ANTI-INFLAMMATORY EFFECT OF THE ETHANOLIC EXTRACT OF *ACANTHOPANAX TRIFOLIATUS* (L.) MERR LEAVES

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ABSTRACT

The leaves of *Acanthopanax trifoliatus* (L.) Merr has been used to treat several diseases such as tuberculosis and lung hemorrhage, and as a tonic to improve general weakness. The objective of this study was to investigate experimentally the possible anti-inflammatory properties of *Acanthopanax trifoliatus* (L.) Merr. The effect of ethanolic extract of leaves of *Acanthopanax trifoliatus* (EAT) was evaluated in acute and chronic experimental models of inflammation. For acute inflammation, EAT 300 mg/kg and piroxicam 30 mg/kg showed significant inhibition in carrageenan-induced oedema in rats with an inhibitory percentage of 46.23% and 84.9% respectively. Whilst, at lower dosage of 30 and 100 mg/kg, EAT did not give any significant anti-inflammatory effect. For chronic inflammation, EAT at 300 mg/kg and indomethacin 10 mg/kg also exhibited significant inhibition in Complete Freund Adjuvant (CFA) –induced arthritis in rat paw for the duration of 28 days with an inhibitory percentage of 45.7% and 83.43% respectively. These results have demonstrated that the ethanolic extract of *Acathopanax trifoliatus* leaves exhibits promising anti-inflammatory activity in both acute and chronic inflammation models

Keywords: Anti-inflammatory, Carrageenan-induced oedema, CFA-induced arthritis, Chronic inflammation, *Acanthopanax trifoliatus*

INTRODUCTION

Inflammation can be acute and if the agent of factor that triggering inflammation is failure to eradicate, it can prolong to chronic inflammation. Acute inflammation is rapid in onset and short duration, lasting from a few minutes to as long as a few days, and is characterized by fluid and plasma protein exudation and a predominantly neutrophilic leukocyte accumulation. Chronic inflammation may be more critical or dangerous because it occurs in longer duration (days to years), and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and fibrosis (scarring) [1]

Acanthopanax trifoliatus is known in Chinese traditional medicine for its ginseng-like activity and has been utilized as a folk-medicine for bruise, neuralgia, impotence, and gout in China, Taiwan, and the Philippines [2,3] The plant is also used to treat leprosy; the roots are used to heal ulcers and to cure ring-worm infection. A decoction of the leaves is drunk to treat tuberculosis and to improve general weakness. In Cambodia, Laos, and Vietnam, an infusion of the bark is used to correct nervous affections and as a stimulant as well as a tonic and believed to ameliorate the memory [4,5]. It has been proven to have a rather good curative effect on treating common cold, jaundice, gastric pain, diarrhoea and ulcer [6]. In Malaysia, this plant can also be used as an ingredient in '*lei cha*', a traditional Hakka Chinese herbal tea which is believed to have powerful tonic effects.

Phytochemical studies on the stem, bark and the leaves of *Acanthopanax trifoliatus* have revealed a high content of diterpenoids such as continentalic acid [7,8], triterpenoid carboxylic acids [9-13], phenylpropanoid glycosides [14-16] and other compounds such as essential oils, lipids, steroids and alkanes [17-20]

Thus far, there have been only few pharmacological studies on *Acanthopanax trifoliatus* in the literature. It was reported to show evidence for anti-oxidative [21, 22] anti-mutagenic [23], anti-nociceptive [6], anti-tumour [24] and anti-ulcer effects [25] when tested on the leaf extract. So far, there is no study on its anti-inflammatory effect been reported on this plant. To treat the inflammation, several anti-inflammatory drugs from the steroidal (corticosteroids) and non steroidal drugs (NSAIDs) have been used. Despite their great number for treatment, it also can cause undesired and serious side effects. Therefore, the development of new and more powerful drugs is still needed especially from the medicinal plants. Hence it is justifiable to screen this plant for anti-inflammatory properties., as plant with anti-inflammatory activities are of particular therapeutic

importance, as it can reduce risks of ulceration caused by NSAIDs treatment [25, 26].

MATERIALS AND METHODS

Plant material and Extraction

The leaves of *Acanthopanax trifoliatus* were collected from Bangi, Selangor, Malaysia in July, 2009 and was deposited as a voucher at the Herbarium of Forest Research Institute in Kepong, Selangor, Malaysia.

The leaves were separated from the stem, washed, air-dried, and then oven-dried at 40°C. The dehydrated leaves were grinded into powdered form and soaked in 90% ethanol for 2 days. The extracted solvent was then filtered and was concentrated by using rotary evaporator until the solvent was completely removed and crude extract was obtained.. The extract was then dissolved in 5% ethanol as its vehicle and was prepared into desired dose concentrations for pharmacological testing.

Animals

Healthy male Sprague *dawley* rats weighing between 250-350 g were provided by Animal Unit of Faculty of Veterinary Medicine, Universiti Putra Malaysia, Malaysia with ethics approval from the Animal Ethics Committee of Faculty Medicine & Health Sciences, Universiti Putra Malaysia (UPM/FPSK/PADS/BR-UUH/00332). The animals were grouped in cages in the animal house at Faculty of Medicine and Health Sciences, UPM with normal laboratory condition (25 + 3°C), 12 hours light and 12 hours dark cycle. The rats were also provided with adequate supply of pellets and water *ad libitum*. They were acclimatized at least one week before starting the experiments. In all experimental model of inflammation, the studies were carried out using six rats in each group.

