

RESEARCH ARTICLE

Analgesic, anti-inflammatory, antipyretic activities and acute toxicity of the ethanolic extract of *Clausena harmandiana* Pierre in animals

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Abstract

Clausena harmandiana Pierre (CH), a local plant used traditionally for pain relief and antipyretic, was used to investigate analgesic, anti-inflammatory, antipyretic activities and acute toxicity from an ethanolic extract of its root bark (CH-EtOH). Chemical analysis by HPLC indicated dentatin and nordentatin contents in CH-EtOH at 1.71 and 2.57% w/w, respectively. Analgesic activity of oral CH-EtOH was tested in mice by thermal threshold tests and acetic acid (AA)-induced writhing response, anti-inflammatory by carrageenan-induced rat paw edema, antipyretic by baker yeast-induced fever rat model and acute toxicity followed OECD Guidelines. Oral CH-EtOH significantly decreased writhing responses in a dose-dependent manner ($p < 0.05$), but slightly affected thermal threshold, suggesting its peripheral analgesic action. It showed no anti-inflammatory activity but significantly reduced the rectal temperature on yeast-induced pyrexia ($p < 0.05$) in a dose-dependent manner comparable to paracetamol. Acute toxicity test of oral CH-EtOH up to 3 g/kg did not show any serious toxic effects during a 7-day period of observations. This evidenced the use of ethanolic extract of root bark of *C. harmandiana* in folk medicine as analgesic and antipyretic agents.

Introduction

Clausena harmandiana Pierre (CH) is a member of the Rutaceae family and has been used in folk medicine for relief of pain and fever (Wangboonskul *et al*, 1984). No scientific report has been carried out to establish these claimed effects. Notably, the toxicity properties of CH also need to be proved systematically. Many members of *Clausena* spp such as *C. lansium* (Adebajo *et al*, 2009), *C. excavate* (Manosroi *et al*, 2005, Rahman *et al*, 2002), *C. hepataphylla* (Sohrab *et al*, 2001, Chakraborty *et al*, 1995) and *C. guillauminii* (Nakamura *et al*, 2009) have pharmacological activities. Five coumarins, clausarin, dentatin, osthol, xanthoxyletin and nordentatin, and one alkaloid, heptaphylline, were first isolated from *C. harmandiana* Pierre by Wangboonskul (1984). Later, two carbazol alkaloids, 2-hydroxy-3-formyl-7-methoxycarbazole and 7-methoxyheptaphylline with biological activity were isolated from *C. harmandiana* (Chaichantipyuth *et al*, 1988). Dentatin and nordentatin have antimicrobial (Wu and Furukawa, 1982, Sunthitikawinsakul *et al*, 2003) and strong antioxidant activities (unpublished data). Heptaphylline, dentatin and clausarin exhibited the antiplasmodial activity against *Plasmodium falciparum* (Yenjai *et al*, 2000) while a recent report showed that carbazoles and coumarins extracted from this plant stimulated glucose uptake in L6 myotubes (Noipha *et al*, 2010). Xanthoxyletin showed an inhibitory effect on the inflammation mediators, iNOS, TNF- α and COX-2 expressions in mouse macrophage RAW 264.7 (Nakamura *et al*, 2009). In addition, the crude ethanolic extract of CH shows antimicrobial activity against bacteria isolated from dogs with otitis externa infection (Chatchawanchooteera *et al*, 2009). However, there is little research regarding the information on the analgesic, anti-inflammatory and

antipyretic activities of the crude extract of CH *in vivo*.

The aim of this study was to investigate the analgesic, anti-inflammatory, antipyretic activities and acute toxicity of the ethanolic extract (CH-EtOH) from the root bark of CH using mice and rat models.

Materials and Methods

Chemicals: Methanol and phosphoric acid were of analytical reagent grade, procured from BDH (VWR International Ltd., Poole, England). The HPLC-grade reagent acetonitrile and methanol were also from BDH, while the HPLC-grade water was obtained from a deionized water treatment system (ELGA, Purelab Option, UK). Diclofenac, acetic acid (AA), paracetamol and aspirin were purchased from a local pharmacy, while morphine was a kind donation from Srinagarind Hospital, Khon Kaen University. Yeast, carrageenan and propylene glycol (PG) were purchased from Sigma. The suspension of CH-EtOH was prepared in PG (vehicle).

