ANTI-INFLAMMATORY EFFECT OF PORTERWEED (*Stachytarpheta Jamaicensis* (L.) Vahl) LEAF EXTRACT TO TNF-α EXPRESSION

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ABSTRACT

Porterweed (Stachytarpheta jamaicensis (L.) Vahl) contain many flavonoids compound. It is a potential candidate to supress the inflammatory process through down regulation of TNF-α level as a potent pro-inflammatory cytokine in chronic inflammation cases. The aim of this research was to improve the effect of Porterweed leaf extract to the TNF-α level and considered the optimum dosage in wistar rats with chronic inflammation model. Twenty five rats was divided into negative control group, positive control group, and treatment groups with dosage 50, 100 and 150 mg/kg BW. Data obtained through microscopical histologic observation on aortic organ after immunohistochemistry staining and was analyzed descriptively. The result revealed that Porterweed leaf extract is able to inhibit the increasing of TNF-α level. This phytochemical substance is predicted capable of preventing the oxidation reaction progession of cyclooxygenase-2 (COX-2) to produce prostaglandins. The decreasing of prostaglandin level causes a negative feedback to TNF-α production and iniciating the declining level of TNF-α. Thus, the Porterweed leaf extract indicates supress chronic inflammatory process through down regulation of TNF-α and prostaglandin activity in optimum dose 150 mg/kg bw.

Key words: *Porterweed (Stachytarpheta jamaicensis (L.) Vahl), antiinflammation, TNF-α*

INTRODUCTION

Inflammation is a protective response for challenging microorganism or tissue damage which leads to the restoration of tissue function. The reactions is involving the secuential release of pro anti-inflammatory mediators and increasing the microvascular permeability which caused the exudation of fluid and plasma proteins into the inflammed tissue. Thus, the activated neutrofils undergo apoptosis, a process that play a central role in the inflammation resolution (Kadl *et al*., 2004). Inflammation is a complex event which is part of the response to all multicellular organisms that indicate on a network that lives and dies due to a foreign agent (Kreier and Richard, 1990). The inflammatory phenomenon can be seen as microvascular damage, the increasing permeability of capillary, and leukocyte migration into the inflammed tissue. TNF-α acts as a major mediator of inflammation in the immune response. It is an acute-phase proteins which initiate the cascade of cytokines and increases the vascular permeability (Janeway *et al*., 1999).

Nowadays, the researcher's attention are focused on arachidonic acid metabolites as the important mediators of inflammation. Arachidonic acid is derived from cell membrane phospholipids which are activated by injury. It can be metabolized in two different pathways, the cyclooxygenase pathway which produce a number of prostaglandins and thromboxane. The second is lipooxygenase pathway which produce leucotrienes (Rustam *et al*., 2007). The treatment that has been devoted to cope with was using of non-steroid anti-inflammatory drugs (NSAIDs). It works by inhibit the synthesis of prostaglandins (PG) (Fauziyah, 2008) and basically causes an undesirable side effects. Thus, people are trying to use some traditional medicine as an anti-inflammatory such as *Eleutherine americana* bulb, *Curcuma xanthorriza* rhizome, and *Carica papaya* root (Sa'roni *et al.*, 1987).

Any kind of herbs which often being utilized is porterweed (*Stachytarpheta jamaicensis* (L.) Vahl) that usually grow on the edge of the road, terrain, and other abandoned places. The leaves is used to treat the inflammed throat by boil and mix it with some spices, and the boiled water can be drunk. Porterweed (*Stachytarpheta jamaicensis* (L.) Vahl) contains chemical substances such as flavonoid, alkaloid, and glycoside used as anti-inflammatory and deuritic. Porterweed contain many flavonoids compound. It is a potential candidate to suppress the inflammatory process through down regulation of TNF-α level as a potent pro-inflammatory cytokine in chronic inflammation. In this study TNF-α was choosen as parameter because TNF-α

acts as a major mediator of inflammation, as a form of immune system response. The TNF-α expression can be seen in tissues such as aorta, especially in tunica media. The utilization of Porterweed (*Stachytarpheta jamaicensis* (L.) Vahl) as anti-inflammatory were suggested due to its flavonoid content which is predicted to have a protective effects against inflammation (Zafar *et al*., 2010). The flavonoids level are large enough to potentially overcome the chronic inflammation by decreasing the TNFα levels as one of the inflammatory mediators.

