Anti-inflammatory and anti-hyperalgesic activities of *Acanthopanax trifoliatus* (L) Merr leaves

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ABSTRACT

Context: *Acanthopanax trifoliatus* is a ginseng-like plant, which has been widely used to treat various diseases including inflammatory-related diseases. **Aims:** The present study has been designed to investigate the anti-inflammatory and anti-hyperalgesic effects of various fractions of *Acanthopanax trifoliatus* leaves ethanolic extract in rats. **Materials and Methods:** Anti-inflammatory activity was studied by using carrageenan-induced edema on rat paw whilst anti-hyperalgesic was assessed by using carrageenan-evoked thermal hyperalgesia on plantar test. **Statistical Analysis Used:** Data were analyzed using Student *t*-test to compare with control.Multiple comparisons for difference between control and extract-treated groups were evaluated by Tukey HSD (Honestly Significant Difference) test. *P* values less than 0.05 ($P<0.05$) is considered significant. **Results:** Among three different fractions i.e., hexane, dichloromethane, and methanol tested, methanolic fraction displayed the most potent fraction amongst those three. It gave significant anti-inflammatory effect at highest dose, 500 mg/kg, with 77.24% of inhibition. Whilst for anti-hyperalgesic activity, methanolic fraction showed the highest efficacy at 375 mg/kg. Administration of methanolic fraction of *Acanthopanax trifoliatus* inhibited paw edema in a dose- dependent manner. The inhibition for both activities might be due to possible composition of polar compounds, which are flavonoids and phenolics content. **Conclusions:** Methanol fraction of *Acanthopanax trifoliatus* leaves has potential effect as anti-inflammatory and anti-hyperalgesia in acute inflammation model.

Key words: *Acanthopanax trifoliatus*, anti-hyperalgesic carrageenan-evoked thermal hyperalgesia, plantar test

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INTRODUCTION

Inflammation is a non-specific response of the microcirculation to tissue injury caused by physical, chemical, or biological stimuli or some combination of these. However, when there is loss of homeostatic control of this process of defense, an inflammation plays a damaging role that contributes to the appearance and worsening of diseases.[1] Hyperalgesia is defined as increase sensitivity to pain, which may be caused by damage to nociceptors and peripheral nerves. Hyperalgesia in inflammatory processes corresponds to primary hyperalgesia. Primary hyperalgesia occurs at the site of injury and is characterized by hyperalgesia to mechanical and heat stimuli.[2]

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Acanthopanax trifoliatus (*A. trifoliatus*), locally known as "Sam kar pei" amongst Chinese community, belongs to family Araliaceae. Leaves and root of this plant is commonly used in traditional Chinese medicine (TCM) for its ginseng-like activity. It has been utilized as a folk-medicine for bruise, neuralgia, impotence, and gout in China, Taiwan, and Philippines.[3-4] 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 is believed to ameliorate the memory.[5] It has been proven to have a rather good curative effect on treating common cold, jaundice, gastric pain, diarrhea, 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 leaves of *Acanthopanax trifoliatus* have revealed a high content of diterpenoids such as continentalic acid,[7] triterpenoid carboxylic acids,[8] phenylpropanoid glycosides[9] and other compounds such as essential oils, lipids, steroids, and alkanes.[10-13]

Thus far, there have been only few pharmacological studies on *Acanthopanax trifoliatus* in the literature. It was reported to show evidence for anti-oxidative,[14] anti-mutagenic,[15] anti-nociceptive,[6] anti-tumor,[16] and anti-ulcer effects[17] when tested on the leaf extract. Thus, the present study sought to explore the anti-inflammatory and anti-hyperalgesic effects of various fractionated extract of *A. trifoliatus* (L) Merr leaves using the rat carrageenan model of inflammatory pain.

MATERIALS AND METHODS

Drugs and chemicals

Carrageenan, tween 80 and piroxicam were obtained from Sigma Chemical (USA). All the other chemicals were of analytical grade.