Extraction: Samples of *C. Harmandiana* (CH), collected in year 2008 from Khon Kaen and Kalasin provinces, Thailand, was identified by Mr. Supachai Tiyavorrannun at Faculty of Pharmaceutical Sciences, Khon Kaen University, where the voucher specimen (Reference number: 386) was deposited. The dried root bark of CH was ground and macerated with 70% ethanol and periodically stirred at room temperature for 3 d. The filtrate was then concentrated under low pressure at 40°C to yield a solid brown residue (19.7% yield), stored in an air-tight container at 2-8°C in a refrigerator until use.

Chromatographic analysis: Chromatographic analysis for CH-EtOH was performed with an Agilent 1100 Series HPLC instrument equipped with an

isocratic pump (Agilent G1310A), a variable wavelength detector (Agilent G1314A, USA) and a model 7725i manual injector valve with a 20 μ L sample loop. Chromatographic separation was carried out at room temperature using a Hypersil ODS analytical column (4.0 \times 250 mm, 5 μ m) with a guard column (Agilent, USA). The mobile phase consisted of 35:20:45 v/v of acetonitrile: methanol: phosphoric acid (5mM) in water. The flow rate was 1.0 ml/min and the detector set at 280 nm. As the standards for HPLC chromatography, xanthoxyletin, osthol, nordentatin, heptaphylline, dentatin, clausine-k, lansine, 7-methoxymukonal, 7-hydroxy-hepta-phylline, 7-methoxy-heptaphylline were extracted from CH and purified. The calibration curve of two active compounds, nordentatin and dentatin, were prepared using 5 concentrations ranges from 2.5-100 μ g/ml. The crude extract was dissolved in methanol to make up the concentration to 1 mg/ml. An aliquot of 20 μ l was then injected into the HPLC system. All data were acquired using Agilent Chemstation Rev. A. 10.02 software.

Animal study: Adult male ICR mice (weighing 30 \pm 5 g) and adult male Sprague-Dawley (SD) rats (weighing 175 \pm 25 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon-pathom, Thailand, and the animal handling were under supervision of the certified veterinarian of the Animal Unit of the Faculty of Medicine, Khon Kaen University, Thailand. The study protocol has been reviewed and approved by the Animal Ethics Committee of Khon Kaen University, based on the Ethic of Animal Experimentation of the National Research Council of Thailand (AE KKU 27/2551). The animals were housed under natural conditions (22 \pm 3 $^{\circ}$ C, 55 \pm 5%RH, 12h /12 h light-dark cycle) for 1 wk prior to experiment. They had free access to a rodent diet and clean

water. Experiments were carried out on groups of 5-6 animals. Before experiments, animals were fasted for 6-8 h, but allowed free access to water, and acclimatized in the laboratory unit for at least 1 h before testing.

Analgesic activity by thermal pain with writhing test:

ICR mice were randomly assigned to groups (n = 6 each), i.e. a negative control being given oral PG used as the vehicle, a positive control being given oral diclofenac (20 mg/kg) and 3 treatment groups being given oral CH-EtOH (250, 500 and 750 mg/kg). Intraperitoneal injection of 1% v/v acetic acid (100 mg/kg) to each mouse was given 20 min after administration. The number of writhing behavior such as abdominal constriction and stretching or twisting and turning of the trunk, over a period of 5 min after acetic acid injection was observed and recorded for 30 min.

Analgesic activity by thermal pain with hot-plate test:

ICR mice were randomly assigned to groups (n = 6 each), i.e. a negative control being given oral PG used as the vehicle, a positive control being given subcutaneous injection of morphine (10 mg/kg) and treatment group being given oral CH-EtOH (500 mg/kg). Mice were placed on a hot-plate maintained at 55 \pm 1 $^{\circ}$ C for detecting latency time to pain responses including licking of the hind limb, jumping or shaking the paws, and recorded as indicators of nociception. Each mouse was tested to measure a baseline time of response prior to administration of PG, drug or CH-EtOH. The reaction times or hot-plate latencies were taken at 15, 30, 60 and 90 min after each administration. The antinoci-ception was indicated by the percentage of inhibition which was calculated from the area under the curve (AUC) during a period of 90-min observation after administration, as follows:

$$\% \text{inhibition} = \frac{AUC_{\text{treat}} - AUC_{\text{cont}}}{AUC_{\text{cont}}} \times 100$$

Where AUC_{cont} = area under the curve (AUC) of the graph plotted between the latency time and time after administration of the PG group; AUC_{treat} = AUC of the graph plotted between the latency time and time after administration of the treated group.

