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Expression of ICAM-1, SOD-2, TNF-α, AND IL-6 AORTA Endhotel Cells Hypercholesterolemia Wistar Rats when provided ethanol extracts of *Inocarpus fagiferus* Fosb seeds

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Abstract

In this paper is reported a preclinical test of extract Tahitian chestnut (Inocarpus fagiferus Fosb) seeds by determining several variables as specific biomarkers to prevent early atherosclerosis in wistar rats. The study used a randomized posttest only control group design. Twenty-five rats were randomized into 5 (five) groups i.e: control groups (P_0 and P_1), and treatment groups as like P_2 (high cholesterol diet and 50 mg/kg bw extract), P_3 (high cholesterol diet and 100 mg/kg bw extract), and P_4 (high cholesterol diet and 150 mg/ kg bw extract). After the study all of the rats were euthanized to obtain aorta for immunohistochemistry to be analyzed to obtain data on ICAM-1, SOD-2, TNF-IL, and IL-6. The results of statistical analysis using Anova with α= 0.05 showed that the extract of Tahitian chestnut seeds at a dose of 50 mg/ kg bw was able to prevent atherosclerosis of wistar rats with a decrease in positive ICAM-1, TNF-α and IL-6 expression of aortic endothelial cells, and significantly increase in SOD-2 expression ($p < 0.05$) compared to positive control P_1.

Keywords: Inocarpus fagiferus Fosb, anti-atherosclerosis, oxidative stress and preclinical trial

INTRODUCTION

Seeds of Tahitian chestnut (Inocarpus fagiferus Fosb) are ethnobotanycally be used to antioxidant sources [1-3]. The recent studied show that extract of this seed contained compounds which can increase the levels of Superoxide Dismustase (SOD) and lipid profile improvement in blood plasm of rat so it's potents to inhibit antioxidant and diseases caused by oxidation reactions such as atherosclerosis [4]. Futhermore, the study showed that ethanol extracts of Inocarpus fagiferus Fosb seed able to induce endogenous antioxidant SOD in doses of 50 mg/kg bw by increasing the expression of SOD-3 aorta endhotel cell and decreasing the levels of MDA ($p<0.05$), were biomarker antioxidant and antiatherosclerosis [5]. The ability of ethanol extract Inocarpus fagiferus Fosb seed to induce endogenous antioxidants depends on the levels of SOD in the body that are expressed in a cell.

SOD-2 endogenous antioxidants are expressed in aortic endothelial cells as biomarkers of anti-atherosclerosis [6-11]. The condition of aortic endothelial SOD-2 expression is related or influential on ICAM-1 aortic endothelial expression, so ICAM-1 is also an early biomarker of atherosclerosis. Blood plasma MDA levels associated with the process of inhibiting lipid peroxidation in endothelial cell lipoprotein membranes are thought to be related to the inflammatory mechanism. In this paper also reported the expression of TNF-α and IL-6 aortic endothelial cells by immunohistochemistry.

METHODS

Materials and Equipments

The study used to seed of Tahitian chestnut (Inocarpus fagiferus Fosb), it's herbarium specimen saving in LIPI's Kebun Raya "Eka Karya" Bali. Wistar rats are used to study with ethical clearance as previously reported. Chemicals antibody primer used are Rabbit Anti-ICAM-1 (Bioss, Cat. Bs-0608R), Anti-SOD-2 (Bioss, Cat. bs-1080R), Anti-IL-6 (Bioss, Cat. bs- 0379R), Anti-TNF-α (Bioss, Cat. bs-3895R), kit LSAB (Dako, Denmark), and chemicals usually used to imunohistochemical analysis. While, equipment which is used to study imunnohistochemical analysis as like microscope binoculer Olympus CX41 with camera Olympus DP12, microtom rotary (Jung Histocut Leica 820), microscope slide polylysine, and coverslip

Procedure Extraction seed of Inocarpus fagiferus Fosb and Applied to Rat

Extract Tahitian chestnut (Inocarpus fagiferus Fosb) is concentrated ethanol (EtOH) extract which is obtained to extract seed with ethanol solvent as like previously reported. Ethanol extract applied to rat used to randomized posttest only control group design with 5 groups that were aim to prove the potency of extract as antiatherosclerosis agent. The groups are negative control (P_0 ; rat with feed standard), positive control (P_1; rat with feed high fat diet), P_2; rat with feed high fat diet and ethanol extract in doses of 50 mg/kg bw, P_3; rat with feed high fat diet and ethanol extract in doses of 100 mg/kg bw, and P_4; rat with feed high fat diet and ethanol extract in doses of 150 mg/kg bw.

