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Anti-inflammatory and anti-hyperalgesic effects of *Ardisia crispa* Thunb. D.C

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ABSTRACT

Ardisia crispa Thunb D.C (Myrsinaceae), has long been used in treating various ailments among the local villagers. The objective of this study was to investigate experimentally the possible anti-inflammatory and anti-hyperalgesic properties of *Ardisia crispa*. The effect of hexane fraction of ethanolic extract of root of *Ardisia crispa* (ACRH) was evaluated in experimental models of pain and inflammation. The root extract at 3 - 300 mg/kg showed significant inhibition in carrageenan-induced oedema in rats with a maximum of 93.34% at 300 mg/kg. There was a significant ($p<0.001$) inhibition in carrageenan-induced hyperalgesia with ACRH 30, 100 and 300 mg/kg. The anti-inflammatory observed with the extract were comparable to that of standard. The present study indicates that the hexane fraction of *Ardisia crispa* (ACRH) exhibits significant anti-inflammatory and anti-hyperalgesic effects.

KEY WORDS: Anti-hyperalgesic, anti-inflammatory, carrageenan-induced oedema, carrageenan-induced hyperalgesia, *Ardisia crispa*

INTRODUCTION

The root and leaves part of *Ardisia crispa* are commonly used in folklore medicine (1). The plant *Ardisia crispa* Thunb. D.C belongs to the family Myrsinaceae and it is widely distributed in Asia stretching from Japan and the Himalayas to Java and the Philipines. It can be found in the undergrowth and jungle fringes, dappled shades and shady edges in Malaysia (2).

Its root is reported to be used as one of the traditional ingredient in post-natal syndromes where the root is boiled and the boiled concoction is used to treat pain in the throat and chest as well as to treat rheumatism. The mixture of its leaves and root is used as skin liniment (3). The root juice is useful for treating earache, cough, fever, diorrhea and also for women after-birth. In Canton, it has been marketed as "sin-lo-san", a herbal decoction drunk for sprains and broken bones. In Thailand, the root will be mixed with other plants towash "dirty blood' or in women with dysmenorhea (menstrual pain) (4).

As it has been established by the villagers among the South East Asian countries and China that they consumed the root and leaves part of *Ardisia crispa* to reduce pain and swellings (5)., therefore, it was decided to study the anti-hyperalgesic and anti-inflammatory activities of the root extract of *Ardisia crispa*.

MATERIALS AND METHODS

Plant Material and Extraction

The roots of *Ardisia crispa* (Family: Myrsinaceae) were collected from Tangga Batu, Melaka, Malaysia between July to December 1999 and was deposited as a voucher specimen (no: 20841) in the herbarium of Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia. The samples were cut into small pieces and dried at 60^0C for 3 days. The dried root and leaves were then grounded using Wiley laboratory mill. Grounded dried plant materials were macerated in cold aqueous ethanol (70% ethanol) for 48 hours. The extract was concentrated under reduced pressure in a rotary evaporator. The crude aqueous ethanol extract was then fractionated successively with n-hexane, dichloromethane and methanol. The solvents were removed under reduced pressure in a rotary evaporator at 40^0C and the concentrates dried at room temperature to yield solid residues; hexane fraction (14.1% w/w), dichloromethane fraction (7.62% w/w) and methanol fraction (57.40% w/w). The hexane fraction of *Ardisia crispa* is labelled as ACRH and the dichloromethane fraction is labeled as ACRC.

Animals

Healthy male *Sprague dawley* rats weighing between 170-250 g were obtained from Animal Unit of Faculty of Medicine, Universiti Malaya with ethics approval from the Animal Ethics Committee of Universiti Malaya

(FIS/16/04/02 RAH(R)). The animals were fed on standard laboratory diet and allowed free access to water.

Anti-inflammatory activity

ACRH was evaluated for anti-inflammatory activity by carrageenan-induced oedema on rat paw method (6). Male *Sprague dawley* rats were randomly distributed into 7 groups of 6 animals each. First group served as a control, second group served as the standard (received indomethacin 10 mg/kg ip) while the third, fourth, fifth, sixth and seventh group received 3, 10, 30, 100 and 300 mg/kg body weight of ACRH 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 seven 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 four 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 and was calculated as oedema percentage (7) according to the formula:

