Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(6); 1080-1085

ISSN: 0975-4873

Research Article

Study on the Antiinflammatory Activity of *Artocarpus altilis* Leaves Extract in Mice

Fakhrudin, N[*1,2], Hastuti, S[1], Andriani, A[1], Widyarini, S[3], Nurrochmad, A[1]

[1]*Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, 55281, Indonesia;* [2]*Center for Natural Antiinfective Research, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, 55281, Indonesia;* [3]*Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Sekip Utara, Yogyakarta, 55281, Indonesia.*

Available Online:11th October, 2015

ABSTRACT

In addition to the body's response to injury, inflammation is a physiological process underlying the progression various diseases. Investigation for the discovery and development of antiinflammatory agents from natural sources is a promising strategy for curing inflammatory diseases. Breadfruit or *Artocarpus altilis*, is an Indonesian native plant traditionally used for the treatment of various disease including inflammatory-related diseases such as arthritis, tumors, pain, gastritis and atherosclerosis. Scientific evidence regarding the antiinflammatory effect of *A.altilis* leaf is limited. This study aimed to investigate the antiinflammatory activities of *A.altilis* leaf extract (AAE). The expression and the activity of COX, the enzyme responsible for prostaglandins formation, were also evaluated. The *A.altilis* leaf was extracted in ethyl acetate and the dried extract was used for the experiments in mice models. We employed a carrageenan-induced paw edema in mice to evaluate the antiinflammatory activities. The level of COX-2 expression in the paw tissues were determined using immunohistochemistry, whereas COX-2 inhibitory activity was tested on *in vitro* enzymatic assays. We found that AAE at the doses of 250, 500 and 1000 mg/kgBW significantly reduced the volume of paw edema until 6 hours of observation. *In vitro* enzymatic assays revealed that AAE has a lower IC_{50} against COX-2 compared to COX-1, suggesting the higher selectivity for COX-2. In addition, the level of COX-2 expression in the hind paws was also significantly reduced upon AAE treatment in dose-dependent manner. These indicated that AAE has a potency to be further developed as antiinflammatory agent or as a source of lead compounds acting as antiinflammation.

Keyword: *Artocarpis altilis*, antiinflammation, cyclooxygenase

INTRODUCTION

Inflammation is a body response to injuries induced by a variousstimuli, such as infectious agents from microorganisms, noxious substances, physical damages, and changes induced by malignant cells. Various pathological conditions, including atherosclerosis, sepsis, cancer, arthritis and metabolic syndromes are related with inflammatory condition[1]. Currently, no satisfying drug is available for the treatment of these inflammatory-related diseases. There are two antiinflammatory drugs available in the clinic: corticosteroids and non-steroidal antiinflammatory drugs (NSAID). Despite the fact that corticosteroids and NSAID (non-steroidal antiinflammatory drugs) remain the common choice for the treatment of inflammatory diseases, the usage of these drugs are restricted by their undesirable side effects and the limited potency to reduce the symptoms of inflammation. Moreover, chronic use of corticosteroids antiinflammatory drugs has been limited as they exhibited a weight gain, osteoporosis and immunosuppressive effects. Whereas high dose NSAID medication leads to gastrointestinal tract-related toxicities[2]. Consequently, the development of novel potential antiinflammatory agents with a desirable side effect is a great of interest.

Many therapeutic targets have been identified to interfere the inflammatory processes. One of the most important therapeutic target is cyclooxygenase (COX)[3]. COX-1 is constitutively expressed, whereas COX-2 is an inducible enzyme expressed during the inflammatory processes[4]. COX-2 is responsible for the formation of prostaglandins and has a key role for therapeutic intervention in pain and inflammatory-related diseases[5,6]. Inhibition of COX-2 activity has become an important target for combating inflammatory disorders[3,7,8]. COX-2 was the established therapeutic target in inflammation and aspirin is the fist NSAID inhibitor of COX-2. Inhibition of COX-2 activity interferes the production of prostaglandins, the inflammatory mediators that play a crucial role in the development of inflammatory responses and varios pathophysiological conditions[5,9]. Unfortunately, the use of aspirin is associated with the undisired side effects including renal toxicity and gastrointestinal bleeding[10]. In addition, the newer selective COX-2 inhibitor, the "coxib" derivatives, still exhibits severe cardiovascular side effects[11-13]. Consequently, the exploration for finding

**Author for Correspondence*

the alternative therapeutic agent targeting COX-2 is a promising research.

