ORAL INTAKE OF SARDINELLA LONGICEPS OIL THE DECREASE OF TNF-α AND IL-6 LEVELS IN ATHEROSCLEROTIC WISTAR RAT

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ABSTRACT

Lifestyle changes to consumption of variegated instant food may be associated several heath hazards, such as obesity, dyslipidemia, and atheroschlerosis. This study was conducted to investigate the effects of orally administered Sardinella longiceps oil as an anti inflammatory agent on the serum levels of TNF-α and IL-6 considered as biomarkers for atherosclerosis.

The study design is an true experimental with randomized pretest and posttest control group design, using 50 Wistar rat equaly divided into 5 groups, i.e. placebo control group 0% and 4 treatment groups each treated daily with 10%, 15 %, 20 % and 25 % fish oil respectively for 6 weeks. Before the treatment was started, all rats were orally fed daily with a high cholesterol diet for 13 weeks to induce atherosclerosis. Our study showed that the intake of 20% fish oil had resulted in the significantly greatest decrease of 45,63 % in the TNF-α serum levels,from 28.62 ± 1.25 to 15.56 ± 7.20 pg/mL and similar significant decrease 15,42% in of IL-6 serum levels from 134.64 ± 1.98 to 113.87 ± 4.30 pg/mL. The overall results of our study seemed to imply than in the Wistar rats, oral intake of Sardfinella longiceps oils signifacantly decreased serum levels of TNF-α and IL-6 probably through their anti-inflamatory effects. Futher research to determin the magnitude of effects sardinella longiceps oils on the serum levels TNF-α and IL-6 human.

Keywords: Sardinella longiceps oils, fish oils, anti-inflammatory agent, lifestyle changes. Instant foods, TNF-α and IL-6 in human.

INTRODUCTION

It has been understood that there is a significance correlation between high lipid serum levels and incidens of atherosclerosis, a trigger of coronary heart desease. Coronary heart desease present as a results of blood circulation disturbance and abnormality of cardiac electricity or other forms of arythmia. This leads to unorganized myocardiac contraction, blood flow obstruction, and blood flow regugirtation. All of these conditions resulted in blood flow on each contraction will return back to the heart (shunts), blood flow abnormality and end up with

heart failure (Vinay, *et al*. 2004). Atherosclerosis is a slow rate progressive desease, present in large to medium arterial muscle and elastic artery. The main sites of atheroschlerosis are abdominal aorta, coronary arteri, poplitea arteri, torax aorta desendens , carotic intern artery, and circular Willisi. Risk factors, such as hypertension, chronic hypercholesterolemia, immune system disturbance, toxin and virus are also involved in the arterial endotelial wall destruction. This demage induces permeability changes of endotelial cells and leads to increase of plasma constituen, such as lipoprotein that can easily enter to arteri wall. Demaging of these endotelial cells could also changed thrombosistein lumen artery property that can leads to adhesion of thrombosite to the blood and induce inflammation. If this demaging process exeed continually for long time, will be followed by continous atheroschlerosis and leads to thickness of tunica intima and results in desturbance of blood flow on that site (Szmitko, *et al*., 2003). Managing consumption type is one way to overcome this condition. Decrease of plasma cholesterol can be increased by rising of cholesterol turnover rate. Faster cholesterol replacement can be achieved through intake of polyunsaturated fatty acids. These acids in metabolism act as an antioxidant that could breakdown saturated fatty acid chain of hypercholesterolemia patients (Kanjwal, 2004).

Hypercholesterolemia, atherosclerosis inducer is a multifactorial deasase also correlates to proinflammatory cytokine, such as IFN-γ, IL-1β, IL-6 and TNF-α. Some research reported that atherogenic consumption could increase formation of IL-6 and TNF-α, however, did not significantly changes of increase IL-1β (Stefan, *et al*., 1996; Ahmed ,2001; Han *et al*., 2002).

Based on background explained above, this research was conducted to investigate role of Sardinella longiceps oil as an anti inflamation through decrease of IL-6 and TNF-α levels on atherosclerosis Wistar rat.