$$\% \text{ swelling} = \frac{Vr - Vr0}{Vr0} - \frac{Vl - Vl0}{Vl0} \times 100$$

Vr = Right Paw Volume

Vr0= Right paw initial volume

Vl= Left paw volume

Vl0= Left paw initial volume

Anti-hyperalgesic activity

Carrageenan-induced hyperalgesia

Male *Sprague dawley* rats were assigned into seven groups: control (10% Tween 80), standard (Indomethacin 10 mg/kg ip), ACRH 3, 10, 30, 100 and 300 mg/kg. After half an hour, 0.1 ml of 1% carageenan was injected subcutaneously onto right hind paws of the rats weighing 200~250 g. Then, the same amount of saline was injected onto the plantar surface of left hind paw. A thermocouple (Ugo Basile 7371) was placed under the heel of the hind paws. The withdrawal latencies were recorded every 30 minutes for the duration of 6 hours. Hyperalgesic effect was detected when carrageenan induced hyperalgesia on the right hind paw prolong the reaction time (withdrawal latencies) compared to the baseline values. The baseline value is the recorded withdrawal latencies before the hind paw was injected with the carageenan (8).

Statistical analysis

The data for each experiment were expressed as the mean value ± S.E.M (standard error of mean) (n=6). Unless otherwise specified, differences between vehicle control and treatment groups were tested using one way Analysis of Variant (ANOVA) followed by suitable multiple comparison of either Dunnett's, Dunn's or Fisher LSD Test. A value of p <0.05 was considered statistically significant.

RESULTS

Carrageenan-induced oedema

Carrageenan significantly induced oedema 1 (71.3 ± 10.0%, p<0.05), 2 (87 ± 8.2%, P<0.05), 3 (97.9 ± 2.0%, P<0.05), 4 hours (100.0 ± 0%, p<0.05) after injection of carrageenan compared to vehicle control. ACRH at 3 mg/kg significantly decreased oedema at 1 (26.7 ± 7.8%, p<0.05) and 2 hours (44.9 ± 9.8%, p<0.05). On the other hand, ACRH at 10, 30, 100 and 300 mg/kg showed significant decrease (p<0.05) in paw oedema after 1 (19.5 ± 6.9%; 12.1 ± 6.1%; 3.1 ± 0.1%; 6.7 ± 3.1%), 2 (15.6 ± 8.6%; 22.6 ± 16.1%; 2.6 ± 2.6%; 24.4 ± 13.1%), 3 (44.7 ± 4.1%; 36.3 ± 13.0%;9.9 ± 6.2%; 15.3 ± 10.1%) , 4 hours (48.6 ± 9.8%; 37.4 ± 13.8%; 20.4 ± 13.1%; 14.2 ± 9.8%) of injection of carrageenan respectively when compared with control. At 90 minutes, ACRH exhibited a gradual increment in percent inhibition of oedema when compared to control group (Table 1.0). At 100 mg/kg, its activity was as effective as indomethacin 10 mg/kg after 1 (3.1 ± 0.1%; 8.1 ± 2.2%) and 2 hours (2.6 ± 2.6%; 3.0 ± 1.7%) of carrageenan injection respectively. ACRH 300 mg/kg only showed significant inhibition which is comparable to indomethacin 10 mg/kg at 1 hour (6.7 ± 3.9%; 8.1 ± 2.2%) respectively .All doses showed significant anti-inflammatory effects (Table 1.0). The ED50 of ACRH calculated from the log dose-response curve was 6.8 mg/kg^{-} (graph not shown)

Carrageenan-induced hyperalgesia

Paw withdrawal latency was significantly reduced in carrageenan – induced hyperalgesia from 10.7 + 0.4 seconds (0 hour) to 9.6 ± 1.2 seconds after 1 hour; 2.8 ± 0.6 seconds after 2 hours; 2.2 ± 0.4 seconds after 3 hours; 2.1 ± 0.3 seconds after 4 hours; 2.1 ± 0.4 seconds after 5 hours and 3.9 ± 0.6 seconds after 6 hours respectively. ACRH administered orally at 30 mg/kg significantly reduced carrageenan-induced hyperalgesia after 2 (4.8 ± 0.5 seconds, p<0.05) and 3 hours (3.8 ± 0.9 seconds, p<0.05) when compared with control. Whilst ACRH at 100 and 300 mg/kg significantly increased paw withdrawal latency (p<0.05) after 1 (13.0 ± 1.7 seconds; 10.7 ± 2.3

