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**Antinociceptive and Anti-inflammatory Properties of
Melastoma malabathricum Leaves Chloroform
Extract in Experimental Animals**

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Abstract: The present study was carried out to establish the antinociceptive, anti-inflammatory and antipyretic properties of *Melastoma malabathricum* leaves chloroform extract in experimental animals. The antinociceptive activity was measured using the abdominal constriction, hot plate and formalin tests, while the anti-inflammatory 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 *vacuo* (40°C) to dryness (4.38 g), was dissolved in dimethyl sulfoxide to the doses of 20, 100 and 200% and administered subcutaneously 30 min prior to subjection to the above mentioned assays. The extract, at all concentration used, was found to exhibit significant ($p < 0.05$) antinociceptive and anti-inflammatory activities. Only the effect observed with the formalin test occurred in a concentration-dependent manner. It is concluded that the lipid-soluble compounds within the chloroform extract of *M. malabathricum* leaves possess potential antinociceptive and anti-inflammatory agents that require further attention.

Key words: *Melastoma malabathricum*, chloroform extract, antinociceptive activity, anti-inflammatory activity

Introduction

Melastoma malabathricum, a plant that belongs to the family Melastomataceae, is a common shrub found in previously cleared land and waste places (Burkill, 1966). The plant, also known to the Malays as 'Senduduk', has been used in the traditional medicine to treat various types of ailments (Jaganath and Ng, 2000). For examples, the chewed or pounded leaves of *M. calabura*, were pasted onto the inflamed wound while juices of the finely chopped leaves were squeeze onto the inflamed wound to stop bleeding (Ahmad *et al.*, 1993). In addition, Jaganath and Ng (2000) have also reported the use of the powdered leaves of *M. malabathricum* as an astringent for dysentery or to alleviate the discomfort of hemorrhoids while the Institute of Medical Research (2002) have also listed the leaves application onto wounds and pox scars to enhance the healing process. Other than that the liquid obtained after boiling the leaves can be use to treat diarrhea (Institute of Medical Research, 2002). However, all of the claims above have never been scientifically proven. Recent scientific study by Sulaiman *et al.* (2004) has revealed the potential of the ethanolic extract of *M. malabathricum* leaves as an antinociceptive agent that act at the peripheral and central levels. Opioid receptors have also been

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shown to take part in the reported activity of the extract. Although there is claim on the presence of compounds like quercetin, quercitrin, rutin, β -sitosterol, α -amyrin and sitosterol 3-O- β -D-glucopyranoside in the plant (Sulaiman *et al.*, 2004) there is no scientific publication to confirm that finding.

Despite the fact that the *M. malabathricum* is famously used in the Malays' traditional culture to treat various ailments and due to the lack of scientific study to establish the its pharmacological potentials, the present study was carried out to evaluate the antinociceptive and anti-inflammatory properties of the lipid-soluble compounds of *M. malabathricum* leaves obtained through chloroform extraction.

Materials and Methods

Plant Material

M. malabathricum were collected in August-September, 2005 from its natural habitat in Shah Alam, Selangor, Malaysia. It was identified by Mr. Shamsul Khamis, a botanist at the Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia and a voucher specimen (SK 507/03) was deposited at the Herbarium of the Laboratory of Natural Products, IBS, UPM, Malaysia.

Phytochemical Screening of the M. malabathricum Leaves

The phytochemical screening of *C. malabathricum* leaves was carried out according to the standard screening tests and conventional protocols as described by Ikhiri *et al.* (1992).

Preparation of Chloroform Extract of M. malabathricum (CEMM)

The CEMM was prepared by soaking the air-dried powdered leaves of *M. malabathricum* (20 mg) in chloroform in the ratio of 1: 20 (w/v) for 72 h. 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 (4.38 g). The dried extract was diluted in Dimethyl Sulfoxide (DMSO) (1:50; w/v) and considered as the stock solution with 100% concentration/strength. The stock solution was diluted again with DMSO to the concentrations of 10 and 50% for the antinociceptive and anti-inflammatory studies. Simple calculation has demonstrated that the 10, 50 and 100% concentrations CEMM were approximately equal to the doses of 20, 100 and 200 mg kg⁻¹, respectively.

