Antinociceptive and Anti-inflammatory Properties of *Corchorus capsularis* Leaves

Chloroform Extract in Experimental Animal Models

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The antinociceptive and anti-inflammatory properties of *Corchorus capsularis* leaves chloroform extract were investigated in experimental animal models. The antinociceptive activity was measured using the writhing, hot plate and formalin tests, while the anti-inflammatory activity was measured using the carrageenan-induced paw edema test. The extract, obtained after 72 h soaking of the air-dried leaves in chloroform followed by *in vacuo* evaporation to dryness, was weighed and prepared by serial dilution in DMSO in the doses of 20, 100 and 200 mg/kg. The extract was administered (s.c.) 30 min prior to subjection to the respective assays. The extract was found to exhibit significant (*p* < 0.05) antinociceptive and anti-inflammatory activities. As a conclusion, the present study confirmed the traditional claims of using *C. capsularis* to treat various ailments related to inflammation and pain.

**Key words** — *Corchorus capsularis*; chloroform extract; antinociceptive activity; anti-inflammatory activity

INTRODUCTION

*Corchorus capsularis* L., also known to the Malays as ‘Kancing baju’, is a plant that belongs to the family of Tiliaceae.1) The leaves of *C. capsularis* have been claimed to possess stimulant, demulcent, laxative, appetizer and stomachic and its infusion is traditionally used to treat fevers, constipation, dysentery, liver disorders and dyspepsia.1) In addition, a decoction of the roots or unripe fruits has also been used to treat dysentery.1) Other than that, the leaves of *C. capsularis* are eaten as vegetables in various part of the world such as Bangladesh, Africa, Middle East and Southeast Asia, including Malaysia, for a long time.2) Furthermore, the dry leaves were used as substitute for coffee or tea in Japan and were regarded as health-food.3) Based on the lack of scientific studies to establish its potential pharmacological properties, the present study was aimed at evaluating the antinociceptive and anti-inflammatory properties of chloroform extract of *C. capsularis* leaves.

**MATERIALS AND METHODS**

**Plant Material** The leaves of *C. capsularis* were collected in July-September, 2005 from its natural habitat in Kg. Kuala Kangkong, Simpang Ampat, Alor Setar, Kedah, Malaysia by Mr. Mohd. Suhaimi Ismail. It was identified by Mr. Shamsul Khamis, a botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia, (UPM), Serdang, Selangor, Malaysia, and a voucher specimen (SK 856/05) was deposited at the Herbarium of the Laboratory of Natural Products, IBS, UPM, Malaysia.

**Phytochemical Screening of the *C. capsularis* Leaves** The phytochemical screening of *C. capsularis* leaves was carried out according to the standard screening tests and conventional protocols as described by Ikhiri et al.3)

**Preparation of the Chloroform Extract *C. capsularis* (CECC)** The CESN was prepared by soaking the air-dried powdered leaves of *C. capsularis* (20 g) in chloroform in the ratio of 1 : 20 (w/v) for 72 hrs. The supernatant was collected and filtered using Whatman No. 1 filter paper while the remaining plant residue was discarded. The filtered supernatant obtained was evaporated to dryness and the weight of the crude dried chloroform extract obtained was measured (3.87 g). The dried extract was diluted in dimethyl sulfoxide (DMSO) (1 : 50; w/v) and considered as the stock solution with dose of 200 mg/kg.
The stock solution was diluted with DMSO to the doses of 20 and 100 mg/kg for the antinociceptive and anti-inflammatory studies.

**Preparation of Drugs** One hundred mg/kg acetylsalicylic acid (ASA) (Bayer, Singapore) and 5 mg/kg morphine (Sigma, Germany), used for the purpose of comparison, were prepared by dissolving them in distilled water (dH2O).