Analgesic activity by thermal pain with tail-flick test: Animals were grouped as previously described and each being placed in a Plexiglas container which was used to immobilize the body while allowed free mobility of its tail. The tail was subjected to expose to heat by immersion in water controlled at $55 \pm 1^\circ\text{C}$. Tail-flick latency was to determine the time when animals flick the tails away from the hot water. Before administration of the treatments, each mouse was tested to measure a baseline time of response. The reaction times or tail-flick latencies were determined at 15, 30, 60 and 90 min after administration of each drug. The percentage of inhibition was calculated as previously described.

Anti-inflammatory activity: Adult male SD rats were randomly assigned into groups ($n = 6$ each), a negative control receiving oral PG, a positive control group receiving oral aspirin (300 mg/kg), treatment groups taken oral CH-EtOH (250, 500 and 750 mg/kg). Carrageenan-induced paw edema for testing anti-inflammatory activity (Winter *et al*, 1962) was employed. The paw volume of the rats was measured before treatment as a baseline paw volume. Each rat was subjected to subplantar injection of 0.1 ml of 1% w/v carrageenan of the right hind paw 1 h after treatment. The paw volumes were measured by the displacement technique using a plethysmometer (Ugo Basile Model

7150, Italy) at 1, 2 and 3 h after carrageenan injection.

Antipyretic activity: Adult male SD rats were randomly assigned as previously described for anti-inflammatory activity but oral paracetamol (150 mg/kg) being given to the positive control group. Rectal temperature (T_R) was measured by inserting a lubricated thermistor probe (external diameter 3 mm) into the rectum of the animal. The probe was linked to a digital device (Harvard Universal Oscillograph, USA), which displayed the temperature at the tip of the probe. The values displayed were manually recorded. Each rat was subjected to subcutaneous injection of baker's yeast (0.135 g/kg) to induce pyrexia, and then given the treatment at 2 h after the yeast injection. T_R of each animal was triplicate recorded before the yeast injection and hourly for 6 h after treatment, then averaged.

Acute toxicity test: Single doses (0, 1 and 3 g/kg) of oral CH-EtOH was tested in ICR mice for acute toxicity, the method being adopted from an international guideline (OECD, 2001). A group of mice given oral PG was used as a control. The behavioral changes of each animal were monitored for 6 h after administration on Day 1, and then observed once daily for 7 d. Body weights of the mice were recorded using an electronic balance (Sartorius, Germany) as well as mortality monitored. All mice were sacrificed and examined for any abnormality of the internal organs.

Statistical analysis: Results were expressed as the mean \pm standard error of the mean (SEM). Statistical analysis was performed using SPSS version 11.5 and two-way repeated measures ANOVA (One factor repetition). The criterion for the statistical significance was $p < 0.05$.

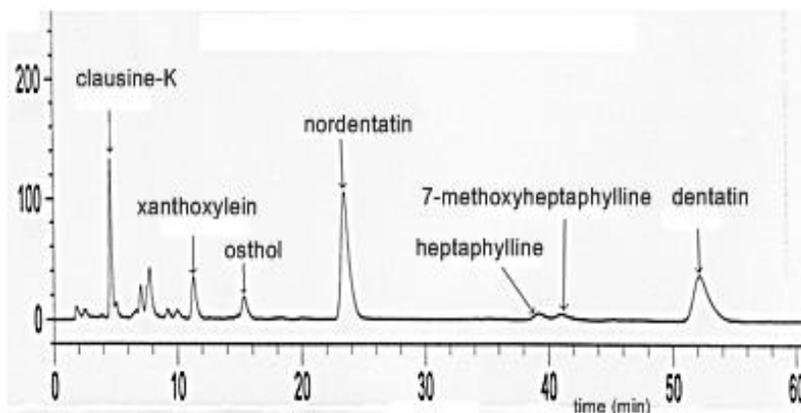


Figure 1 HPLC chromatogram of CH-EtOH: volume ratio of mobile phase 35:20:45 acetonitrile:methanol:phosphoric acid (5mM) in water with a 1.0 ml/min flow rate and detection at 280 nm.