Preparation of Drugs

Hundred mg kg⁻¹ Acetylsalicylic Acid (ASA) (Bayer, Singapore) and 5 mg kg⁻¹ morphine (Sigma, Germany), used for the purposed 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 Malaysia (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 mice and 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 (1983).

All mice were equally divided into 10 groups of 7 mice each ($n = 7$) and received (sc) DH_2O , ASA (100 mg kg^{-1}) or CEMM (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 (sc) dH_2O , 100 mg kg^{-1} ASA, 5 mg kg^{-1} morphine or CEMM (10, 50 and 100% concentrations), respectively 30 min prior to subjection to the test. The second five groups were used in the anti-inflammatory study and received (sc) dH_2O , 100 mg kg^{-1} ASA or CEMM (10, 50 and 100% concentration), respectively 30 min prior to subjection to the test. All of the test solutions were administered in the volume of 10 mL kg^{-1} body weight.

Antinociceptive Assay

Abdominal Constriction Test

The abdominal constriction test (Dambisya and Lee, 1995) with slight modification as described by Zakaria *et al.* (2005) was used to evaluate the chemically-induced antinociceptive activity of CEMM.

Hot Plate Test

The 50°C hot-plate test (Wilson *et al.*, 2003) with slight modification as described by Zakaria *et al.* (2005) was used to evaluate the thermally-induced central antinociceptive activity of CEMM.

Formalin Test

The formalin test described by Hunskaar and Hole (1987) was used but with slight modifications. Pain was induced by injecting $50 \mu\text{L}$ 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 (Mendes *et al.*, 2000), 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 min while the late phase of nociception, indicating an inflammatory type of pain response, was measured 15-30 min after formalin injection.

Anti-inflammatory Assay

The carrageenan-induced paw edema test (Chakraborty *et al.*, 2004) with slight modification as described by Zakaria *et al.* (2006) was used to determine the anti-inflammatory activity of CEMM.

Statistical Analysis

The results are presented as Mean \pm 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 M. malabathricum Leaves

The phytochemical screening of the leaves of *M. malabathricum* has demonstrated the present of flavonoids, saponins, tannins, steroids and triterpenes but no alkaloids.

Pharmacological Studies on the CEMM

Figure 1 shows the antinociceptive profile of CEMM assessed using the acetic acid-induced abdominal constriction test in mice. The extract, at all concentrations used, exhibited a significant

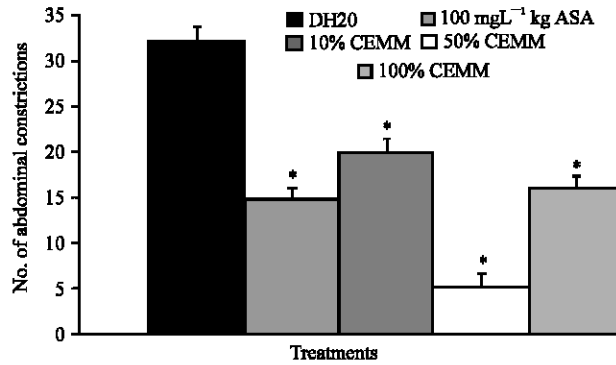


Fig. 1: The antinociceptive profile of CEMM assessed by the abdominal constriction test in mice.
*Significant ($p < 0.05$) when compared to the control group

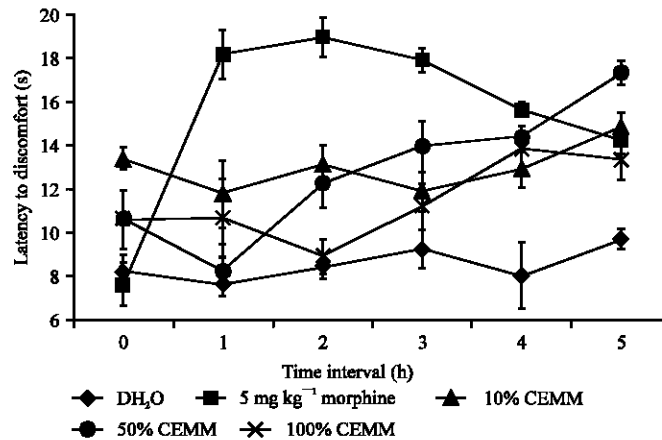


Fig. 2: The antinociceptive profile of CEMM assessed by the hot plate test in mice