Medicinal plants have significant contribution to the drugs development and discovery and still provide abundant and promising source for lead structures as drug candidates. Many plants have been used by folk for decades to treat various human diseases including inflammatory diseases. *Artocarpus altilis* (Moraceae), commonly known in Indonesia as Sukun, is a flowering tree native to Indonesia and New Guinea, and spreading throughout Southeast Asia and Africa. Indonesian people consume the starchy fruit part after boiling and frying it at all stage of growth as it provides high amount of fibers and carbohydrates as well as contains protein, vitamins, calcium, magnesium, potassium, copper, iron, niacin, thiamin, riboflavin, lutein, and phenolics[14,15]. Although the fruit of *A. altilis* has been acknowledges as a potential food source for food security for the growing global population[15], the leaf part is underutilized and known to be a non-toxic suggesting the safety in therapeutic uses[16].

The leaves of *A. altilis* have been traditionally used by folk for treating various disorders such as hypertension, liver cirrhosis, diabetes, hypercholesterolemia, and also used in inflammatory conditions such as arthritis, pain, gastritis and stroke[17,18]. Previous studies showed that *A. altilis* contains various non-polar a non-glycoside prenylated flavonoids[19-22] responsible for several pharmacological activities including anticancer[23,24], antiausteric[19], antioxidant[25], antiinflammation[26], antiplatelet[22] and antiatherosclerotic[27]. The leaves were also known to contain lutein, sitosterol, squalene, unsaturated triglycerides, polyprenol, unsaturated fatty acids[28]. Previous study demonstrated that AAE showed a promising antihypertension activity via inhibition of angiotensin converting enzyme activity[29] and exhibited antiatherosclerosis activity[30]. However, only little scientific data is available regarding the antiinflammatory activity of AAE both *in vitro* and *in vivo* studies. In this study, we investigated the antiinflammatory activity of AAE in mice and the effects on the expression and the activity of cyclooxygenase-2 (COX-2).

MATERIALS AND METHODS

Plant material

The main material used in this study is *Artocarpus altilis* leaves. The leaves were harvested from Melati district, Sleman, Yogyakarta, Indonesia. Plant identification was done by the botanist at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. Freshly harvested leaves were dried at 50°C in the oven for 48 h and further grinded before the extraction.

Reagents and Chemicals

Indomethacin and carrageenan were purchased from Sigma Aldrich (Saint Louis, MO, USA), ethyl acetate and aquadest were obtained from local supplier (Brataco Chemika, Yogyakarta, Indonesia), whereas dimethyl sulfoxide and the PBS components were obtained from EMerck (Darmstadt, Germany). Colorimetric-based COX Inhibitor Screening Assay was purchased from Caymann Chemical (Michigan, USA, catalog number 760111) and COX-2 antibody was obtained from Santa Cruz Biotechnology (Texas, USA, catalog number sc-1747-R).

Extraction

The extraction of plant material was done using maceration method. The dried *A. altilis* leaves (500 gram) were macerated three times in ethyl acetate (2.5 L). After filtration, the solvent was evaporated using a vacuum rotary evaporator instrument under a reduced pressure to dryness.

Animals

Male BALB/c mice (20-30 g) were used in this study. They were housed in a controlled environment and fed with standard pellet diet and water ad libitum. After acclimatization for at least one week before the experimental session, the mice were randomized and divided into 6 groups. All the experimental protocols were performed *according* to the guidelines approved by Institutional Animal Ethic Committee (number 192/KEC-LPPT/IX/2014), Universitas Gadjah Mada, Indonesia.

Carrageenan-induced paw edema

The acute inflammatory activity was evaluated using Carrageenan-induced paw edema assay as previously described[31]. Paw edema was induced by subplantar injection of freshly prepared 200 μL carrageenan 1% (solution in distilled water) in to the right hind paws. The animals were randomly divided into 5 groups of 5 animals each. All groups, except the untreated group, were given single dose of extracts (250, 500 or 1000 mg/kgBW), solvent or indomethacin (5 mg/kgBW), 30 minutes prior to paw edema induction using carrageenan. The volume of the paw edema was measured using plethysmometer every 30 minutes for 6 hours after carrageenan administration. The antiinflammatory activity (percentage activity) was determined based on the paw edema volume differences between the extracts-treated and the solvent-treated groups after 6 hours.