The experiment was conducted for four months then aorta endhotelium cell of each rat groups were analyzed for their expression ICAM-1, SOD-2, TNF-α and IL-6. These data were analysed using one way Anova (SPSS version 19.0 for Windows) with Shapiro-Wilk and Levene Test each to, . normality and homogeneity, while to see the difference between groups was followed by Post Hoc Test LSD. All tests with significance level of 5% (α = 0.05).

RESULTS AND DISCUSSION

Expression of ICAM-1 Aortic Endhotelial Cell

Adhesion molecule ICAM-1 is one of the mediators of inflammation are expressed by endothelial cells due to oxidative stress conditions (hypercholesterolemia), which formed lipid peroxide will increase the expression of ICAM-1 and contribute to the formation of atherosclerotic plaque [12]. It's simillar with research [13] that high-

cholesterol diet in Wistar rats for 16 weeks, increased the expression of ICAM-1 in the aortic wall as well as it can be used as a biomarker of early stage development of atherosclerosis [13-15]. Research in cultured endothelial cell line also proved that lipid peroxide increased the expression of ICAM-1 [16-18] and correlated to monocyte infiltration [19-24]. Instead decreased expression of ICAM-1 in experimental animals shown to reduce the formation of vasculitic lesions and atherosclerosis [25-29]. Thus the positive expression of ICAM-1 can be used as the initial stage of the development of atherosclerosis maker [13-15]. Figures 1 shown that the greater the dose of the ethanol extract of the Inocarpus fagiferus Fosb seeds given to the tested rats as in the treatment group increasingly decreased significantly the expression of ICAM-1 positive endothelial cells.

The results of the inter-group mean difference test showed that ICAM-1 expression in the negative control group (P_0) differed significantly from the positive control group (P_1) ($p < 0.05$), treatment group P_2 significantly different from P_1 ($p < 0.05$), treatment group P_3 was significantly different from P_1 ($p < 0.05$), treatment group P_4 was significantly different from P_1 ($p < 0.05$), treatment group P_2 was not significantly different from P_3 ($p > 0.05$), treatment group P_2 is not significantly different from P_4 ($p > 0.05$), and treatment group P_3 is not significantly different from P_4 ($p > 0.05$). This means that at a dose of 50 mg / kg bw it has been able to reduce the expression of ICAM-1, a larger dose will have no effect on expression.

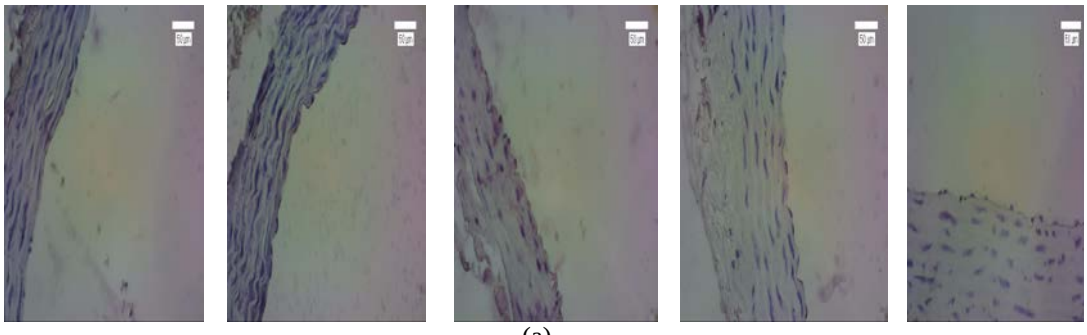
Expression of SOD-2Aortic Endhotelial Cell

Superoxide dismutase-2 (SOD-2) acts as the first defense against oxidative stress [30]. In blood vessels, SOD-2 acts as a protective vascular mitochondrial damage and the development of atherosclerosis [31], reducing the levels of superoxide anion and improve endothelial function in hypercholesterolemia [32,33], so that endothelial cells express the SOD-2 with higher levels [34]. Figures 2 shown an overview of expression and the average of SOD-2 rat aortic endothelial cells.