Chemicals

Complete Freund Adjuvant, piroxicam and indomehacin (Sigma Chemical, USA) were used in this study

Anti-inflammatory activity

Carrageenan-induced paw oedema

EAT was evaluated for acute anti-inflammatory activity by carrageenan-induced oedema on rat paw method [27]. Male *Sprague dawley* rats were randomly distributed into 5 groups of 6 animals each. First group served as a vehicle control (5% EtOH), second

group served as the standard (piroxicam 30 mg/kg p.o) while the third, fourth, and fifth group received 30, 100 and 300 mg/kg body weight of EAT respectively. After 30 minutes, 0.1 ml of 1% w/v suspension of carrageenan was injected subcutaneously onto the plantar surface of right hind paw to all the five groups. Equal volume of saline was injected onto the plantar surface of the left hind paw. The volumes of both hind paws of each rat were measured using a Plethysmometer (Model 7140, Ugo Basile) at every half-hourly interval until the period of five hours after the injection of the carrageenan. For a consistent measurement, a line was marked just above the ankle joint of both rat's hind limbs. Hind paw swelling was measured when the paw was immersed at the line marked. Oedema formation was expressed as the difference between left and right hind rat paw. The inhibition percentage for each group was calculated by comparison with the control group, considered as 100% inflammation.

The percentage of anti-inflammation was calculated using the formula given below [28]:

Percentage of anti-inflammation = (Vf – Vo) control – (Vf – Vo) treated x 100

(Vf – Vo) control

Vf = Final volume

Vo = Initial volume

Complete Freund Adjuvant (CFA)-induced arthritis

Chronic arthritis was induced by injection of 0.1ml of Complete Freund Adjuvant

(CFA) onto the right hind paw of the rat subcutaneously. The rats were divided into five groups of six rats; Control group (5% ethanol), EAT (30, 100, and 300mg/kg) and indomethacin (10mg/kg). The treatment was given orally after 14 days from the day of CFA injection for 14 days. Weight and oedema was measured on rats before induction, before treatment, and after treatment. After that, the percentage of inhibition was determined [29]. On day 0, rats were injected with CFA and acclimatized for 14 days. At day 14, the entire groups were force fed daily according to their treatment for 14 days. The experiment was completed in 28 days. The percentage

of anti-inflammation was calculated by using the same formula as in acute inflammation method [2]

Statistical analysis

Statistical analysis was done by using SPSS software version 16.0. Data was expressed as mean + standard error of mean (S.E.M) (n=6). The results of carrageenan-induced rat paw oedema and CFA-induced arthritis were expressed as changes of percentage from control values. The data obtained were analysed by using one-way analysis of variance (ANOVA) to determine the significance of the difference between the controls and rat treated with the test compounds. Student's t-test was applied to the results to evaluate the significance or to compare between two groups. Multiple comparisons for difference between drug-treated groups and control group were evaluated by Tukey HSD (Honestly Significant Difference) test. P values less than 0.05 $(p<0.05)$ is considered significant.

RESULTS

Carrageenan-induced oedema

Carrageenan significantly induced oedema 1 (15.74 + 3.30 %, 2 (19.79 + 5.82 %,), 3 (26.07 + 5.75 %), 4 (32.42 + 6.50 %), 5 hours (38.89 + 7.02%). On the other hand, only EAT at higher dosage ie 300 mg/kg showed a significant decrease (p<0.05) in paw oedema after 4.5 (19.57 + 1.89%) and 5 hours (20.63 + 1.89%) of injection of carrageenan respectively when compared with control (Fig 1) Whilst EAT at lower dosage only showed significant difference in reducing oedema when compared with piroxicam (Table 1). In the meantime, piroxicam exhibited its onset of action after 2 hours of carrageenan injection and remained throughout the experimental period of 5 hours (Fig 1) Carrageenan-induced rat paw oedema on the right hind paw for control group reached its peak at 300 minutes. Therefore, the optimum percentage inhibition of oedema of EAT and piroxicam were calculated at 300 minutes as compared to optimum oedema effect in control group as shown in Table 1. At 300 mg/kg, EAT only supressed 46.23% inhibition of oedema compared to 84.99% inhibition by piroxicam. Therefore, the ED_{50} for EAT cannot be calculated as at highest dosage used (300 mg/kg), as the oedema inhibition produced was still less than 50%.
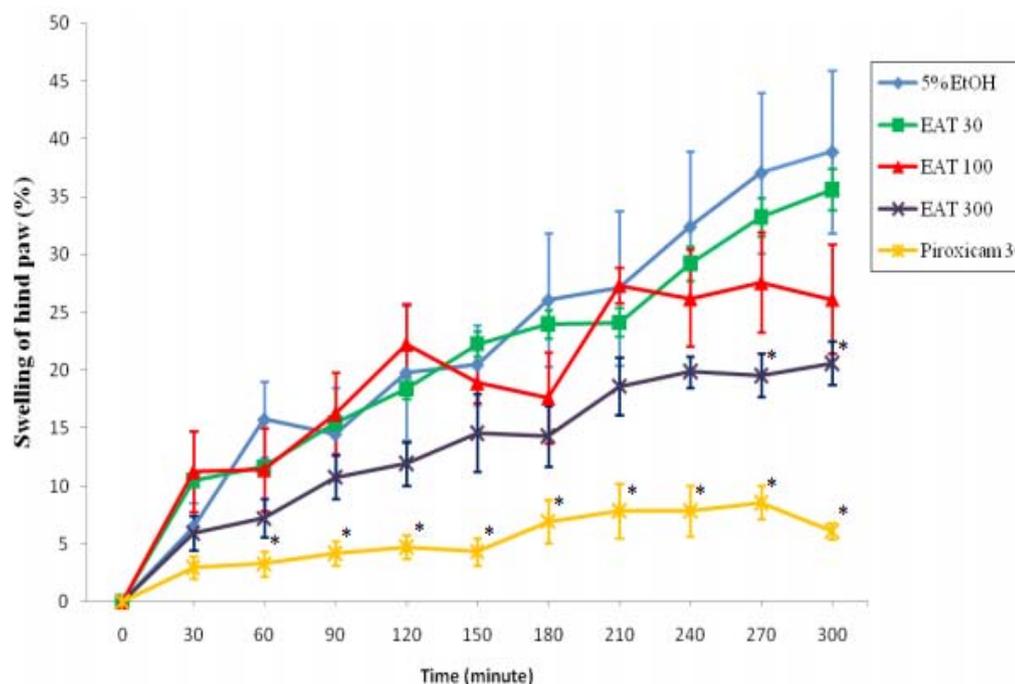