Immunohistochemistry of COX-2

After the measurement of paws edema, the mice were sacrified and the soft plantar region sections of the hind paw were cut and fixed in 10% buffered formalin for 24 hours and further embedded with parafin[32]. The parafin-embedded blocks were cut with a thickness of 4 mm, flattened and then attached to the glass slides coated with poly-lysine. Antigen recovery was done by addition of xylol and ethanol to deparafinize and dehydrate the sections, respectively, and further washed in a phosphate buffered saline (PBS) solution. The sections were incubated for 10 minutes in a peroxidase blocking solution containing 3% H_2O_2 to abolish endogenous peroxidase activity and further incubated 10 minutes in a bloking buffer containing 5% bovine serum albumin (BSA). The sections were incubated overnight at 4°C with diluted mice primary antibody (anti COX-2, 1:250 dilution in PBS-BSA) and then washed in PBS for 5 minutes. The secondary biotinylated universal antibody (antiIgG) at 1:200 dilution was added and the sections were incubated for 5 minutes at room temperature. Following washingwith PBS for 5 minutes, the sections were incubated for 10 minutes in the conjugated-

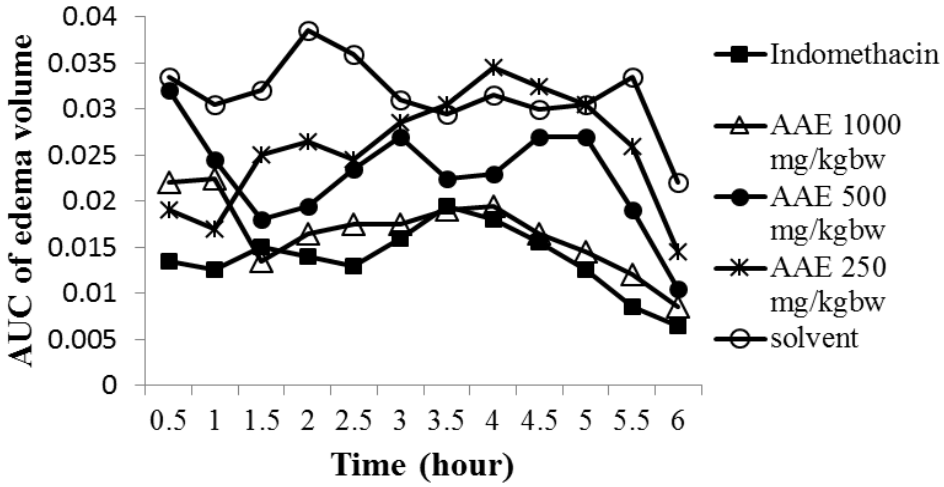

Figure 1. The AUC for time-response curves of paw edema on carrageenan-induced paw edema in mice (5 animals per group). The *A. altilis* extract (AAE) were tested at 250, 500 and 1000 mg/kgBW and indomethacin (5 mg/kgBW) was used as a positive control.

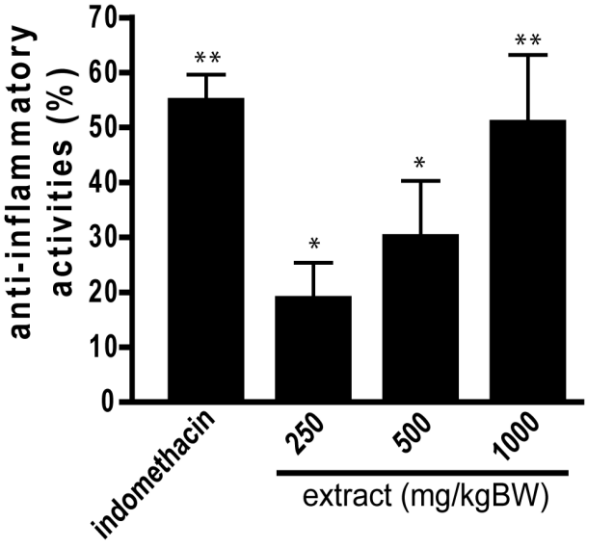

Figure 2. Antiinflammatory activity of *A. altilis* extract on carrageenan-induced paw edema in mice (5 animals per group). The extracts were tested at 250, 500 and 1000 mg/kgBW and indomethacin (5 mg/kgBW) was used as a positive control. The values are mean ± standard errors. * $p< 0.05$; ** $p< 0.01$ (ANOVA/Dunnett, compared to solvent-treated group).