Preparation of plant extract

Acanthopanax trifoliatus leaves were collected from Semenyih, Selangor, Malaysia and authenticated by Dr. Roslida Abdul Hamid. Voucher specimen (SK2004/12) was deposited at the herbarium of Institute of Biological Sciences, Universiti Putra Malaysia. The leaves are separated, washed, and then dried in an oven at 38-42°C for several days. The dried leaves were ground into powdered form and weighed. The powdered leaves were next soaked in 90% aqueous ethanol for 48 hours. The solvent was filtered after 48 hours, and new aqueous ethanol was added and continued to soak the leaves. The step was repeated until the solvent became colorless. The ethanolic solution of *A. trifoliatus* leaves was then filtered, and then evaporated by rotary evaporator to get crude ethanolic extract. The crude ethanolic extract was later fractionated successively with hexane, dichloromethane, and methanol to yield hexane extract (ATHE), dichloromethane extract (ATDE), and methanolic extract (ATME). The fractionated extracts were then prepared into several desired dose concentration for pharmacological tests.

Animals

The male Sprague Dawley rats, which weighed 180-350 grams and aged between 2-4 months, were obtained from Faculty of Veterinary Medicine, UPM with ethics approval from the Animal Ethnics Committee of University Putra Malaysia (Ethnics Approval No.: UPM/FPSK/PADS/BR-UUH/00371). The animals were grouped in cages in the animal house at Faculty of Medicine and Health Sciences, UPM. In all pharmacological tests, the studies were carried out using six rats in each group.

Carrageenan-induced paw edema

ATHE, ATDE (500 mg/kg), ATME (50, 100, 250, 375, 500 mg/kg), and piroxicam (30 mg/kg) were administered orally one hour prior to testing. The rats received intraplantar injections of 0.1ml of 1% suspension of carrageenan onto the right hind paw, and the contralateral paw received 0.1ml of normal saline, which was used as control. A line was marked right above the ankle joint of both rats' hind limbs in order to get the consistent measurements. Edema was measured using a plethysmometer (Ugo-Basile 7340, Italy). The measurements of paw volume were determined immediately after carrageenan injection. Both paws of each rat were measured at every half-hourly interval until period of six hours. The swelling of hind paw was measured when the hind paw was immersed at the line marked. Anti-inflammatory activity was calculated according to the formula[18]: Edema inhibition (%)=(1-D)/C × 100 where D represents the difference in paw volume after ATME administered compared to control group and C represents the volume in control group.

Carrageenan-evoked thermal hyperalgesia

ATHE, ATDE (500 mg/kg), ATME (50, 100, 250, 375, 500 mg/kg), and piroxicam (30 mg/kg) were administered orally one hour prior to testing. The rats received intraplantar injections of 0.1ml of 1% suspension of carrageenan on the right hind paw, and the contralateral paw received 0.1ml of normal saline, which was used as control. Anti-hyperalgesia was assessed by the Hargreaves model of thermal hyperalgesia.[19] A plantar test (Ugo Basile 7370, Italy) was used to measure the paw withdrawal latencies (PWLs) of the hind paws from a radiant heat stimulus to evaluate thermal hyperalgesia every 30 mins for 3.5 hours after injection of carrageenan. PWL was defined as the time required for the paw to show an abrupt withdrawal. Data was calculated as a mean of three repeated measurements.

Statistical analysis

The data obtained were analyzed by using student's paired *t*-test to evaluate the significance or to compare between the control and extract-treated groups. Multiple comparisons for difference between control and extract-treated groups were evaluated by Tukey HSD (Honestly Significant Difference) test. *P* values less than 0.05 ($P < 0.05$) is considered significant.

RESULTS

Evaluation of anti-inflammatory effect on carrageenan-induced edema, as well as anti-hyperalgesic effect on carrageenan-evoked thermal hyperalgesia was assessed in hexane (ATHE), dichloromethane (ATDE), and methanol (ATME) fractionated extracts at 500 mg/kg. The most potent extract was further experimented in both activities in various doses to obtain its ED_{50}.