MATERIALS AND METHOD

This research is applying a true experimental randomized pre and posttest control group design to determine the role of Sardinella longiceps oil for anti inflammation. Research was conducted using 50 Wistar rat and grouped into 5 groups, i.e. P0 for control with 0% fish oil, P1 for treatment with 10% fish oil, P2 for treatment with 15% fish oil, P3 for treatment of 20%, and P4 for treatment of 25%. Rats were fed with a high cholesterol diet for 13 weeks to achieved atheroschlerosis, then was treated with various concentration of fish oil for 6 weeks. TNF-α and

IL-6 levels rats serum for atheroschlerosis (pretest) and after treated (posttest) were then detected. All data obtained were analyzed statistically to determine the mean different of treatment using one way anova at 5% significant level.

RESULTS

Decrease of TNF-α levels

Mean of TNF-α serum levels pre and postest data were presented in Table 1.

Table 1
Mean of TNF-α serum levels data

Treatment	TNF-α (pg /mL)	
	Pretest	Postest
SFO 0 % (control)	28.98 ± 6.00	28.11 ± 5.94
SFO 10 %	29.12 ± 5.79	27.32 ± 5.01
SFO 15 %	29.02 ± 5.34	24.42 ± 5.74
SFO 20 %	28.62 ± 4.72	15.56 ± 7.20
SFO 25 %	29.02 ± 5.06	26.02 ± 8.34

SFO = Sardinella longiceps fish oil

Data on Table 1 were normally distributed with $p > 0.05$ and their variance were also homogen with $p > 0.05$. All pretest data were comparable ($p > 0.05$), therefore, the mean different of various treatment of fish oil can be only performed based on posttest data and analyzed using one way anova. It was obtained that there are diffirent between all treatment. Then, followed by Post Hoc test (LSD) to determine the different. The Post Hoc Test results were presented on Table 2. Table 2

Resume of Post Hoc Test of TNF-α Levels

Treatment		Mean Different of TNF-α (pg/mL)	*p**
SFO 0% (control)	- SFO 10%	0.79	0.770
	- SFO 15%	3.69	0.177
	- SFO 20%	12.55	0.001
	- SFO 25%	1.10	0.686
SFO 10%	- SFO 15%	2.90	0.287
	- SFO 20%	11.76	0.001
	- SFO 25%	0.30	0.911
SFO 15%	- SFO 20%	8.86	0.002
	- SFO 25%	- 2.59	0.339
SFO 20%	- SFO 25%	- 1.45	0.001

SFO = Sardinella longiceps fish oil
*significant $p < 0.05$

Decrease of IL-6 Levels

Mean of pre and postest data of IL-6 serum levels were presented on Table 3.

Table 3
Mean of IL-6 serum levels Data Pre and Posttest

Treatment	IL-6 (pg/mL)	
	Pretest	Postest
SFO 0% (control)	134.58 ± 2.21	133.15 ± 4.01
SFO 10 %	134.24 ± 2.64	130.28 ± 3.59
SFO 15 %	134.75 ± 2.51	127.20 ± 5.56
SFO 20 %	134.64 ± 1.98	113.87 ± 4.30
SFO 25 %	135.34 ± 4.57	120.87 ± 7.89

SFO = Sardinella longiseps fish oil

Data on Table 3 were normally distributed with $p > 0.05$ and their variance were also homogen with $p > 0.05$. The mean different of various fish oil treatment can be performed on the basis of posttest data, if, however, all pretest data are comparable. It was obtained that all pretest data are comparable with $p < 0.05$, therefore, mean different of the treatment were obtained based on posttest data and analyzed using one way anova. There were significant different of treatment obtainedwith $p < 0.0$, then the data were analyzed using Post Hoc Test to measured the different. Post Hoc test results were sumerized in Table 4.

Table 4
Resume of Post Hoc Test of IL-6 Levels

Treatment		Mean Different of IL-6 (ρg/mL)	*p**
SFO 0% (control)	- SFO 10%	2.87	0.232
	- SFO 15%	5.95	0.016
	- SFO 20%	19.28	0.001
	- SFO 25%	12.28	0.001
SFO 10%	- SFO 15%	- 3.09	0.201
	- SFO 20%	16.41	0.001
	- SFO 25%	9.41	0.001
SFO 15%	- SFO 20%	13.33	0.001
	- SFO 25%	6.33	0.011
SFO 20%	- SFO 25%	- 7.00	0.001

SFO = Sardinella longiceps fish oil

*Significant $p < 0.05$

DISCUSSIONS

The research results indicate that the higherst decrease of 12.55 pg/mL of TNF-α was obtained for intake of 20% SFO. Increase of SFO to 25% could not increase the decrease of TNF-α levels. This condition indicates that concentration of 25% SFO has already saturated, therefore, it could not decrease of TNF-α levels any further. This was supported by the finding of Chen and Goeddel (2002), they found that there is no transcription of NF-Kβ, so that, no further production of TNF-α due to saturation.

