Antinociceptive activity of the leaves of *Ficus deltoidea* aqueous extract

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Abstract

*Ficus deltoidea* or ‘mas cotek’ has been widely known to possess therapeutic property in traditional medicine. Hence, an investigation was carried out on the aqueous extract of *Ficus deltoidea* leaves for antinociceptive activity in several in vivo experimental models. In this study we investigated the effect of *F. deltoidea* in adult male Balb C mice and adult male Sprague-Dawley rats at doses of 100, 300, and 1000 mg/kg against acetic acid-induced abdominal writhing, formalin and hot plate tests to evaluate its analgesic property. The results showed that intraperitoneal administration of aqueous extract of *Ficus deltoidea* leaves at all doses indicated the present of both peripheral and central mediated activities. Moreover, in the hot plate test, it significantly raised the pain threshold. In the formalin test, the extract produced a significant reduced paw licking caused by the formalin-induced pain. The results demonstrate that *Ficus deltoidea* presents antinociceptive activity in mice, which supports its folkloric use as an analgesic.

Keywords: *Ficus deltoidea*, Antinociceptive, Acetic acid-induced abdominal test, Formalin test, Hot plate test

Introduction

*Ficus deltoidea* is an epiphytic shrub which is native and widely distributed in several countries of the Southeast Asia. In Malaysia, it is commonly known as Mas Chotek, Serapat Angin, Telinga Beruk and other names \cite{1}. Different parts of the plant are used traditionally to treat various kinds of ailments. The fruit are chewed to relief headache, toothache and cold, powdered root and leaves of the plants has been applied externally to wounds and sores, and around the joints for relief of rheumatism.

Decoction or infusion from the whole plants is a well known traditional herbal drink for women after childbirth to help strengthen the uterus. Moreover, it improve blood circulation, regain energy and it is believe to enhance sexual desire (personal communication from local people where the plants was collected). Besides being one of the popular herbs used in Malay traditional medicine, the pharmacological properties of this plant have not yet been studied, only one report of blood glucose lowering effect has been published \cite{2}. The basis form the traditional use of this herb in the management of painful related conditions has not been scientifically verified to the best of our knowledge. The present study therefore, is aimed at investigating possible antinociceptive activity of the aqueous extract from the leaves of *F. deltoidea* and to explore its possible mechanism of action using chemical and thermal nociception models.

Materials and Method

Plant Material

Fresh *Ficus Deltoidea* leaves were collected from their natural habitat around Lata Kinjang, Tapah Perak. A voucher specimen (SK 1510/2007) was deposited at the Phytomedical Herbarium, Institute of Biosciences, Universiti Putra Malaysia, Serdang, Selangor.

Aqueous extract

The leaves of *F. deltoidea* were dried at room temperature until constant weights were gained (2 weeks). The dried leaves were grinded into small particle, weighed (700g) and added with distilled water in the ratio of 1:20 (w/v). The mixture was left for 72 hours at room temperature and the supernatant was filtered using filter paper. The aqueous supernatant obtained, considered as stock solution with 100% concentration was later diluted to various concentrations (100, 300, 1000mg/kg).

Animals

Male Sprague-Dawley rats (180-200g; 8-10 weeks old) and Male Bald-C mice (25-30g; 5-7 weeks) were used in this study. The animals were kept under room temperature (27 ± 2°C; 70-80% humidity; 12 h light/darkness cycle) with access to food and water.
librium. The animals were acclimatized to the laboratory for at least 1 hour before testing and were used only once throughout the experiments. Experiments reported in this study were performed in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [4].

Antinociceptive tests

Acetic acid-induced abdominal writhing test
The acetic acid-induced abdominal writhing test was performed according to procedures described previously [3,5]. This test evaluate the chemically induced peripheral antinociceptive activity of AEFD. The acetic acid prepared as 0.6% (v/v) solution in dH2O and used to induce pain in mice peritoneal cavity, was administered (i.pl) in the volume of 10ml/kg, 30 min after the sc administered of the respective dH2O, Aspirin, and AEFD(100, 300, 1000mg/kg). The abdominal writhing resulting from the injection of acetic acid consists of a contraction of the abdominal region together with a stretching of hind limbs. The behavioral responses to nociception including biting, licking and scratching of the injected paw were noted and the time spent was recorded up to 30 min. Antinociceptive activity was indicated by the reduction in the mean of the number of abdominal constriction in the test groups compared to the control group.

