Antinociceptive and Anti-inflammatory Activities of Dicranopteris linearis Leaves
Chloroform Extract in Experimental Animals

Zainul Amiruddin Zakaria,1,a,b,c Zuleen Delina Fasya Abdul Ghani,1,e Raden Nur Suraya Raden Mohd. Nor,1,e Hanan Kumar Gopalan,e Mohd. Roslan Sulaiman,b and Fatimah Corazon Abdullahd

1Faculty of Biotechnology and Life Sciences, Universiti Industri Selangor, Jalan Zirkon A7/A, Sekyen 7, 40000 Shah Alam, Selangor, Malaysia, bDepartment of Biomedical Sciences, Faculty of Medicine and Health Science, and 3Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

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The present study was carried out to establish the antinociceptive and anti-inflammatory properties of Dicranopteris linearis leaves chloroform extract in experimental animals. The antinociceptive activity was measured using the abdominal constriction, formalin and hot plate tests, while the anti-inflammatory activity was measured using the carrageenan-induced paw edema. The extract, obtained after 72 h soaking of the air-dried leaves in chloroform followed by evaporation under vacuum (40°C) to dryness, was dissolved in dimethyl sulfoxide to the doses of 20, 100 and 200 mg/kg and administered subcutaneously 30 min prior to subjection to the above mentioned assays. The extract, at all doses used, was found to exhibit significant ($p < 0.05$) antinociceptive activity in a dose-dependent manner. However, the significant ($p < 0.05$) anti-inflammatory activity observed occur in a dose-independent manner. As a conclusion, the chloroform extract of D. linearis possesses antinociceptive and anti-inflammatory activity and thus justify its traditional uses by the Malays to treat various ailments.

Key words——Dicranopteris linearis; chloroform extract; antinociceptive activity; anti-inflammatory activity

INTRODUCTION

Dicranopteris linearis (L.), known to the Malay’s as “Resam”, is a plant that belongs to the family Gleicheniaceae.1,2 D. linearis is common in secondary forests and grows well on poor clay soils. It belongs to a family of ferns3,4 that possess enormous economic utility attributed to their medicinal, food and aesthetic values.5 The leaves of D. linearis were used in the Malay’s traditional medicine as a cooling drink and also to reduce fever.1,6 Furthermore, the plant is also used by the people of Papua New Guinea to treat external wound, ulcers and boils,7 by the people of Indochina to overcome the intestinal worms infection8 and by the tribes on Indian mountain in the treatment of asthma and for woman’s sterility.5

Phytochemical study has revealed the present of various types of flavonoids, particularly of the flavonol 3-O-glycosides types, in the leaves of D. linearis.8 Other than that, reports involving D. linearis focused on the rare earth elements9 and allergenicity.10 Based on the traditional medicinal values described earlier and the lack of exploration on the potential pharmacological properties of D. linearis, the present study was aimed at elucidating the antinociceptive and anti-inflammatory properties of chloroform extract of D. linearis leaves.

MATERIALS AND METHODS

Plant Material The leaves of D. linearis were collected in June-July 2005 from its natural habitat in Shah Alam, Selangor, Malaysia and a voucher specimen (SK 855/05) was deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia.

Phytochemical Screening of the D. linearis Leaves

The phytochemical screening of D. linearis leaves was carried out according to the standard screening tests and conventional protocols as described by Ikhiiri et al.11

Preparation of Chloroform Extract of D. linearis (CEDL) The CEDL was prepared by soaking the air-dried powdered leaves of D. linearis (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 (4.71 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 further diluted with DMSO to the doses of 20 and 100 mg/kg and used together for the antinociceptive and anti-inflammatory studies.

Preparation of Drugs 100 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 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, Universiti Putra (UPM), Selangor, Malaysia, were used in this study. They were kept under room temperature (27±2°C; 70—80% humidity; 12 h light/darkness cycle) in the Animal Unit, Faculty of Biotechnology and Life Sciences, Universiti Industri Selangor for at least 48 h before use. The UPM ethical guidelines for investigations of experimental pain in conscious animals adopted from Zimmermann12 were used throughout the experimental duration. Food and water were supplied ad libitum up to the beginning of the experiments.