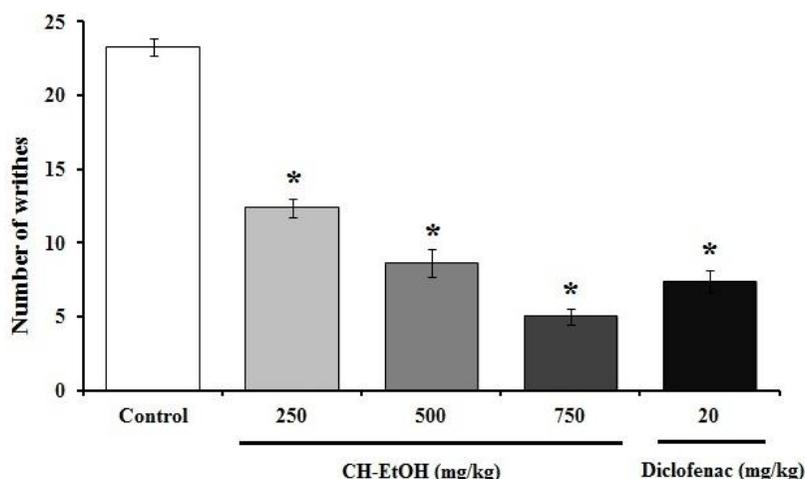


Figure 2 Analgesic activity of CH-EtOH evaluated by the acetic acid-induced abdominal writhing model in mice. Nociception was evaluated by counting the number of writhes during a 30 min period after ip injection of acetic acid. Groups of animals were pre-treated with PG (control) or diclofenac (20 mg/kg) or CH-EtOH (250–750 mg/kg), po, 20 min prior to acetic acid injection. Each column represents the mean±SEM (n=6) *- $p < 0.05$ compared to the control group.

Results and Discussion

The isocratic elution used in this study proved to be suitable to separate the compounds. HPLC fingerprint of CH-EtOH and the biomarkers. Figure 1 shows identifiable bioactive compounds which was comparable to relative

retention times and the UV spectra obtained from the diode array detector of 10 pure biomarkers. These included clausine-k, lansine, 7-methoxy-mukonal, xanthoxyletin, 7-hydroxy-heptaphylline, osthol, nordentatin, heptaphylline, 7-methoxyheptaphylline and dentatin

with respective retention times at 4.7, 7.8, 8.0, 11.6, 15.2, 15.7, 24.1, 40.1, 42.0 and 53.5 min.

The quantitative analytical method for the active constituents, dentatin and nordentatin was validated with a relative standard deviation of 2.5 and 1.8% (n=11) of the precision between days for dentatin and nordentatin at the concentration of 100 µg/ml, respectively. Accuracy was determined from recovery of 40 µg/ml in repeated assays and found to be 100.51±0.49 and 100.32± 0.58 for dentatin and nordentatin, respectively (n=5). The linear relationship between concentrations and measurements was shown by the r² of 0.9996 and 0.9995 for dentatin and nordentatin, respectively. The limit of quantitation of dentatin and nordentatin was 130.1 and 113.7 ng/ml, respectively, whereas the detection limit of dentatin and nordentatin was 39.04 and 34.10 ng/ml, respectively. The yield of dentatin and nordentatin in the sample calculated in the dried ground root bark was identified to be 1.71% and 2.57% w/w, respectively.

The analgesic activity of CH-EtOH by acetic acid-induced writhing responses

in mice are presented in Figure 2. Overall, the oral administration of CH-EtOH reduced the number of abdominal constrictions induced by acetic acid in the dose-dependent manner and this inhibitory effect was maintained throughout the examining period (p<0.05).

It appeared that CH-EtOH had an analgesic action comparable to diclofenac. The analgesic activity of CH-EtOH showed a dose-dependent pattern. CH-EtOH at a dose of 750 mg/ml had a statistically significant higher effect than the dose of 500 and the dose of 500 mg/kg had also higher effect than the dose of 250 mg/kg (p<0.05).

In the hot-plate test in mice model, the oral administration of CH-EtOH (500 mg/kg) exhibited an analgesic activity as early as 15 and 30 min after treatment (Table 1). The inhibition was 31.9% as compared to the negative control (p<0.05). An analgesic activity of morphine (10 mg/kg, sc) was much more potent and longer duration than that of CH-EtOH, with the inhibition rate of 98.4% when compared to the control (p<0.05).