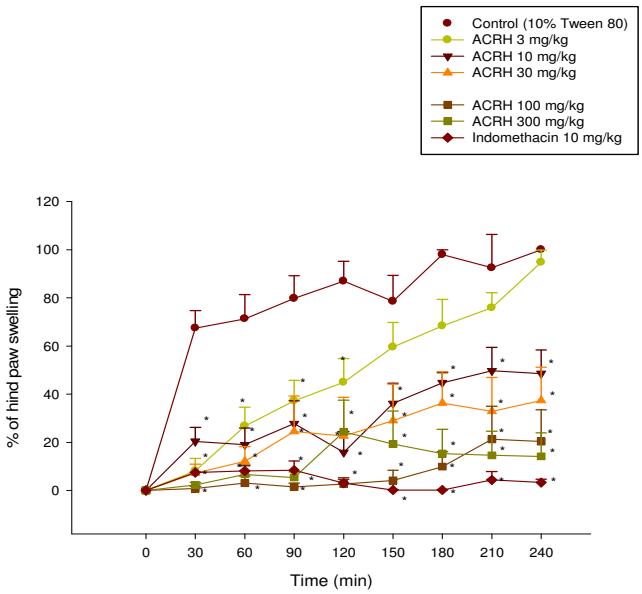

Figure 1: Percentage inhibition of carrageenan-induced paw oedema in rats on various doses of ACRH and indomethacin obtained from the optimum value at 90 minutes as comparison to 100% of swelling in control group. *P<0.001 indicated significant difference when compared with control group using ANOVA followed by Dunnett's Multiple Comparison Test.

Table 1. Percentage inhibition of carrageenan-induced paw oedema in rats on various doses of ACRH obtained from the optimum value at 90 minutes as comparison to 100% of swelling in control group.

Group	% of oedema (Mean ± S.E.M)	% inhibition of oedema (Obtained from average value)
Control (10% Tween 80)	79.79 ± 9.35	0
ACRH 3 $mgkg^{-1}$	37.24 ± 8.48	43.66*
ACRH 10 $mgkg^{-1}$	27.74 ± 9.49	65.23*
ACRH 30 $mgkg^{-1}$	24.33 ± 14.68	69.26*
ACRH 100 $mgkg^{-1}$	1.58 ± 1.58	98.09*
ACRH 300 $mgkg^{-1}$	5.39 ± 2.34	93.34*
Indomethacin 10$mgkg^{-1}$ (i.p)	8.44 ± 3.87	89.4*

**p<0.001 indicated significant difference when compared with control group using ANOVA followed by Dunnett's Multiple Comparison Test.*

seconds), 2 (17.3 ± 3.2 seconds; 10.9 ± 0.9 seconds), 3 (15.3 ± 3.1 seconds; 10.4 + 1.2 seconds), 4 (12.5 ± 3.1 seconds; 6.8 ± 0.8 seconds), 5 (12.1 ± 2.1 seconds; 9.6 ± 1.9 seconds), 6 hours (11.9 ± 2.1 seconds; 8.5 ± 1.7 seconds) respectively. At 100 mg/kg, ACRH is as potent as indomethacin 10 mg/kg at the early hours of hyperalgesia. However, the activity of ACRH 100 mg/kg was more than indomethacin 10 mg/kg after 3 (15.3 ± 3.1 seconds; 11.1 ± 3.3 seconds), 4 (12.5 ± 3.1 seconds; 8.9 ± 1.9 seconds), 5 (12.1 ± 2.1 seconds; 6.7 ± 1.9 seconds) and 6 hours (11.9 ± 2.1 seconds; 8.5 ± 2.1 seconds) of carrageenan-induced hyperalgesia respectively (Figure 1.1). Nevertheless, anti-hyperalgesic effect of ACRH was not exhibited in a dose dependent manner. At 100 $mgkg^{-1}$ its anti-hyperalgesic effect was more effective than at 300 mg/kg (F=0.366) (Table 1.2). The ED50 obtained from log-dose response curve was about 64.6 mg/kg (graph not shown)

DISCUSSION

To study the effect of 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 (9). The injection of carrageenan to the hind paw of rats is a common model to study inflammation and inflammatory pain. Moreover, here is a good correlation between efficacy

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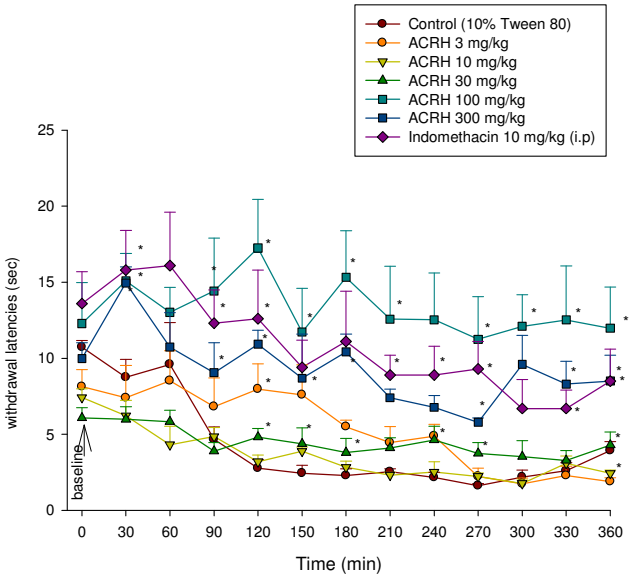