Formalin test
The formalin test was performed according to Dubuisson and Dennis [6]. 100, 300, 1000mg/kg of AEFD were administered into rats (sc) in the volume of 10ml/kg. After 30 minute, 50ml of 5% formalin were injected into the subplantar region of the left hind paw on rats for antinociceptive studies. The rats then were rested 

Hot plate test
The hot plate test described in [3] with slight modification was used to assess the thermal-induced central antinociceptive activity of AEFD. In these experiment, the hot plate (Ugo Basile, model-7280) was maintained at 50±0.2°C. Mice were placed into the Plexiglas walls on the heated surface to constrain their locomotion on the palate and latency to a discomfort reaction (licking of the paws or jumping) was recorded at 30, 60, 120, 180, 240 and 300 min following administration of the test agents. The cut off time of 20s was chosen to indicate complete analgesia and to avoid tissue injury. The prolongation of the latency time compared with the values of the control was used for statistical comparison. Control animals were treated with dH2O (10mg/kg; sc) while morphine (5mg/kg) was used a references .

Statistical analysis
The statistical analyses were performed by ANOVA, followed by Dunnett’s multiple comparison tests. The results were expressed as the mean ± S.E.M. to show variation in groups. Differences are considered significant when P<0.05.

Results and Discussion
The present study was carried out to assess the potential pharmacological properties of AEFD based on traditional claim reported earlier of Ficus deltoidea. It was found to display an antinociceptive activity when accessed using the acetic acid-induced writhing, formalin-induced paw licking, and thermal nociception in the hot plate test. These methods investigated both the peripheral and central antinociceptive mechanisms. The acetic acid induced abdominal constriction is believed to show the involvement of peripheral mechanisms, whereas the hot plate test is believed to show that of central mechanisms and the formalin is used to investigate both peripheral and central mediated mechanism [8].

Results of the present study demonstrated that the AEFD possessed antinociceptive activity evident in all the nociceptive models, which suggestive of the present of the both central and peripheral mediated activities.

The acetic acid-induced writhing test is a chemical stimulus widely used for the evaluation of peripheral antinociceptive activity. In the acetic acid induced abdominal constriction test, the results showed that the AEFD extract (300 and 1000 mg/kg) potently and significantly reduced the number of abdominal writhing in a dose dependant manner as compared to control animals. It has been postulated that acetic acid acts directly by inducing the release of endogenous mediators, such as PGE2.
Figure 1 The antinociceptive profile of AEFD assessed by the acetic acid-induced writhing test in mice.

Figure 2 The antinociceptive profile of AEFD assessed using the formalin test in rats.
(prostaglandin E2) and PGF2α in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs [9,10]. Therefore the result of the acetic acid induced writhing strongly suggests that the mechanism of this extract may be linked partly to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissue, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor. AEFD of 100 mg/kg did not display any significant reduce in the abdominal writhing test. Figure 1 showed the antinociceptive profile of AEFD assessed using the acetic acid induced abdominal constriction test in mice.

The formalin test which is used to assess various classes of analgesic drugs [11], is able to discriminate between central and peripheral pain components. It produce a distinct biphasic response and may act differently in the early and late phase of the test. Figure 4.2 show the antinociceptive profile of AEFD assessed using the formalin test in rats. Significant (P<0.05) antinociceptive activity can be seen in the early and late phase of the formalin-induced nociception. In the early phase, administration of 300mg/kg showed the highest inhibition in then licking response followed by 1000 mg/kg and 100 mg/kg of AEFD whereas in the late phase, 100 mg/kg of AEFD showed the highest inhibition followed by 300 mg/kg and 1000 mg/kg. This observation indicated that high dose of AEFD extract does not ensure high inhibition. Morphine (5 mg/kg), the reference drug used showed significant inhibition of both the early and late phase of the formalin test. It is also well known that the formalin test may involve sensorial C-fibers [12] in early phase and a combined process generated by peripheral inflammatory tissue and functional changes in the dorsal horn in late phase [15]. Inhibition of both phases of pain as observed with the extract in this study showed that they contain active analgesic principles acting both centrally and peripherally.

The central analgesic effect of the aqueous extract may be supported by the results recorded in the hot plate test which is a selective method able to screen centrally acting opiate analgesic drugs [7]. It was demonstrated that intraperitoneal administration of the 1000 mg/kg of AEFD extract exerts significant prolonged in the response latency time to the heat stimulus. 1000 mg/kg This effect begun early at 60 min after administration of aqueous extract and persists until the following fifth hour. This pattern can also be observed in 100 and 300 mg/kg extracts. As expected, morphine (5 mg/kg, i.p.) significantly increased the latency time to the nociceptive response compared with control group. These results suggest that activation of central opioid receptors are involved in the antinociceptive action of the AEFD extract. Figure 4.3 show the antinociceptive profile of AEFD assessed using the hot plate test in mice.

Figure 3 The antinociceptive profile of AEFD assessed by the hot plate test in mice
Conclusions

In conclusion, this study indicated that the aqueous extract of *F. deltoidea* (AEFD) possess significant antinociceptive effects in laboratory animals at the doses investigated. The results support the traditional use of this plant in some painful conditions and also suggest the presence of biologically active principles which may be worth further investigation.

References