Table 1 The reaction times measured by the hot-plate test in mice at different times after treatment with control, CH-EtOH and morphine at 15, 30, 60 and 90 min after each treatment as a test of analgesic effect of CH-EtOH. Data are presented as mean ± SEM (n=6); *p<0.05 compared to control (PG only)

Treatment	Dose (units/kg), route of admin.	Reaction time (sec.) after treatment					Inhibition (%)
		0 min	15 min	30 min	60 min	90 min	
PG	10 ml, po	5.00±0.00	4.17±0.31	4.33±0.21	4.67±0.21	4.17±0.31	0
CH-EtOH	500 mg, po	4.67±0.21	6.33±0.42*	7.00±0.37*	5.83±0.54	4.33±0.33	31.9
Morphine	10 mg, sc	5.50±0.22	9.83±0.40*	12.00±0.77*	7.67±0.33*	7.00±0.37*	98.4

Table 2 Analgesic effect of CH-EtOH by the tail-flick test in mice at different times after treatment recorded as tail-flick latency at 15, 30, 60 and 90 min after each treatment. Data are presented as mean ± SEM (n=6); *p<0.05 compared to control (PG only)

Treatment	Dose (/kg)	Reaction time (sec.) after treatment					Inhibition (%)
		0 min	15 min	30 min	60 min	90 min	
PG	10 ml, po	5.17±0.17	5.50±0.43	5.50±0.22	5.33±0.21	5.17±0.31	0
CH-EtOH	500 mg, po	4.33±0.33	6.00±0.37	8.33±0.61*	7.00±0.82	6.17±0.65	26.9
Morphine	10 mg, sc	4.83±0.31	12.67±0.33*	14.83±0.17*	13.00±0.52*	8.33±0.42*	122.8

Table 3 Anti-inflammatory activity of CH-EtOH on carrageenan-induced paw edema in rats at different times after carrageenan injection. Carrageenan edema was induced 1 h after drug treatments. Data are presented as mean ± SEM (n=6); *p<0.05 compared to control (PG only)

Treatment	Dose (per kg)	Paw volume (ml)				Inhibition (%)
		0 h (baseline)	1 h	2 h	3 h	
PG	10 ml	0.85±0.05	1.08±0.07	1.27±0.14	1.29±0.14	-
CH-EtOH	250 mg	0.87±0.04	1.12±0.07	1.33±0.16	1.35±0.16	-
CH-EtOH	500 mg	0.91±0.04	1.18±0.07	1.30±0.14	1.28±0.13	-
CH-EtOH	750 mg	0.89±0.04	1.10±0.07	1.22±0.15	1.25±0.16	-
Aspirin	300 mg	0.87±0.04	0.97±0.03	0.99±0.07*	0.96±0.07*	67.4

Table 4 Effect of the extract of *C. harmandiana* (Pierre) and paracetamol on yeast-induced fever in rats. Values are expressed as mean±SEM. Oral treatment given 2 h after yeast injection (n=6). * - P <0.05 from control group

Treatment	Dose (/kg)	Average rectal temperature (°C)						
		Baseline	Time after yeast injection		Time after CH-EtOH given (h)			
			1 h	2 h	1	2	3	4
PG	10 ml	36.23 ± 0.06	36.68 ± 0.09	37.05 ± 0.12	37.62 ± 0.19	37.20 ± 0.24	36.95 ± 0.23	36.60 ± 0.05
CH-EtOH	250 mg	36.22 ± 0.03	36.60 ± 0.04	36.82 ± 0.03	36.80 ± 0.23*	36.75 ± 0.16*	36.47 ± 0.10*	36.17 ± 0.06*
CH-EtOH	500 mg	36.13 ± 0.06	36.60 ± 0.06	36.83 ± 0.04	36.60 ± 0.19*	36.45 ± 0.13*	36.20 ± 0.07*	36.10 ± 0.06*
CH-EtOH	750 mg	36.15 ± 0.04	36.52 ± 0.05	36.73 ± 0.04	36.40 ± 0.10*	36.37 ± 0.13*	36.28 ± 0.05*	36.07 ± 0.06*
Paracetamol	150 mg	36.15 ± 0.06	36.55 ± 0.09	36.78 ± 0.08	36.55 ± 0.34*	36.47 ± 0.16*	36.22 ± 0.04*	36.30 ± 0.07*