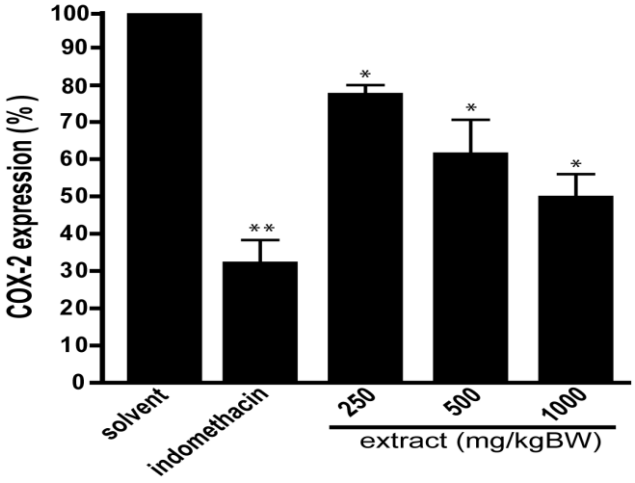

Figure 3. Effect of *A. altilis* extract on the inhibition of COX-2 expression on carrageenan-induced paw edema in mice (5 animals per group). Mice paws were cut and fixed in buffered formalin and COX-2 expression was quantified. The extracts were tested at 250, 500 and 1000 mg/kgBW and indomethacin (5 mg/kgBW) was used as a positive control. The values are mean ± standard errors. * p< 0.05; ** p< 0.01 (ANOVA/Dunnett, compared to solvent-treated group).

streptavidin peroxidase complex. After another 5 minutes washing with PBS, the sections were stained by incubation (10 minutes) in peroxidase substrate solution containing 3,3'diaminobenzidine-peroxide (DAB) at 1:9 dilution, and counter-stained with Mayer hematoxylin (100 µl for 2 minutes). The stained sections were then dehydrated using ethanol and xylol, added mounting media and embedded in microscope slides for immunohistochemistry analisis. When the cytoplasm was stained brown, COX-2 immunostaiting was considered positive. COX-2 expression was evaluated based on the percentage of positive cells according to the previous method[32,33]. The percentage of COX-2 expression was determined by calculating the COX-2 expressing cells (positive cells) in the extracts-treated groups compared to the solvent-treated group. The cell counting was done in a light microscope observed under 1000X magnification at 5 different fields.

COX-1 and COX-2 enzymatic assays

The COX-1 and COX-2 inhibitory activities of the extracts were evaluated using a colorimetric-based COX Inhibitor Screening Assay (Caymann, catalog number 760111) that employs peroxidase component of cyclooxygenase. The activity of peroxidase is measured colorimetrically at 590 nm based on the generation of oxidized N,N,N',N'-tetramethyl-p-phenylenediamine