**Experimental Animals** Male Balb-C mice (25—30 g; 5—7 weeks) and Sprague-Dawley rats (180—200 g; 8—10 weeks old), obtained from the Animal Source Unit, Faculty of Veterinary Medicine, University Putra (UPM), Serdang, Selangor, Malaysia, were used in this study. All of the animals were kept under room temperature (27±2°C; 70—80% humidity; 12 h light/darkness cycle) in the Animal Holding Unit, Faculty of Medical and Health Sciences, UPM for at least 48 h before use. Food and water were supplied ad libitum up to the beginning of the experiments. At all times the rats were cared for in accordance with current UPM principles and guidelines for the care of laboratory animals and the UPM ethical guidelines for investigations of experimental pain in conscious animals as adopted from Zimmermann.4

All mice were equally divided into 10 groups of 7 mice each (n=7) and received (s.c.) dH2O, ASA (100 mg/kg) or CECC (10, 50 and 100% strength) 30 min prior to subjection to the abdominal constriction or hot plate tests, respectively. On the other hand, all rats were equally divided into 11 groups of 5 rats each (n=5). The first six groups were used in the formalin test and received (s.c.) dH2O, 100 mg/kg ASA, 5 mg/kg morphine or CECC (20, 100 and 200 mg/kg), respectively 30 min prior to subjection to the said test.

The second five groups were used in the anti-inflammatory study, and received (s.c.) dH2O, 100 mg/kg ASA or CECC (20, 100 and 200 mg/kg), respectively 30 min prior to subjection to the test. All of the test solutions were administered in the volume of 10 ml/kg body weight.

**Antinociceptive Assay**

**Abdominal Constriction Test** The abdominal constriction test6 as described by Zakaria et al.6 was used to evaluate the chemically-induced peripheral antinociceptive activity of CECC.

**Hot Plate Test** The 50°C hot-plate test7 with slight modification as described by Zakaria et al.6 was used to evaluate the thermally-induced peripheral antinociceptive activity of CECC.

**Formalin Test** The formalin test described by Hunskaar and Hole8 was used but with slight modifications. Pain was induced by injecting 50 µl of 5% formalin in the subplantar region of the left hind paw. Rats were given (s.c.) test solutions 30 min prior to formalin injection. The rats were individually placed in transparent Plexiglass cage observation chamber. The amount of time the animal spent licking the injected paw, considered as an indicator of pain, was recorded for duration of 30 min following the formalin injection. The early phase of nociception, indicating a neurogenic type of pain response, was measured between 0—5 minutes while the late phase of nociception, indicating an inflammatory type of pain response, was measured 15—30 minutes after formalin injection.

**Anti-inflammatory Assay** The carrageenan-induced paw edema test9 with slight modification as described by Zakaria et al.10 was used to determine the anti-inflammatory activity of CECC.

**Statistical Analysis** The results are presented as Mean±Standard Error of Mean (SEM). The one-way ANOVA test with Dunnett post-hoc test was used to analyze and compare the data, with p<0.05 as the limit of significance.

**RESULTS**

**Phytochemical Screening of the C. capsularis Leaves** The phytochemical screening of the leaves of C. capsularis has demonstrated the present of flavonoids, saponins, tannins, steroids and triterpenes but no alkaloids.

**Pharmacological Studies on the CECC** The antinociceptive profile of CECC assessed using the acetic acid-induced abdominal constriction test in mice is shown in Fig. 1. The extract, at all concentrations used, exhibited a significant (p<0.05) and concentration-independent antinociception. The 20 mg/kg CECC was found to give unexpectedly remarkable decrease in the number of abdominal constrictions (=10 folds reduction) when compared to the control group while the 100 and 200 mg/kg CECC were found to give ≈3 to 4 folds reduction in the number of abdominal constrictions, respectively. Interestingly, the 100 mg/kg CECC produced an equipotent antinociceptive activity when compared to the 100 mg /kg ASA. Five mg/kg morphine was found to cause ≈6 folds decreased in the number of abdominal con-
Fig. 1. The Antinociceptive Profile of CEDL Assessed by the Abdominal Constriction Test in Mice
Data were presented as mean ± S.E. (n=7). * Significant (p<0.05) when compared to DH2O-treated group.

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strictions when compared to the control group.

