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Oriental Pharmacy and Experimental Medicine 2007 **7(1)**, 34-40

Analgesic and antipyretic actions of *Muntingia calabura* leaves chloroform extract in animal models

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SUMMARY

The present study was carried out to elucidate the potential of *Muntingia (M.) calabura* leaves chloroform extract (MCCE) as antinociceptive, anti-inflammatory and antipyretic agents using various animal models. The dried powdered leaves of *M. calabura* (20 g) were soaked in chloroform for 72 h and the supernatant obtained was then evaporated to dryness. The crude dried extract (0.912 g), dissolved in dimethyl sulfoxide (1 : 20; w/v) and considered as a stock solution (100% concentration/strength), was then diluted to the concentrations of 10 and 50% and used together in all experimental models. The MCCE was found to show significant ($P < 0.05$) antinociceptive and antipyretic activities, but less remarkable anti-inflammatory activity. Only the antinociceptive activity of MCCE measured using the abdominal constriction test and in the first phase of the formalin test occurred in a concentration-dependent manner. The anti-inflammatory activity of 50 and 100% concentrations MCCE was observed only at the range of time interval of 60 - 120 and 60 min, respectively. Based on the results, we conclude that the *M. calabura* leaves chloroform extract possessed remarkable antinociceptive and antipyretic, but less effective anti-inflammatory, activities and thus justifies the Peruvian folklore claims of its medicinal values.

Key words: *Muntingia calabura*; Chloroform extract; Antinociceptive; Anti-inflammatory; Antipyretic

INTRODUCTION

Muntingia (M.) calabura L., known to the Malays as '*Kerukup Siam*', is a plant that belongs to the Elaeocarpaceae family. The leaves of *M. calabura* has been used in the Peruvian folklore medicine to treat ailments such as to alleviate headache, cold, gastric ulcers and swelling of the prostate gland (Morton, 1987; Verheij and Coronel, 1992; Jensen, 1999). However, the plant medicinal value is not well documented in the Malays folklore medicine. The leaves and roots of *M. calabura* have been scientifically reported to exhibit antitumour activity (Kaneda *et al.*, 1991; Su *et al.*, 2003), an activity that is attributed to their flavonoids and flavones constituents. Our earlier studies on the *M. calabura* leaves aqueous extract pharmacological activities have demonstrated that the extract possessed antinociceptive (Zakaria *et al.*, 2006a) and antibacterial (Zakaria *et al.*, 2006b,c) activities. The antinociceptive activity of the *M. calabura* extract was assessed using the abdominal constriction (Zakaria *et al.*, 2006a), hot plate and formalin tests. Furthermore, the antinociceptive activity of *M.*

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calabura aqueous extract assessed using the abdominal constriction test was also demonstrated to involve stimulation of the L-arginine/nitric oxide/cyclic guanosine monophosphate pathway (Zakaria *et al.*, 2006a) and activation of the opioid receptor. Other than that, we have also demonstrated the anti-inflammatory and antipyretic properties of the said extract. The objective of the present study is to determine the antinociceptive, anti-inflammatory and antipyretic activities of *M. calabura* leaves chloroform extract (MCCE) in rats using various animal models.

MATERIALS AND METHODS

Plant material

The leaves of *M. calabura* were collected in July-August, 2005 from its natural habitat in Shah Alam, Selangor, Malaysia and a voucher specimen (SK 964/04) was deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia.

Preparation of *Muntingia calabura* chloroform extract

The MCCE was prepared by soaking the residues of air-dried powdered leaves of *M. calabura* (14.6 mg), which were previously soaked in distilled water (Verheij *et al.*, 1992), 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 (0.912 g). The dried extract was diluted in dimethyl sulfoxide (DMSO) (1 : 20; w/v) and considered as the stock solution with 100% concentration/strength. The stock solution was diluted with DMSO to the concentrations of 10 and 50% for antinociceptive, anti-inflammatory and antipyretic studies.

Preparation of drugs

100 mg/kg acetylsalicylic acid (ASA) (Bayer, Singapore), 5 mg/kg morphine sulfate (Sigma, Germany), used for the purposed of comparison, were prepared by dissolving them in distilled water (dH_2O).