MATERIALS AND METHODS

Rats were fasted for 18 hours and divided into five groups A, B, C, D (positive control group) and E (negative control group). The A, B, and C groups were treated with Porterweed extract and the dosages were 50 mg/kg bw, 100 mg/kg bw, and 150 mg/kg bw. The rats' feet volume was measured by platysmometer before and after the treatment. It was injected with 0.1 ml carrageen on the toes between 5th and 4th segment of the right foot and waited for 1 week to produce chronic inflammation (Sulaiman *et al*., 2009). In this study, the chronic inflammation is prolonged in 2 weeks. Rats were threated for 1 week and conducted by gavages. On the 7th day, rats were dislocated and surgeried so that the aortic can be taken. The aortic was made into slides with immunohistochemical staining to observe the expression of TNF-α.

RESULTS

At the 7 day after treatment with Porterweed leaf extract, the aorta from 24 rats were dissected and made into slides for TNF-α immunohistochemistry staining. The slides were grouped by its dosage treatment. The aortic slides reveal that every dosage has different level of TNF-α expression. The negative control group (D group) shows that there is TNF-α expression which is showed by the light brown and purple color on the slides. The positive control group (E group) shows that the expression of TNF-α increased due to the expression of dark brown color. The A group shows that the brown color is dominant which indicates the high expression of TNF-α. The B group shows the brown color but lower than A group. The C group shows that the slides color almost purple which means that the expression of TNF-α is lower that A and B group (Figure 1). This result reveals that the higher dosage given to the inflammed rats will surpress the expression of TNF-α.

DISCUSSION

The Porterweed leaf extract (*Stachytarpheta jamaicensis (L.) Vahl*) *given to the Wistar rats with carrageenan-induced inflammation was to determine its effect to the aortic* TNF-α level. TNF-α was used due to its role as the main inflammation mediator on immune response (Janeway *et al*., 1999). From previuos researchs, there was some animal which is used as the research object such as rats chemical agent-induced inflammation. One of the chemical agent is carrageenan (Sulaiman, *et al*., 2009). Carrageenan-induced inflammation of localized tissues has been shown to affect systemic blood cell reactivity. Carrageenan pretreatment appeared to sensitize leucocytes to lipopolysaccharide challenge and enhance production of tumour necrosis factor-a (TNF-α). In conclusion, we have shown that a neurolytic nerve block can limit the systemic inflammatory response observed in mice after intraplantar injection of carrageenan (Pham-Marcou *et al*., 2005).

The utilization of carrageenan has several advantage such as left no marks, cause no tissue damage, and give more sensitive respond to anti-inflammatory drugs than the other irritant compound (Siswanto and Nurulita, 2005 in Fauziyah, 2008). According to Radhakrishnan et al. (2009), the 1% injected carrageenan can caused chronic inflammation. It is used in this study to induce chronic inflammation model in vivo by injecting 1,5% carrageenan for two weeks. Then, the therapy using porterweed leaf axtract (*Stachytarpheta jamaicensis (L.) Vahl)* with variation dosages (50 mg/kg bw, 100 mg/kg bw, dan 150 mg/kg bw) was treated to the rats.

Porterweed leaf extract (*Stachytarpheta jamaicensis (L.) Vahl) has a potent as antonociceptive and antinflammation (Sulaiman et al. 2009). The extract contain active compounds such as alkaloid, flavonoid, and glicoside. The compound which is expected as the antiinflammation agent is flavonoid. Flavonoid in its aglikon form is nonpolar and in its glicosidic form is polar. Based on its nature, the solvent used in this study was ethanol 70% because of its semipolar nature. Furthermore, ethanol 70% cause no inflammation in cell membrane and improve the solute stability* (Harborne, 1987 in Fauziyah, 2008).

Porterweed (*Stachytarpheta jamaicensis* (L.)Vahl) leaf extracts contains an active substance in the form of alkaloids, flavonoids, and glycosides. Several flavonoids are biochemically active compounds with known anti-inflammatory (Liang *et al*., 1999). Certain flavonoids modulate the enzyme activities of arachidonic acid (AA) metabolizing enzymes such as phospholipase A2 (PLA2),

cyclooxygenase (COX), and lipoxygenase (LOX) and the nitric oxide (NO) producing enzyme, nitric oxide synthase (NOS). An inhibition of these enzymes by flavonoids reduces the production of AA, prostaglandins (PG), leukotrienes (LT) and nitric oxide NO, crucial mediators of inflammation (Lafuente *et al*. 2009, Alshalmani, 2011). Thus, the inhibition of these enzymes by flavonoids may be one of the most important mechanism of their anti-inflammatory activity.

Flavonoids can also inhibit production of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and interferon-γ (Alshalmani, 2011). TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells. Additionally, TNFα stimulates neutrophils to transcribe and release cytokines, and chemokines biosynthesis. Interaction between these mediators thus enhances further inflammatory reaction and inhibition of TNF-α release can reduce the severity of inflammation (Laupattarakasem *et al*., 2006). Flavonoid in the extract is able to inhibit the inflammation by inhibiting the formation of prostaglandin as the inflammation mediator. Flavonoid supressed the prostaglandin discharge by inhibit the metabolism of arachidonic acid and non-steroid compound which works through the inhibition of cyclooxygenase mechanism which has a role in prostaglandin biosynthesis (Muschler, 1991 in Fauziyah, 2008).