The antinociceptive profile of CECC assessed using the formalin test in rats is shown in Fig. 2. The extract exhibited significant (p<0.05) antinociceptive activity in both phases of the formalin test, as can be seen with 5 mg/kg morphine but not 100 mg/kg ASA. Interestingly, the 100 and 200 mg/kg CECC exhibited complete analgesia in the second phase of the test.

The antinociceptive profile of CECC assessed using the hot plate test in mice is shown in Fig. 3. The CECC, at all concentrations used, was also found to show a concentration-independent antinociception. Interestingly, the significant (p<0.05) activity was observed after 30 min of the extract administration compared to the 5 mg/kg morphine that showed significant (p<0.05) activity after 1 h of its administration. Although the extract activity could be observed until the end of the experiment, the morphine-produced antinociceptive activity was significantly higher than the extract between the interval time of 1—3 hrs.

Figure 4 shows the anti-inflammatory profile of CECC assessed using the carrageenan-induced paw edema test in rats. The extract, at all concentrations used, caused a significant (p<0.05) decrease in the thickness of edematous paw for the first 6 hrs when compared to the control group. This activity was found to diminish in the last 2 hrs of the experimental time. As a comparison, the 100 mg/kg ASA produced
Discussion

The present study has confirmed the possible antinociceptive and anti-inflammatory properties of the CECC. The ability of CECC to reduce the number of abdominal constrictions indicates the presence of antinociceptive activity but did not specify the involvement of peripheral or central mechanisms.\(^{11}\) However, according to Ballou et al.\(^{12}\) the acetic acid-induced abdominal constrictions were brought about by the release of cyclo-oxygenase (COX)-synthesized prostacyclin within the abdominal cavity of the mice and thus the observed antinociceptive activity could plausibly be due to inhibition of peripheral COX. Further studies using the formalin and hot plate assays are usually performed before a final conclusion could be drawn on the type of mechanism involved in the extract-induced antinociceptive activity.

The formalin test produced a distinct biphasic nociceptive response generally regarded as the early and late phases.\(^{13}\) According to Tjølsen et al.,\(^{14}\) the early phase is a result of direct stimulation of nociceptors by formalin and is an acute reaction observed immediately after the administration of formalin and persists for 5 minutes. The late phase, on the other hand, appears between 15 and 60 min after the for-
malin administration and is due to the inflammatory processes as well as activation of the neurons located in the dorsal horns of the spinal cord.\textsuperscript{14} In term of the activity, drugs that act centrally (i.e., opioids) have been demonstrated to affect both phases while drugs that act peripherally (i.e., NSAIDs) only influence the said late phase.\textsuperscript{11} The ability of the CECC to inhibit both phases of the formalin test suggested the involvement of central mechanism and is concomitant with the activity shown by centrally acting analgesic drugs (morphine).\textsuperscript{11}

Study using the hot plate test, which is usually used to determine the involvement of central antinociceptive mechanism\textsuperscript{15} has, at least, confirmed the ability of the extract to influence the central mechanism as seen with the formalin test. Additionally, this study has also demonstrated the extract ability to reduce nociception related to the non-inflammatory, acute nociceptive stimuli. Hosseinzadeh and Younesi\textsuperscript{30} has demonstrated that drugs acting centrally inhibit the abdominal constriction and hot plate tests while those acting peripherally inhibit only the abdominal constriction test.\textsuperscript{17} However, Pini et al.\textsuperscript{15} have earlier reported on the central antinociceptive activity of paracetamol, which is due to inhibition of the central COX.\textsuperscript{12} Taking into account the finding made by Pini et al.,\textsuperscript{30} we suggested that the centrally-mediated antinociceptive activity of CECC involved, at least in part, inhibition of the central COX activity. Other than that, the ability to reduce the thermal-induced nociceptive stimulus indicates the extract ability to cross the blood-brain barrier (BBB)\textsuperscript{18} since the said nociceptive mechanism involved the stimulation of a region within the central nervous system (i.e., spinal cord activation).\textsuperscript{15}

