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 typified 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).

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. 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 heal ulcers and to cure ring-worm infection. A decoction of the leaves is drunk to treat tuberculosis and 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 Acanthopanax 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

MATERIALS AND METHODS

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 powder 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/FPDK/PADS/BR-UIH/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.

Chemics

Complete Freund Adjuvant, piroxicam and indomethacin (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. 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 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:

\[
\text{Percentage of anti-inflammation} = \frac{(V_f - V_o) \text{ control} - (V_f - V_o) \text{ treated}}{(V_f - V_o) \text{ control}} \times 100
\]

\(V_f = \text{Final volume}\)

\(V_o = \text{Initial volume}\)

**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. 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.

**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 suppressed 46.23% inhibition of oedema compared to 84.99% inhibition by piroxicam. Therefore, the ED50 for EAT cannot be calculated as at highest dosage used (300 mg/kg), as the oedema inhibition produced was still less than 50%.

**Carrageenan-induced arthritis**

Carrageenan significantly induced arthritis 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 suppressed 46.23% inhibition of oedema compared to 84.99% inhibition by piroxicam. Therefore, the ED50 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

<table>
<thead>
<tr>
<th>Group</th>
<th>% of oedema (mean ± S.E.M)</th>
<th>% of oedema inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5% ethanol)</td>
<td>21.69 ± 2.04</td>
<td>0</td>
</tr>
<tr>
<td>EAT 30mg/kg</td>
<td>20.42 ± 1.45</td>
<td>10.50</td>
</tr>
<tr>
<td>EAT 100mg/kg</td>
<td>18.66 ± 1.39</td>
<td>32.43</td>
</tr>
<tr>
<td>EAT 300mg/kg</td>
<td>13.05 ± 0.97*</td>
<td>46.23</td>
</tr>
<tr>
<td>Piroxicam (30mg/kg)</td>
<td>5.17 ± 0.50*</td>
<td>84.99</td>
</tr>
</tbody>
</table>

* P<0.05 indicated significant difference when compared to control group using ANOVA followed by Tukey’s Multiple Comparison Test.

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)

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage change in body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0 Before Induction</td>
</tr>
<tr>
<td>5 % Ethanol</td>
<td>0</td>
</tr>
<tr>
<td>EAT 30 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>EAT 100 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>EAT 300 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>Indomethacin 10 mg/kg</td>
<td>0</td>
</tr>
</tbody>
</table>

Data presented as mean ± S.E.M (n=6). *Significant (p<0.05) when compared to control (5% ethanol); + 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 by t-test.
The acute inflammation is produced when water and plasma proteins extravasate into the tissue in response to injury. The exudative phase, characterized by an intense infiltration of neutrophils, is followed by a proliferative phase, characterized by the infiltration of monocytes and macrophages. The proliferative phase is associated with the expression of pro-inflammatory cytokines and chemokines, which recruit these cells to the site of injury. The third phase, characterized by the resolution of inflammation, is associated with the resolution of cellular infiltration and the remodeling of the injured tissue.

The inflammatory response is mediated by a variety of pro-inflammatory and anti-inflammatory signals. Pro-inflammatory signals include pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), as well as reactive oxygen species and oxygen free radicals. Anti-inflammatory signals include anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), as well as anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids.

The anti-inflammatory effects of ethanolic extract of Acanthopanax trifoliatus (L.) Merr (EAT) leaves in adjuvant induced arthritis

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

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>Post Treatment</th>
<th>Day 14</th>
<th>Day 16</th>
<th>Day 19</th>
<th>Day 21</th>
<th>Day 23</th>
<th>Day 25</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5% ethanol)</td>
<td></td>
<td>46.05 + 2.18a</td>
<td>50.43 + 4.72a</td>
<td>48.90 + 4.93a</td>
<td>44.96 + 1.59a</td>
<td>33.38 + 2.07a</td>
<td>48.90 + 3.62a</td>
<td>44.96 + 4.28a</td>
</tr>
<tr>
<td>EAT 30</td>
<td></td>
<td>37.64 + 4.90a</td>
<td>49.60 + 4.01a</td>
<td>40.54 + 3.96a</td>
<td>38.82 + 4.32a</td>
<td>34.32 + 4.64a</td>
<td>34.32 + 4.32a</td>
<td>25.50 + 3.34a</td>
</tr>
<tr>
<td>EAT 100</td>
<td></td>
<td>34.06 + 2.98a</td>
<td>44.96 + 4.01a</td>
<td>40.54 + 3.96a</td>
<td>38.82 + 4.32a</td>
<td>34.32 + 4.64a</td>
<td>34.32 + 4.32a</td>
<td>25.50 + 3.34a</td>
</tr>
<tr>
<td>Indomethacin (10)</td>
<td></td>
<td>43.68 + 5.24</td>
<td>40.11 + 3.77</td>
<td>21.90 + 5.17</td>
<td>24.36 + 2.94</td>
<td>26.65 + 4.62</td>
<td>15.84 + 2.17</td>
<td>8.52 + 2.18a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>% of swelling (mean + S.E.M) (n=6)</th>
<th>% of arthritis inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5% ethanol)</td>
<td>52.26 + 4.34</td>
<td>0</td>
</tr>
<tr>
<td>EAT 30 mg/kg</td>
<td>43.27 + 4.04</td>
<td>9.57</td>
</tr>
<tr>
<td>EAT 100 mg/kg</td>
<td>39.93 + 3.56</td>
<td>23.71</td>
</tr>
<tr>
<td>EAT 300 mg/kg</td>
<td>25.50 + 3.34*</td>
<td>47.57</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>8.52 + 2.18a</td>
<td>83.43</td>
</tr>
</tbody>
</table>

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. 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. The observation that NSAIDs inhibit COX activity attests to the contribution of prostaglandins to the inflammation.

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. The acute inflammation is produced when water and plasma increases in tissues during arachidonic acid metabolism via cyclooxygenase (COX) and lipooxgenase (LOX) enzyme pathways. 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. The later phase that begins more than four hours after carrageenan injection showed significant anti-inflammatory effects in carrageenan-induced inflammation, substantial induction of COX-2 was observed after three hours with elevated level of thromboxane B2 (TXB2) and local oedema. Therefore, the ability of EAT leaves in oedema inhibition may result 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.

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. 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. 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 elevated level of thromboxane B2 (TXB2) and local oedema. Therefore, the ability of EAT leaves in oedema inhibition may result 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.

The possible mechanism which involved in the reduction of oedema 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. Besides CFA, reagents such as type II collagen and formaldehyde 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 46. 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 loss 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 lost 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, it was found that 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 52.

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 53.

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 and 300 mg/kg) (Table 3). However, only 300 mg/kg of EAT showed significant difference from the control group starting from day 14 until the day 21 (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 53. 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 54. 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 trifoliatu 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 flavonoids 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 trifoliatu 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.

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