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ANTI-INFLAMMATORY EFFECTS OF THE ETHANOLIC EXTRACT OF ACANTHOPANAX TRIFOLIATUS (L) MERR LEAVES IN ACUTE AND CHRONIC ANIMAL MODELS OF INFLAMMATION

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Anti-inflammatory Effects of The Ethanolic Extract of *Acanthopanax trifoliatus* (L.) Merr Leaves in Acute and Chronic Animal Models of Inflammation

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Abstract

The leaves of *Acanthopanax trifoliatus* (L.) Merr have been used to treat several diseases such as tuberculosis and lung hemorrhage, and as a tonic to improve general weakness. This study was conducted to determine the effect of EAT leaves on both acute and chronic anti-inflammatory activity. For acute inflammation, EAT gave significant anti-inflammatory effect at EAT 300 mg/kg and piroxicam 30 mg/kg as compared to control group and the percentage of inhibition for the treated group of 30, 100, and 300 mg/kg were 10.50%, 32.43%, and 46.23% respectively. For chronic inflammation, EAT exhibited significant effect also at 300 mg/kg and indomethacin 10 mg/kg as compared to control group and the percentage of inhibition for treated group of 30, 100, 300 mg/kg were 9.57%, 23.71%, and 47.57% respectively. It can be concluded that EAT exhibited a dose dependent inhibitory effect towards oedema in both acute and chronic inflammation. These result has shown that EAT leaves have anti-inflammatory properties.

Key Words: Anti-inflammation, Carrageenan, Freund's Complete Adjuvant (FCA)

1.0 Introduction

The leaves of *Acanthopanax trifoliatus* have been used to treat several diseases. In China, decoctions of the leaves and young shoots have been used to treat tuberculosis and lung haemorrhage, and as a tonic to improve general weakness (Perry, 1980). In Vietnam, five species have been detected and among these, *Acanthopanax trifoliatus* has been used in folk medicine (Museli *et al.,* 1999) as a drug with ginseng-like activity.

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) (Kumar *et al.,* 2007).

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.

2.0 Materials and Methods

2.1 Preparation of Plants Extracts

The leaves part of *Acanthopanax trifoliatus* was collected in July to August 2009 from its natural habitat in Bandar Baru Bangi, Selangor, Malaysia. The leaves were separated before washed and then leaves were dried in an oven at 38-42°C for several days and were grounded into powdered form. The leaves were weighed and macerated in 90% aqueous ethanol for 48 hours and run for rotary evaporator. The crude extract (EAT) were air dried in a fume hood at room temperature for several days and was weighed again before being dissolved in 5% ethanol as its vehicle and were prepared into several desired dose concentrations for pharmacological tests.

2.2 Animals

Male *Sprague Dawley* rats weighing 250-350 grams were provided by Faculty of Veterinary Medicine, UPM with ethics approval from the Animal Ethics Committee of University Putra Malaysia (Ethics Approval No.: 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 also were provided with adequate supply of pellets and water *ad libitum*. The rats were acclimatized at least one week before starting the experiments. In all experimental models of inflammation the studies were carried out using six rats in each group.

2.3 Carrageenan-Induced Paw Oedema

The anti-inflammatory effect was evaluated by carrageenan induced rat paw oedema according to the method of Winter *et al.,* (1962) with slight modification. Oedema was

induced by injection of 0.1ml of 1% suspension of carrageenan in 0.1ml of distilled water into the right plantar region of the rat. On the left plantar, it was injected with same volume of distilled water.

The rats were divided into five groups of six rats; Control group (5% ethanol solution), EAT (30, 100, and 300mg/kg) and Piroxicam (30mg/kg). The volumes of both hind paws of each rat were measured by using Plethysmometer. The paw volume measurements were determined immediately before carrageenan injection. Then, at every half hour the paw volume will be measured until the period of five hours.

The oedema component of inflammation was measured when the hind paw was immersed at the line marked and was expressed as a percentage by the formula below (Saso *et al.,* 2001):

$$\text{Percentage of swelling} = \left(\frac{(V_r - V_{ro}) - (V_l - V_{lo})}{V_{ro} \qquad V_{lo}} \right) \times 100$$

V_r = Right paw volume
V_{ro} = Right paw initial volume
V_l = Left paw volume
V_{lo} = Left paw initial volume

2.4 Adjuvant Induced Arthritis

Chronic arthritis was induced by injection of 0.1ml of Freund's Complete Adjuvant (FCA) 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 adjuvant 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 (Babu *et al.,* 2009).

The percentage of anti-inflammation for both acute and chronic was calculated using the formula given below (Sulaiman *et al.,* 2009):

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

V_f = Final volume
V_o = Initial volume

2.5 Statistical Analysis

Data was expressed as mean $\pm$ standard error of mean (S.E.M) (n=6). 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.

3.0 Results

3.1 Carrageenan Induced Paw Oedema

Table 3.1: Percentage inhibition of carrageenan-induced paw oedema in rats on various doses of EAT leaves.

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

Table above shows the percentage inhibition of carrageenan-induced paw oedema in rats at 300 minutes on various doses of EAT. The entire group has been given through the oral administration. The inhibition was compared to optimum oedema formation at 300 minutes induced by carrageenan. *$p<0.05$ indicated significant difference from control group using 1-way ANOVA followed by Tukey's multiple comparison test.

3.2 Adjuvant-Induced Arthritis

Table 3.2: Percentage inhibition of arthritis induced by FCA at day 28 on various doses of EAT leaves.

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

Table above shows the percentage inhibition of arthritis induced by FCA at day 28 on various doses of EAT. EAT and indomethacin was given orally as compared to optimum swelling that induced by FCA at day 28. *$p<0.05$ indicated significant difference from control group using 1-way ANOVA followed by Tukey's multiple comparison test.

Table 3.3: Percentage change in body weight of rats-induced arthritis.

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

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

4.0 Discussion

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 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, 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 2.

In chronic study, post treatment of EAT, it showed the reduction of foot swelling for all treated group (30, 100, 300 mg/kg). However, only 300 mg/kg of EAT showed significant difference from the control group. In the present study, it was found that the ethanolic extract

of *Acanthopanax trifoliatu*s exhibited antiarthritic 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 (Sithisarn & Jarikasem, 2009).

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