Fig. 1: Figure shows attenuating effect of various doses of EAT and piroxicam (30 mg/kg) given orally in carrageenan-induced oedema.

Data presented as Mean + S.E.M (n=6 animals)*$p<0.05$ indicated significant difference when compared with control group as determined by t-test

Table 1: Table shows percentage inhibition of carrageenan-induced paw oedema in rats on various doses of EAT obtained from the optimum value at 300 minutes as comparison to 100% of swelling in control group

Group	% of oedema (mean + S.E.M)	% of oedema inhibition
Control (5% ethanol)	21.69 + 2.04	0
EAT 30mg/kg	20.42 + 1.45	10.50
EAT 100mg/kg	18.66 + 1.39	32.43
EAT 300mg/kg	13.05 + 0.97*	46.23
Piroxicam (30mg/kg)	5.17 + 0.50*	84.99

. *$P<0.05$ indicated significant difference when compared with control group using ANOVA followed by Tukey's Multiple Comparison Test.
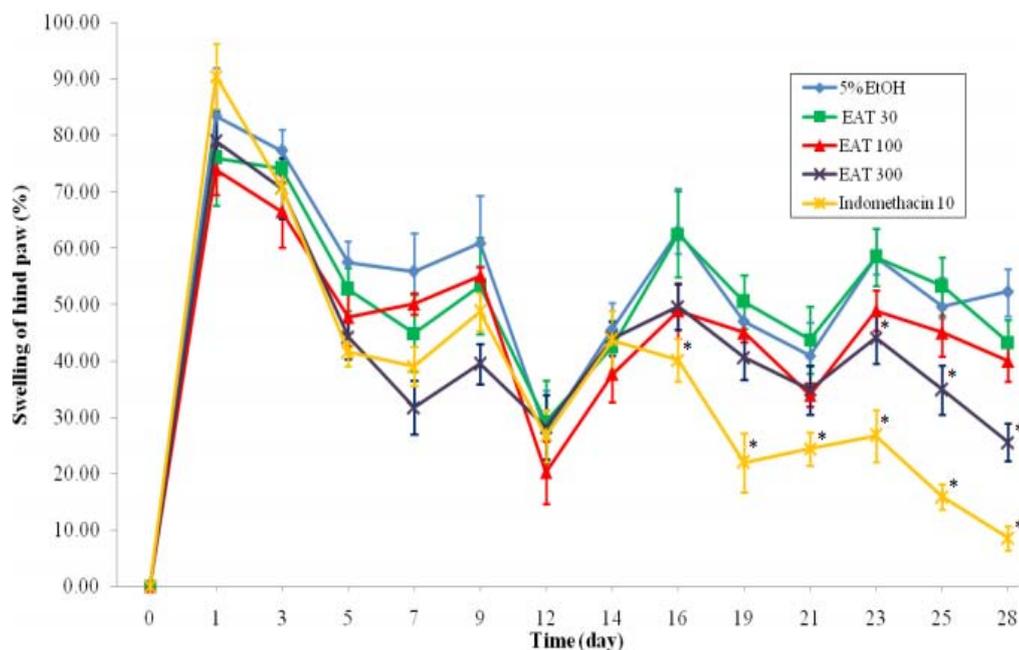
CFA- induced arthritis