Experimental animals

Male ICR mice (25 - 30 g; 5 - 7 weeks old), obtained from the Veterinary Animal Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), Malaysia were handled according to the procedures described in Zakaria *et al.* (2006a). At all times the rats were cared for in accordance with current UPM principles and guidelines for the care of laboratory animals set by the Animal Care and Use Committee, Faculty of Medicine and Health Sciences, UPM (Reference Code: UPM/FPSK/USH/0088) 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) or MCCE (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 ASA, 5 mg/kg morphine or MCCE (10, 50 and 100% concentrations), respectively 30 min prior to subjection to the said test. The second and third five groups were used in the anti-inflammatory and antipyretic studies, and received (*sc*) dH_2O, 100 mg/kg ASA or MCCE (10, 50 and 100% concentrations), respectively 30 min prior to subjection to the said tests. 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 (Dambisya and Lee, 1995) was used to evaluate the chemically-induced antinociceptive activity of MCCE as described Zakaria *et al.* (2005).

Hot plate test: The 50°C hot plate test (Wilson *et al.*, 2003) was used to assess the thermal-induced antinociceptive activity of MCCE as described by Zakaria *et al.* (2005).

Formalin test: The formalin test (Hunskaar and Hole, 1987) was used with slight modifications. Pain was induced by administration of 50 µl of 5% formalin into the subplantar region of the right hind paw. Rats were given (*sc*) extract/drugs 30 min before the administration of formalin. The rats were individually placed in transparent Plexiglass cage observation chamber and the amount of time each of them spent licking the injected paw was recorded for duration of 30 min following the formalin injection. The early phase of nociception was measured between 0 - 5 min while the late phase of nociception was measured 15 - 30 min after formalin injection. 100 mg/kg ASA and 5 mg/kg morphine were used as reference drugs.

Antipyretic assay

The pyretic activity (Reanmongkol *et al.*, 2002) was used with slight modification as described by Zakaria *et al.* (2006d).

Anti-inflammatory assay

The carrageenan-induced paw edema test (Chakraborty *et al.*, 2004) was used with slight modification as described by Zakaria *et al.* (2006d).

Statistical analysis

The results are presented as mean ± standard error of the 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

Fig. 1 shows the antinociceptive activity of MCCE assessed using the abdominal constriction test in mice. The extract exhibited a concentration-dependent antinociceptive activity with the highest concentration

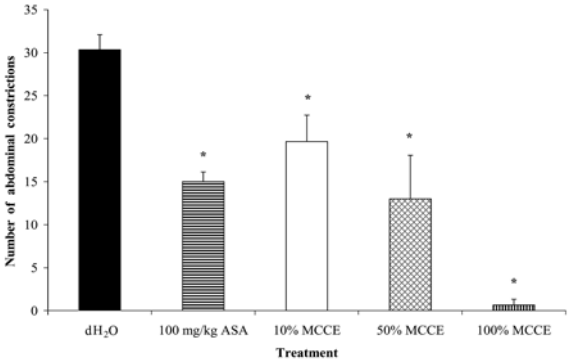

Fig. 1. The antinociceptive profile of MCCE assessed by the abdominal constriction test in mice. *Significant ($P < 0.05$) when compared to the control group.

of MCCE produced almost complete antinociceptive activity. Other than that, the 50% concentration MCCE was also found to produce an equieffective acrivity when compared to the 100 mg/kg ASA.

Fig. 2 shows the antinociceptive activity of MCCE assessed using the hot plate test in mice. The extract showed a concentration-independent antinociceptive activity that is inconsistent throughout the period of experiment. The 10 and 50% concentrations MCCE antinociceptive activity was found to increase significantly ($P < 0.05$) after 1.5 h of its administration while the 100% concentration MCCE exhibited significant ($P < 0.05$) activity after 30 min of its administration. There is inconsistency in the MCCE activity with the 10% concentration MCCE

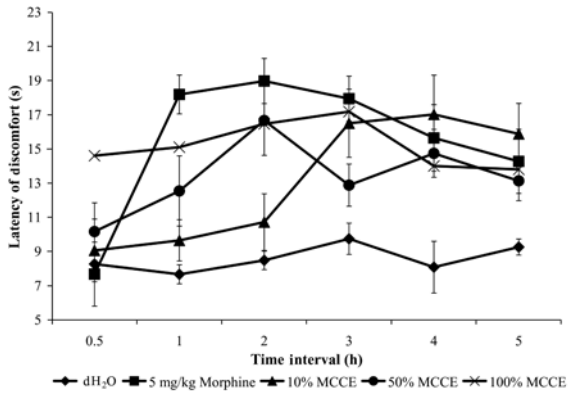

Fig. 2. The antinociceptive profile of MCCE assessed by the hot plate test in mice.

