ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF BEN-CHA-MOON-YAI REMEDY

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ABSTRACT: Ben-Cha-Moon-Yai remedy is an antipyretic and anti-inflammatory agent in Thai traditional medicine which includes roots of Ma-Toom, Phe-Ka, Lam-Yai, Chare-Tare and Khad-Linn. The antinociceptive activity of the root extract of Ben-Cha-Moon-Yai remedy (BMY) was initially determined using hot-plate, formalin and acetic acid-induced writhing models in mice. Hot-plate latencies were determined in male ICR mice prior to the administration of 0.9% normal saline solution (10 ml/kg, i.p.), morphine (10 mg/kg, i.p.), 2% Tween 80 (10 ml/kg, p.o.) and various doses of BMY (125-500 mg/kg, p.o.) and were subsequently determined at 15, 30, 45, 60, 90, 120 and 240 min. The mean percent maximum possible effect (%MPE) was calculated and used in the determination of the area of analgesia (%MPE-min). All doses of BMY produced a significant analgesic response in the hot-plate test. In formalin test, mice were induced with intraplantar injection of 2.5% formalin solution 30 min after intraperitoneal administration of 0.9% normal saline solution (10 ml/kg) or morphine (10 mg/kg) or 1 hr after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) or various doses of BMY (125-500 mg/kg) and the number of paw licks were determined at 0-5 min and 15-30 min. All doses of BMY significantly decreased the number of paw licks compared to vehicle controls in the late phase. In acetic acid-induced writhing, mice were induced with intraperitoneal injection of 0.6% acetic acid 60 min after the oral administration of 2% Tween 80 (10 ml/kg), IND (10 mg/kg) or various doses of BMY (125-500 mg/kg) and the mean writhing response was determined for 30 min. All doses of BMY significantly decreased the number of writhes compared to vehicle controls. Study then determined the anti-inflammatory property of orally administered BMY (125-500 mg/kg) using carrageenan-induced mouse paw edema and compared with that of IND. Mice treated with IND and all doses of BMY showed a significant reduction in carrageenan-induce paw edema in the second phase. Taken together these results demonstrated that BMY possesses both antinociceptive and anti-inflammatory activities. These results support the antinociceptive effects through both central and peripheral mechanisms. Additional studies are required to determine the mechanism of these actions of BMY.

Keywords: Ben-Cha-Moon-Yai, Hot-plate, Formalin test, Writhing Test, Carrageenan-induced paw edema

INTRODUCTION

Since ancient times people have used plants as herbal medicine to restore and maintain health. Many plants are well known as sources of biologically active compounds. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants, as a valuable source of new pharmaceutical products [1]. While the use of plant extracts with medicinal properties is a popular alternative for the treatment of different pathologies, the lack of scientific analysis may lead to several adverse effects.

Ben-Cha-Moon-Yai remedy consists of five herbal roots in equal parts by weight, including roots of *Aegle marmelos* Corr. (Ma-Toom), *Oroxylum indicum* Vent. (Phe-Ka), *Dimocarpus longan* Lour. (Lam-Yai), *Dolichandrone serrulata* (DC) Seem. (Chare-Tare) and *Walsura trichostemon* Miq. (Khad-Linn). While this formula has been used as an antipyretic and anti-inflammatory agent in Thai traditional medicine for many centuries, there is scarce scientific evidence involving its biological activity. Recently, Bansuttee et al. [2] first reported the antipyretic effect of Ben-Cha-Moon-Yai remedy using the lipopolysaccharide (LPS)-induced fever

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assay in rats, however there is no scientific evidence to support Ben-Cha-Moon-Yai remedy as an anti-inflammatory agent. Furthermore, many investigators have demonstrated the biological activity of various components of these five plants and some evidence for the extracts from *Aegle marmelos* Corr. [3-5], *Oroxylum indicum* Vent. [6-8] and *Dimocarpus longan* Lour. [9, 10]. The present study was aimed to systematically investigate the antinociceptive and anti-inflammatory effects of Ben-Cha-Moon-Yai remedy.