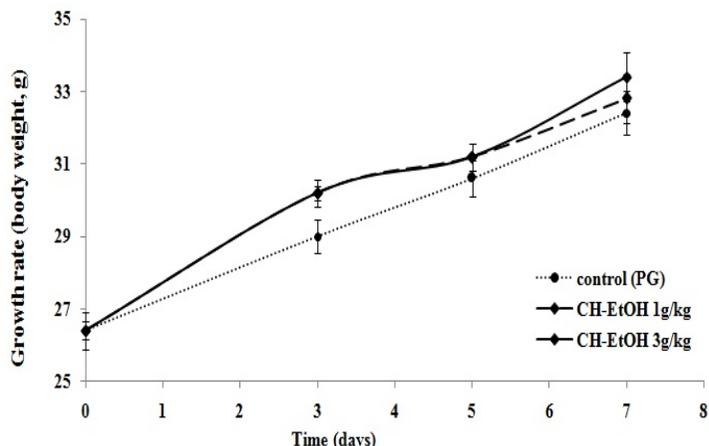


Figure 3 Body weight changes of mice observed during a 7-day period after a single administration of CH-EtOH of doses of 0, 1 and 3 g/kg. All treatments were given orally. Data is presented as mean ± S.E.M. (n=5); *p<0.05 compared to control (PG only).

Analgesic effect of CH-EtOH was examined further by the tail-flick test in mice. Morphine (10 mg/kg, sc) significantly increased the pain latency against heat stimulus at every time examining point (Table 2) with the inhibition rate of 122.8% as compared

to PG control (p<0.05). Although CH-EtOH (500 mg/kg, po) induced the significant tolerance to pain by heating at 30 min after administration, its efficacy was only 26.9% inhibition as compared to PG control.

The subplantar injection of the carrageenan produced inflammatory edema which increased gradually, reaching its maximum by the 2 h after carrageenan injection (Table 3). Aspirin at a dose of 300 mg/kg, orally, exhibited an anti-inflammatory activity that became significant 1 h after carrageenan injection and its effect was maintained throughout the examining time. The edema inhibition of aspirin was 67.4% ($p < 0.05$). The oral administration of CH-EtOH had no effect on carrageenan-induced rat paw edema ($p > 0.05$).

Antipyretic activity of CH-EtOH was examined by the baker's yeast fever test in rats. The results are shown in Table 4. In the control group, rat rectal temperature (T_R) increased gradually and reached its maximum at 3 h after yeast injection. Paracetamol (150 mg/kg, po) and CH-EtOH treatments prevented yeast-induced fever which became significant 1 h after drug administration and maintained its activity throughout the experiment ($p < 0.05$). The antipyretic pattern of CH-EtOH was similar to that of paracetamol.

During the 7-day period of observations, there was no death of animal in any dose groups (0, 1 and 3 g/kg CH-EtOH). Therefore, the lethal dose of oral administration of CH-EtOH in mice was estimated to be higher than 3 g/kg. In addition, normal body weight gains were observed in all groups as shown in Figure 3. No behavioral changes or tissue abnormalities (by gross pathology of internal organs) was noted in any groups of treatment. The obtained data imply that oral administration of CH-EtOH at the doses used in this study is safe.

The present study showed the antinociceptive and antipyretic effects of the ethanolic extract from the root bark of *C. harmandiana* Pierre (CH-EtOH). These effects of CH-EtOH were observed at doses that had no toxicity

or the overall behavioral changes of the animals.

C. harmandiana, particularly, the root bark, has long been used in Thai traditional medicine for relief of aches and fever. In this study, we evaluated the antinociceptive activity of CH-EtOH with three different ways, a chemical anti-nociceptive test (writhing test) and the two thermal antinociceptive tests (hot-plate and tail-flick tests). Acetic acid-induced writhing response is a model commonly used for screening peripheral analgesics, whereas hot-plate and tail-flick tests are for screening central analgesics. Acetic acid-induced represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid (Ahmed *et al*, 2006). In the experiment, we induced pain by injection of acetic acid into the peritoneal. Animals react with a characteristic stretching behavior which is called writhing. In our results, CH-EtOH exhibited significant analgesic effects in acetic acid-induced abdominal writhing in a dose-dependent manner and the duration of action was comparable to diclofenac. However, CH-EtOH had very weak central analgesic action of slight extension of the animal's reaction times to heat stimulus. Analgesic activity by tail-flick model, this model is central model in which opioid agent exert analgesic effects via spinal receptor. The antinociceptive effect on the tail-flick and hot-plate test indicates that CH-EtOH causes inhibition on both spinal reflex and supraspinal centers (Dewey *et al*, 1970). No significant anti-inflammatory effect was observed in CH-EtOH at the oral doses up to 750 mg/kg to the acute rat paw edema induced by carrageenan.