Giving a dose of 50 mg/kg bw of ethanol extract in rat have a significant effect when compared to positive control rat (P_1), or in other words increasing non-significant SOD-2 expression on P_1, but the greater the dose of ethanol extract Gayam seeds were given to rats, so the tendency of positive SOD-2 expression in aortic endothelial cells increased.

The results of the mean difference test between groups showed that the SOD-2 expression in the negative control group (P_0) was not significantly different from the positive control group (P_1) ($p > 0.05$), treatment group P_2 was not significantly different from P_1 ($p > 0.05$), treatment group P_3 was significantly different from P_1 ($p < 0.05$), treatment group P_4 was significantly different from P_1 ($p < 0.05$), treatment group P_2 was not significantly different from P_3 ($p > 0.05$), treatment group P_2 was not significantly different from P_4, ($p > 0.05$), and treatment group P_3 was not significantly different from P_4 ($p > 0.05$).

Increased expression of SOD-2 in P_3 and P_4 treatment even though it does not differ significantly from P_2 this was due to the antioxidant compounds contained in the extract can inhibit the oxidation reaction by the way it reacts with superoxide anion ($\cdot O_2^-$) molecules to form molecules that are relatively more stable as like hydrogen peroxide (H_2O_2), oxygen (O_2) and water (H_2O). Decreased superoxide anion radicals will be inducing endogenous antioxidant SOD so that its expression is increased. Mechanism of the antioxidant compounds are SOD inducer.



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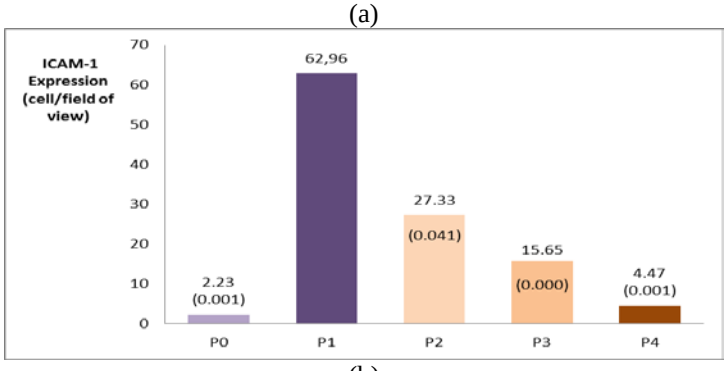
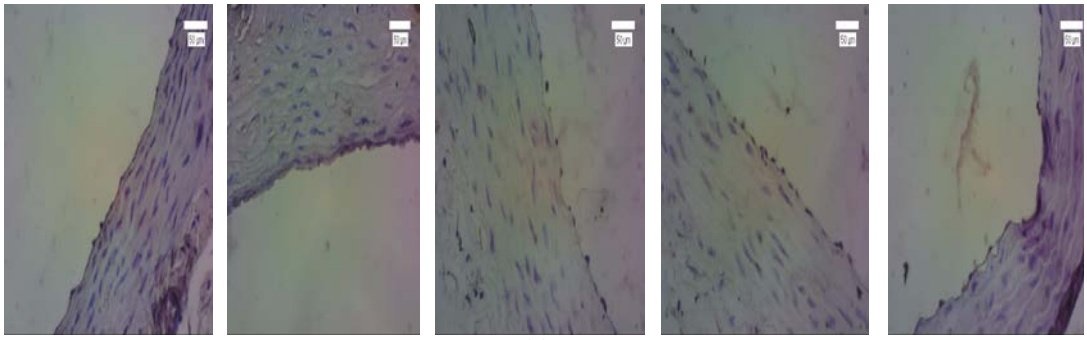


(b)

Figure 1. (a) ICAM-1 expression on aortic endothelial cell, (b) the average of expression treatment and control group