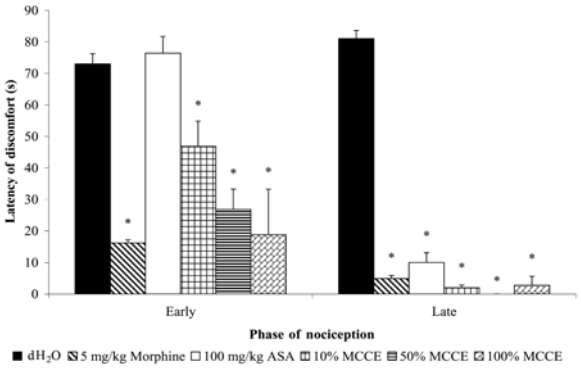

Fig. 3. The antinociceptive profile of MCCE assessed by the formalin test in rats. *Significant ($P < 0.05$) when compared to the respective control group.

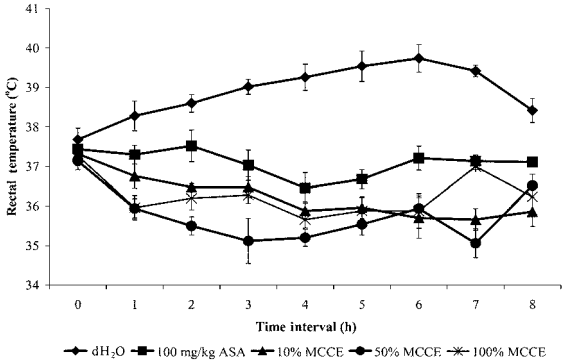

Fig. 5. The antipyretic profile of MCCE assessed by the brewer's yeast-induced pyrexia test in rats.

activity increased while the 50 and 100% concentrations MCCE activity diminished at the end of the experiment.

Fig. 3 shows the antinociceptive activity of MCCE assessed using the formalin test in rats. The extract exhibited significant ($P < 0.05$) antinociceptive activity in both phases of nociception. The concentration-dependent activity was observed in the early, but not late, phase of the formalin test.

Fig. 4 shows the anti-inflammatory activity of MCCE assessed using the carrageenan-induced paw edema test in rats. The extract anti-inflammatory activity, at all concentrations used, was less effective than that of 100 mg/kg ASA and was inconsistent throughout the period of experiment. The 50 and 100% concentrations MCCE activity were observed at the range of time interval of 60 - 120 and 120 min after the carrageenan administration, respectively. The 100 mg/kg ASA was found to be more effective than the MCCE throughout the experiment.

Fig. 5 shows the antipyretic activity of MCCE assessed using the brewer's yeast induced pyrexia test in rats. The extract exhibited a concentration-independent antipyretic activity, which is more effective than the 100 mg/kg ASA. Interestingly, the activity lasted until the end of the experiment.

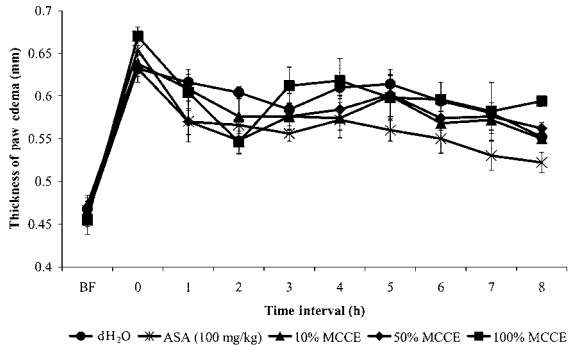

Fig. 4. The anti-inflammatory profile of MCCE assessed by the carrageenan-induced paw edema test in rats.

DISCUSSION

The present study has demonstrated that the MCCE was more effective as antinociceptive and antipyretic agents than as an anti-inflammatory agent and thus confirmed the traditional uses of its leaves in the treatment of ailments like headache, cold and gastric ulcers (Morton, 1987; Verheij and Coronel, 1992; Jensen, 1999). Although the less remarkable anti-inflammatory activity of MCCE seems to contradict the traditional claim of its use to treat gastric ulcers, the present of activity within a short time range do indicate the present of an anti-inflammatory activity in the said extract. Furthermore, the present of various pharmacological activities in the chloroform extract indicated the present of lipid-soluble bioactive compounds in

the MCEE. We have earlier reported on the aqueous extract of *M. calabura* antinocieptive activity (Zakaria *et al.*, 2006a) and demonstrated its anti-inflammatory and antipyretic activities in our laboratory. Based on the recent findings, it is plausible to suggest that the water-soluble, but not lipid-soluble, bioactive compounds were more effective as an anti-inflammatory agent. The reasons for carrying the pharmacological studies on its chloroform extract were to generally identify the best solvent system for future isolation of bioactive compounds from *M. calabura* leaves since we have earlier reported that its aqueous extract was more effective as an antibacterial agent than its chloroform extract (Zakaria *et al.*, 2006b) and to compare indirectly their effectiveness based on their pharmacological profiles.

