

**THE EFFECT OF EVOO IN REDUCING ICAM-1 AND  
INCREASING eNOS IN HIGH FAT DIET RATS**

**PENGARUH EVOO UNTUK MENURUNKAN ICAM-1 DAN  
MENINGKATKAN eNOS PADA TIKUS DENGAN DIET  
TINGGI LEMAK**



**Thesis**

**Submitted as partial fulfilling of the requirement  
for Master Degree of Biomedical Science**

**KHALED MOHAMED SALEM BLHAJ  
22010112419053**

**FACULTY OF MEDICINE  
DIPONEGORO UNIVERSITY  
SEMARANG  
2014**

## *APPROVAL PAGE*

# **THE EFFECT OF EVOO IN REDUCING ICAM-1 AND INCREASING eNOS IN HIGH FAT DIET RATS**

Arranged by

# **KHALED MOHAMED SALEM BLHAJ**

## **22010112419053**

Semarang July 2014

**Approved by:**

### **Supervisor I**

Supervisor II

Prof. Dr. Lisyani B Suromo. Sp. Pk(K)  
NIP. 130354869

Dr .dr.RA Kisdjamiatun. RMD. MSC  
NIP. 196401301990032001

**Approved by,**

Head of Master Degree Program in Biomedical Science

Faculty of Medicine Diponegoro University

Prof. Dr. dr. Tri Nur Kristina, DMM, M.Kes  
NIP 195905271986032001

## **DECLARATION**

I hereby declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education, there are no elements belonging Plagiarism forth in Decree No. 17 of 2010. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of reference is given.

Semarang, July, 2014

Khaled Blhaj

## **ACKNOWLEDGEMENT**

I am deeply grateful to my supervisor, Prof. Dr. Lisyani B Suromo. Sp. Pk(K) and co-supervisor, Dr. dr.RA Kisdjamiyatun. RMD. MSC, for the time they spent for guidance and proofreading this thesis and for constant concern to support this study as well.

My special thanks to Prof. Dr. dr. Tri Nur Kristina. DMM. M.Kes, the Head of Master Degree Program in Biomedical Science Faculty of Medicine of Diponegoro University, and as my examiner for the patient and kindness in encouraging me to do everything in order to finish my thesis.

I would like to say my gratitude to my examiner Prof. dr. Edi Dharmana, M.Sc, PhD, Sp. Park who spends his time to examine me when I was doing my thesis proposal exam.

Special thanks go to University of Diponegoro Semarang, Biomedic Department, and Kariadi Hospital.

My colleagues from the Department of Biomedicine Science of Diponegoro University. I want to thank them for all their help, support, interest and valuable hints. I also express my special thanks to Miss Nata Sulastri, Miss Fika and Abdul Chaqim for helping me.

My gratitude to LBBT of Gajah Mada University, the place where the experiment of this study has been done.

I would like to express my gratitude to all those who gave me the possibility to complete this thesis.

I would like to thank my family, my father Mohamed and my mother and all my brothers and sisters and my friends Ibrahim, Salem, Amer, Ahmed, Ahmed Muazeib, Abdulrahman, Yabes, Leny, Fitri, Hana, Diana and Asma and all my friends.

Semarang, July, 2014

Khaled Blhaj

## TABLE OF CONTENT

	Page
TITLE PAGE .....	i
APPROVAL PAGE .....	ii
DECLARATION.....	iii
ACKNOWLEDGEMENT .....	iv
TABLE OF CONTENT .....	vi
LIST OF ABBREVIATION .....	ix
LIST OF FIGURES.....	xii
LIST OF TABLES .....	xiii
LIST OF APPENDIXS.....	xiv
ABSTRACT .....	xv

<b>CHAPTER I. INTRODUCTION</b> .....	<b>1</b>
Background .....	1
Research Question .....	4
Research Objectives .....	4
Research Benefits .....	5
Research Originality .....	5

## **CHAPTER II**

High-fat diet .....	9
Complication of high fat diet .....	10
Risks caused by high fat diet .....	10
Diabetes mellitus .....	11
Obesity .....	12
Lipids Metabolism of high fat diet .....	13
Roles of Lipids .....	13
Digestion of lipids .....	13

	page
Lipoproteins .....	14
Types of Dietary Fat .....	15
Saturated fat .....	15
Trans fat .....	16
Monounsaturated fatty acids .....	16
Polyunsaturated fatty acids .....	17
Extra virgin olive oil .....	17
Active Compound of Extra virgin olive oil .....	18
The benefits of Extra olive oil .....	19
Endothelial cells .....	20
Endothelial nitric oxide synthase (eNOS).....	20
Nitric oxide.....	22
Intercellular adhesion molecule-1 (ICAM-1).....	24
Role ICAM-1.....	25
Theoretical Framework .....	26
Conceptual Framework .....	27
Hypothesis .....	27
 <b>CHAPTER III. RESEARCH METHODOLOGY</b> .....	 28
Scope of study .....	28
Place and time of the research.....	28
Research Design .....	28
Population and Sample .....	29
Population .....	29
Inclusion Criteria.....	29
Exclusion Criteria .....	29
Drop out .....	30
Randomization .....	30
Sample size.....	30