It is well-known that tissue damage causes inflammation to cause the release of endogenous mediators such as histamine, serotonin, brady-kinin and prostaglandins, by which in turn

stimulate the nociceptors. Arachidonic acid is released from the damaged cell membrane by an enzyme phospholipase A₂ and is then continuously metabolized to prostaglandins and leukotrienes by cyclooxygenase and lipoxygenase, respectively (Sweetman, 2011). In the acetic acid-induced abdominal writhing model, prostaglandin E₂ and F_{2α} were released, and the prostaglandin biosynthesis inhibitors such as NSAIDs, non-narcotic analgesics and antioxidants prevented the release of prostaglandin (Deraedt *et al*, 1980). Therefore, the peripheral analgesic activity of CH-EtOH may be mediated, at least in part, through inhibition of local arachidonic acid pathways. However, neither the blockage of synthesis nor the release of other inflammatory mediators such as bradykinin, histamine, could be excluded. It remains unclear why CH-EtOH possessed peripherally mediated analgesic action but was ineffective in relieving inflammation. Nakamura *et al* (2009) studied the inhibitory effect of two oxycoumarins (osthol, xanthoxyletin) isolated from the root bark of *C. guillauminii* on the inflammatory mediators. Since the cyclooxygenase-2 (COX-2) was inhibited by xanthoxyletin, they expected the anti-inflammatory activity of oxycoumarins. Besides this, strong antioxidant activity of nordentatin (unpublished data) suggested its potential to prevent the release of prostaglandin (Deraedt *et al*, 1980). Both xanthoxyletin and nordentatin are the compounds found in *C. harmandiana* but our results showed that CH-EtOH had no anti-inflammatory activity. There are several possible explanations for the failure of CH-EtOH in anti-inflammation tests. Firstly, the level of nordentatin and xanthoxyletin in our sample might be low and did not reach the effective level to elicit anti-inflammatory activity. Secondly, CH-EtOH might be poorly distributed into the inflammatory tissues. Thirdly, there may be some contradictions among the

compounds within the extract. Related to this, Nakamura *et al* (2009) reported that oxycoumarins have different chemical structures, so that careful selection of oxycoumarins is necessary to evaluate the anti-inflammatory activity of the oxycoumarins.

In the present study, antipyretic activity of CH-EtOH was evaluated by a baker's yeast-induced hyper-thermic rat model. CH-EtOH showed excellent antipyretic activity in a dose-dependent manner comparable to paracetamol. This drug has both the analgesic and antipyretic effects with weak anti-inflammatory action (Graham and Scott, 2005). These features were similar to those of CH-EtOH. The mechanism of analgesic action of paracetamol is not fully understood but supposed to involve the inhibition of prostaglandin bio-synthesis (Sweetman, 2011). In the fever condition, production of endogenous pyrogens such as interleukins, interferons and tumor necrosis factor, was accelerated and these substances increase the synthesis of prostaglandin E₂ (Moltz, 1993). CH-EtOH extract may inhibit the production and/or biological activities of some of these endogenous pyrogens.

In terms of toxicity, CH-EtOH at high dose (1 and 3 g/kg, po.) did not cause the death of mice nor affect the behavior. In addition, the body weight gain of the CH-EtOH-treated mice was similar to controls. Since the lethal dose (LD) of CH-EtOH was estimated to be more than 3 g/kg, doses for antinociceptive and antipyretic effects are far lower than the lethal dose and supposed to be safe. From these results, we assume that the use of CH-EtOH as a peripheral analgesic agent in human would be safe. Further study on the chronic toxicity induced by CH-EtOH should be carried out.

Conclusion

The ethanolic extract from the root bark of *C. harmandiana* (CH-EtOH) has significant antinociceptive and antipyretic activities without acute toxicity. The precise mechanisms underlying these actions or the identification of the pure compounds having those activities was out of the scope of this study but is currently under investigation. These findings will give a scientific explanation for the continued use of this plant by people who prefer to use alternative medicines for the treatment of some painful and fever-related conditions.

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