The abdominal constriction and hot plate tests have been claimed as models for studying the peripheral and central analgesic properties of extracts/drugs, respectively (Amabeoku *et al.*, 2001). Thus, based on the ability of the MCCE to attenuate the chemically- and thermally-induced nociception, it is plausible to suggest that the extract is exhibiting their effects both peripherally and centrally. To clarify the antinociceptive mechanism of MECC, we carried out the formalin test, which is one of the widely used methods to study the non-anti-inflammatory, antinociceptive properties of extracts/drugs (Tjølsen *et al.*, 1992). Tjølsen *et al.* (1992) even claimed that the formalin test produced better results than the ones using the mechanical or thermal stimulus. A distinct biphasic phase seen in formalin test, generally labeled as early and late phases, represent the irritating effect of formalin at the C-type sensory nerve fibers and tonic inflammatory pain response, respectively (Tjølsen *et al.*, 1992). Centrally acting drugs, like morphine, inhibit both phases while peripherally acting drugs, like non-steroidal anti-inflammatory drugs, inhibit only the late phase (Hunskaar *et al.*, 1985).

The MCCE also exhibited an antipyretic activity, which confirmed the traditional uses of the plant in the treatment of headache and cold (Morton, 1987). The fact that pyretic activity involved inhibition of the activity of prostaglandins synthesized within the central nervous system (CNS) (Uzcátegui *et al.*, 2004) and that the blood-brain barrier (BBB) prevents drug's molecules or other chemicals from entering the CNS (Begley *et al.*, 2000), our finding indicated the extract ability to enter the CNS and thus could also be used to explain the extracts' morphine-like ability seen with the formalin test as well as the hot plate test.

The ability of MCCE to produce a weak anti-inflammatory activity is not well understood since the extract was found to show significant antinociceptive and antipyretic activities. Although it has not been proven yet, it is plausible to suggest that the extract was less active in a prostaglandin-induced inflammatory processes (Damas *et al.*, 1986) and exhibited its antipyretic activity via a prostaglandin-independent mechanism, such as the one induced by tumour necrosis factor-α (TNF-α), as reported by Kluger (1991).

The MCCE concentration-independent activities seen in most assays might be attributed to the phenomenon known as therapeutic window (Tripathi, 2001). According to Tripathi (2001) certain extract's/drug's desired therapeutic effect could be seen only over a narrow range of drug doses or plasma drug concentrations and if this doses/concentrations were outside the narrow therapeutic range, a suboptimal beneficial effects or decline in effect will be produced.

Various bioactive compounds of flavonones and flavones types have been isolated and identified from the leaves and roots of *M. calabura* using the ethanol solvent Su *et al.* (2003) and Kaneda *et al.* (1991). In addition, Chen *et al.* (2005) have also reported on the present of flavonoids with cytotoxic activity isolated from the chloroform extract of *M. calabura* stem barks. Although their antinociceptive, anti-inflammatory and antipyretic effects have not yet proven, it is plausible to suggest the involvement of the flavonoids in the said activities of MCCE

based on several studies published previously (Ramesh *et al.*, 1998; Kupeli *et al.*, 2006). It is believed that those flavonoids ability to influence the said activities occur through modulation of the pro-inflammatory gene expression, such as inducible NO synthase and cyclooxygenase-2 (Dawson and Snyder, 1994). Furthermore, the ability of the MCCE to exhibit remarkable antinociceptive and less remarkable anti-inflammatory activities was in line with previous report made by Kupeli *et al.* (2006). Finally, we conclude that the MCCE possess potential antinociceptive and antipyretic, but less promising anti-inflammatory, activities and, at least, justify the traditional uses of the plant to alleviate headache and cold.

ACKNOWLEDGEMENTS

These studies were supported by the research grant of Universiti Industri Selangor, Malaysia (Project Code Number: 03013; Project Vote Number: 3090103013). The authors would like to thanks Universiti Putra Malaysia for the facilities.

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