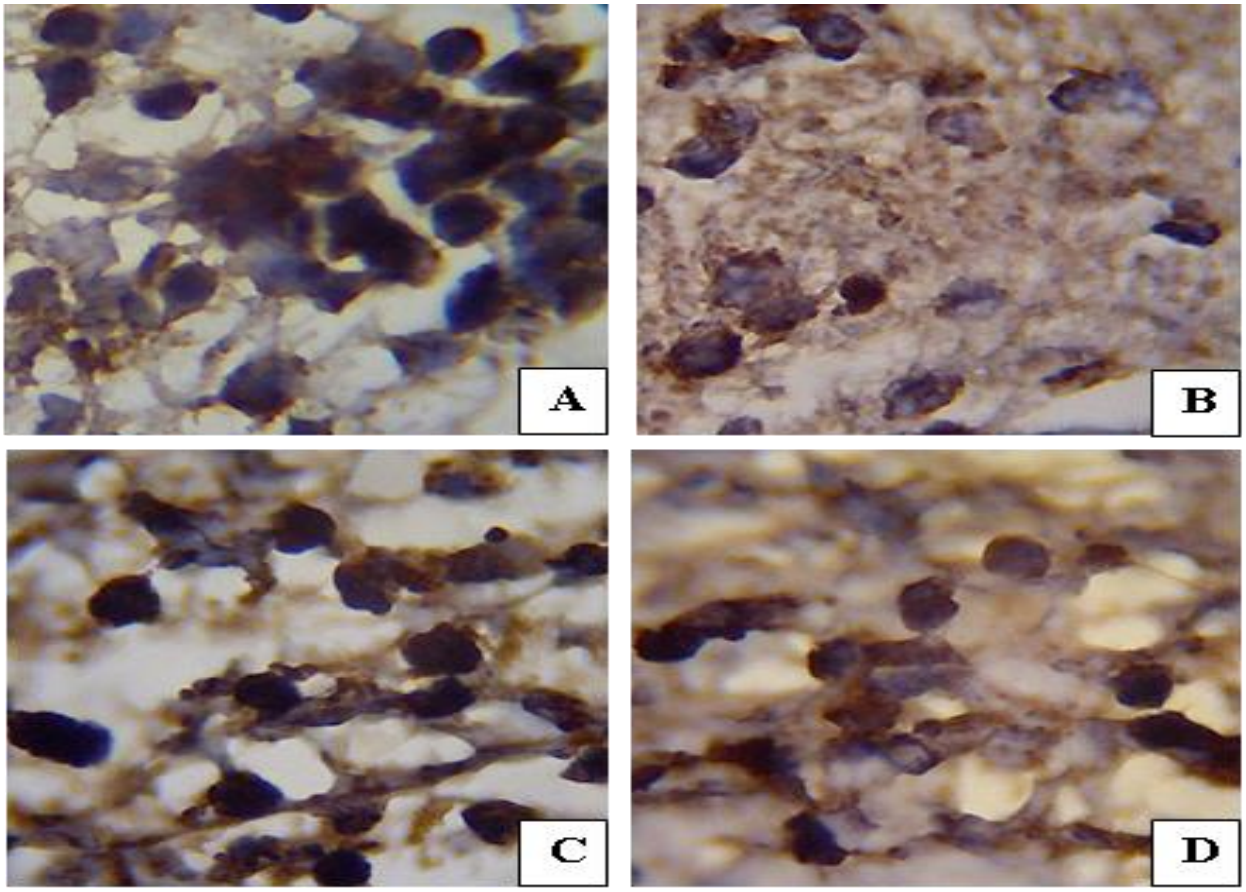

Figure 4. Representative pictures showing COX-2 expression in the soft plantar region sections of the hind paw. The pictures were taken in a light microscope observed under 1000X magnification at 5 different fields. The cox-2 expressing cells were colored in dark brown. A: solvent-treated goup, B: indomethacin-treated group, C: AAE 250 mg/kgBW, D: AAE 250 mg/kgBW.

(TMPD)[34]. The assay was performed according *to the manufacturer's spesified protocol*.

Statistical Analysis

Data from all experiments were presented as mean values with ± standard error mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) with Dunnet post hoc test, $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The leaf of *A. altilis* is used in Indonesia as an herbal medicine preparation to treat various diseases including inflammatory diseases. Although the leaves were traditionally prepared for the medication as a decoct dosage form, our preliminary study revealed that the *A. altilis* leaves ethyl acetate extract (AAE) demonstrated a higher antiinflammatory activity compared to the aquoeus extract[35]. In this present study, we examined the antiinflammatory effects of AAE in mice using acute experimental model of inflammation. We employed carrageenan-induced paw edema which represents a common simple method for antiinflammatory evaluation and then we studied the more specific antiinflammatory effect on the COX-2 expression and activity. We found that AAE exhibited antiinflammatory activity by reducing carrageenan-induced paw edema in dose-dependent manner (Figure 1 and 2). Paw edema is a common feature for an acute inflammatory process[36]. Thus, the reduction of carrageenan-induced edema volume upon AAE treatment indicated that the extract has an acute anti-inflammatory activity. However, the activity is lower compared to the positive control, indomethacin.

