

EFFECT OF *ACANTHUS ILICIFOLIUS* L EXTRACT ON THE REDUCTION OF TNF- α EXPRESSION IN WISTAR STRAIN *RATTUS NORVEGICUS*

I Ketut Sudiana¹, Elyana S Asnar²

¹Department of Anatomic Pathology

²Department of Physiology

Airlangga University School of Medicine
Surabaya

ABSTRACT

Vascular lesion is an important factor in the occurrence of atherosclerosis. Vascular lesion can be triggered by collagenase-2 (matrix metalloproteinase-8 or MMP-8). MMP-8 is produced by endothelium resulting from the induction of TNF- α . TNF- α is one of proinflammatory cytokines produced by macrophage. One effort to overcome vascular lesion is the use of the extract of the fruit *Acanthus ilicifolius* L. However, the effects of this fruit's extract on the prevention of endothelial lesion were unclear. An experimental study was conducted to find those effects by using post test only control group design, involving 15 experimental animals, comprising 10-week old male Wistar strain *Rattus norvegicus*. These animals were divided randomly. Group I (5 rats) served as control, group II (5 rats) were induced with *Helicobacter pylori* LPS in a dose of 0.5 ug/200 gr BW, three times, once for three days, and control group III (5 rats) were induced with *H. pylori* LPS similar to that of group II but combined with the extract of the fruit *Acanthus ilicifolius* L in a dose of 70 mg/kg BW in 0.5% CMC per oral/day, for 10 days. Three days after the administration of *Acanthus ilicifolius* L extract, TNF- α -producing macrophage in the rats' cardiac tissue were examined using immunohistochemistry. The results revealed that TNF- α -producing macrophage in control group and that in LPS-induced group showed significant difference ($p < 0.05$). The significant difference ($p < 0.05$) was also found between group receiving LPS and that receiving the combination of LPS and *Acanthus ilicifolius* L extract. However, control group and the group receiving LPS with *Acanthus ilicifolius* L extract showed no significant difference ($p > 0.05$). In conclusion, the fruit *Acanthus ilicifolius* L has suppressive effect on TNF- α production in macrophage.

Keywords: atherosclerosis, inflammation, *Acanthus ilicifolius* L

Correspondence: I Ketut Sudiana, Department of Anatomic Pathology, Airlangga University School of Medicine, Jl Prof Dr Moestopo 47, Surabaya 60131, phone 62-31-5020251 ext. 153.

INTRODUCTION

Tumor necrotic factor alpha (TNF- α) is one of proinflammatory cytokines that play role in the process of cellular apoptosis. TNF- α is also a cytokine that can induce endothelium to express e-selectin (Kuby 2000). E-selectin is an adhesive molecule against neutrophil. Therefore, if e-selectin increases at the endothelial surface, this event may result in neutrophil aggregation in blood vessels. Neutrophil bound on endothelial surface by the adhesive molecule may secrete protease enzyme (collagenase-2), designated as Matrix Metalloproteinase-8 (MMP-8). This enzyme has capability to damage extracellular matrix protein, particularly collagen types I, II, III, and IV (Mamohara 2004). Since basal membrane of blood vessel constitutes of collagens type I, IV, and VIII, the presence of neutrophil-secreted MMP-8 may result in the damage of collagen types I and IV in blood vessel (Darnell 1990). The damage of collagens type I and IV in blood vessel

resulted in vascular lesion, and, furthermore, the lesion may trigger the occurrence of atherosclerosis. One type of plants that can be used to make medication for treating vascular lesion is the plant *Acanthus ilicifolius* L. This plant grows in marshy areas and can be easily obtained in Indonesia. The fruit of this plant is efficacious, as it has anti-inflammatory and regulative effect on vascular system. The contents of *Acanthus ilicifolius* L. are Acanthicifoline, oleanolic acid, P-sitosterol, lupeolquercetin, glucopyranoside, frigonelin and hydroxy benzoxazinoid (Kanchanapoom 2001). However, until today the effect of *Acanthus ilicifolius* L fruit extract on the reduction of TNF- α has not been proved. Since this study could not be applied to human, this study used Wistar rats (*Rattus norvegicus*) as experimental animals. Materials used to induce inflammatory reaction in the experimental animals were LPS (lypopolysaccharide) of *Helicobacter pylori*, which was given intramuscularly, three times, once in three days, in a dose of 0.5 ug/200 gr BW. To suppress the

activity of TNF- α expressing macrophage, we used the extract of *Acanthus ilicifolius* L fruit given orally in a dose of 70 mg/kg BW for ten consecutive days.

MATERIALS AND METHODS

This was a true experimental study using posttest only control group design. Experimental animals involved in this study were male Wistar strain *Rattus norvegicus* white rats, aged ten weeks. Material used to trigger the inflammation was *Helicobacter pylori* LPS, given intramuscularly as much as 0.5 ug/200 gr BW solved within physiological salt (volume 0.2 ml). The animals were divided into three groups, group I (not receiving *Helicobacter pylori* LPS), group II, receiving *Helicobacter pylori* LPS three times, once in three days, and group III, receiving *Helicobacter pylori* LPS three times in the same way, also receiving the extract of *Acanthus ilicifolius* L per oral in a dose of 70 mg/kg BW in 0.5% CMC/day every day for ten days. Three days after the administration of the fruit's extract, the three experimental animals were sacrificed for examining TNF- α expressing macrophages in cardiac tissue using immunohistochemical method.