($p < 0.05$) antinociceptive activity in a concentration-independent manner. The 50% concentration CEMM produced a more effective activity than that of the 100% concentration CEMM when compared to the control group with the former caused approximately 6 folds decrease in the number of abdominal constriction while the latter caused only 2 folds decrease. The 10 and 100% concentrations CEMM produced an equi-effective activity when compared to the 100 mg kg⁻¹ ASA.

The antinociceptive profile of CEMM assessed using the hot plate test in mice was shown in Fig. 2. The CEMM, at all concentrations used, also exhibited a significant ($p < 0.05$) antinociceptive activity in a concentration-independent manner. The 10% concentration CEMM was found to produce constant antinociceptive activity throughout the experimental time while the other two concentrations showed somewhat inconsistency activity between the interval 1-2 h. The 50% concentration CEMM lost its activity at the interval time of 1 h while the 100% concentration CEMM lost its activity at the interval time of 2 h after their administration. Interestingly, the activity of both concentrations of the extract were found to increase significantly ($p < 0.05$) after that and maintained until the end of the experiment. Throughout the study, the 5 mg kg⁻¹ morphine antinociceptive activity was found to be greater than that of the extract except at the last interval time (5 h) where its activity was significantly ($p < 0.05$) lower than the 50% concentration CEMM.

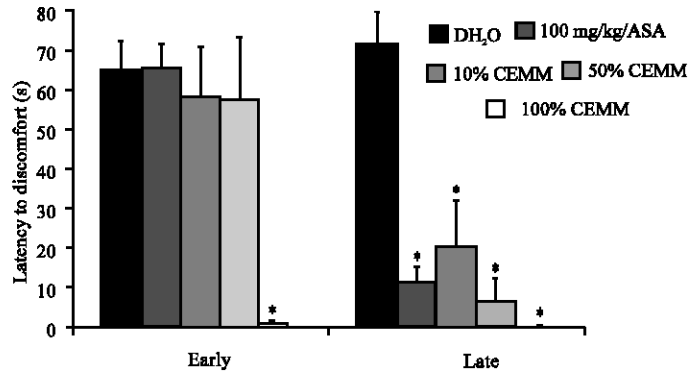


Fig. 3: The antinociceptive profile of CEMM assessed by the formalin test in rats. * Significant ($p < 0.05$) when compared to the respective control group

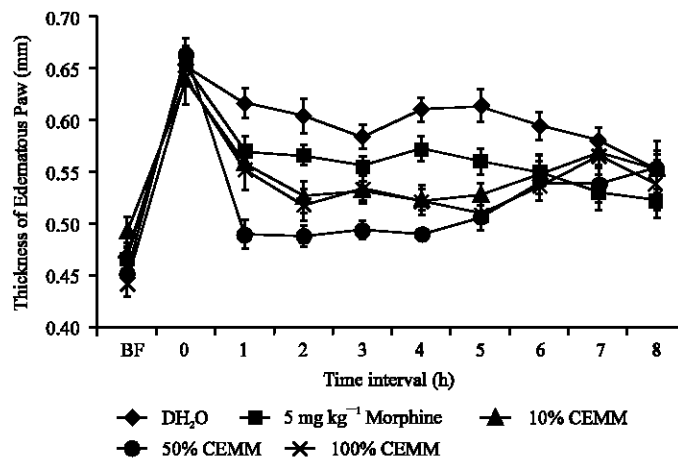


Fig. 4: The anti-inflammatory profile of AEMM assessed by the carrageenan-induced paw edema test in rats

The antinociceptive profile of CEMM assessed using the formalin test in rats was shown in Fig. 3. The extract, at all concentrations used, exhibited significant ($p < 0.05$) antinociception in both phases of nociception with a more effective activity seen in the late phase. The extract was found to block the early phase nociception only at the highest concentration used whereas the inhibition of the late phase was observed in all of the concentrations used. Interestingly, the 100% concentration CEMM caused almost complete and complete inhibition of the nociception in the early and late phases, respectively.