Oedema was significantly increased 14 (45.65 + 3.93 %) , 16 (63.01 + 4.08[a]%), 19 (46.95 + 2.18%), 21 (40.89 + 1.82%), 23 (58.34 + 2.99%), 25 (49.62 + 2.07%), 28 days (52.26 + 4.34%) after CFA induction. At the highest dose of 300 mg/kg, EAT significantly reduced the odema 14 (43.96 + 2.98, P,0.05), 23 (44.05 + 4.64, p<0.05), 25 (34.82 + 4.32, p<0.05) and 28 day (25.50 + 3.34, p<0.05) post-induction. On the other hand, other dosages ie 30 and 100 mg/kg of EAT did not significantly reduce the oedema in CFA-induced arthritic model. Indomethacin at 10 mg/kg started its onset of action at day 16 and the duration of period remained through out the experiment (Fig 2). Indomethacin significantly reduced the

oedema 16 (40.11 + 3.77, p<0.05), 19 (21.90 + 5.17, P<0.05), 21 (24.36 + 2.94, p,0.05), 23 (26.65 + 4.62, p<0.05), 25 (15.84 + 2.17, p<0.05) and 28 days (8.52 + 2.18, p<0.05) post-induction (Table 2). EAT at 300 mg/kg significantly inhibited the arthritis by 47.57%, which is only half fold of the inhibition by indomethacin by 84.43% (Table 4). In terms of body weight, Table 2 showed mean percentage change in body weight that measured before induction of CFA (day 0), before treatment (day 14) and after treatment (day 28) of EAT. At day 14, all the rats' weight was decreased due to present of arthritis induced by CFA. At the last day of experiment (day 28), treated group of EAT 100 mg/kg and 300 mg/kg showed significant difference in weight when compared to control group but not to indomethacin treatment (Table 2.)

Table 2: Table shows percentage change in body weight of rats-induced arthritis before induction (day 0), before treatment (day 14) and after treatment (day 28)

Group	Percentage change in body weight (%)		
	Day 0 **Before Induction**	**Day 14** **Before Treatment**	**Day 28** **After Treatment**
5 % Ethanol	0	-4.00+1.11[b]	-6.95+1.54[b]
EAT 30 mg/kg	0	-1.82+1.14[b]	-1.15+2.45[b]
EAT 100 mg/kg	0	-2.23+0.44[b]	4.55+1.73*[a]
EAT 300 mg/kg	0	-3.15+0.49[b]	7.05+2.87*[a]
Indomethacin 10 mg/kg	0	-2.34+0.44	-3.8+0.98

Data presented as mean + S.E.M (n=6). *Significant (*p<0.05*) when compared to control (5% ethanol); [a] Significant (*p<0.05*) when compared to indomethacin; [b] Not significant (*p>0.05*) when compared to indomethacin

Fig. 2: Figure shows the effect of CFA-induced arthritis on different dose of EAT and indomethacin that given orally. Daily treatments of EAT were started on day 14 till the end of the experiment.

Data presented as mean + S.E.M swelling of right hind paw (n=6). *p<0.05 indicated significant difference from control group as determined by t-test.

Table 3: Table shows anti-inflammatory effects of ethanolic extract of *Acanthopanax trifoliatus* (L.) Merr (EAT) leaves in adjuvant induced arthritis

Group (mg/kg)	Percentage of oedema (Mean + S.E.M) (n=6) Post Treatment						
	Day 14	Day 16	Day 19	Day 21	Day 23	Day 25	Day 28
Control (5% ethanol)	45.65 + 3.93[b]	63.01 + 4.08[a]	46.95 + 2.18[a]	40.89 + 1.82[a]	58.34 + 2.99[a]	49.62 + 2.07[a]	52.26 + 4.34[a]
EAT 30	42.47 + 4.57[b]	62.48 + 7.58[a]	50.43 + 4.72[a]	43.72 + 5.90[a]	58.39 + 5.11[a]	53.21 + 5.11[a]	43.27 + 4.04[a]
EAT 100	37.64 + 4.90[b]	48.90 + 4.93[b]	44.96 + 1.59[a]	33.88 +2.07[*a]	48.90 + 3.61[a]	44.96 + 4.28[a]	39.93 + 3.56[a]
EAT 300	43.96 + 2.98[*b]	49.60 + 4.01[b]	40.54 + 3.96[a]	34.82 + 4.32[b]	44.05 +4.64[*a]	34.82 +4.32[*a]	25.50 +3.34[*a]
Indomethacin (10)	43.68 + 5.24	40.11 + 3.77[*]	21.90 + 5.17[*]	24.36 + 2.94[*]	26.65 + 4.62[*]	15.84 + 2.17[*]	8.52 + 2.18[*]

[*] Significant *(p<0.05)* when compared to the control (5% ethanol)

[a] Significant *(p<0.05)* when compared to indomethacin

[b] Not significant *(p>0.05)* when compared to indomethacin

Table 4: Table shows percentage inhibition of arthritis induced by FCA at day 28 on various doses of EAT and indomethacin (10 mg/kg) given orally as compared to optimum swelling at day 28

Group	% of swelling (mean + S.E.M)	% of arthritis inhibition
Control (5% ethanol)	52.26 + 4.34	0
EAT 30mg/kg	43.27 + 4.04	9.57
EAT 100mg/kg	39.93 + 3.56	23.71
EAT 300mg/kg	25.50 + 3.34*	47.57
Indomethacin (10mg/kg)	8.52 + 2.18*	83.43

*$p<0.05$ indicated significant difference from control group using 1-way ANOVA followed by Tukey's multiple comparison test

DISCUSSION

The anti-inflammatory effects of ethanolic extract of *Acanthopanax trifoliatus* leaves were evaluated on carrageenan (acute) and CFA (chronic) induced inflammation in rat's model. To study the effect of acute anti-inflammation, we decided to use the carrageenan induced paw inflammation model, which is very useful in the search for oral anti-inflammatory drugs acting peripherally via inhibiting the mediator of acute inflammation [30]. The injection of carrageenan to the hind paw of rats is a common model to study inflammation and inflammatory pain. .Moreover, there is a good correlation between efficacy in this model and activity in humans [31]. The observation that NSAIDs inhibit COX activity attests to the contribution of prostaglandins to the inflammation.