Carrageenan-induced paw edema

The results of carrageenan-induced rat paw edema, which indicated the anti-inflammatory activity of different extracts

of *A. trifoliatus*, are presented in Table 1. At 500 mg/kg, ATME significantly attenuated carrageenan-induced rat paw edema and showed higher efficacy when compared with other extracts [Table 2]. Results on anti-edematogenic at various doses of ATME showed significant attenuation at all doses tested [Figure 1]. Vehicle-treated animals exhibited a time-dependent paw edema response that was modestly attenuated in animals treated with piroxicam 30 mg/kg and ATME at various doses. The vehicle control exerted optimal inflammation at 210 min. Thus, the percentage of inflammatory inhibition at 210 min was calculated in various doses of ATME [Table 2]. ATME displayed anti-inflammatory activity in a dose-dependent manner. Interestingly, there was a big difference of inhibitory activity between two doses of ATME i.e., 375 mg/kg and 500 mg/kg, i.e., 37.9% and 77.2%. ED_{50} calculated was 415 mg/kg (graph not shown).

Carrageenan-evoked thermal hyperalgesia

A similar pattern was observed in carrageenan-evoked thermal hyperalgesia model. Injection of carrageenan into the rats' right hind paws evoked thermal-hyperalgesia with a significant decrease of paw withdrawal latencies (PWLs) that began at 30 min when compared with the basal level [Figure 2]. Contralateral left hind paw withdrawal latencies remained constant at a basal level for the entire experiment (data not shown). Comparison amongst various fractionated extracts (ATHE, ATDE, and ATME) showed that ATME (500 mg/kg) showed the highest efficacy as it significantly increased paw withdrawal latencies (PWLs) in the right hind paw, followed by ATHE [Table 3].

Oral administration of ATME (100-500 mg/kg, p.o) produced a significant reduction in the hyperalgesic effect induced by carrageenan. Interestingly, ATME at 375 mg/kg showed better anti-hyperalgesic effect in terms of increasing PWL than ATME at 500 mg/kg [Figure 2]. Piroxicam 300 mg/kg also showed significant anti-hyperalgesic activity.

DISCUSSION

It has been a well-accepted concept that all pain, whether acute or chronic, peripheral or central, originates from inflammation and the inflammatory response.[20] The carrageenan model is a well-established paradigm for studying an inflammatory pain.[21] This model is commonly used for determining anti-inflammatory

Table 1: Anti-inflammatory effect of different fractionated extracts of *Acanthopanax trifoliatus* leaves in carrageenan-induced rat paw edema

Group (mg/kg)	Volume of edema (ml)											
	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min	330 min
Negative control (1% Tween 80)	0.00± 0.00	0.27± 0.06	0.33± 0.08	0.37± 0.09	0.43± 0.07	0.49± 0.07	0.62± 0.07	0.73± 0.11	0.67± 0.07	0.55± 0.08	0.49± 0.07	0.44± 0.06
Hexane (500 mg/kg)	0.00± 0.00	0.11± 0.05[b]	0.15± 0.04	0.16± 0.05	0.22± 0.04*	0.20± 0.06*	0.25± 0.05*	0.26± 0.07*	0.27± 0.06*	0.17± 0.06*	0.15± 0.03*	0.07± 0.04*
Dichloromethane (500 mg/kg)	0.00± 0.00	0.09± 0.01*	0.12± 0.02	0.16± 0.03	0.22± 0.03*	0.35± 0.05	0.45± 0.05	0.60± 0.06	0.68± 0.05	0.57± 0.04	0.46± 0.05	0.34± 0.03
Methanol (500 mg/kg)	0.00± 0.00	0.09± 0.02	0.12± 0.03	0.12± 0.03*	0.14± 0.03*	0.17± 0.03*	0.16± 0.02*	0.17± 0.02*	0.15± 0.03*	0.14± 0.02*	0.13± 0.02*	0.10± 0.02*
Positive control (Piroxicam 30 mg/kg)	0.00± 0.00	0.04± 0.01*	0.05± 0.02*	0.06± 0.02*	0.07± 0.02*	0.08± 0.02*	0.12± 0.01*	0.14± 0.02*	0.10± 0.02*	0.08± 0.01*	0.06± 0.01*	0.04± 0.01*

Mean±S.E.M.; *n*=6;*Significant ($P < 0.05$) when compared with control (1% Tween 80); b???