All mice were equally divided into 10 groups of 7 mice each (n=7) and received (sc) dH2O, ASA (100 mg/kg) or CEDL (10, 100 and 200 mg/kg) 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 from the formalin test and received (sc) dH2O, 100 mg/kg ASA, 5 mg/kg morphine or CEDL (10, 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 (sc) dH2O, 100 mg/kg ASA or CEDL (10, 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 test described by Dambisya and Lee (1995)13 was used to evaluate the chemically-induced antinociceptive activity of CEDL.

Formalin Test The formalin test described by Hunskar and Hole14 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 (sc) 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,13 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.

Hot Plate Test The 50°C hot-plate test16 with slight modification as described by Zakaria et al.17 was used to evaluate the thermally-induced central antinociceptive activity of CEDL.

Anti-inflammatory Assay The carrageenan-induced paw edema test18 with slight modification as described by Zakaria et al.19 was used to determine the anti-inflammatory activity of CEDL.

Statistical Analysis The results are presented as Mean ± Standard Error of Mean (S.E.M.). 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 D. linearis Leaves The phytochemical screening of the leaves of D. linearis has demonstrated the present of flavonoids, saponins, tannins, steroids and triterpenes, but not alkaloids.

Pharmacological Studies on the CEDL Figure 1 shows the antinociceptive profile of CEDL assessed using the acetic acid-induced abdominal constriction test in mice. The extract, at all doses used, exhibited a significant (p<0.05) antinociceptive activity in a dose-dependent manner. The 20 mg/kg CEDL showed an equieffective activity when compared to the 100 mg/kg ASA, which is approximately 2 folds decreased in the number of abdominal constrictions. Interestingly, the 100 and 200 mg/kg CEDL caused approximately 10 folds decreased in the number of abdominal constrictions.
Figure 2 shows the antinociceptive profile of CEDL assessed using the formalin-induced nociceptive test in rats. The extract was found to exhibit significant ($p < 0.05$) antinociceptive activity in the early and late phases of the test in a dose-dependent manner. However, the 20 mg/kg CEDL was effective in the late, but not the early, phase. Furthermore, the extract activity, particularly at the doses of 100 and 200 mg/kg, was more effective than that of 5 mg/kg morphine in both phases.

Figure 3 shows the antinociceptive profile of CEDL assessed using the hot plate test in mice. The CEDL, at all doses used, also exhibited a dose-dependent ($p < 0.05$) antinociceptive activity with the 100 and 200 mg/kg extract showed an activity that lasted until the end of the experiment. The 20 mg/kg CEDL activity was observed only for the first 4 hrs before it completely diminished at the end of the experiment. Generally, the 5 mg/kg morphine produced an antinociceptive activity that was greater than the extract in the first 2 hrs before it started to decline gradually for the next 3 hrs. Interestingly, the 100 and 200 mg/kg CEDL activity were found to be greater than that of the morphine when measured at the last interval time (5 hrs).

Figure 4 shows the anti-inflammatory profile of CEDL assessed using the carrageenan-induced paw edema test in rats. Interestingly, the CEDL produced significant ($p < 0.05$) anti-inflammatory activity that did not depend on the doses of extract used. The ex-
tract, at all doses used, exhibited an activity that was greater than that of the 100 mg/kg ASA. However, this activity lasted only for the first 6 hrs after the carrageenan administration.

**DISCUSSION**

The present study has demonstrated the potential of *D. linearis* lipid-soluble extract as antinociceptive and anti-inflammatory agents, and at least confirmed its use in the treatment of ulcers and wound. The CEDL ability to reduce the nociceptive effect when assessed using the abdominal constriction and hot plate tests indicate its ability to reverse the chemical- and thermal-induced nociceptive activity with the activity seen with the latter assay also indicates the extract involvement in the central antinociceptive mechanism. In addition, the ability of CEDL to inhibit the chemically- and thermally-induced nociceptive response suggested that the extract possessed a characteristic of strong analgesics like opioid agonists. On the other hand, the ability of the CEDL to inhibit both phases of the nociceptive activity induced by the formalin administration also indicates its potential central antinociceptive activity. Other than that, the activity seen with the formalin test suggested that the extract inhibited nociceptive activity via direct action on...
the nociceptor (early phase) or indirectly through the inhibition of inflammatory mediators’ release (late phase).