From the result, only piroxicam (100 mg/kg) and EAT at 300 mg/kg showed significant anti-inflammatory effects in carrageenan-induced oedema model.. Changes in paw volume after the injection of carrageenan corresponding to oedema occurred rapidly and in a biphasic manner [32].

The biochemical mechanism for the inflammatory reaction induced by carrageenan in animals, however, is not clear. On the other hand, chemical mediators such as histamine, serotonin, prostaglandins and kinin are presumed to be involved in the occurrence and development of inflammation [33].

The acute inflammation is produced when water and plasma increases in tissues during arachidonic acid metabolism via cyclooxygenase (COX) and lipooxygenase (LOX) enzyme pathways [34]. The first phase of inflammation is characterized by the release of histamine and serotonin that begins immediately after injection and last for one hour. The second phase is characterized by the bradykinin release via prostaglandins mediator, oxygen-derived free radicals and cyclooxygenase that begin after one hour and last for three hours [35, 36]. The later phase that begins more than four hours was reported to be sensitive to most clinically effective anti-inflammation agents [37].

From Figure 1, it shows that the swelling of rat's hind paw induced by carrageenan on the treated groups (30 mg/kg, 100 mg/kg and 300 mg/kg) were decreased when compared to control group started from 0 minute to 60 minutes. Between this first hour, the compounds that present in EAT may involve in the reduction of histamine and serotonin release as first phase of acute inflammation.

The second phase that started from 90 minutes to 240 minutes showed a decreasing in swelling of rat hind paw. Thus, the compounds of EAT may also involve inhibiting the prostaglandin production. From the present study, the reduction of swelling of the group treated with EAT is exhibited in a dose-dependent manner.

The onset of inflammation and increased prostaglandin production are usually related to COX-2 expression. After injection of carrageenan, substantial induction of COX-2 was observed after three hours with enhanced level of thromboxane B_2 (TxB_2) and local oedema [38]. Therefore, the ability of EAT leaves in oedema inhibition may resulted from the action on COX-2. It can therefore be postulated that EAT inhibited the production of second phase of chemical mediators such as prostaglandin and bradykinin, and/or antagonized the actions of these chemical mediators [33].

From all three different doses of ethanolic extracts of *Acanthopanax trifoliatus* leaves, only dose of 300 mg/kg showed significant reduction in the swelling of carrageenan-induced rat paw oedema as compared to control group. The onset began as early as 30 minutes and lasted until the end of the experimental period which is five hours. (Fig 1)

The observed inhibitory effect was compared to the drug piroxicam, which its onset was also started at 30 minutes and the duration of action lasted more than five hours (Fig 1). Piroxicam basically inhibited the oedema, the total leukocyte infiltration and the mononuclear cell infiltration into the carrageenan-injected pleural cavity of the rat [39]. Piroxicam as a positive control was used because it strongly inhibits both COX-1 and COX-2 at therapeutic concentrations, and shows some preference for COX-1 *in vitro* [40, 41].

The possible mechanism which involved in the reduction of oedema volume may come from the inhibition or antagonism of actions of chemical mediators such as histamine (phase 1) and prostaglandins (phase 2) via COX-2. From the graph (Fig 1), the inhibition of the carrageenan-induced oedema in treated group was only significant after 4 hours when compared to control group. Thus, this showed that the oedema inhibition of acute inflammation mostly occurred during phase 1.

In chronic inflammation study, complete Freund adjuvant (CFA) is a reagent that frequently used for induction of rheumatoid arthritis as a model for chronic inflammation[42]. Besides CFA, reagents such as type II collagen[43] and formaldehyde[44] are also used as inducers to

induce rheumatoid arthritis in rats. Rheumatoid arthritis (RA) is an inflammatory disorder characterized by infiltration of leukocytes into the synovial tissue and synovial fluid of joints, eventually leading to cartilage and bone destruction [45]. The most abundant cells in the synovial membrane are macrophages and T lymphocytes, but plasma cells, dendritic cells, and activated fibroblasts were also found [46].

RA that cause by adjuvant (AIA) is a T-cell mediated that cause chronic inflammatory stress [47]. AIA involved an immune response to an antigen that present on the capsule of the mycobacterium. Following adjuvant arthritis induction with CFA, rats not only develop arthritis but also systemic features of inflammation, such as uveitis, inflammation of the gastrointestinal tract and body weight loss due to clinical onset of arthritis [48].

The weight of all animal groups was reduced at day 14 which was the last day before treatment started due to development of arthritis (Table 2). When the treatments started, the weight in all treated groups (EAT 30, 100 and 300 mg/kg) were increased until the end of the experiment but not in control group and positive group. The percentage change in body weight of treated groups of EAT 100 and 300 mg/kg showed significant difference when compared to control group. The lost of body weight that was found in the control group could be due to reduction of absorption of glucose and leucine in rat intestine in arthritic condition [49].

