ANTIPYRETIC EFFECT OF BEN-CHA-MOON-YAI REMEDY

Somkit Bansuttee[1], Rawiwan Manohan[2], Chanida Palanuvej[2], Nijsiri Ruangrungsi[2,3] and Pasarapa Towiwat[1,*]

[1]Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330 Thailand [2]College of Public Health Sciences, Chulalongkorn University, Bangkok 10330 Thailand [3]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330 Thailand

ABSTRACT: Ben-Cha-Moon-Yai (BMY) herbal remedy is an antipyretic and anti-inflammatory drug in Thai traditional medicine which includes roots of Ma-Toom, Phe-Ka, Lam-Yai, Chare-Tare and Kad-Linn. We determined the antipyretic activity of the root extract of BMY remedy using lipopolysaccharide (LPS)-induced fever in rats compared to that of acetylsalicylic acid (ASA). Fever was induced in animals with an intramuscular injection of LPS (50 μg/kg) 1 hr after oral administration of 2% Tween 80, ASA 300 mg/kg or various doses of BMY (125-500 mg/kg). Rectal temperature was measured before the pretreatment and at 1 hr intervals for 7 hr after LPS injection. All doses of BMY significantly (p<0.05) attenuated the increased rectal temperature produced by LPS and were found to be as potent as ASA. These results demonstrated that the root extract of BMY remedy at all doses tested possesses antipyretic activity.

Keywords: Ben-Cha-Moon-Yai, LPS-induced fever, antipyretic effect

INTRODUCTION: Fever is one of the most common complaint and signs of illness. Acetylsalicylic acid (ASA) and acetaminophen are equally effective in reducing fever in humans. Nonsteroidal anti-inflammatory agents (NSAIDs) such as indomethacin and ibuprofen are also excellent antipyretics. Although ASA and NSAIDs are effective antipyretics, they have several adverse effects including gastrointestinal toxicity (e.g., dyspepsia, heartburn, abdominal discomfort, nausea, and vomiting), allergic reactions, inhibition of platelet aggregation, fluid retention, drowsiness, and dizziness. Therefore, a natural antipyretic agent with reduced or no toxicity is essential. There are several Thai herbal formulas that have been used as antipyretic agents including Chan-Tha-Lee-La, Pra-Sa-Chan-Dang and Ben-Cha-Lo-Ka-Wi-Chian.

Ben-Cha-Moon-Yai (BMY) is an herbal remedy used in Thailand as an antipyretic and anti-inflammatory drug. The formula is composed of five herbal roots in an equal part by weight including roots of *Aegle marmelos* (L.) Corr. (Ma-Toom), *Oroxylum indicum* Linn. (Phe-Ka), *Dimocarpus longan* Lour. (Lam-Yai), *Dolichandrone serrulata* DC. (Chare-Tare) and *Walsura trichostemon* Miq. (Kad-Linn). Many researches had been done to investigate various pharmacological effects of several parts of this five herbal plants including root, leaf and stem bark. Recently, Arul *et al.*[1)] investigated the antipyretic effect of the serial extracts of the leaves of *A. marmelos* utilizing yeast-induced fever model in rats. All the extracts derived from the leaves of *A. marmelos* dose of 50 mg/kg showed antipyretic activity.

Although BMY remedy is widely used as an antipyretic by many Thai traditional practitioners, there is no scientific data that supports its use. Therefore, this study was designed to investigate the antipyretic effect of the root extract of BMY remedy compared with ASA utilizing lipopoly-saccharide-induced fever model in rats in order to provide scientific evidence to support its use in Thai traditional medicine.

MATERIALS AND METHODS:

Plant material and preparation of plant extract

All five herbal roots of BMY remedy were collected from Nakhon Ratchasima Province, Thailand. Voucher specimens were authenticated by one of the authors, N.R. and deposited at the College of Public Health Sciences, Chulalongkorn University. Roots were washed, air-dried under shade and ground to coarse powders. The individual dried-root powder was exhaustively macerated with absolute ethanol in a closed

*To whom correspondence should be addressed.
E-mail: pasarapa.c@chula.ac.th
Tel. +66 2218 8319, fax +66 2218 8324

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 each ethanolic and aqueous extract of each herbal root was recorded. These extracts were stored at -20°C. The root extract of BMY remedy 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 for the study.