	page
Variables of Study .....	30
Independent Variables .....	30
Dependent Variables.....	30
Operational Definition .....	31
Data Analysis .....	31
Research Instrument and Substance .....	32
Instrument .....	32
Material.....	32
Experiment methods .....	33
Research Flow .....	35
Research Ethics .....	36
 <b>CHAPTER IV. RESULTS</b> .....	 37
Level Of ICAM-1 .....	37
Level Of eNOS .....	38
 <b>CHAPTER V. DISCUSSION</b> .....	 42
Limitations of the Study .....	42
 <b>CHAPTER VI. CONSLUSION AND SUGGESTION</b> .....	 43
Conclusion .....	43
Suggestion .....	43
 <b>REFERENCE</b> .....	 44

## LIST OF ABBREVIATION

<b>AA</b>	: Arachidonic acid
<b>ACS</b>	: Acute coronary syndrome
<b>ASCVD</b>	: Atherosclerotic cardiovascular disease
<b>ADMA</b>	: Asymmetric dimethyl arginine
<b>CABG</b>	: Coronary artery bypass graft
<b>CHD</b>	: Coronary heart disease
<b>CD54</b>	: Cluster of Differentiation 54
<b>DHA</b>	: Docosahexaenoic
<b>Ecs</b>	: Endothelial cells
<b>ELISA</b>	: Enzyme-linked immuno sorbent assay
<b>eNOS</b>	: Endothelial nitric oxide synthase
<b>EPA</b>	: Eicosapentaenoic
<b>EVOO</b>	: Extra-virgin olive oil
<b>FAO</b>	: Food and Agriculture Organization
<b>FMD</b>	: Flow mediated dilation
<b>HDL</b>	: High density lipoproteins
<b>HDL-C</b>	: HDL cholesterol
<b>HT</b>	: Hydroxytyrosol
<b>ICAM-1</b>	: Intercellular adhesion molecule-1
<b>IL</b>	: Interleukin
<b>INOS</b>	: Inducible nitric oxide synthase

<b>kD</b>	:	Kilo Dalton
<b>LDL</b>	:	Low density lipoprotein
<b>LDL-C</b>	:	LDL cholesterol
<b>LLC</b>	:	Lectro Life Technologies
<b>LH</b>	:	Lipid hydro peroxide
<b>LPPT</b>	:	Laboratorium Penelitian dan Pengujian Terpadu
<b>MCP-1</b>	:	Monocyte chemotactic protein-1
<b>MI</b>	:	Myocardial infarction
<b>MUFA</b>	:	Monounsaturated fatty acids
<b>NF-κB</b>	:	Nuclear factor-κB
<b>NK</b>	:	Natural killer
<b>NO</b>	:	Nitric oxide
<b>NOS</b>	:	Nitric oxide synthase
<b>NNOS</b>	:	Neuronal nitric oxide synthase
<b>Ob</b>	:	Obesity
<b>OL</b>	:	Oleuropein
<b>OOPL</b>	:	Olive oil polar lipid
<b>Ox-LDL</b>	:	Oxidized LDL
<b>PBMC</b>	:	Peripheral blood mononuclear cell
<b>PC</b>	:	Phenolic compound
<b>PGs</b>	:	Prostaglandins
<b>PGE2</b>	:	Prostaglandin E2
<b>PPL</b>	:	pomace polar lipid

<b>PUFAs</b>	: Polyunsaturated fatty acids
<b>ROS</b>	: Reactive oxygen species
<b>SD</b>	: Standard deviation
<b>SMCs</b>	: Smoot muscle cells
<b>SPSS</b>	: Statistical Package for the Social Sciences
<b>TC</b>	: Total cholesterol
<b>TGs</b>	: Triglycerides
<b>TMD</b>	: Traditional Mediterranean diet
<b>TNF</b>	: Tumor necrosis factor
<b>TNF-a</b>	: Tumor necrosis factor -a
<b>VCAM1</b>	: Vascular cell adhesion molecular 1
<b>VLDL</b>	: Very-low density lipoproteins
<b>WHO</b>	: World Health Organization

## **LIST OF FIGURES**

Figure 1. Summary of vasoprotective effects of nitric oxide in atherosclerosis .....	24
Figure 2. Theoretical framework .....	26
Figure 3. Conceptual framework of study .....	27
Figure 4. Research Flow .....	35

## LIST OF TABLES

<b>Table 1 :</b> Previous report related to study on protective effect olive oil on HFD .....	6
<b>Table 2 :</b> Operational definition .....	31
<b>Table 3 :</b> Serum ICAM-1 levels of HFD rats with and without EVOO treatment .....	37
<b>Table 4 :</b> Serum eNOS levels of HFD rats with and without EVOO treatment .....	38

