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Effects of Sacha Inchi (*Plukenetia volubilis*) Oil on Serum Interleukin 6 Levels and Foam Cell Count in Male Wistar Rats (*Rattus norvegicus*) Fed a High Fat and Fructose Diet

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ABSTRACT
A high-fat, high-fructose diet induces pro-inflammatory agents such as Interleukin-6 (IL-6) and hyperlipidemia, leading to atherosclerosis. Sacha inchi oil, rich in alpha-linolenic acid (ALA), has anti-inflammatory and lipid-modulating properties. This study aimed to examine the effect of sacha inchi oil on serum IL-6 levels and foam cell count in the aorta of wistar rats on a high-fat, high-fructose diet. This experiment is a true experimental study with a post-test-only control group design, 30 male wistar rats were divided into five groups: normal control (K0), high fat and fructose diet (HFFD) (K1), and three treatment groups receiving HFFD with sacha inchi oil at doses of 0.27 ml/200gBW/day (P1), 0.54 ml/200gBW/day (P2), and 1.08 ml/200gBW/day (P3) for 28 days. On day 29, serum IL-6 levels were measured using ELISA. Mean serum IL-6 levels were K1 (8.465 ng/L), P1 (4.294 ng/L), P2 (3.197 ng/L), P3 (1.379 ng/L), and K0 (1.340 ng/L), with significant differences among groups ($p<0.05$) except between K0 and P3 ($p>0.05$). Mean foam cell counts were K1 (55.3 cells/fov), P1 (47.1 cells/fov), P2 (53.2 cells/fov), P3 (52.6 cells/fov), and K0 (44.4 cells/fov), with significant differences between K0 and K1, K1 and P1, K0 and P2, and K0 and P3 ($p<0.05$). Sacha inchi oil significantly reduced IL-6 levels at all doses compared to the negative control ($p<0.05$), with the 1.08 ml/200 g/day dose being most effective. The 0.27 ml/200 g/day dose inhibited foam cell formation in HFFD rat. In conclusion, sacha inchi oil reduces serum IL-6 levels and inhibits foam cell formation in male wistar rats on a high-fat, high-fructose diet.
Keywords: High Fat and Fructose Diet, Sacha Inchi Oil, IL-6, Foam Cell Count.

INTRODUCTION

Aging process is a continuous process that will be experienced by everyone and will cause a gradual decline in the body functions. The development of advanced medical science, including anti-aging medicine, makes a new paradigm in the world of medicine with scientifically-based technology and science, where aging can be detected early, prevented, treated and corrected so that humans have a good quality of life with increasing age (Pangkahila, 2019). One external factor that can accelerate the aging process is an unhealthy diet, such as a diet high in fat and fructose. This unhealthy diet can trigger chronic diseases such as cardiovascular disease, one of which is atherosclerosis (Pangkahila, 2007; Perazza et al., 2020). Atherosclerosis is a buildup of substances in the form of fats, platelets, macrophages, leukocytes, and other substances that begin in the intima lining of arterial blood vessels. Atherosclerosis can occur in all arteries, such as the aorta and coronary arteries of the heart. Atherosclerosis also causes a chronic low-grade inflammatory response, which will attract innate and adaptive immune cells, causing the progression of atherosclerosis plaques (Wolf & Ley, 2019).

Atherosclerosis is one of the causes of cardiovascular disease and one of the highest causes of death and disability in the world (Roth et al., 2020; Vos et al., 2020). Based on data from The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) in 2019, the highest cause of death in Indonesia is caused by stroke followed by ischemic heart disease and both of these diseases are caused by the progression of atherosclerosis. A diet high in fat and fructose can lead to increased lipogenesis and inflammatory levels, one of which is interleukin 6 (IL-6), which plays a role in triggering atherosclerosis (Javadifar et al., 2021). Research data also suggest that IL-6 is not only a contributor to the onset of atherosclerosis formation but also a sign of atherosclerosis lesion progression (Dimosiari et al., 2023). Foam cells have an important role in the formation and development of atherosclerosis (Javadifar et al., 2021). Foam cells are macrophages or smooth muscle cells that contain cholesterol. The foam cells will then form a collection of fat along the endothelium, referred to as a fatty streak, which is the primary lesion of atherosclerosis (Libby, 2021; Wolf & Ley, 2019).