The weight of rats in indomethacin group also decreased although the drug is very effective in inhibiting swelling of the arthritis. The weight loss occurred because the stomach and intestinal tract were injured due to toxicity of indomethacin. The absorption of the foods will be diminished. From other studies that have been done by using drug indomethacin, there was evidence of gastrointestinal haemorrhage, suggested by the presence of dark colouration of the faeces [50]. Indomethacin is a potent nonselective cyclooxygenase inhibitor with inhibit both COX-1 and COX-2 that upregulates prostaglandin production. It also inhibits phospholipase A and C and reduces neutrophil migration [51] The long term inhibition of both COX isoforms through the use of NSAIDs may produce ulceration of the stomach lining and gastric mucosa [51].

CFA injection was observed and showed that the swelling and redness of the rat foot developed over twenty-four hours of period (Fig 2). This inflammatory reaction subsided slightly during the next 9-12 days and increased at the time when disseminated arthritis appeared [52] (Fig 2). The treatment was started at day 14 after the day of adjuvant induction indicated that the secondary increased in swelling of injected foot with the appearance of polyarthritis [29].

From day 16 to day 21 and day 23 to day 28 post treatment of EAT, it showed the reduction of foot swelling for all treated group (30, 100, 300 mg/kg) (Table 3). However, only 300 mg/kg of EAT showed significant difference from the control group from day 23 (10 days of post treatment of EAT leaves) until the final day (day 28). Indomethacin as positive control group also showed significant difference from the control group starting from day 14 until the day 28 (Table 3). Therefore, it can be concluded that EAT exhibited a dose dependent inhibitory effect towards oedema as well.

Several mediators, including proinflammatory cytokines and chemotactic cytokines (chemokines), have been implicated in the inflammatory events underlying RA [53, 54]. Cytokines expression in RA synovial tissue can be divided into several categories which are proinflammatory, anti-inflammatory, chemotactic and mitogenic cytokines. The example of proinflammatory cytokines such as IL-1 (α and β), TNF-α and IFN (α, β and γ). For anti-inflammatory cytokines (also known as immunoregulatory cytokines) included TGF-β, IL-4, IL-10 and IL-13 [44].

The early phase (before day 14 post adjuvant injection) and later phase of adjuvant induced arthritis (AIA) could be distinguished. Cytokine and chemokine production was increased in AIA versus normal rats [54]. In the previous study, it showed that the extract of *Acanthopanax trifoliatus* contains high levels of flavonoid compounds [21]. This compounds may have effects in reducing the number of proinflammatory cytokine such as TNF-α production.

However, the exact mechanism should be determined in the future study.

Cytokines play a pivotal role in many processes including inflammatory cell recruitment, adhesion and activation. In addition, prostaglandins are secreted into the synovial cavity and are involved in perpetuation of local inflammation, vasodilation and vasoconstriction, and also with bone resorption [55]. The ability of EAT to reduce oedema formation may also relate to its inhibitory action of prostaglandin synthesis.

In the present study, it was found that the ethanolic extract of *Acanthopanax trifoliatus* exhibited anti-arthritic effect evidenced by increase in body weight and decreased oedema formation in comparison with arthritic control group. The exact mechanism on how the EAT leaves involved in the reduction of paw swelling for both acute and chronic inflammation is still unknown. The active compounds such as phenolic and flavanoids that present in EAT leaves may play a role in reducing the oedema because these compound besides possess anti-inflammatory effects, it also play a role in antioxidant activity [21]

CONCLUSION

The ethanolic extract of *Acanthopanax trifoliatus* leaves exhibits promising anti-inflammatory effect in both acute and chronic inflammation model at higher dosage of 300 mg/kg. The effect is almost comparable to that of the standard NSAID vis piroxicam and indomethacin. However, the exact mechanism of action for its pharmacological activity has not been determined yet. Studies are underway to evaluate the mediators and pathways involved in the inflammatory activity. Also, it is worthwhile to isolate the bioactive compounds which are responsible for those activities.

REFERENCES

1. Kumar V, Abbas AK, Fausto N, Mitchell RN. Acute and Chronic Inflammation. In Robbins Basic Pathology: Eighth Edition, Saunders Elsevier. Philadelphia 2007 pp31-32.
2. Perry LM, Metzger J. Medicinal plants of East and Southeast Asia: attributed properties and uses. MIT Press, Cambridge, MA. 1980 pp. 40
3. Loi DT. Glossary of Vietnamese Medical Plants. Science and Technics, Hanoi 1998.pp 392.
4. Agence de Coop´eration Culturelle et Technique. Les plantes m´edicinales au Vietnam: m´edecine traditionelle et pharmacop´ ee, Livre 1. Agence de Coop´ eration Culturelle et Technique, Paris 1990a
5. Wiart C. Ethnopharmacology of Medicinal Plant: Asia and The Pacific. Humana Press, Totowa, NJ.2006. pp 117.
6. Sainan S, Sulaiman MR, Somchit MN, Hidayat MT, Zakaria ZA. Evaluation of antinociceptive activities of *Acanthopanax trifoliatus* in mice. Proceedings of the 20th . Scientific Meeting of The Malaysian Society of Pharmacology and Physiology 2005. pp. 26.
7. Phan VK, Cai XF, Chau VM, Lee JJ, Kim YH**,** Kaurane-Type Diterpene Glycoside from the Stem Bark of *Acanthopanax trifoliatus.*Planta Med 2004; 70: 282–284.
8. Nguyen TP, Kyung AL, Su JJ, Cai XF, Jung KC, Kim YH, Kang JS. Capillary electrophoretic method for the determination of the diterpenoid isomers in *Acanthopanax* species. J Pharmaceutic Biomed Analysis 2006; 40:56-61.
9. Ty PD, Lischewski M, Phiet HV, Preiss A, Nguyen PV, Adam, G. 3α,11α-Dihydroxy-23-oxo-lup-20(29)-en-28-oic acid from *Acanthopanax trifoliatus.* Phytochem 1985; 24: 867-869.
10. Ty PD, Lischewski M, Phiet HV, Preiss A, Sung TV, Schmidt J, Adam G. Two triterpenoid carboxylic acids from *Acanthopanax trifoliatus.* Phytochem 1984; 23:2889-2891.
11. Kutschabsky L, Pfeiffer D, Lischewski M, Ty PD, Adam G. Molecular and crystal structure of a new 24-nor-thterpenoid carboxylic acid from *Acanthopanax trifoliatus.* Croatica Chem Acta 1985; 58: 427-434.
12. Lischewsk M, Kuttschabsky L. Two 24-nortriterpenoid Carboxylic acids from *Acanthopanax trifoliatus.* Phytochem 1985; 24:.2355-2357.