Arachidonic acid release is a starting point for a general inflammatory response. Arachidonic acid is released from membrane phospholipids in cells by the action of PLA2 and metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) pathways to prostaglandins. Cyclooxygenase (COX) exists in two different isoforms COX-1 and COX-2. Thus, COX-1 is a constitutive enzyme existing in almost every cell type, while COX-2 is an inducible enzyme that produces large quantities of PG, and is highly expressed in the inflammation related cells when they are stimulated with proinflammatory cytokines and/or bacterial lipopolysaccharide.

Lipoxygenases (LOXs) are responsible for generating hydroxyl acids and leukotrienes from AA. One of the isoforms, 12-LOX, synthesizes 12-HETE which aggregates platelets and induces inflammatory response. Selected phenolic compounds such as flavonols were found to onhibit these enzymes, reducting the release and metabolism of arachidonic acid and thus, diminishing the formation of inflammatory mediators (Lafuente *et al*., 2009). If the COX-2 synthesis is inhibited, so does the prostaglandin synthesis. Prostaglandin role is making the pain related to the tissue damage or inflammation. Prostaglandin works slow but has a greater potent when combined with the other mediator or substances which is released locally such as histamine, serotonine, leucotrine, and cytokine. Prostaglandin can caused vasodilatation and increase the local blood flow (Ganiswara, 1995 in Lumbanraja, 2009).

Tumor necrosis factor-α (TNF-α), is a kind of pleiotropic cytokine implicated in many physiological and pathological reactions including cell death, cell survival, immune response, and inflammation (Rossard, 2009). It induce the inflammation which is caused by prostaglandin as the inflammation initiator. Nowadays, the expression and synthesis of TNF is not restricted only to *hematopoietic cells*, it is also applied to the other cytokine (Gonzales, 2008).

When the inflammation occured, TNF-α will be mediated by the releasing of prostaglandin E2 and the expression of COX-2 will occure in human gingival fibroblast (HGF) (Nakao *et al*., 2002). The releasing and level of TNF-α induced by Prostaglandin E2 and accumulation of COX-2 mRNA at the same time is depend on the HGF. As prostaglandin E2 isn't produced, it caused TNF-α cannot be mediated and decreasing the TNF-α level in blood. If the prostaglandin is inhibited in its cyclooxygenase pathway, there is a negative feedback that make the decreasing of TNF-α production.

The aortic slides reveal that every dosage has different level of TNF-α expression. The negative control group (D group) shows that there is TNF-α expression which is showed by the light brown and purple color on the slides. The positive control group (E group) shows that the expression of TNF-α increased due to the expression of dark brown color. The A group shows that the brown color is dominant which indicates the high expression of TNF-α. The B group shows the brown color but lower than A group. The C group shows that the slides color almost purple which means that the expression of TNF-α is lower that A and B group. This result reveals that the higher dosage given to the inflammed rats will surpress the expression of TNF-α due to the flavonoids that may inhibit the cyclooxygenase in the formation of prostaglandins. Inhibition of prostaglandin is indicated by the decreasing of TNF-α level in the aortic-stained TNF-α slides.

Porterweed (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract influence the decreasing of TNF-α expression with optimum dosage of 150 mg/kg bw due to the flavonoid content which has the ability to reduce the level of TNF-α as inflammation agent by inhibit the metabolism of prostaglandins in the cyclooxygenase pathway.

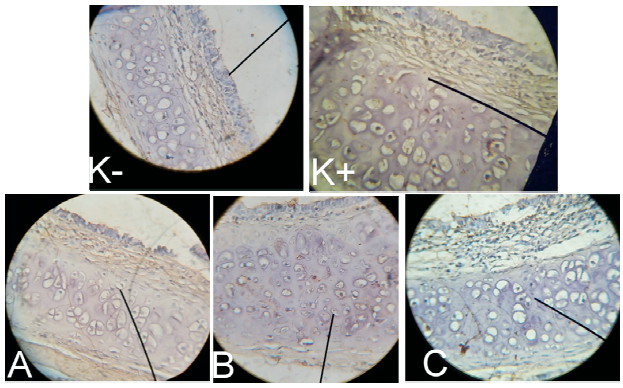

Figure 1. The aortic slides from each group treatment. (A) group was treated with 50 mg/kg bw Porterweed leaf extract, (B) group was treated with 100 mg/kg bw, (C) group was treated with 150 mg/kg bw, (K-) negative control group, (K+) positive control group.

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