Based on the above, atherosclerosis can have a detrimental impact, so prevention is necessary. One food source that can have the effect of suppressing inflammation, regulating cholesterol in the blood, inhibiting platelet aggregation and suppressing oxidative stress is *alpha linolenic acid* (ALA) or essential omega 3 derived from plants (Pandohee, 2022). ALA compounds have anti-inflammatory effects by suppressing the NF-kB pathway, thereby inhibiting the production of proinflammatory cytokines, including IL-6. Based on meta-analysis data, increasing ALA consumption reduces cardiovascular disease risk by 10% and reduces fatal coronary heart disease risk by 20% (Sala-Vila et al., 2022).

One plant that is believed to have a high ALA content is *Plukenetia volubilis* or sacha inchi. This plant comes from the forest in the Amazon and has traditionally been consumed as a healthy food (Goyal et al., 2022). Sacha inchi also known as inca beans, have begun to be cultivated in Indonesia and can be found in Java and Bali. This plant is a natural material that can be a potential source as a supply of various macro and micronutrients. Some of the ingredients in sacha inchi are alpha linolenic acid (ALA) or omega 3, linoleic acid (LA) or omega 6, oleic acid (OA) or omega 9, tocopherol, flavonoids, and phytosterols (Rodzi & Lee, 2022). The ALA content in sacha inches is one of the highest compared to other nuts, such as walnuts or chia nuts. Based on the content of sacha inchi, it is hypothesized to have effects as hypolipidemic, immunomodulating, anti-glycemic and antioxidant and emollient (Cárdenas et al., 2021).

Based on the explanation above, it is expected that the active substances contained in sacha inchi oil can act as anti-inflammatory agents to prevent the formation of atherosclerosis and increase life expectancy. This made the researchers want to find out whether giving sacha inchi oil could inhibit the increase in interleukin 6 levels and the number of foam cells in the aorta of rat fed a diet high in fat and fructose.

RESEARCH METHODS

This research has received approval from the Veterinary Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, with no. B/201/UN14.2.9/PT.01.04/2023. The research was conducted at the Integrated Biomedical Laboratory of the Faculty of Medicine University of Udayana. This research is true experimental with a post-test-only control group design. The study sample was 30 male wistar rats (*Rattus norvegicus*) weighing 180-250 grams, and the sample was divided into 5 groups randomly. Acclimatization was carried out for 7 days at the beginning of the study. The normal control group (K0) was given standard feed 594; The negative control group (K1) was given high-fat and fructose diet (HFFD); treatment group 1 (P1), treatment 2 (P2) and treatment 3 (P3) were given HFFD with different doses of sacha inchi oil 0.27ml/200g/day, 0.54ml/200gBW/day and 1.08ml/200gBW/day. Sacha inchi oil is produced by House of Sachi, Cikarang, West Java, with ALA content of 49.1%, LA 35.7%, and OA of 8.1%. HFFD given is a mixture of 17g standard feed 594 and 3g lard (85:15) ad libitum and 2g duck egg yolk given by oral gavage 1x a day and an additional 30% fructose in rat drinks. The study was carried out for 28 days after acclimatization. On the 29th day, blood collection from the medial canthus of the orbital sinus was as much as 2cc, and surgery was performed to take the aortic arch organ to examine the number of foam cells. Results are analyzed with the SPSS program. The Kruskal-Wallis test was used to see the difference in IL-6 levels and the number of foam cells between

groups of rat and continued with the mean difference test to see the difference between groups and was said to be meaningful if $p<0.05$.

RESULTS AND DISCUSSION

Based on the results of research data obtained after treatment for 28 days, a normality test was carried out with *Shapiro Wilk*, serum IL-6 levels, and abnormally distributed foam cell count ($p<0.05$). The results of the homogeneity test, both data are homogeneous ($p>0.05$). Then continued with the Kruskal-Wallis non-parametric test, which showed a difference in the average results of serum IL-6 levels and the number of foam cells ($p < 0.05$). The test is continued with the mean difference test to determine the differences between groups. The test results obtained can be seen in Table 1.