Animals

Male Wistar rats weighing 140-180 g obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom, Thailand served as experimental subjects in the study. The animals were housed in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under standard conditions of temperature (25±2°C) and 12 hr/12 hr light/dark cycles. The animals were kept under laboratory conditions for one week prior to the start of the experiments and allowed food and water *ad libitum*. 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

Acetylsalicylic acid (ASA; Sigma Chemical Co., USA) was suspended in 2% (w/v) Tween 80 solution. Lipopolysaccharide from *Escherichia coli* (LPS; Sigma Chemical Co., USA) was dissolved in sterile pyrogen-free normal saline at 500 µg/ml. ASA (300 mg/kg) was used as a standard antipyretic drug. The control animals were given with an equivalent volume of vehicle via the same route.

Lipopolysaccharide-induced fever

The method of Santos and Rao[2] was modified and used for the assessment of the antipyretic activity of the BMY. Animals (N=36) were fasted overnight prior to the experiments and on the day of testing. Animals were kept singly in restrainers for 1 hr to acclimate to their new environment. The animals were pretreated orally with 2% Tween 80 solution, ASA (300 mg/kg) or various doses of BMY (125, 250 and 500 mg/kg) for 1 hr before injection of LPS. Fever was induced with 50 µg/kg of LPS injected intramuscularly into the right thigh of the rats. Rectal temperature was measured before the pretreatment of animals and at 1 hr intervals for 7 hr after the administration of the bacterial endotoxin with a lubricated digital thermometer (YSI Precision TM 4000A, USA) inserted 3-4 cm deep into the rectum of the rats. The rectal temperature of normal rats was also measured at 1 hr intervals for 7 hr. The control arm of the experiment involved animals treated with 2% Tween 80 solution plus LPS. All experiments were carried out between 08.00 hr and 18.00 hr in a quiet laboratory with an ambient temperature of 25+ 2°C.

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) followed by a *post-hoc* Tukey HSD test for multiple comparisons. Statistical significance was assessed as $p<0.05$.

RESULTS: Lipopolysaccharide injected intramuscularly significantly ($p<0.001$) produced a time-dependent increase in rectal temperature in vehicle pretreated rats starting from 2 hr, and this effect was maintained for 7 hr after LPS injection. The maximum increase in rectal temperature was reached at 3 hr (1.06°C) giving a maximum observed mean rectal temperature of 38.82 ±0.10°C after which there was a decrease. During the same period, the maximum mean rectal temperature of normothermic rats was 37.66 ±0.14°C. Thus, LPS significantly ($p<0.001$) increased the rectal temperature (Table 1 and Figure 1).

ASA 300 mg/kg significantly ($p<0.05$) attenuated the increase in rectal temperature produced by LPS at 2 hr and the antipyretic effect was maintained over the 7 hr period. The maximum mean rectal temperature in the presence of ASA was 37.76±0.21°C. All doses of the root extract

Table 1 Effect of the root extract of BMY remedy (125-500 mg/kg) on lipopolysaccharide-induced fever in rats