13. Kiem PV, Cai ZF, Minh CV, Lee JJ, Kim YH. Lupane-triterpene Carboxylic Acids from the Leaves of *Acanthopanax trifoliatus.* Chem. Pharm. Bull. 2003; 51:1432-1435.
14. Yook CS, Kim IH, Hahn DR. Nohara T, Chang SY. A lupane-triterpene glycoside from leaves of two *Acanthopanax.* Phytochem 1998; 49: 839-843.
15. Yook CS, Chang SY, Lai JH, Ko SK, Jeong JH, Nohara T. Lupane-Glycoside of *Acanthopanax trifoliatus* forma tristigmatis Leaves. Arch Pharm Res 1999; 22:629-632.
16. Phan VK, Chau VM, Nguyen TD, Cai XF, Lee JJ, Kim YH. Two new phenylpropanoid glycosides from the stem bark of *Acanthopanax trifoliatus*. .Arch. Pharm. Res. 2003; 26: 1014–1017.
17. Chen FC., Lin YM, Lin S. Constituents of Three-leaved *Acanthopanax.* Phytochem 1972; 11:1496-1497.
18. Chen FC, Lin YM, Yu PL. Constituents of *Acanthopanax trifoliatus*. Phytochem 1973; 12: 467-468.
19. Muselli A, Hoi TM, Cu LD, Moi LD, Bessiere JM, Bighelli A, Casanova J. Composition of the essential oil of *Acanthopanax trifoliatus* (L.) Merr. (Araliacaea) from Vietnam. Flavour and Fragrance Journal 1999; 14(1):41-44.
20. Du J, Gao L. Chemical constituents of the leaves of *Acanthopanax trifoliatus (Linn) Merr*. China J Chinese Mat Medica 1992; 17:356-357
21. Sithisarn P, Jarikasem S. Antioxidant Activity of *Acanthopanax trifoliatus*. Med Princip Pract 2009; 18: 393-398
22. Praphasawat R, Klungsupya P, Ezure Y, Suwanagul A, Theangtrong N, Himakhoun L, Arunpairojana V. Phytochemistry and antioxidant activities of the leaf extracts of *Acanthopanax trifoliatus*. Thai J Pharmaceutical Sci 2005 ; 2: 13-16
23. Meng Z, Sakai Y, Ose Y, Sato T, Nagase H, Kito H, Matsuda H, Sato M, Mizuno M. Anti-mutagenic activity of medicinal plants in traditional Chinese medicines. Mutation Research/Environmental Mutagenesis and Related Subjects 1988; 30(5): 378-379
24. Chen CH. Study of Anti-Tumor Effect of *Acanthopanax trifoliatus*; Understand of Knowledge and Attitude of Chinese Herb between Nurses and Society in Eastern Taiwan. Ph.D Thesis. Graduate Institude of Medical Sciences, Tzu Chi University, Taiwan 2006
25. Roslida AH, Siti Nurusaadah H, Fezah O. Gastroprotective effect of *Acanthopanax trifoliatus* on experimentally induced acute ulcer in rats. Pharmacologyonline 2010; 2:828-841.
26. Borrelli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies. Phytother Res 2000; 14:581–591
27. Winter CA, Risley EA, Nuss GW. Carageenin induced oedema in the hind paw of the rat as an assay for anti-inflammatory drugs. Proc.Soc. Exp. Biol 1962;. 3: 544-547.
28. Sulaiman MR, Zakaria ZA, Chiong HS, Lai SK, Israf DA, Azam Shah TM. Antinociceptive and anti-inflammatory effects of Stachytarpheta jamaicensis (L.) Vahl (Verbenaceae) in experimental animal models. *Med Princ Pract 2009; 8(4): 272-279.*
29. Babu NP, Pandikumar P, Ignacimuthu S. Anti-inflammatory activity of *Albizia lebbeck* Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. J. Ethnopharmacol 2009; 125(2):356-60.
30. Mossa JS, Rafatullah S, Galal AM, Al-Yahya MA. Pharmacological studies of *Ruus retinorrhea*. Int. J. Pharmacognosy 1995; 33: 242-246.
31. Otterness IG, Bliven ML.. Laboratory models for testing NSAIDs In: J.G. Lombardio's : NSAIDs..Wiley, New York 1985 pp 111-252.
32. Hargreaves KM, Dubner R, Brown F, Flores C, Joris J.. A new sensitive method for measuring thermal nocicepton in cutaneous hyperalgesia. Pain 1988; 32: 72-88.
33. Matsuda H, Yoshikawa M, Linuma M, Kubo M. Antinociceptive and anti-inflammatory activities of limonin isolated from the fruits of *Evodia rutaecarpa* var. bodinieri. Planta Med 1997; 64: 339-342.
34. Moura ACA, Silva ELF, Fraga MCA, Wanderley AG, Afiatpour P. Anti-inflammatory and chronic toxicity study of the leaves of *Ageratum conyzoides* L. in rats. Phytomed 2005; 12: 138–142.
35. Garcia-Pastor P, Randazzo A, Gomez-Paloma L, Alcaraz MJ, Paya M. Effects of petrosaspongiolide M, a novel phospholipase A2