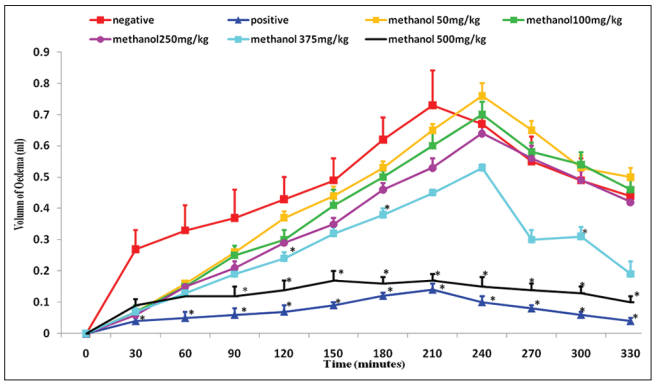

Figure 1: Attenuating effect of edema of carrageenan-induced hind paw of rats treated with different doses of methanol fraction and piroxicam (30 mg/kg) given orally

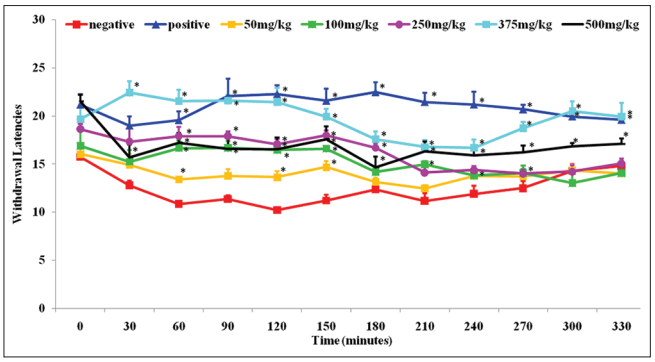

Figure 2: Effects of radiant heat on withdrawal latencies of carrageenan-induced right hind paw of rats treated with different doses of methanol fraction and piroxicam (30 mg/kg) given orally

properties for natural drugs as well as for searching for new anti-inflammatory agents. In addition, this model also helps in detecting orally active anti-inflammatory agents and evaluating the development of non-steroidal anti-inflammatory drugs. Therefore, this model was selected in the experimental study since the model shows very promising sensitivity, especially in acute phase of inflammation.[22] In this model, the development of edema and hyperalgesia in rat hind paw was described as a biphasic event.[23] The initial phase observed during the first hour was attributed to a release of histamine, serotonin, bradykinin, platelet activating factors (PAF), and leukotrienes; the second phase occurring within the first and third hours (peak of the inflammatory responses) is due to the release of prostanoids, especially prostaglandins and prostacyclins, by the action of 1 and 2-cyclooxygenases (COX-1 and COX-2).[24] During any inflammatory response, usually innocuous stimuli produce pain, which is caused by the stimulation of afferent nervous fibers by action of kinins, serotonin, histamine, prostanoids, protons, and reactive oxygen species (ROS) released during inflammation.[25]

In the present study, we first demonstrate that pre-treatment with ATME significantly inhibits carrageenan-induced edema and carrageenan-evoked thermal hyperalgesia, indicating that *Acanthopanax trifoliatus* is a potent preventive agent for inflammatory pain. Consistent with other reports, carrageenan-induced edema, and hyperalgesia peaked at 3.5-4 hour post injection.[26] Treatment with piroxicam significantly blocked the hyperalgesic response and modestly attenuated the edema response. These findings are consistent with earlier reports that NSAIDs affect inflammatory nociception.[27] It has also been reported that, during the development of carrageenan-evoked inflammatory pain, peripheral constitutive COX-1 and constitutive NOS play a primary role in the early phase (1h); in the late phase (4 h) in which COX-2 an iNOS are fully activated. The over production of prostaglandin and NO mainly synthesized by COX-2 and iNOS is a key mediator for the maintenance of inflammation.[28] Moreover, evidence exists that central and peripheral COX-2 are equally involved in mechanical hyperalgesia, while central COX-2 is predominantly involved in thermal hyperalgesia.[29]

