGTTTCCCGT-3'
(Wang et al., 2017).

2.3 Statistical Analysis

The result of the study (relative ratio of gene expression) is presented as mean ± SEM. Statistical analysis was done using SPSS 26.0 statistical software (IBM, United States). ANOVA continued with LSD post hoc analysis was used for testing the difference between the groups, and $p<0.05$ is considered as significant.

3 RESULTS AND DISCUSSION

We presented the result One Way Anova analysis for the mean relative ratio of FATP3 gene expression normalized by GAPDH in table 1 below.

Table 1: One Way Anova of FATP3 Gene Expression in Aorta of Wistar Rats.

Groups	FATP3 Relative Ratio ± SEM	N	*F*	*p*
Negative control	0.88 ± 0.06	4	1.524	0,219
Positive control	0.74 ± 0.07	4		
Allium sativum	0.97 ± 0.06	4		
Punica granatum	0.89 ± 0.04	4		
Curcuma longa	1.01 ± 0.07	4		
Rosuvastatin	1.02 ± 0.09	4		
Curcuma xanthorrhiza	0.97 ± 0.07	4		

The result of semi-quantitative PCR and the graphical result of the study is presented in figure 1 below

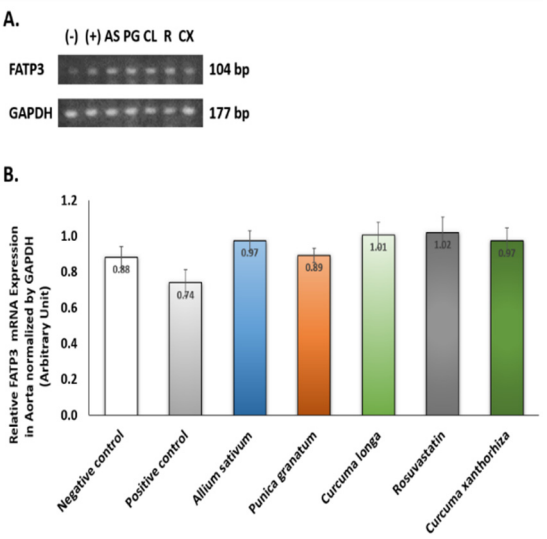

Figure 1: A. FATP3 and GAPDH Gene Expression from Aorta of High Fat Diet-fed Rats; B. Relative Ratio of FATP3 Gene Expression from Aorta of High Fat Diet-fed Rats. (-) = Negative control, (+) = Positive control, AS = *Allium sativum*, PG = *Punica granatum*, CL = *Curcuma longa*, R = Rosuvastatin, CS = *Curcuma xanthorrhiza*

Protein-mediated transport active has been proven to be the major route for the entering of LCFA into the cells. Therefore, a comprehensive understanding of FATPs would guide the possibility of making them a promising target therapy for treating metabolic diseases (Glatz, Luiken, & Bonen, 2010). FATP3 and FATP4 are endothelial fatty acid transport, both required for effective LCFA transport through the barrier of the vascular endothelium (C. Hagberg et al., 2013). Research showed that a high-fat diet induces an increase of FATP1 in soleus muscle, but a decrease in gastrocnemius muscles and this contrary effect might be caused by different fiber types correlated with distinct PPAR gamma sensitivity (Marotta et al., 2004). In the intestine of humans, FATP4 is upregulated after 3 days of a high-fat diet (Tremblay et al., 2013). But research about the effect of a high-fat diet on FATP3 gene expression is still limited.

In this study, we found no significant difference in FATP3 gene expression between groups (figure 1). This is a preliminary study that aims to investigate whether FATP3 in the aorta might change after given a high-fat diet and vitamin D3 that might induce atherosclerosis and after supplementation of herbal ingredients. This result might show that a high-fat diet and herbal ingredients might not influence FATP3 gene expression in the aorta, but there is a tendency

of increase of FATP3 gene expression in treatment groups compared to control. This tendency to increase might show increased activity of FATP3 on LCFA uptake as compensation to prevent lipid deposition in non-adipose tissue. While in the positive control, high fat diet and vitamin D3 that potentially induce atherosclerosis decrease FATP3 gene expression. We hypothesize this effect (table 1) may occur because of endothelial dysfunction that might potentially found in early atherosclerosis (Gimbrone Jr & García-Cardeña, 2016), but further investigation needs to be conducted to support this hypothesis.

The limitation of this study is: (1) time duration of HFD and vitamin D3 induce atherosclerosis might be too short, therefore we suggest a longer time duration to achieve a better perspective of the molecular mechanism behind fatty acid transport protein alteration in aorta after supplementation of herbal ingredients; (2) herbal ingredients formulation might not be appropriate and need to be adjusted for better result of the experiments; (3) microscopic evaluation to confirm atherosclerosis, such as Weigert and van Kossa staining (Pang et al., 2010) is not provided in this study.

4 CONCLUSIONS

In summary, herbal ingredients (*Allium sativum, Punica granatum, Curcuma longa,* and *Curcuma xanthorrhiza*) do not influence FATP3 gene expression in the aorta of high-fat diet-fed Wistar rats. Further study needs to be conducted to investigate the detailed mechanism of LCFA transport change in hyperlipidemia states and the role of herbal ingredients as anti-atherosclerotic agents.

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