One of the key factor responsible for the progression of inflammatory process is cyclooxygenase (COX) enzymes, especially COX-2. This inducible enzyme is over expressed in inflammation and is known to be responsible for the formation of prostaglandins from arachidonic acid. Thus, we investigated the effect of AAE on the COX-2 expression and activity. COX-2 expression in the soft plantar region sections of the hind paw was investigated using immunohistochemistry, whereas the COX-2 enzymatic activity was evaluated using a colorimetric-based COX inhibitor assay. Our study indicated that COX-2 expression was reduced (Figure 3 and 4) and the COX-2 activity was also inhibited (Figure 5) upon AAE treatment in dose-dependent manner. Although indomethacin (5 mg/kgBW) demonstrated stronger activity, the higher concentration of indomethacin or the extract might be required to completely inhibit COX-2 expression to the lower level. These results indicated that

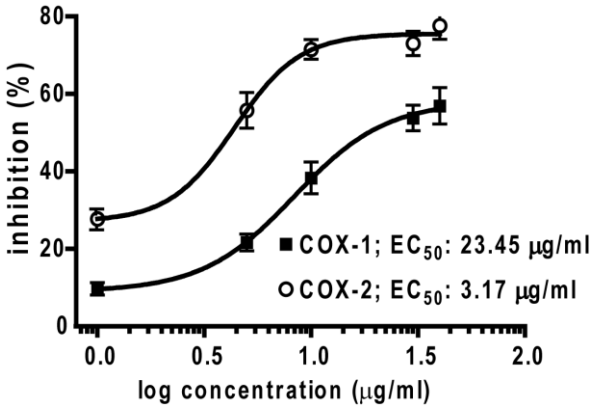

Figure 5. *Artocarpus altillis* extract inhibited COX-1 and COX-2 activity *in vitro*. The extract was was dissolved in DMSO and prepared in 5 different concentrations, each in a triplicate (n=3). The values are means ± standard errors.

AAE, at least in part, exerts antiinflammatory activity via targeting both the expression and activity of COX-2.

To determine the selectivity of AAE on COXs inhibition, we also investigated the inhibitory effect of the extract on COX-1 (Figure 5). Interestingly, AAE demonstrated a potent COX-2 inhibition activity (IC_{50}: 3,17μg/ml) in comparison to that of COX-1 (IC_{50}: 23,45), indicating that this extract contains a promising compound with a high potency to be developed as a selective COX-2 inhibitor. These indicate that AAE exerted antiinflammatory activity, at least partly by inhibiting the activity and the expression of COX-2. As COX-2 has a crucial role in inflammation and it still represents a potential therapeutic target for inflammation[37,38], the inhibition of COX-2 activity and expression upon AAE treatment makes *A. altilis* as a potential source of natural compounds with a promising antiinflammatory activity.

To determine the selectivity of AAE against COX-1 and COX-2, we tested the potency of AAE in inhibiting COX-1 and COX-2 in *in vitro* enzymatic assays. Figure 5 demonstrates that AAE inhibited both COX-1 (IC_{50}: 23.45 μg/ml) and COX-2 (IC_{50}: 3.17 μg/ml). Interestingly, AAE shows more potent inhibition against COX-2 compared to COX-1, indicating that this extract contains a promising compound with a high potency to be developed as a selective COX-2 inhibitor.

Our study clearly demonstrates that the AAE exhibited a promising antiinflammatory activity in an experimental model of acute inflammation in mice. These results are in accordance with the previous study showing that *A. altilis* contains several antiinflammatory flavonoids with a distinct mechanism of action[39]. Artocarpin, a prenylated flavonoid isolated from *A. altilis* was also claimed to be a compound responsible for the antiinflammatory activities as it decreased the level of TNFα and IL-1β, a major pro-inflammatory transcription factor and a pro-inflammatory cytokine, respectively[26]. These findings provide the scientific evidence for the traditional use of *A. altilis* leaves for the treatment of inflammatory diseases[17].