Table 2: Percentage inhibition of carrageenan-induced paw edema in rats in various fractions of A. *trifoliatus* obtained from the optimum value at 210 minutes

Group	% of edema (mean±S.E.M)	% of inhibition of edema
Control (1% Tween 80)	72.5	0
ATME 50 mg/kg	65.0	10.34
ATME 100 mg/kg	59.8	17.52
ATME 250 mg/kg	53.3	26.48
ATME 375 mg/kg	45.0	37.93
ATME 500 mg/kg	16.5*	77.24
Piroxicam 30 mg/kg	14.2*	80.41
ATHE 500 mg/kg	26.0*	66.14
ATDE 500 mg/kg	68.0	5.56

Priorly, *Acanthopanax trifoliatus* have been reported to possess antioxidant properties.[14] Therefore, the action of ATME as a radical scavenger of superoxide may also contribute to its anti-inflammatory and anti-hyperalgesic effect. Furthermore, research done in different models of inflammation has indicated that *A.trifoliatus* exerted anti-inflammatory activity, not only in the acute model of inflammation, but also in a subchronic model, such as FCA-induced arthritis in rats.[30] The presence of lupine triterpene saponins and kaurane diterpenes including 16-αH, 17-isovalerate-ent-kauran-19-oic acid, in the plant which can strongly inhibit the enzymatic action of cyclooxygenase,[6] may also contribute to its action in mitigating inflammatory pain.

In summary, our findings showed that the methanol fraction of *Acanthopanax trifoliatus* leaves had relevant, beneficial anti-inflammatory and anti-hyperalgesic effects,

Table 3: Anti-hyperalgesic effect of various fractionated extracts of *Acanthopanax trifoliatus* leaves in carrageenan-evoked thermal hyperalgesia

Group (mg/kg)	Paw withdrawal latency (PWL) (s)											
	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min	330 min
Negative control (1% Tween 80)	15.7± 1.08	12.78± 0.51	10.83± 0.25	11.35± 0.41	10.20± 0.31	11.20± 0.61	12.37± 0.93	11.17± 0.77	11.90± 0.82	12.48± 0.73	14.28± 0.67	14.78± 0.35
Hexane (500 mg/kg)	15.0± 1.09*	15.38± 1.50	15.22± 1.03*	15.9± 1.02*	16.95± 0.61*	16.82± 0.68*	15.20± 0.51[a]	16.77± 1.05*	15.87± 0.94*	16.52± 0.69*	18.37± 0.58*	17.93± 0.51*
Dichloromethane (500 mg/kg)	16.33± 0.87	18.27± 0.90	18.20± 0.62	17.78± 0.62*	17.10± 0.33*	12.20± 0.76	12.15± 0.56	13.28± 0.70	12.97± 0.41	13.52± 0.92	15.00± 0.28	16.32± 0.29
Methanol (500 mg/kg)	21.54± 0.67*	15.70± 1.33*	17.23± 1.01*	16.6± 1.04*	16.53± 1.22*	17.58± 1.33*	14.67± 1.08*	16.33± 0.99*	15.93± 0.85*	16.25± 0.67*	16.85± 0.31*	17.10± 0.55*
Piroxicam (30 mg/kg)	21.17± 1.08	19.00± 0.93	19.55± 0.92*	22.03± 1.82*	22.28± 0.91*	21.58± 1.23*	22.47± 1.03*	21.45± 0.97*	21.18± 1.35*	20.68± 0.47*	19.95± 0.52*	19.60± 0.53*

Mean±S.E.M. (*n*=6); when compared with control,* *P*<0.001; a???

in accordance with its ethnopharmacological use. Further studies should be proceeded in order to determine the bioactive compounds responsible for those effects as well as elucidating the mechanisms involved in those activities.

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