The above mentioned findings have also revealed the extract effectiveness in inhibiting the chemically- and thermally-induced nociception. Furthermore, the ability of CECC to affect both the chemically- and thermally-induced nociceptive response is a characteristic of strong analgesics like opioid agonists.\textsuperscript{3,19} It has also been reported that chemically- and thermally-induced tests elicit the selective stimulation of C and A\(\delta\) fibers, respectively.\textsuperscript{20,21} Furthermore, the extract was also suggested to be able to cause direct action to the nociceptor and inhibit the inflammatory mediators release based on the fact that it blocked both phases of the formalin test. It is claimed that the early phase is due to direct formalin effect on the nociceptor while the late phase is caused by the release of inflammatory mediators like prostaglandins.\textsuperscript{13,14}

Other than that, the extract was also suggested to produce its antinociceptive activity by directly inhibiting the prostaglandin synthesis/release or indirectly blocking the peripherally- or centrally-mediated COX enzymes.\textsuperscript{12,15}

The ability of the CECC to reduce the thickness of edematous paw suggested the present of an anti-inflammatory activity.\textsuperscript{11} This finding could be supported by earlier claimed made by Attaway and Zaborsky\textsuperscript{22} that compounds with anti-inflammatory activity might also possess antinociceptive activity. According to Vinegar et al.,\textsuperscript{23} the carrageenan-induced inflammation could be divided into the early and late phases in which the former could be associated the release of histamine and serotonin while the latter could be due to the release of prostaglandin-like compounds.\textsuperscript{24,25} Di Rosa et al.\textsuperscript{26} have also demonstrated the ability of the steroidal and non-steroidal anti-inflammatory drugs to inhibit the late phase of inflammation. These findings are in line with our observation on the ability of the extract to block the inflammatory phase (late phase) of the formalin test. Furthermore, this finding has scientifically confirmed the folklore used of \textit{C. capsularis} leaves as demulcent.

Although DMSO was found to show significant activity in the abdominal constriction and late phase of the formalin tests, as well as in the paw edema test, its effects were less remarkable when compared to the extract. Thus, it is plausible to suggest that all of the activities observed were attributed to the extract, but not the DMSO. Furthermore, the ability of the extract, but not the DMSO, to show antinociceptive activity in the hot plate test, seems to support the above suggestion. However, these observations were against several reports made earlier.\textsuperscript{27,28}

Other than that, the concentration-independent antinociceptive activity of CECC seen with the abdominal constriction test is not well understood. However, it is well known that certain drugs exhibited desired therapeutic effect only within a narrow drug doses or plasma concentration range.\textsuperscript{29} Drugs with this type of phenomenon, known as therapeutic windows, will exhibit suboptimal beneficial effects or even decline in effects if the dose/concentration were below or above this narrow therapeutic range. However, factors such as strains of animals, polarity of extracts and routes of administration could also be the contributing fac-
tors.

Although extensive study on the pharmaco-chemical property of *C. capsularis* has never been carried out, earlier study by Manzoorkhuda and Habermehl has traced the present of corosolic acid, ursolic acid and oxo-corosin in the plant as well as its counterpart, *C. olitorius*. Further studies on the active constituents of the various part of *C. olitorius* have demonstrated the present of dammarane-type triterpene, glycoside, xylans, proteins and amino acids.

Of these compounds, dammarane triterpene glycoside (3-glucoside of 20, 24-epoxy-3β, 12β, 25, 30-tetrahydroxy-dammarane) and glycoside (25, 30-O-beta-diglucopyranoside) have been reported to present in the leaves of *C. olitorius*. Study carried out by Yoshikawa et al. has demonstrated the present of dammarane-type triterpene oligoglycosides in the seeds of *Zizyphus jujube*, which was also found to inhibit the release of histamine from rat peritoneal exudate cells induced by antigen-antibody-reaction. Although the present of dammarane-type triterpene glycoside in *C. capsularis* and its relation to the observed anti-inflammatory activity of CECC is yet to be proven, the observed activities could plausibly be due to the presence of the said compound. Finally, we conclude that the CECC possessed antinociceptive and anti-inflammatory activities, which justify its traditional uses in the treatment of various ailments.

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