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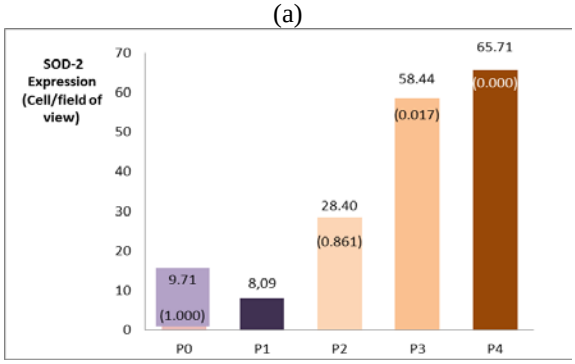
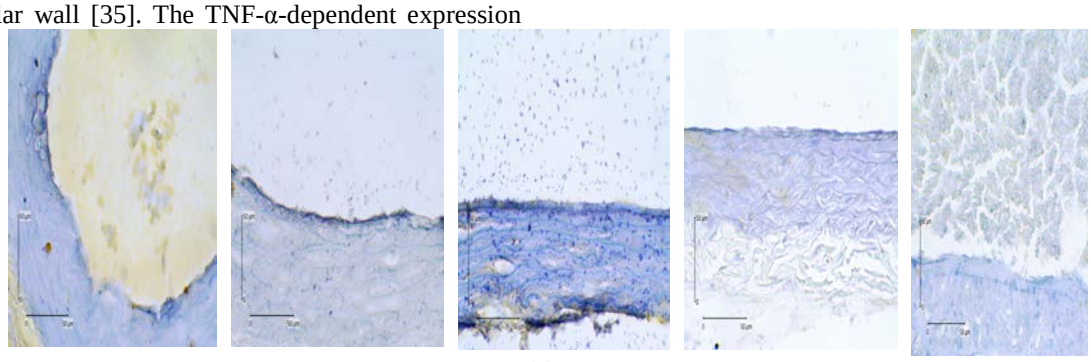


(b)

Figure 2. (a) SOD-2 expression on aortic endothelial cell, (b) the average of expression treatment and control group

Expression of TNF-α Aortic Endhotelial Cell

TNF-α induces the rapid expression of cell adhesion molecules (CAM) such as E-selectin, vascular cell adhesion molecule 1 (VCAM-1) and intercellular cell adhesion molecule 1 (ICAM-1) at the endothelial surface. These molecules mediate the attachment and transmigration of leukocytes from the blood stream into the vascular wall [35]. The TNF-α-dependent expression of ICAM-1/ VCAM-1 on EC varies with the anatomical location and the hemodynamic condition. A significantly higher and more sustained increase in endothelial CAM expression occurs in the greater curvature of the aortic arch as compared to the contralateral flatter curvature [36]. Figures 3 shown an overview of expression and the average of TNF-α rat aortic endothelial cells.



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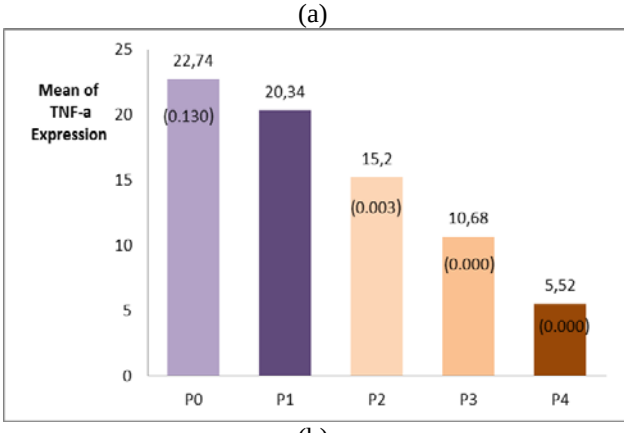
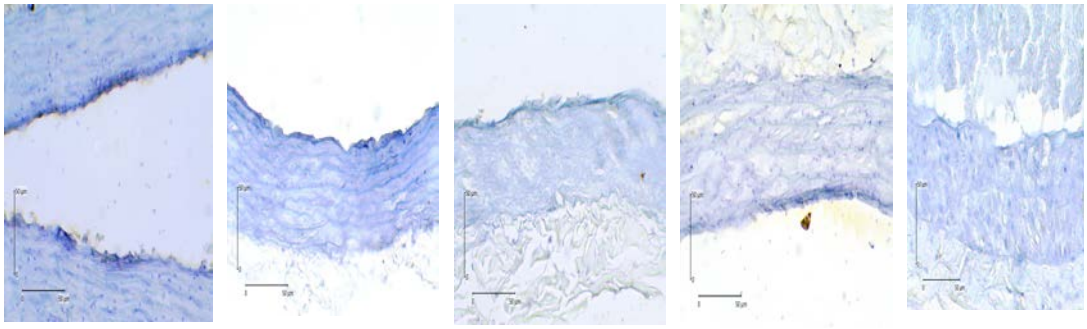