The anti-inflammatory profile of CEMM assessed using the carrageenan-induced paw edema test in rats can be seen in Fig. 4. The CEMM exhibited significant ($p < 0.05$) activity at all of the concentrations used but in a concentration-independent manner. The activity can be seen as early as 1 h after the extract administration and lasted for the first 6 h. Except for the 50% concentration CEMM which still maintained its significant ($p < 0.05$) activity, the other concentrations of CEMM were found to lose their anti-inflammatory activity. Except at the interval time of 4 h where it lost the activity, the 100 mg kg⁻¹ ASA was found to produce significant ($p < 0.05$) anti-inflammatory activity for the first 6 h before the activity diminished for the next 2 h. Throughout the experimental time, the 100 mg kg⁻¹ ASA anti-inflammatory activity was found to be lower than that of the extract.

Discussion

The present study demonstrated the ability of CEMM to produce the antinociceptive and anti-inflammatory activities in experimental animals. The ability to produce antinociceptive activity in those assays indirectly indicates the extract ability to inhibit chemically-and thermally-induced nociception. According to Hunskaar *et al.* (1986) and Hunskaar and Hole (1987) the ability to inhibit both types of nociceptive stimulus indicate the extract's characteristic as strong analgesics such as opioid agonists (morphine).

In addition, the ability to prolonged latency to discomfort/pain in the hot plate test coupled with the reversed of early phase nociception seen in the formalin test indicates the extract antinociceptive activity involved central mechanism (Pini *et al.*, 1997; Amanlou *et al.*, 2005). According to Hosseinzadeh and Younesi (2002), the ability to inhibit both types of tests, the abdominal constriction and hot plate tests, also suggested that the extract possessed a centrally mediated activity. Although the exact mechanism of antinociceptive action of the CEMM is not yet determined, it is plausible to suggest the involvement of opioid receptor (Sulaiman *et al.*, 2004) as part of the mechanism involved. The involvement of Cyclo-Oxygenase (COX) in the central antinociceptive mechanism of the CEMM is also worth mentioned based on the earlier report made by Pini *et al.* (1997) on the paracetamol-induced central antinociceptive activity involved the central COX inhibition and the later report by Ballou *et al.* (2000) on the present of central COX, which also contributes to the central nociceptive processes.

Other than that, the abilities to block the late phase nociception as well as to reverse the edema formation in the carrageenan-induced paw edema test suggested that the CEMM-mediated antinociceptive activity particularly involved, in part, inhibition of the COX action or prostaglandin synthesis (Amanlou *et al.*, 2000; Damas *et al.*, 1986). According to Damas *et al.* (1986), the edema development could be attributed to the presence of kinins and polymorphonuclear leucocytes, which have been associated with the release of pro-inflammatory mediators like prostaglandins. This suggestion is supported by finding made Ballou *et al.* (2000) that the abdominal constrictions induced by the acetic acid were due to the release of COX-synthesized prostacyclin within the peritoneal cavity.

The abdominal constriction test, although was very sensitive in detecting an antinociceptive property of compounds/dose levels that may be inactive in other tests (Bentley *et al.*, 1981), is not specific because it could no be use to specify the involvement of peripheral and/or central mechanism in the observed antinociceptive activity of compounds/CEMM (Chan *et al.*, 1995). It has been demonstrated that the acetic acid-induced irritation of the peritoneal cavity (Deraedt *et al.*, 1980) caused stimulation of the local receptors located at the surface of the cells lining the peritoneal cavity (Bentley *et al.*, 1983), which in turn caused release of prostaglandins, particularly the PGE₂ and PGE_{2α}, that lead to inflammatory pain (Vogel and Vogel, 1997). Due to its inability to specify the actual mechanism that take part in the CEMM antinociceptive activity, additional studies using the hot plate and formalin assays have to be carried out before a final conclusion could be draw on the actual mechanism involved in the extract antinociceptive activity.