*Fig 1.1: Effects of radiant heat on withdrawal latencies of right hind paw injected with 0.1 ml carrageenan, treated with various doses of ACRH given orally and indomethacin. Data presented as mean ± S.E.M of 6 animals. *P<0.05 indicates significant difference from control analysed by t-test*

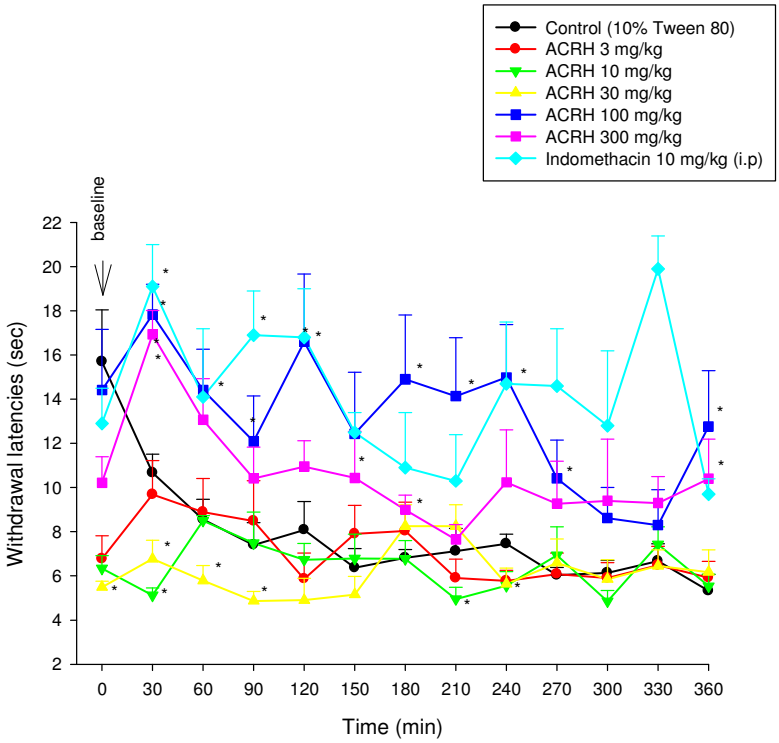

*Fig 1.2: Effects of radiant heat on withdrawal latencies of left rat hind paw injected with 0.1 ml saline after oral administration of various doses of ACRH and indomethacin. Data presented as mean ± S.E.M of 6 animals. *P<0.05 significantly differed from control analysed by t-test.*

Table 1.1 : Effects of ACRH at various doses administered orally on paw withdrawal latency of rat left and right hind paw injected with 0.1 ml saline and carrageenan respectively taken at 6 hours.

Group	Paw latency (sec)			
	Left hind paw (saline injected)		Right hind paw (Carrageenan injected)	
	Baseline (At 0 hour)	At 6 hours	Baseline (At 0 hour)	At 6 hours
Control (10% Tween 80)	15.7 ± 2.3	5.3 ± 0.7	10.7 ± 0.4	3.9 ± 0.6
ACRH 3 mg/kg	6.8 ± 1.0	5.9 ± 0.7	8.1 ± 1.1	1.9 ± 0.2
ACRH 10 mg/kg	6.3 ± 0.6	5.5 ± 0.5	7.4 ± 0.6	2.4 ± 0.2
ACRH 30 mg/kg	5.5 ± 0.3	6.2 ± 1.0	6.1 ± 0.7	4.3 ± 0.9*
ACRH 100 mg/kg	14.4 ± 2.7	12.8 ± 2.5*	12.3 ± 2.7	11.9 ± 2.7*
ACRH 300 mg/kg	10.2 ± 1.2	10.4 ± 1.8*	9.9 ± 1.0	8.5 ± 1.7*
Indomethacin 10 mg/kg	12.9 ± 1.6	9.7 ± 0.7*	13.6 ± 2.1	8.5 ± 2.1*