In summary, we demonstrate that AAE exhibited antiinflammatory activity in mice experimental models. It inhibited carrageenan-induced paw edema and reduced the expression and activity of COX-2. Additionally, it showed a higher selectivity against COX-2 in comparison to COX-1. These findings suggested that *A. altilis* leaves could be a potential source for the discovery of novel antiinflammatory compounds for drugs or dietary supplements.

ACKNOWLEDMENT

This research was supported by Hibah Penelitian Unggulan Perguruan Tinggi (PUPT), the Directorate General of Higher Education, Ministry of Education and Culture, Republic of Indonesia, (Grant number: LPPM-UGM/344/LIT/2014). We also thank Setiono for an excellent technical support.

REFERENCES

1. Weiss U. Inflammation. Nature. 2008;454(7203):427.
2. Dequeker J. NSAIDs/corticosteroids--primum non nocere. Advances in experimental medicine and biology. 1999;455:319-25.
3. FitzGerald GA. COX-2 and beyond: approaches to prostaglandin inhibition in human disease. Nat Rev Drug Discov. 2003;2(11):879-90.
4. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, A. Van De Putte LB, et al. Cyclooxygenase in biology and disease. The FASEB Journal. 1998;12(12):1063-73.
5. Ricciotti E, FitzGerald GA. Prostaglandins and Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31(5):986-1000.
6. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203):428-35.
7. Flower RJ. The development of COX2 inhibitors. Nat Rev Drug Discov. 2003;2(3):179-91.
8. Maroon JC, Bost JW, Maroon A. Natural anti-inflammatory agents for pain relief. Surg Neurol Int. 2010;1:80.
9. Simmons DL, Botting RM, Hla T. Cyclooxygenase Isozymes: The Biology of Prostaglandin Synthesis and Inhibition. Pharmacological reviews. 2004;56(3):387-437.
10. Thiagarajan P, Jankowski JA. Aspirin and NSAIDs; benefits and harms for the gut. Best practice & research Clinical gastroenterology. 2012;26(2):197-206.
11. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. Cardiovascular Events Associated with Rofecoxib in a Colorectal Adenoma Chemoprevention Trial. New England Journal of Medicine. 2005;352(11):1092-102.
12. Silverstein FE, Faich G, Goldstein JL, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: The class study: a randomized controlled trial. JAMA. 2000;284(10):1247-55.
13. Rainsford KD. Anti-inflammatory drugs in the 21st century. Sub-cellular biochemistry. 2007;42:3-27.