The hot plate test has been regarded as one of the best methods to study on the central antinociceptive effect of a compound/extract (Pini *et al.*, 1997). The thermal stimulus is also described as acute, non-inflammatory nociceptive stimulus as it caused direct stimulation of the nociceptors without causing any inflammatory-mediated nociception. Centrally, but not peripherally, acting drugs increased the latency to discomfort (Hosseinzadeh and Younesi, 2002; Amanlou *et al.*, 2005) and this activity is also observed with the CEMM indicating that the extract also possessed centrally-mediated action.

The formalin test, usually used to study the non-anti-inflammatory, antinociceptive properties of a compound/extract (Hunskaar *et al.*, 1985), produces a distinct biphasic nociceptive response

described as the early and late phases. The early phase, caused by a direct effect of formalin on nociceptors and does not involve the inflammatory process, occurred almost immediately following the formalin administration and continues for 5 min while the late phase, involving the inflammatory process and activation of the neurons located in the dorsal horns of the spinal cord, can be seen between 15 and 60 min after the formalin administration (Tjølsen *et al.*, 1991). According to Chan *et al.* (1995), drugs acting centrally affect both phases while those acting peripherally were effective only in the late phase of the formalin test. Therefore, the ability of CEMM to affect both phases of the formalin test indicates its potential central antinociceptive mechanism (Chan *et al.*, 1995) and confirmed the earlier observation using the hot plate test.

Carrageenan-induced rat paw edema test has been accepted as one of the methods to screen for new anti-inflammatory compounds/extracts (Chan *et al.*, 1995; Joseph *et al.*, 2005; Di Meglio *et al.*, 2005). The development of edema has been associated with the presence of kinins and polymorphonuclear leucocytes, with the latter demonstrated to release pro-inflammatory factors, particularly prostaglandins. Thus, the edema reducing ability of CEMM could be attributed to the extract ability to inhibit prostaglandin release or COX-mediated prostaglandin synthesis (Damas *et al.*, 1986).

Except for the antinociceptive effect assessed using the formalin test, the rest of the effects occur in a concentration-independent manner. The 100% concentration CEMM activity was less effective than the other extract might be associated with the phenomenon known as 'therapeutic windows' (Tripathi, 2001) or receptor deactivation (Katzung, 1995). Certain drugs have been demonstrated to exhibit desired therapeutic effects only over a narrow range of doses or plasma drug concentrations and suboptimal beneficial activities or even decline in activities would be produced if the dose used were below or above the narrow therapeutic range (Tripathi, 2001). On the other hand, certain drugs have been reported to cause deactivation of the receptor when present at high concentration within the biological system (Katzung, 1995). However, further studies need to be carried out before we could clarify on those phenomenon involvements in the mechanism of action of CEMM.

Based on the findings, it is plausible to suggest that lipid-soluble/non-polar compounds could also take part in the observed activities as chloroform is an organic solvent that dissolved lipid-soluble compounds (Vogel *et al.*, 1989). Some compounds, such as those of flavonoid types, have been reported to be isolated through chloroform extraction (Chen *et al.*, 2005). Other than the flavonoids, tannins have also been isolated from the leaves of *M. malabathricum* (Yoshida *et al.*, 1992a; Lohézic-Le Dévéhat *et al.*, 2002). Flavonoids, in particular, have been demonstrated to possess an anti-inflammatory activity (Kim *et al.*, 2004). Our observations on the antinociceptive and anti-inflammatory activities of CEMM are concurrent with claimed made Attaway and Zaborksy (1993). Finally, it is concluded that the CEMM possessed an antinociceptive, which is mediated via peripheral and central mechanisms and anti-inflammatory activities and justify the folklore uses of the plant in treating ailments associated with pain and inflammation.

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