MATERIALS AND METHODS

Plant material and preparation of plant extract

All five herbal roots of Ben-Cha-Moon-Yai remedy were collected from Chiangrai, Tak, Nakhon Ratchasima and Surin provinces of Thailand. Voucher specimens were certified by one of the authors, (N.R.) and deposited at the College of Public Health Sciences, Chulalongkorn University. All five roots were washed, air-dried under shade and ground to coarse powders. Each dried-root powder was exhaustively macerated with absolute ethanol in a closed conical flask at room temperature, and filtered. The filtrate was evaporated to dryness under vacuum. Maceration was continued with water until exhaustion, and the filtrate was lyophilized to dryness. The percent yield of the ethanolic and aqueous extract of each herbal root was recorded. These extracts were stored at -20 °C. The root extract of Ben-Cha-Moon-Yai remedy (BMY) was prepared by mixing each extract in the quantity (based on the yield of each root extract) equivalent to the remedy. A weighed amount of BMY was suspended in 2% aqueous Tween 80 solution and used in this study.

Animals

Male ICR mice (18-25 g) were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakornpathom. Animals were maintained in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under standard conditions of temperature (25±2°C), humidity (50-60%), 12 hr/12 hr light/dark cycles and provided with food and water *ad libitum*. The animals were kept under these laboratory conditions for one week prior to the start of the experiments. At the end of each experiment, the animals were sacrificed with carbon dioxide asphyxiation. This study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Drugs and chemicals

Morphine sulfate (MO; Thai FDA), formaldehyde (Merck, Germany), acetic acid (Merck, Germany), and λ-carrageenan (Sigma Chemical Co., USA) were dissolved in 0.9% sodium chloride solution (NSS; General Hospital Products Public Co., Thailand). Indomethacin (IND; Sigma Chemical Co., USA) and BMY were suspended in 2% (w/v) Tween 80 solution (Srichansahaosoth Co., Thailand). Morphine sulfate and indomethacin were used as standard analgesic drugs. Indomethacin was also used as a standard anti-inflammatory drug. The control animals were given with an equivalent volume of vehicle via the same route.

Antinociceptive activity test

Hot-plate Test

Hot-plate test was carried out according to the method previously described by Woolfe and MacDonald in 1944 [11]. Male ICR mice weighing 18-25 g were used (N=10 per group). In these experiments, the hot-plate (Harvard apparatus, USA) measuring 28×28 cm was maintained at 55±0.5°C and surrounded by a clear Plexiglas wall cylinder, 20 cm in diameter and 30 cm in height to confine the animal to the heated surface during testing. On the day of testing, animals were randomly assigned to one of six treatment groups and underwent 3 pre-drug baseline trials on the hot-plate spaced 5-10 min apart. Only those animals which had a pretreatment hot-plate latency time less than 45 sec were utilized in these studies. After pre-drug baseline trials, mice were administered various treatments and retested. Each mouse was placed on the hot-plate from an elevation of 5 cm and the latency between placement and licking of a hind paw or vigorous jumping up from the surface of the metal plate was used as the end point and recorded with a stopwatch. If this behavior was not observed within 45 sec the animal was removed from the hot-plate, given a score of 45 sec for its hot-plate latency and returned to its home cage. The average of the last two trials served as the baseline pre-drug latency.

Immediately, after the third baseline trial on the hot-plate, the animals were treated with NSS (10 ml/kg) and MO (10 mg/kg) intraperitoneally or 2% Tween 80 (10 ml/kg) and various doses of BMY (125, 250 and 500 mg/kg) orally. The postdrug latency was measured for 7 subsequent trials at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. To avoid tissue damage the cut-off time was set at 45 sec. The time-course of hot-plate latency was expressed as the mean percent

maximum possible effect (%MPE) according to the following formula:

$$\%MPE=\frac{\text{(post drug latency) - (pre-drug latency)}}{\text{(cut-off time) - (pre-drug latency)}}\times 100$$

The area of analgesia for the hot-plate assays was derived by computing the area under the corresponding 0-240 min time-course-%MPE curves; areas were calculated using the trapezoidal rule [12].

Formalin test

The test was based on the method described earlier by Hunskaar and Hole in 1987 [13]. Male ICR mice weighing 18-25 g were used (N=8 per group). Twenty microliter of 2.5% formalin solution was injected subcutaneously into the plantar surface of the left hind paw of each mouse 30 min after intraperitoneal administration of NSS (10 ml/kg) and MO (10 mg/kg) or 1 hr after oral administration of 2% Tween 80 (10 mg/kg), indomethacin (IND; 10 mg/kg) and various doses of BMY (125, 250 and 500 mg/kg). Following the formalin injection, animals were immediately placed in an observation cylinder. The number of paw licks or bites of the injected paw were recorded at 0-5 min and 15-30 min and expressed as the total number of paw licks in the early phase (0–5min) and the late phase (15–30 min) after formalin injection.