36. Panthong A, Kanjanapothi D, Taesotikul T, Phankummoon A, Panthong K, Reutrakul V. Anti-inflammatory activity of methanolic extracts from *Ventilago harmandiana* Pierre. J Ethnopharmacol 2004; 91: 237-242.
37. Sinha S, Murugesan T, Maiti K, Gayan JR, Pal M, Saha BP. Evaluation of anti-inflammatory potential of *Berjenia ciliate* Sternb. Rhizome extracts in rats. J Pharm Pharmacol 2001; 53(2): 193-196.
38. Seibert K, Zhang Y, Leahy K, Hauser S, Masferer JL, Perkins W, Lee L, Isakson PC. Pharmacological and biochemical demonstration of the role of cyclooxygenase-2 in inflammation. PNAS 1994; 12013-12017.
39. Otterness IG, Larson DL, Lombardino JG. An analysis of piroxicam in rodent models of arthritis. J Inflamm Res 1982; 12(3): 308-312.
40. Meade EA, Smith WL, DeWitt DC. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isoenzymes by aspirin anad other non-steriodal anti-inflammatory drugs. J Biol Chem 1993; 266: 6610-6614
41. Mitchell JA, Akarasereemont P, Thiemermann C, Flower RJ, Vane JR. Selectively of non-steriodal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxgenase. PNAS 1993; 90: 11693-11697.
42. Choi J, Lee KT, Jung HJ, Park HS, Park HJ. Antirheumatoid arthritis effect of Kochia scoparia fruits and activity comparison of momordin Ic, its prosapogenin and sapogenin. Arch Pharm Res 2002; 25: 336-324.
43. Leela Krishna V, Chitra V, Soujanya Reddy J. Anti-arthritic activity of whole plant Acalypha indica on type II collagen induced arthritis in wistar rats. Int J Pharm Pharmaceut Sci 2011; 3(5): 99-102
44. Waheed MA, Farha Deeba, Rabbani Syed, Yousuf Ali M, Zeeyauddin K, Alshatwi AA. Animal studies on unani drug formulation for its analgesic, antipyretic, anti-inflammatory and non-toxic effect. Int J Pharm Pharmaceut Sci 2011; 3(4): 66-72
45. Harris ED. Rheumatoid arthritis: pathophysiology and implications for therapy. New Engl J Med 1990; 332: 1277-1287.
46. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Ann Rev Immunol 1996; 14: 397-440.
47. Harbuz MS, Perveen-Gill Z, Lalies MD, Jessop DS, Lightman SL, Chowdrey HS. The role of endogenous serotonin in adjuvant-induced arthritis in the rat. Br J Rheumatol 1996; 35: 112-116.
48. Prakken BJ, Roord S, Ronaghy A, Wauben M, Albani S, van Eden W. Heat shock protein 60 and adjuvant arthritis: a model for T cell regulation in human arthritis. Springer Sem Immunopathol 2003; *25*: 47-63.
49. Geetha T, Varalakshmi P. Anti-inflammatory activity of lupeol linoleate in adjuvant induced arthritis. Fitoterapia 1998; 69: 13-19.
50. Egan CG, Lockhart JC, Ferrell WR. Pathophysiology of vascular dysfunction in a rat model of chronic joint inflammation. J Physiol 2004; 557(2): 635-643.
51. Ferrari RA, Ward SJ, Zobre CM, Van Liew DK, Perrone MH, Connell MJ, Haubrich DR. Estimation of the *in vivo* effect of cyclooxygenase inhibitors on prostaglandin E2 levels in mouse brain. Eur J Pharmacol 1990; 179: 25-34.
52. Roth SH. NSAID gastopathy. A new understanding. Arch Intern Med 1996; 156: 1623-1628.
53. Ward JR, Cloud RS. Comparative effect of antirheumatic drugs on adjuvant induced polyarthritis in rats. J Pharmacol Exp Ther 1996; 152: 116-121.
54. Koch AE, Kunkel SL, Strieter RM. Cytokines in rheumatoid arthritis. J Invest Med 1995; 43: 28-38.
55. Szekanecz Z, Kim J, Koch AE. Chemokines and chemokine receptors in rheumatoid arthritis. Sem Immunol 2003; 15(1): 15-21.
56. Barton NJ, Stevens DA, Hughes JP, Rossi AG, Chessell IP, Reeve AJ, McQueen DS. Demonstration of a novel technique to quantitatively assess inflammatory mediators and cells in rat knee joints. J Inflamm 2007; 4: 13-20.