Inflammation is a response of tissue demage during vascularization. This response is followed by an important process, such as endotelial process. Endotel is an important parts of blood vein that play an important role in atheroschlerosis. Endotel is a main target of mechanical and chemical demage due to dislipidemia risk factor. Chronic, continues, and prolong dislipidemia resulted in proinflammation response and prothrombic which are intially acute becoming chronic. This will be followed by infiltration of leucocyte cells, mainly, monocyte cells to lower subendotelial tissue to form macrophage cells. These cells will destroy all remains LDL-C and oxydized to form foam cells and change to ateroma (Baraas, 2006).

The last two decades research obtained that fish oil is effective as an anti inflammation. This is due to fish oil rich of eicosapentanoic acis (EPA) and dokohexanoic acid (DHA). These fatty acids are group as poly unsaturated fatty acid with double bond at the third carbon atom (from metil group) which are known as omega-3 (Han, *et al*., 2002; Ahmed, 2001; Stefan, *et al*., 1996). In this study, sardinella longiceps oil which is rich of omega-3 was applied and proved to exeed anti inflammation effect. This anti inflammation effect is due to activation endothelium nuclear factor-kapa beta (ENF-κβ) on perifer vein. ENF-κβ is a transcription factor distributed on all endotelial cells that have a role in controlling of vascularization.

Simopolous (2002) obtained that the role of omega-3 as an anti inflammation is due to their action as immunomodulator. In addition, their role as antiinflammation is as a results of arachidonic acids effect. These acids are substrate for triggering formation of cyclooxyginase

and 5-lipooxyginase. These two oxyginases have vasodilator endothelium-dependent behaviour to cause relaxation of ordinary coronary arteri and paradoxal vasoconstruction on atheroschlerosis arteri.

Data in Table 4 indicate that there is a decrease of IL-6 levels since the intake of 10% of SFO. Eventhough, there is a decrease of IL-6 levels caused by treatment of 10% SFO which is about 2.87 pg/mL, the decrease is not significant statistically with $p > 0.05$. Then, intake of 15% SFO resulted in significantly decrease of 5.95 pg/mL of IL-6 levels with $p < 0.05$. In addition, intake of 20% SFO have also a similar trend to significantly decrease of 19.28 pg/mL of IL-6 levels with $p < 0.05$. However, increase of concentration to 25% SFO intake did not significantly decrease IL-6 levels, indicates by $p > 0.05$.

Sardinella longiceps oil are rich of eicosapentaenoic acid (EPA) and dokohexanoic acids (DHA). These two acids are goruped to omega-3 has an ability as an antiinflammation. During endotel cells experiencing of activated inflammation leads to increase selectin and VCAM-1 expression. VICAM-1 induce monocyte adhesion. This adhesion was also induced by proinflammation cytokine, such as 1L-1β and TNF-α. These cytokines were induced by CRP protein produced as a results of IL-6 response by protease activated receptor signaling, uptake of oxLDL, through oxLDL receptor-1 (LOX-1) and by interaction of CD40/CD40 ligand in arteri intima (Bonetti, *et al*., 2003). IL-6 has an important role in inflammation response and this cytokine is secreted by activated-macrophage, leads to phebric and known as pyrogen endogen. IL-6 was also initiate phase acute response marked by protein phase acute production by hepatocyte (Coico, *et al*., 2003).

CONCLUSSIONS

1. Intake of 20% SFO decrease TNF-α serum levels of atheroschlerosis Wistar rat around 45.63%, i.e. from 28.62±4.72 to 15.56±7.20 pg/mL.
2. Intake of 20% SFO decrease IL-6 serum levels of atheroschlerosis Wistar rat around 15.42%, i.e. from 134.64±1.98 to 113.87±4.30 pg/mL.

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