Acetic acid-induced writhing test

Male ICR mice weighing 18-25 g were used (N=8 per group). Analgesic activity testing was determined utilizing the acetic acid-induced writhing method described by Koster et al. in 1959 [14]. On the day of testing animals were randomly assigned to one of five treatment groups. Mice were pretreated with 2% Tween 80 (10 ml/kg), IND (10 mg/kg) or various doses of BMY (125, 250 and 500 mg/kg) orally 1 hr before intraperitoneal injection of 0.6% acetic acid at the volume of 10 ml/kg. Animals were then placed in an observation cylinder. The number of writhes (contraction of abdominal muscle together with hind limb extension) were observed and counted at 5 min intervals for a period of 30 min after acetic acid administration. Antinociceptive activity was reported as the percentage of inhibition of the writhing response compared with the vehicle control group. The percentage of inhibition of the writhing response was calculated using the following formula:

$$\%\text{ Inhibition of writhing response} = \frac{\text{Wr (control)} - \text{Wr (test)}}{\text{Wr (control)}}\times 100$$

with Wr = mean number of writhing response.

Rota-rod test

Male ICR mice weighing 18-25 g were tested on the rota-rod (N = 8 per group) as previously described by Dunham and Miya [15]. The animals were placed on a horizontal rod (3.5 cm diameter) rotating at 16 rpm (Ugo Basile, Italy). Mice capable of remaining on the rotating rod for 60 sec or more in three successive trials were selected for the study. Each mouse was treated with 2% Tween 80 (10 ml/kg) or BMY 500 mg/kg orally and placed on the rotating rod at 30, 60, 90, 120 and 240 min after drug administration. The results were expressed as the time in seconds the animal remained on the rota-rod during 60 sec.

Anti-inflammatory activity testing

Carrageenan-induced paw edema

The anti-inflammatory activity of BMY was determined using the carrageenan-induced paw edema test in the hind paw according to the method of Winter et al. [16] with some modifications. Male ICR mice weighing 18-25 g were used (N = 8 per group). Animals were randomly assigned to one of five treatment groups. On the day of testing, mice were treated with 2% Tween 80 (10 ml/kg), IND (10 mg/kg) or various doses of BMY (125, 250 and 500 mg/kg) orally. One hour later, 1% carrageenan solution at the volume of 50 µl was injected subcutaneously into the plantar surface of the left hind paw of each mouse. The paw volume was measured at 1 hr prior to injection of carrageenan and at 1, 2, 3, 4, 5 and 6 hr after injection using a plethysmometer (Ugo Basile, Italy). Edema was expressed as a mean increase in paw volume in relation to control. The percentage of inhibition of edema was analyzed using the following formula:

$$\%\text{ Inhibition of edema} = \frac{\text{(Vc - Vt)}}{\text{Vc}}\times 100$$

with Vc = the edema volume in control group; Vt = the edema volume in tested group.

Acute toxicity

Animals employed in the rota-rod test were observed during 72 hrs and morbidity or mortality was recorded, if happens, for each group at the end of observation period.

Analysis of Data

The results are expressed as means±S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) and Student's *t*-test followed by a *post hoc* Tukey's test for multiple comparisons. The minimum level for significance was set at $p<0.05$.

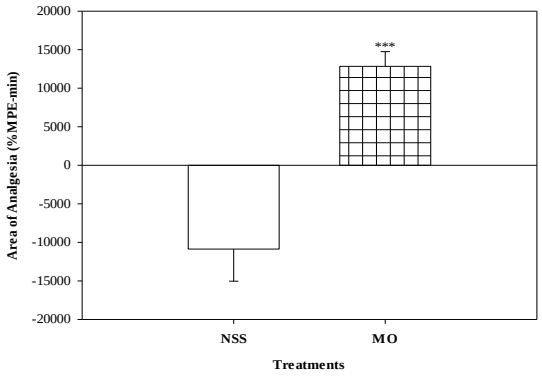

Figure 1 Mouse Hot-plate Test. Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulfate (MO; 10 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. $^{***}p<0.001$ significantly different compared to control animals.