Table 1. Average yield of IL-6 levels and number of foam cells

Group	n	Average results ± SD	
		IL-6 (ng/L)	Number of foam cells (cell/fov)
K0	6	1,40±0,08*	44,42±6,06*
K1	6	8,47±0,18	55,28±6,95
P1	5	4,29±0,16*	47,06±4,08*
P2	6	3,20±0,17*	53,25±8,67
P3	6	1,40±0,05*	52,62±9,76
p		*p*<0,001	*p*<0,042

Note: K0: group fed a normal diet; K1: Negative control, HFFD administered group; P1: HFFD + SIO 0.27ml/200gBW/day, P2: HFFD + SIO 0.54ml/200gBW/day, P3: HFFD + SIO 1.08ml/200gBW/day; *significant difference through mean difference test against K1 group

Based on Table 1, there are significant differences between group K1 with groups K0, P1, P2 and P3 for serum levels of IL-6 and significant differences between groups K1 with K0 and P1 for number of foam cells. Group K1 had higher serum IL-6 levels and aortic foam cell counts than the other groups. These results prove that HFFD administration for 28 days can increase serum IL-6 levels and the number of aortic foam cells in male wistar rats. Based on the mean difference test for serum IL-6, it is known that there is a significant difference in all group ($p<0.05$) except between groups K0 and P3. This showed that increasing the dose of sacha inchi oil given lowered IL-6 levels better. As for the results of the difference test between groups on the number of foam cells, a significant difference was found in the P1 group with the K1 group ($p < 0.05$). So that with the use of P1 treatment doses, the number of foam cells is lower than that of the K1 group. Figure

1 and Figure 2 show the difference in mean serum IL-6 levels and the number of foam cells between groups.

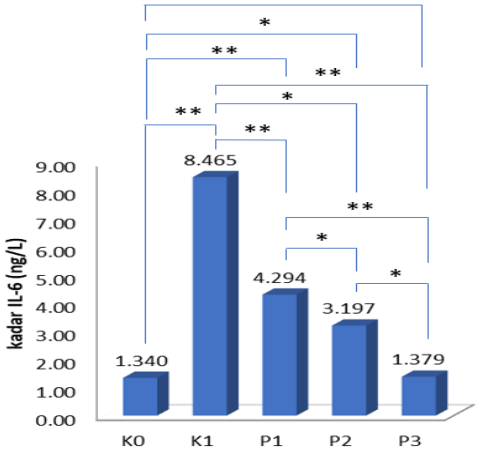

Figure 1. Comparison of differences in mean IL-6 levels between groups (*p<0.05; **p<0.001)

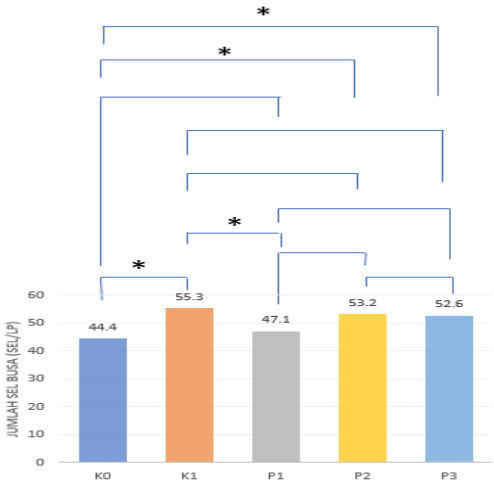

Figure 2. Comparison of the difference in the average number of foam cells between groups (*p<0.05)

Discussion

In this study, a diet high in fat and fructose for 28 days significantly induced IL-6 and foam cell formation, as evidenced by an increase in IL-6 levels of 8.546ng/L (p<0.05) and foam cell formation of 55.3 cells/fov (p<0.05) in the negative control group compared to the normal group. A high-fructose diet causes activation of the NFkB and MAPK pathways, thereby increasing IL-6

production and also its effects de novo lipogenesis. It can also cause the secretion of systemic inflammatory factors increase triglyceride levels, and lower HDL levels in the blood, which are risk factors for foam cell formation (Busnatu et al., 2022; Zhang et al., 2017). A high-fat diet leads to increased IL-6 levels with increased activation of NFkb and decreased expression of PPAR-y, resulting in impaired lipid modulation and increased free fatty acids while increasing LDL levels in the blood (Cortez et al., 2013; Jebari-Benslaiman et al., 2022; Maramis et al., 2014).