Although the abdominal constriction test is a very sensitive test that is thought to involve stimulation of, at least in part, the local peritoneal receptors, the test was considered as a non-specific test since it did not signify the involvement of the peripheral and/or central mechanism in the observed activity. Thus, the use of other tests like the hot plate and formalin tests are required to be carried out before a final conclusion could be made on the actual mechanism involved in the CEDL antinociceptive activity. However, recent findings by Ballou et al.24 involved in the CEDL antinociceptive activity. The exposure of animals paw to thermal stimuli in the hot plate test will lead to the development of a non-inflammatory, acute nociceptive response and the ability of the extract to inhibit the thermal-induced nociceptive response indirectly indicates its ability to also inhibit non-inflammatory pain. The fact that the extract inhibits both types of tests suggested that it’s possessed a centrally mediated activity like morphine.26 However, other than the well known involvement of the opioidergic and non-opioidergic systems in the central antinociceptive mechanisms, the inhibition of central COX could also be suggested as part of the mechanism that leads to the observed CEDL central antinociceptive activity. This suggestion is based on the report made by Ballou et al.24 on the present of central COX that also contributes to the central nociceptive processes and that paracetamol-induced central antinociceptive activity involved the central COX inhibition.25 On the other hand, formalin injection has been demonstrated to produce a distinct biphasic nociceptive response, characterized as an early (0—5 minutes after the formalin injection) and late (between 20 and 60 min after the formalin injection) phases.27 According to Tjølsen et al.,29 the early phase involved a direct effect of formalin on nociceptors, which did not involve an inflammatory mediators release, whereas the late phase is involved an inflammatory processes. Due to these characteristics, the formalin test is usually used in determining the non-anti-inflammatory, antinociceptive effect of extracts/drugs27 as well as for elucidating the extracts/drugs mechanisms of analgesia. The ability of the CEDL to block the early nociceptive phase does indicate that the extract possessed a non-anti-inflammatory, antinociceptive activity.

Carrageenan-induced rat paw edema test is regarded as one of the best methods for screening of anti-inflammatory properties of extracts/drugs.23,30 The carrageenan-induced edema production is associated with the presence of kinins and polymorphonuclear leucocytes, which involved in the release of pro-inflammatory mediators like prostaglandins.31 The ability of the CEDL to reduce the thickness of the edematous hind paw32,33 indicates the anti-inflammatory properties of the extract. This finding seems to justify the traditional use of the plant in the treatment of broiler, ulcers or asthma by the people in Papua New Guinea and India.5,6 The fact that certain drugs exhibit desired therapeutic effects only over a narrow range of doses or plasma drug concentrations could be used to explain our recent findings on the CEDL concentration-independent anti-inflammatory activity. This activity might be associated with a phenomenon known as therapeutic window in which certain compounds/drugs will only produce suboptimal beneficial activities or even decline in activities when the dose used was below or above the narrow therapeutic range.34

The antinociceptive and anti-inflammatory properties of the CEDL could be attributed, at least in part, to the present of flavonoids or tannins in the leaves of the plant. The ability of the flavonoid compounds to produce an anti-inflammatory activity has been proven recently.35 It is also believed that the flavonoid compounds were also responsible for the antinociceptive activity of the CEDL based on the claimed made by Attaway and Zaborsky that compounds with anti-inflammatory activity will also possess antinociceptive activity. Furthermore, the ability of the CEDL to exhibit remarkable antinociceptive activity when compared to morphine indicates that the extract possessed extremely active compounds. As a conclusion, the lipid-soluble compounds of the CEDL possessed potential antinociceptive and anti-
inflammatory activities that require further attention and thus justify the use of the plant in the treatment of various ailments.

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REFERENCES