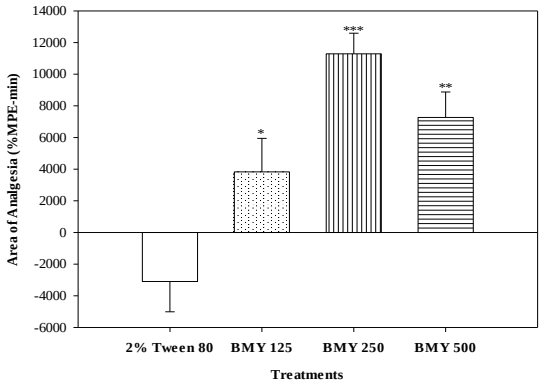

Figure 2 Mouse Hot-plate Test. Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to control animals.

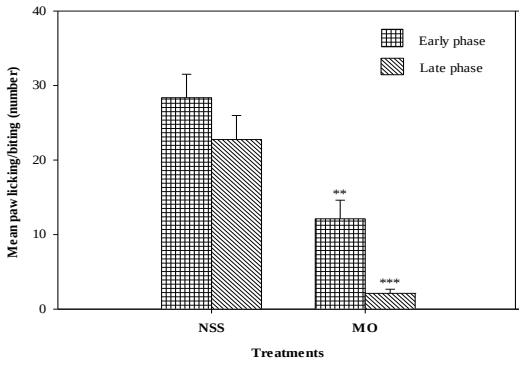

Figure 3 Formalin Test. Mean paw licking/biting (number) after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=8 for all groups. Values represent the mean±S.E.M. $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to control animals.

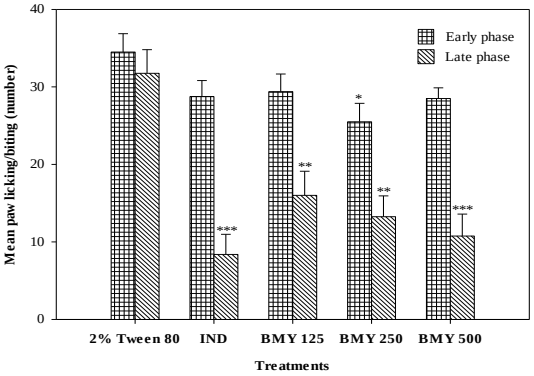

Figure 4 Formalin Test. Mean paw licking/biting (number) after oral administration of 2% Tween 80, Indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8 for all groups. Values represent the mean±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to control animals.

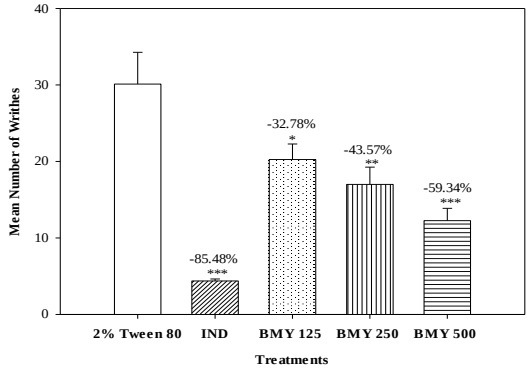

Figure 5 Acetic Acid-induced Writhing Test. Mean number of writhes after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8 for all groups. Values represent the mean±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to control animals. Inhibition is reported as a percentage compared to 2% Tween 80.

RESULTS

Hot-plate test

Morphine 10 mg/kg significantly ($p<0.001$) increased the hot-plate latency producing an area of analgesia at 12,835±1,909 %MPE-min compared with that of normal saline solution (NSS) (-10,873± 4,166 %MPE-min; Figure 1). All doses of BMY (125, 250 and 500 mg/kg) significantly increased the hot-plate latencies when compared to the vehicle group, Figure 2 ($p<0.05$, $p<0.001$ and $p<0.01$, respectively).

Formalin test

MO 10 mg/kg significantly decreased the number

Table 1 Changes in edema volume (ml) from 1-6 hr after carrageenan administration following oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8 for all groups.

Treatments	Paw edema ± S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.028±0.0051	0.0525±0.0031	0.0912±0.0058	0.0775±0.0082	0.0675±0.0118	0.0400±0.0091
IND 10 mg/kg	0.0250±0.0019 (13.04%)	0.0312±0.0029* (40.48%)	0.0400±0.0060*** (56.16%)	0.0237±0.0037*** (69.35%)	0.0187±0.0023** (72.22%)	0.0337±0.0053 (15.62%)
BMY 125 mg/kg	0.0337±0.0046 (-17.39%)	0.0500±0.0063 (4.76%)	0.0612±0.0069* (32.88%)	0.0675±0.0053 (12.90%)	0.0750±0.0082 (-11.00%)	0.0550±0.0057 (-37.50%)
BMY 250 mg/kg	0.0337±0.0053 (-17.39%)	0.0575±0.0070 (-10.00%)	0.0462±0.0080*** (49.31%)	0.0375±0.0049*** (51.61%)	0.0400±0.0073 (40.74%)	0.0462±0.0073 (-16.00%)
BMY 500 mg/kg	0.0375±0.0031 (-30.43%)	0.0512±0.0040 (2.38%)	0.0312±0.0040*** (65.75%)	0.0337±0.0056*** (56.45%)	0.0337±0.0068* (50.00%)	0.0325±0.0041 (18.75%)