In this study, the rat group given sacha inchi oil treatment doses of 0.27ml/200gBW/day and 0.54ml/200gBW/day had significant IL-6 levels ($p<0.05$) lower than the negative control group but higher than the normal group. Administration of sacha inchi oil at a dose of 1.08ml/200gBW/day gave a significant difference ($p<0.05$) to the negative control group but not significantly different from the normal group ($p = 0.301$). This showed that administering sacha inchi oil can inhibit the increase in IL-6 levels in rats given HFFD, and with the largest dose of sacha inchi, the average serum IL-6 levels are almost the same as the normal group. Researchers suspect that the active components in sacha inchi oil can function as an anti-inflammatory to modulate the inflammatory process. The results of this study are in line with previous research that the supplementation of sacha inchi oil can reduce IL-6 levels in experimental animals (Ambulay et al., 2020; Rojanaverawong et al., 2023).

Alpha Linolenic Acid (ALA) in sacha inchi oil can decrease the production of pro-inflammatory agents by decreasing apoptosis protein expression, *transcription factor C/EBP homologous protein (CHOP),* and expression modulation *X-Box Binding Protein-1*(XBP1) which causes decreased activation of the JNK inflammatory pathway and inhibits the NF-kB pathway, thereby regulating inflammatory mediators and decreasing IL-6 production. In addition, ALA also has anti-inflammatory effects from oxylipin which is a derivative of ALA, with activation in *Peroxiaome Proliferator-Activated Receptors* (PPAR-y) thus causing inactivation of *NOD-, LRR- and pyrin domain-containing protein 3* (NLRP3) inflamasomes (Cambiaggi et al., 2023; Candido et al., 2020). The content of OA in sacha inchi oil plays a role in reducing IL-6 by increasing the AMP/ATP ratio and activation of AMPK, and inhibition of the NFkB pathway. In addition, OA can also increase the expression of Nrf-2, which has a role as an antioxidant-forming gene, and an increase in Nrf-2 can also increase the activation of PPAR-y so that it can also reduce inflammation levels (Santa-María et al., 2023).

The ratio of omega 6 / omega 3 contained in the sacha inchi oil used in this study is <1, which is a good comparison to get the maximum anti-inflammatory effects. This effect is caused by omega 3 and omega 6 being mutually competitive. Higher omega 3 can suppress the pro-inflammatory effect of omega 6 by inhibiting the conversion of omega 6 to reduce the level of arachidonic acid production (Dinicolantonio & O'Keefe, 2018; Tortosa-Caparrós et al., 2017). This study,

evidenced by the administration of Sacha inchi oil, can inhibit the increase in IL-6 levels in Wistar rats fed a diet high in fat and fructose.

The results showed that treatment with a dose of 0.27ml/200gBW/day significantly inhibited the increase in the number of foam cells in the aortic arch of male Wistar rats compared to the negative control group ($p<0.05$). Based on research by (Zhang et al., 2012), ALA administration can activate *nuclear receptor farnesoid-X-receptor*, which will improve the expression *Small heterodimer partner* (SHP), and decreased transcription of SREBP-1c, thereby preventing expression of Stearoyl CoA desaturase 1 (SCD1). The decrease in SCD1 expression increases cholesterol efflux from macrophage cells and decreases cholesterol accumulation in foam cells. This is one of the mechanisms of omega 3 to promote regression of atherosclerosis. In addition, the content of ALA, which inhibits the production of SREBP-1c, will also cause a decrease in de novo lipogenesis, thereby lowering triglyceride and LDL levels in the blood, this is a vital factor in lowering the risk associated with cardiovascular disease and metabolic inflammation (Candido et al., 2020). The content of LA in sacha inchi oil can reduce total cholesterol and LDL in the blood. This is due to the upregulation of liver LDL receptors, which causes an increase in LDL metabolism. LA also increases transcription factors through PPAR. LA will promote SREBP activation through SCAP activity, resulting in the upregulation of LDL receptor expression, leading to the elimination of LDL cholesterol from circulation. LA ability to lower LDL cholesterol levels leads to a decrease in the incidence of cardiovascular diseases such as atherosclerosis and mortality (Djuricic & Calder, 2021). These results are also supported by the results of a meta-analysis by replacing 5% of energy from saturated fatty acids with LA associated with reducing the risk of coronary heart disease events by 9% and reducing the risk of death from coronary heart disease by 13% (Sanders, 2019). According to Shramko et al., (2020), OA can shorten the time of LDL particles on artery walls thereby lowering the risk of atherosclerosis. OA also lowers the expression of proteins associated with cholesterol transport, decreases cholesterol biopsies, and lowers oxLDL levels, inhibiting atherosclerosis. In addition, OA can also cause hypomethylation so that it can improve the inflammatory profile and hypomethylation agents can slow the progress of vascular lesions (Santa-María et al., 2023)