The method to make *Acanthus ilicifolius* L was as follows: the fruit *Acanthus ilicifolius* L was dried in room temperature and scaled. The fruit was ground to become fine, and mixed with 100 ml of 98% ethanol, and kept in closed condition for 24 hours. After 24 hours, the fined fruit was filtrated to obtain brownish black filtrate. This procedure was repeated several times until the ethanol used to obtain filtrate became colorless. Obtained filtrate was put within oven in a temperature of 50°C until pellet was formed. From the pellet, solution of 70 mg/BW was made in 0.5% CMC.

RESULTS

Table 1. Discriminant test of TNF- α expression in macrophages of Wistar rats' cardiac tissue (Mann-Whitney Test)

n	Groups	Sig.
5	C (1.480 $\pm$ 1.791)	Hp (10.784 $\pm$ 3.305) 0.006
		Hp-J (1.5200 $\pm$ 0.855) 0.476
5	Hp (10.784 $\pm$ 3.305)	C (1.480 $\pm$ 1.791) 0.006
		Hp-J (1.5200 $\pm$ 0.855) 0.007
5	Hp-J (1.5200 $\pm$ 0.855)	C (1.480 $\pm$ 1.791) 0.476
		Hp (10.784 $\pm$ 3.305) 0.007

Notes:

C : control group

Hp : Rattus group receiving H. pylori LPS

Hp-J : Rattus group receiving H. pylori LPS and *Acanthus ilicifolius* L extract

Unit : Macrophage count expressing TNF- α per 100 macrophage cells

Results obtained in this study were subjected to homogeneity test, and it was found that the data were not homogeneous ($p < 0.05$), so that to conduct discriminant test between control and treatment group we used Mann-Whitney Test. Table 1 shows that statistical test on TNF- α expressing macrophages in control group (1.480 $\pm$ 1.791) and in group induced with intramuscular H. pylori LPS (10.784 $\pm$ 3.305) revealed significant difference ($p < 0.05$). The result of statistical test, however, showed no significant difference ($p > 0.05$) in TNF- α secreting macrophage between control group (1.480 $\pm$ 1.791) and group receiving intramuscular H. pylori LPS combined with per oral *Acanthus ilicifolius* L. fruit extract (1.520 $\pm$ 0.855). Discriminant test between group induced by H. pylori LPS combined with the fruit extract (1.520 $\pm$ 0.855) and the group induced with H. pylori LPS only (10.784 $\pm$ 3.305) apparently revealed significant difference ($p < 0.05$).

DISCUSSION

Control group (1.480 $\pm$ 1.791) and group receiving induction with H. pylori LPS (10.784 $\pm$ 3.305) statistically showed significant difference ($p < 0.05$). It can therefore be inferred that intramuscular LPS administration can trigger the increase of macrophage count that express TNF- α in the cardiac tissue of *Rattus norvegicus*. This is because macrophage is able to phagocytose antigen (H. pylori LPS) that enters the body, so that the cells become activated and macrophage will express various proinflammatory cytokines, such as TNF- α , IL-6, IL-1 and IL-8. TNF- α may trigger the endothelium to express e-selectin as an adhesive molecule to neutrophil (Kuby 2000). The presence of e-selectin at endothelial surface results in neutrophil attachment to the surface of endothelium. If neutrophil aggregation occurs at endothelial surface of blood vessels, the neutrophil may secrete a protease enzyme (collagenase-2), which is recognized as Matrix Metalloproteinase-8 (MMP-8). This enzyme has a capability to damage extracellular matrix protein, particularly collagen types I, II, III and IV (Mamohara 2004). Since blood vessel's basal membrane is constituted of collagen types I, IV and VIII, the presence of MMP-8 secreted by neutrophil may result in the damage of collagen types I and IV in blood vessel (Darnell 1990). This damage results in vascular lesion, and this lesion is an important factor in the process of atherosclerotic formation.

Therefore, we suggested an effort to prevent endothelial lesion. The effort was the suppression of TNF- α secretion by the macrophages by the administration of the extract of *Acanthus ilicifolius* L. fruit. In villages, this material has been used as medication for hemorrhoid (one of vascular abnormalities). After the experimental animals were given with the fruit's extract, we obtained results as can be seen in Table 1, in which TNF- α producing macrophages in control group revealed 1.480 ± 1.791 , while group that was induced intramuscularly with *H. pylori* LPS and received the fruit's extract per oral revealed 1.520 ± 0.855 . Statistical test showed no significant difference ($p > 0.05$). This indicated that the extract of the fruit *Acanthus ilicifolius* L. could suppress the secretion of TNF- α by the macrophage. However, the action of this fruit's extract in suppressing macrophage activity in TNF- α production should be further investigated to find whether the action of *Acanthus ilicifolius* L. extract can also regulate TNF- α synthesis regulation at genetic level or at its synthesis level. Nevertheless, it can be reported from this study that the reduction of macrophage activity in synthesizing TNF- α was truly resulted from the administration of *Acanthus ilicifolius* L. extract, and this can be viewed as a new method for preventing the occurrence of atherosclerosis. The reduction of TNF- α production by macrophage would inhibit the endothelium to express e-selectin, so that neutrophil aggregation in blood vessel wall will be prevented. If neutrophil aggregation does not occur in blood vessel wall, there will be no MMP-8 release by neutrophil, so blood vessel collagen damage will not occur and, finally, vascular lesion can be prevented.

CONCLUSIONS

The extract of the fruit *Acanthus ilicifolius* L. can inhibit macrophage to express TNF- α and it can be used as a basis for developing medication to treat endothelial lesion. Further studies should be undertaken to investigate active material isolated from the fruit *Acanthus ilicifolius* L. The action of the active material in the process of suppressing TNF- α production should also be studied.

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