(b)

Figure 3 a) TNF-α expression on aortic endothelial cell, (b) the average of expression treatment and control group



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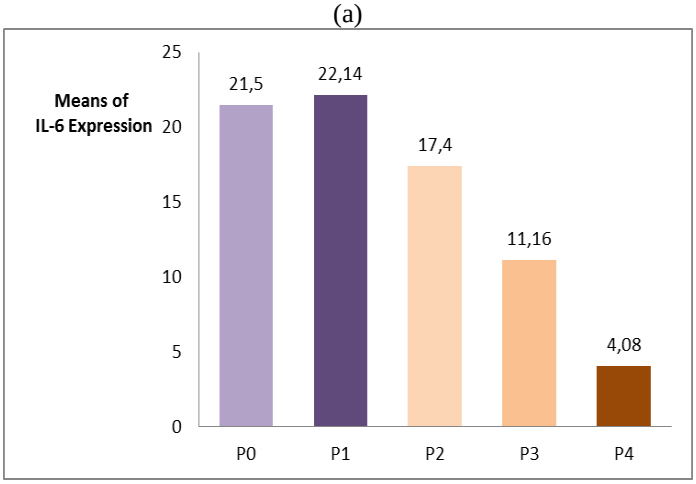

(b)

Figure 4. a) IL-6 expression on aortic endothelial cell, (b) the average of expression treatment and control group

Giving in dose of 50 mg/kg bw (P_2 group) ethanol extract had a significant effect when compared with positive control (P_1 group), or its significantly reduced TNF-α expression against P_1, and if larger dose given to treatment group P_3 and P_4, shown tends to decreases of TNF-α expression in aortic endothelial cells.

The results of the mean difference test between groups showed that the expression of TNF-α negative control group (P_0) was not significantly different from the positive control group (P_1) ($p > 0.05$), P_2, P_3, and P_4 treatment groups differed significantly against P_1 ($p < 0.05$), treatment group P_2 was significantly different from P_3 ($p < 0.05$), treatment group P_2 was significantly different from P_4, ($p < 0.05$), and treatment group P_3 differed significantly against P_4 ($p < 0.05$). This means that in fact only with the lowest dose of 50 mg/kg bw has actually been able to significantly reduce TNF-α expression or inflammation of the aortic cell, and the higher the dose, the inflammatory expression is also comparable, which is decreasing.

Expression of IL-6 Aortic Endhotelial Cell

IL-6 plays a role in the development of an atherosclerotic lesion, which is largely responsible for coordinating the influx of inflammatory cells. Endothelial cells respond to the binding of a complex of IL-6 and soluble IL-6 receptor by elaborating chemokines and increasing the expression of ICAM-1, which together permit the recruitment and intimal transmigration of leukocytes [37]. IL-6 stimulates growth of promoters of macrophage and vascular smooth muscle cells, which are major components of plaque [38-40]. Figures 4 shown an overview of expression and the average of IL-6 rat aortic endothelial cells.

Giving in dose of 50 mg/kg bw (P_2 group) ethanol extract had a significant effect when compared with positive control (P_1 group), or its significantly reduced IL-6 expression against P_1, and if larger dose given to treatment group P_3 and P_4, shown tends to decreases of IL-6 expression in aortic endothelial cells.

The results of the mean difference between groups showed that IL-6 negative control group (P_0) expression was not significantly different from the positive control group (P_1) ($p > 0.05$), treatment group P_2, P_3 and P_4 difference significantly from P_1 ($p < 0.05$), treatment group P_2 was significantly different from P_3 ($p < 0.05$), treatment group P_2 was significantly different from P_4, ($p < 0.05$), and treatment group P_3 was significantly different from P_4 ($p < 0.05$). This also shows that actually only with the lowest dose of 50 mg / kg bw has actually been able to significantly reduce the expression of IL-6 or inflammation of the aortic cell, and the higher the dose, the inflammatory expression is also comparable, which decreases. Figure 4 shows the same tendency as TNF-α expression.

CONCLUSION

The ethanol extract of Tahitian chestnut (Inocarpus fagiferus Fosb) seeds in doses of 50 mg/ kg bw was able to prevent atherosclerosis of wistar rats with significantly ($p < 0.05$) decrease in ICAM-1, TNF-α and IL-6 so increase in SOD-2 expression of aortic endothelial cells.

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