The doses of 0.54ml/200gBW/day and 1.08ml/200gBW/day did not provide a significant difference compared to the K1 group in the aortic foam cell count, probably because there are other processes that occur, such as oxidation or increased levels of oxLDL which is one of the causes of foam cell formation. According to Lawrence, (2021), an excessive consumption of PUFAs can cause lipid peroxidation in lipoproteins such as LDL causing oxLDL, which can increase the risk of atherosclerosis. Although consuming oils high in PUFAs can lower total and LDL cholesterol

levels, LDL oxidized due to PUFAs is a problem and can be deposited into artery walls if consumed in excess (Burdge, 2006).

In this study, higher doses of sacha inchi oil cause a higher decrease in IL-6 levels, but there was no decrease in the number of foam cells when compared to the K1 group. This may be because although IL-6 plays a role in the process of atherosclerosis, it does not mean that if IL-6 is low, then atherosclerosis does not occur. The formation of atherosclerosis is complex and multifactorial. Therefore, it is still possible to form foam cells even if IL-6 levels are low or normal (Barrett, 2020; Rafieian-Kopaei et al., 2014; Reiss et al., 2017). Therefore, although IL-6 plays a role in the formation of foam cells, IL-6 is not the only determinant; there are other factors that contribute to this process. Another factor that plays a role in the formation of foam cells, even though low or normal IL-6 levels are found, is the presence of alternative cytokines. IL-6 is only one of the cytokines that play a role in the formation of foam cells, other pro-inflammatory factors that play a role are TNF-α, IL-1β and IL-8, which can also promote the formation of foam cells independently of IL-6. In addition, there may occur a cellular crosstalk where different immune cells and non-immune cells in the artery wall communicate and interact with each other so that foam cells formation is affected by factors secreted by the cells next to them. The expression of LDL receptors that play a role in ox-LDL absorption, such as CD36 and SR-A1 receptors, can be found in macrophages and contribute to the formation of foam cells, even though IL-6 levels are normal. Genetic factors also play a role in fat metabolism, cholesterol secretion, and foam cell formation, not influenced by IL-6 levels and other non-cytokine factors because the formation of foam cells depends not only on the presence of cytokines but also other factors such as the presence of ox-LDL, fat metabolism, and cholesterol secretion mechanisms (Aherrahrou et al., 2020; Chistiakov et al., 2016; Mahdinia et al., 2023; Nishimura et al., 2013). According to Wu et al., (2023), macrophages can be classified into M1/M2 based on their characteristics; M1 is proinflammatory and can stimulate the formation of IL-6 and TNf-α, while M2 has an anti-inflammatory response and can secrete IL10. These two types of macrophages can still form foam cells with different characteristics. Early in foam cell formation, more M2 macrophages are found in normally more stable plaques and M1 in unstable plaques. The more the lesion progresses, the M2 will decrease and the M1 will increase in number. In addition, both M1 and M2 macrophages can change dynamically during the formation of atherosclerosis, and their subtypes can be one or another (Hou et al., 2023; Lin et al., 2021; Poznyak et al., 2021; Theofilis et al., 2023). In this study, IL-6 levels have decreased, but the number of foam cells is still large, so it is possible that the macrophages containing foam cells are M2 macrophages, not M1, which can secrete IL-6. Therefore, further research is needed that can examine the types of macrophages present in foam cells formed, such as by using immunohistochemical techniques that detect M2 receptors such as CD163, arginase

1 or CD206 (Jinnouchi et al., 2020). Based on the results of the study, the best dose of sacha inchi oil to inhibit the increase in IL-6 levels and the formation of foam cells is at a dose of 0.27ml / 200gBW / day.

CONCLUSION

The conclusion of this study is that the administration of sacha inchi oil at doses of 0.27 ml/200 gBW/day, 0.54 ml/200 gBW/day, and 1.08 ml/200 gBW/day significantly inhibited the increase in serum IL-6 levels in male Wistar rats fed a high-fat, high-fructose diet. However, only the dose of 0.27 ml/200 g/day could prevent the formation of foam cells in the aorta of these rats. This indicates that while higher doses are more effective in reducing inflammation, lower doses are sufficient to prevent early atherosclerotic changes. Therefore, sacha inchi oil has the potential to be a dietary intervention for managing inflammation and preventing atherosclerosis.

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