14. Appiah F, Oduro I, Ellis WO. Proximate and Mineral Composition of Artocarpus altilis Pulp Flour as Affected by Fermentation. Pakistan Journal of Nutrition. 2011;10(7):653-7.
15. Jones AMP, Ragone D, Tavana NG, Bernotas DW, Murch SJ. Beyond the Bounty: Breadfruit (Artocarpus altilis) for food security and novel foods in the 21st Century. Ethnobotany Research and Applications. 2011;9.
16. Sairam S, Urooj A. Safety Evaluation of Artocarpus altilis as Pharmaceutical Agent in Wistar Rats. Journal of Toxicology. 2014;2014:980404.
17. Jagtap UB, Bapat VA. Artocarpus: a review of its traditional uses, phytochemistry and pharmacology. Journal of ethnopharmacology. 2010;129(2):142-66.
18. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. Journal of ethnobiology and ethnomedicine. 2006;2:45.
19. Nguyen MT, Nguyen NT, Nguyen KD, Dau HT, Nguyen HX, Dang PH, et al. Geranyl dihydrochalcones from Artocarpus altilis and their antiausteric activity. Planta medica. 2014;80(2-3):193-200.
20. Patil AD, Freyer AJ, Killmer L, Offen P, Taylor PB, Votta BJ, et al. A new dimeric dihydrochalcone and a new prenylated flavone from the bud covers of Artocarpus altilis: potent inhibitors of cathepsin K. J Nat Prod. 2002;65(4):624-7.
21. Shamaun SS, Rahmani M, Hashim NM, Ismail HB, Sukari MA, Lian GE, et al. Prenylated flavones from Artocarpus altilis. Journal of natural medicines. 2010;64(4):478-81.
22. Weng J-R, Chan S-C, Lu Y-H, Lin H-C, Ko H-H, Lin C-N. Antiplatelet prenylflavonoids from Artocarpus communis. Phytochemistry. 2006;67:824-9.
23. Jeon YJ, Jung SN, Chang H, Yun J, Lee CW, Lee J, et al. Artocarpus altilis (Parkinson) Fosberg Extracts and Geranyl Dihydrochalcone Inhibit STAT3 Activity in Prostate Cancer DU145 Cells. Phytotherapy research : PTR. 2015.
24. Kuete V, Nkuete AH, Mbaveng AT, Wiench B, Wabo HK, Tane P, et al. Cytotoxicity and modes of action of 4'-hydroxy-2',6'-dimethoxychalcone and other flavonoids toward drug-sensitive and multidrug-resistant cancer cell lines. Phytomedicine : international journal of phytotherapy and phytopharmacology. 2014;21(12):1651-7.
25. Lan WC, Tzeng CW, Lin CC, Yen FL, Ko HH. Prenylated flavonoids from Artocarpus altilis: antioxidant activities and inhibitory effects on melanin production. Phytochemistry. 2013;89:78-88.
26. Lee CW, Ko HH, Lin CC, Chai CY, Chen WT, Yen FL. Artocarpin attenuates ultraviolet B-induced skin damage in hairless mice by antioxidant and anti-inflammatory effect. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2013;60:123-9.
27. Wang Y, Deng T, Lin L, Pan Y, Zheng X. Bioassay-guided isolation of antiatherosclerotic phytochemicals from Artocarpus altilis. Phytotherapy research : PTR. 2006;20(12):1052-5.
28. Ragasa CY, Ng VA, Park JH, Kim DW, Cornelio K, Shen CC. Chemical Constituents of Artocarpus altilis and Artocarpus odoratissimus. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014;5(4):1081-7.
29. Siddesha JM, Angaswamy N, Vishwanath BS. Phytochemical screening and evaluation of in vitro angiotensin-converting enzyme inhibitory activity of Artocarpus altilis leaf. Natural product research. 2011;25(20):1931-40.
30. Wang Y, Deng T, Lin L, Pan Y, Zheng X. Bioassay-guided isolation of antiatherosclerotic phytochemicals from Artocarpus altilis. Phytotherapy Research. 2006;20(12):1052-5.
31. Morris C. Carrageenan-Induced Paw Edema in the Rat and Mouse. In: Winyard P, Willoughby D, editors. Inflammation Protocols. Methods in Molecular Biology. 225: Humana Press; 2003. p. 115-21.
32. Lucetti D, Lucetti E, Bandeira M, Veras H, Silva A, Leal L, et al. Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated from Himatanthus drasticus (Mart.) Plumel. Journal of Inflammation. 2010;7(1):60.
33. Hussein SZ, Mohd Yusoff K, Makpol S, Mohd Yusof YA. Gelam honey attenuates carrageenan-induced rat paw inflammation via NF-kappaB pathway. PloS one. 2013;8(8):e72365.
34. Badieyan ZS, Moallem SA, Mehri S, Shahsavand S, Hadizadeh F. Virtual Screening for Finding Novel COX-2 Inhibitors as Antitumor Agents. The open medicinal chemistry journal. 2012;6:15-9.
35. Ajiningtyas RJ. Relationship study of total flavonoid content with the antiinflammatory activity of ethyl acetate, ethanol and aqueous extracts of Artocarpus altilis (park.) Fosberg leaves [Undergraduated Thesis]. Yogyakarta: Undergraduate Thesis, Universitas Gadjah Mada; 2015.
36. Wiig H. Pathophysiology of tissue fluid accumulation in inflammation. The Journal of Physiology. 2011;589(12):2945-53.
37. Mendes RT, Stanczyk CP, Sordi R, Otuki MF, Santos FAd, Fernandes D. Selective inhibition of cyclooxygenase-2: risks and benefits. Revista Brasileira de Reumatologia. 2012;52:774-82.
38. Melnikova I. Future of COX2 inhibitors. Nat Rev Drug Discov. 2005;4(6):453-4.
39. Wei BL, Weng JR, Chiu PH, Hung CF, Wang JP, Lin CN. Antiinflammatory flavonoids from Artocarpus heterophyllus and Artocarpus communis. J Agric Food Chem. 2005;53(10):3867-71.