Each value represents mean±S.E.M.
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to control animals.
Inhibition is reported as a percentage compared to 2% Tween 80.

of licks of both early and late phases producing mean number of licks of 12.8±2.5 and 2.1±0.6 compared with that of NSS (28.4±3.2 and 22.8±3.2; $p<0.01$ and $p<0.001$, respectively; Figure 3). Only BMY 250 mg/kg produced a significant antinociceptive action in the early phase when compared to the vehicle group ($p<0.05$). However, all doses of BMY produced significant antinociceptive activity against inflammatory pain in the late phase ($p<0.01$, $p<0.01$ and $p<0.001$, respectively; Figure 4). The reference drug, IND (10 mg/kg) also caused significant ($p<0.001$) inhibition of the late phase of formalin-induced nociception when compared to the vehicle group (Figure 4).

Acetic acid-induced writhing test

IND (10 mg/kg) significantly ($p<0.001$) decreased the writhing response by 85% producing a mean number of writhes of 4.4±0.3 when compared to the vehicle control (30±4.2). All doses of BMY (125, 250 and 500 mg/kg) significantly reduced the writhing response when compared with the vehicle group ($p<0.05$, $p<0.01$ and $p<0.001$, respectively) and the highest percentage of inhibition of the writhing response (59%) was generated with the highest dose of BMY, Figure 5 (500 mg/kg).

Rota-rod test

The results demonstrated that oral administration of BMY at the highest dose tested (500 mg/kg) did not affect the motor responses of the animals on the rota-rod test at 30, 60, 90, 120 and 240 min after treatment.

Carrageenan-induced paw edema

BMY 125 mg/kg significantly ($p<0.05$) decreased paw edema by 32.9% at 3 hr. BMY 250 mg/kg significantly ($p<0.001$ and $p<0.001$) decreased paw edema by 49% and 51.6% at 3 and 4 hr, respectively. BMY 500 mg/kg significantly ($p<0.001$, $p<0.001$ and $p<0.05$) decreased paw edema by 65.75%, 56.45% and 50% at 3, 4 and 5 hr, respectively. A significant anti-inflammatory effect of IND (10 mg/kg) was also observed at 2, 3, 4 and 5 hr after carrageenan injection (Table 1).

Acute toxicity

The acute toxicity study carried out to evaluate the safety of BMY indicated no acute toxicity or mortality was observed with oral administration of all doses of BMY tested over the 72 hrs observation period.

DISCUSSION

These studies have demonstrated the antinociceptive and anti-inflammatory effects of BMY in various models. Antinociceptive activity was assessed utilizing thermally- and chemically- induced pain models in mice. The anti-inflammatory activity was assessed utilizing carrageenan-induced mouse paw edema.

Firstly, we investigated analgesic activity utilizing hot-plate test, a well-validated model for detection of centrally-acting analgesics. Peripherally-acting analgesics show little to no activity in this test. The hot-plate test produces two basic behavioral components in mice including hind paw licking and jumping with all four feet. Both are considered to be supraspinally integrated responses [17]. This model usually employed MO as a reference drug. MO demonstrated a potent analgesic effect in this model indicating the sensitivity of this test. Our results demonstrated that BMY presented significant analgesic activity in the hot-plate test. The animals treated with the BMY at all doses presented an area of analgesia greater than the

control group with the highest antinociceptive effect produced at the 250 mg/kg dose. The results supported that BMY has central analgesic effect.