## **LIST OF APPENDIXS**

<b>APPENDIXS 1 : Statistical analysis .....</b>	<b>53</b>
Normality test of ICAM-1.....	54
Normality test of eNOS .....	60
<b>APPENDIXS 2: Ethical clearance .....</b>	<b>67</b>
<b>APPENDIXS 3: Photo of the experiment .....</b>	<b>69</b>

## ABSTRACT

**Background:** High fat diet (HFD) induces low chronic inflammation and subsequently leads to the development of endothelial dysfunctions, which are considered as the first pathological symptom of atherosclerosis. Atherosclerosis is marked by increasing sICAM-1 and decreasing eNOS levels, which both of them released by endothelial cells. Consuming EVOO having anti-inflammation effect may retain normal endothelial functions. This study therefore aimed to prove the effect of EVOO in maintaining endothelial cells of wistar rats during HFD consumption.

**Methods:** A randomized post-test only control group design was performed on wistar rats, which were divided into 5 groups, every group consists of 6 rats. Negative and positive control groups received normal diet and HFD, respectively. Three treated groups received combination of HFD and EVOO in different dose (1ml, 2ml, and 3ml/kg BW/day, respectively) for period of 60 days. Serum ICAM-1 and eNOS levels were measured using ELISA method.

**Results:** No significant different of ICAM-1 levels (ANOVA test;  $p=0.202$ ), but significant different of eNOS levels were observed among all groups studied (ANOVA test;  $p=0.001$ ). Further analysis proved that the eNOS levels of positive controls (mean  $\pm$  SD;  $0.32\pm0.14$  ng/ml) were significantly lower than those of negative controls ( $0.72\pm0.32$ ;  $p=0.006$ ) and group of HFD rats receiving EVOO in dose of 3ml/kg BW/day ( $1.01\pm0.45$ ;  $p=0.022$ ). Post Hoc tests of serum eNOS levels between other groups were not significantly different.

**Conclusions:** The result of this study indicates that all conducted treatments have no effect on ICAM-1 levels. Unlike EVOO (3ml/kg BW/day) able to improve eNOS levels of HFD rats.

**Key words:** EVOO, HFD, ICAM-1, eNOS.

## ABSTRAK

**Latar Belakang:** Diet lemak yang tinggi (HFD) menyebabkan inflamasi kronik, kemudian mengarah pada berkembangnya disfungsi endotel yang dipertimbangkan sebagai yang pertama gejala patologik aterosklerosis. Aterosklerosis ini ditandai dengan meningkatnya sICAM-1 dan menurunnya tingkatan eNOS, yang keduanya dikeluarkan oleh sel endotelial. Mengkonsumsi EVOO memiliki efek anti-inflamasi yang mungkin memelihara fungsi endotelial normal. Penelitian ini bertujuan untuk membuktikan efek EVOO dalam mempertahankan sel-sel endotelial dari tikus wistar selama konsumsi HFD.

**Metode:** suatu post-test acak hanya digunakan dan diperlakukan bagi kontrol group (kelompok) design yang kelakuan pada tikus wistar yang terbagi dalam 5 kelompok, setiap kelompok terdiri dari 6 tikus.

Baik kelompok kontrol negatif dan positif telah mendapat diet normal dan HFD, berturut-turut. Tiga kelompok yang dirawat mendapat kombinasi HFD dan EVOO pada berbeda dosis (1ml, 2ml, dan 3ml/kg BW / hari, berturutan) selama 60 hari. Serum ICAM-1 dan kadar eNOS diukur menggunakan metode ELISA.

**Hasil:** Tidak terdapat perbedaan yang bermakna dari kadar ICAM-1 (ANOVA tes;  $p=0.202$ ), tetapi yang berbeda dari kadar Enos yang digunakan dalam semua kelompok studi ( uji ANOVA,  $p= 0,001$ ). Analisis yang lebih lanjut dibuktikan bahwa kadar eNOS kontrol positif ( $mean \pm SD$ ;  $0,32 \pm 0,14$  ng / ml) signifikan lebih rendah daripada kontrol negatif ( $0,72 \pm 0,32$ ,  $p = 0,006$ ) dan grup tikus HFD yang mendapat EVOO pada takaran 3ml / kg BB / hari ( $1,01 \pm 0,45$ ,  $p = 0,022$ ). Tes Post Hoc kadar eNOS serum antara kelompok-kelompok yang lain tidak berbeda signifikan.

**Kesimpulan:** Dari hasil penilitian ini semua pengobatan-pengobatan tidak ada efek kepada kadar ICAM-1. Berbeda dengan EVOO (3ml/kg BW / hari) sanggup memperbaiki kadar eNOS tikus HFD.

**Kata kunci:** EVOO, HFD, ICAM -1, eNOS.