**p<0.001 indicated significant difference between control and treated groups determined with ANOVA followed by Dunnett's Multiple Comparison Test and Fisher LSD*

in this model and activity in humans (10). The observation that NSAIDs inhibit COX activity attests to the contribution of prostaglandins to the inflammation. From the result it is apparent that ACRH showed a significant anti-inflammatory effect in carragegenan-induced oedema model, which are comparable to that of the standard. Changes in paw volume after the injection of carrageenan corresponding to oedema occurred rapidly and in a biphasic manner (11). Similarly, in this study injection of carrageenan increased the paw volume of rats substantially during the 4 hours; an initial phase of oedema formation within 30 minutes followed by a second phase that was sustained up to 4 hours. However, the second phase of oedema was found to be not ubiquitous as the duration of action was continued from its initial phase. Nevertheless, it was observed that the optimum peak of the oedema curve for every dose given to rats would occur within 90 to 150 minutes.

In this study, we also utilized another model for pain on paw withdrawal test that is also known as thermal hyperalgesia, which was designed by Hargreaves et al (8). This model was also used to study hyperalgesic phenomena resulting from inflammation. Carrageenan causes oedema, an increase in paw volume, and an exacerbated sensitivity to thermal and mechanical stimuli which is known as hyperalgesia (12). Paw withdrawal test (Plantar test) is an example of phasic pain model with thermal stimuli. The Hargreaves model (8) is actually a suitable model in determining the anti-inflammatory effect and its mechanism of action either acting on COX-1 or COX-2 and via either the peripheral or CNS response (13). This model is also a sequel from carrageenan induced oedema model. Thus, we can use rat's hind paw injected with carrageenan to evaluate both anti-inflammatory and anti-hyperalgesic effects.

Results showed that ACRH can produce significant suppression on pain induced by the radiant heat applied to the plantar surface of the heel of the right hind paw injected with carrageenan (Figure 1.1) and left hind paw injected with saline (Figure 1.2). At higher doses of 100 and 300 mg/kg, ACRH significantly prolonged the paw withdrawal latencies (PWL) on the right hind paw injected with carrageenan as well as the left hind paw injected with saline. Therefore, based on the results, we suggested that ACRH at higher doses of 100 and 300mg/kg exerted its antinociceptive effect via peripheral as well as central mechanisms, which is in accordance to Hargreaves et al (14). On the other hand, at lower dosage, ACRH only significantly prolonged the PWL on the rat's right hind paw injected with carrageenan, which suggested that it exerted its anti-hyperalgesic effect via peripheral effect alone. These observations may be due to the close relationship between stimulus intensity and response to acute stimuli in the uninjured state, tissue injury and inflammation are associated with hypersensitivity reported by Raja et al (15). This hypersensitivity can be expressed as an increased response and a decrease response latency to noxious stimulus (hyperalgesia), or nocifensive response (i.e pain report or escape attempts) to an innocuous stimulus (allodynia). This altered stimulus-response relationship maybe the

result of peripheral as well as central mechanisms. It is generally accepted that increased activity in sensory afferent fibers after injury associated with nociceptor sensitization may change the spinal processing of sensory input (16).

In summary, it is suggested that the anti-inflammatory and anti-hyperalgesic effects of ACRH is mediated via COX-2 inhibition and acts at both peripheral and central sites. This postulation is suggested by the report from Ballou et al (17) who hypothesized that COX-2 is involved in mediating both a peripheral and central neurologic component of inflammatory pain or thermal hyperalgesia. In addition Zhang et al (18) reported that the continuous production of PGE2 by this isozyme could play a key role in the maintenance of inflammatory hyperalgesia. PGE2 also promotes inflammatory pain by sensitizing afferent nerve endings by the actions of bradykinin and histamine (19). Being the prostanoid, most generally associated with the inflammatory responses, the formation of PGE2 at inflammatory sites is often taken as an indicator of local COX activity. Phytochemical screening done on the hexane fraction indicated that it contains saponin, triterpenoid, flavonoid and tannins. Therefore, we postulated that flavonoids in the fraction may correlate appropriately for the present activities (20).

CONCLUSION

The hexane fraction of ethanolic extract of *Ardisia crispa* Thunb. D.C comprises anti-inflammatory and anti-hyperalgesic effects. Both effects are comparable to that of the standard NSAID vis indomethacin. It is postulated that both activities act at peripheral and central sites. However, the exact mechanism of action for both pharmacological activity have not been determined yet. Studies are underway to evaluate the antiulcerogenic property of the extract. And it is worthwhile to isolate the bioactive compounds which are responsible for those activities.

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