The effectiveness of BMY was investigated using the formalin test which has been introduced as a model for chronic pain [18]. It has been considered an effective technique to measure inflammatory and non-inflammatory pain [13], as well as to elucidate the mechanism of analgesia [13, 19]. In this test, animals displayed nociceptive behaviors consisting of shaking, licking and biting the affected paw in two distinct phases. The early phase (acute pain) initiates immediately after formalin injection which lasts for five minutes as a result of chemical stimulation of primary afferent nociceptors, and the late phase (inflammatory pain) initiates 20 min after formalin injection and lasts for 10 min arising from peripheral inflammation and functional changes in the dorsal horn of the spinal cord [19]. Previous studies reported that substance P and bradykinin participate in the early phase, whereas histamine, serotonin, prostaglandins, nitric oxide and bradykinin are involved in the late phase [13, 19]. Each phase of the formalin test reflects different mechanisms; drugs that act predominantly on the central nervous system inhibit both phases equally, while peripherally-acting drugs inhibit only the late phase [13, 20, 21]. In addition, the late phase is selectively attenuated by cyclooxygenase inhibitors [22]. We employed MO and IND as reference drugs. MO, a central analgesic drug, demonstrated potent analgesic effect in both phases while IND, a peripheral acting drug, demonstrated analgesic response only in the late phase. The present study demonstrated that BMY produced inhibition of the late phase at all doses tested, whereas the early phase was slightly attenuated only at the 250 mg/kg dose. Consequently, the results indicated antinociceptive activity against inflammatory pain and a central action of BMY.

The acetic acid-induced writhing test, used as a screening tool for the assessment of analgesic property of a test compound, is based on the postulation that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons [23]. The writhing response is presumed to be induced by local peritoneal receptor activation [24] as a result of prostanoids mediators [25]. In mice there is an increase in the peritoneal fluid levels of PGE_2 and PGF_2, as well as lipooxygenase products [25], and release of sympathetic nervous system mediators [26]. Furthermore, the nociceptive properties of acetic acid may be due to the release of cytokines, including TNF-α, interleukin-1β, and interleukin-8 by resident peritoneal macrophages and mast cells [27]. In the present writing test, there was a significant reduction in the number of writhes in the animals treated with IND and BMY at all doses tested. The efficacy of BMY 500 mg/kg was comparable to IND. The mechanism of BMY's activity may be due to the reduction on the liberation of those inflammatory mediators or by direct blockade of receptors resulting in a peripheral analgesic action of BMY. The results obtained from both formalin and writhing tests indicated that BMY has peripheral analgesic property. Additional studies are required to determine the mechanisms underlying the analgesic property of BMY.

Furthermore, to exclude the possible cause of non-specific disturbances of motor coordination caused by BMY, the rota-rod test was performed. This test has been used to determine a compound's ability to produce skeletal muscle relaxation, convulsions and depression of the central nervous system. In the present rota-rod test, the results indicated a lack of any detectable relaxant and sedative effects even at the highest dose of BMY tested. Therefore, the behavioral responses observed in the hot-plate, formalin and writhing tests were likely not the motor dysfunction but rather a true antinociceptive effect.

Carrageenan-induced rat paw edema was described by Winter in 1962 [16], and the carrageenan-induced mouse paw edema test established in 1969 by Levy [28]. Since then, the mouse paw edema test has been increasingly used to evaluate anti-inflammatory drug candidates [29]. For determining the anti-inflammatory activity of BMY we performed the carrageenan-induced paw edema test, which is a standard experimental model of acute inflammation characterized by a biphasic response. The first phase (1-2 h after carrageenan injection) is due to liberation of histamine and serotonin in paw tissue, whereas the second phase is sustained by the liberation of prostaglandins [30]. Continuity between the two phases is believed to be mediated by kinins [31, 32].

The carrageenan-induced mouse paw edema test was utilized to examine the anti-inflammatory effect of BMY. The present paw edema test demonstrated that pre-treatment of animals with BMY resulted in inhibition of the second phase of the carrageenan-induced mouse paw edema test at 3, 4 and 5 hrs. This effect may be due to the interference by BMY on the liberation of prostaglandins, or the blockade of the prostaglandin receptors. Further studies are needed to determine the specific mechanism (s) of action of BMY. The acute toxicity assessment has revealed that all doses

of BMY used in this work were safe.

CONCLUSION

The root extract of Ben-Cha-Moon-Yai remedy displayed antinociceptive property in both central and peripheral models of nociception in mice. Moreover, the root extract of Ben-Cha-Moon-Yai remedy also demonstrates anti-inflammatory property in an acute inflammation model. Additional studies are required to better understand their potential antinociceptive and anti-inflammatory mechanism of actions. This study helps clarifying the pharmacological action of this herbal remedy and provides additional scientific support for this Thai traditional medicine.

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