Treatments	Rectal Temperature (˚C) before and after LPS injection								
	-1 hr	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**6 hr**	**7 hr**
Normothermic rats[a]	37.59 ± 0.11	37.62 ± 0.10	37.64 ± 0.21	37.79 ± 0.14	37.66 ± 0.14	37.61 ± 0.07	37.46 ± 0.15	37.33 ± 0.15	37.15 ± 0.15
Control LPS[b]	37.82 ± 0.10	37.76 ± 0.93	38.34 ± 0.04	38.81 ± 0.15#	38.82 ± 0.10#	38.71 ± 0.07#	38.62 ± 0.06#	38.55 ± 0.74#	38.44 ± 0.10#
ASA 300 mg/kg	37.64 ± 0.21	37.74 ± 0.21	37.68 ± 0.23	37.68 ± 0.22*	37.75 ± 0.17*	37.76 ± 0.21*	37.56 ± 0.19*	37.55 ± 0.23*	37.58 ± 0.24*
BMY 125 mg/kg	37.85 ± 0.88	37.74 ± 0.11	37.78 ± 0.08	38.08 ± 0.13*	37.94 ± 0.13*	38.01 ± 0.12*	37.97 ± 0.06*	37.73 ± 0.13*	37.62 ± 0.12*
BMY 250 mg/kg	37.61 ± 0.92	37.38 ± 0.11	37.59 ± 0.21	38.00 ± 0.18*	37.84 ± 0.14*	37.91 ± 0.19*	37.87 ± 0.17*	37.74 ± 0.16*	37.64 ± 0.16*
BMY 500 mg/kg	37.77 ± 0.11	37.81 ± 0.17	37.80 ± 0.19	38.11 ± 0.83	37.86 ± 0.15*	37.91 ± 0.12*	38.02 ± 0.05*	37.87 ± 0.87*	37.72 ± 0.10*

Each value represents mean± S.E.M., N=6 for all groups.
[a]Normothermic rats received 0.9% NSS.
[b]Control LPS received 2% Tween 80 solution.
#p<0.001 significantly different compared to normothermic rat values for the corresponding hour.
*p<0.05 significantly different compared to control LPS values at the corresponding hour.

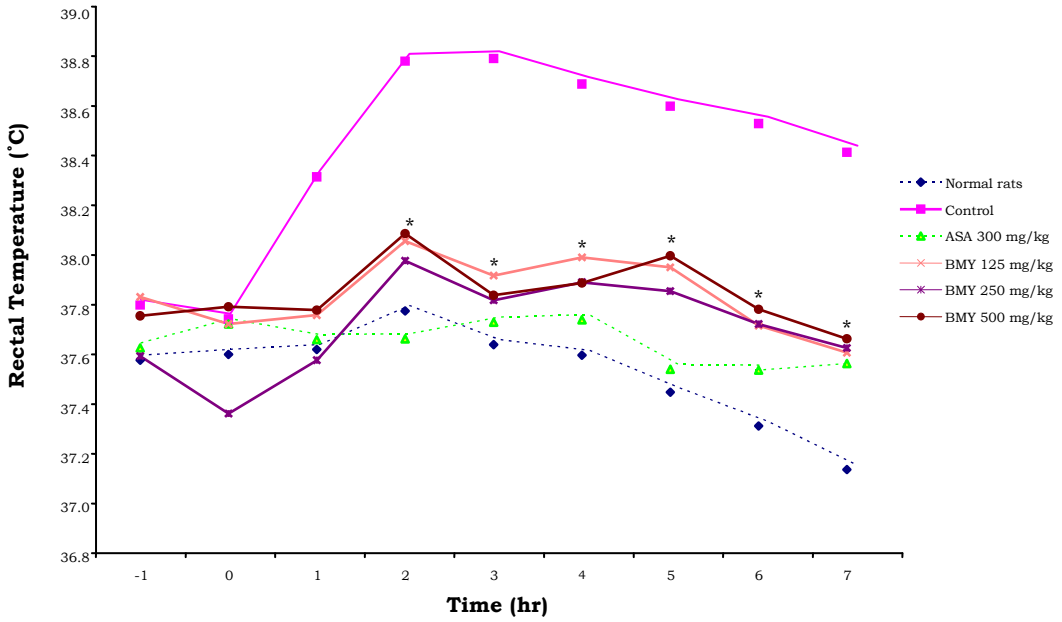

Figure 1 Changes in rectal temperature after oral administration of 2% Tween 80 (control) , acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125- 500 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr prior to LPS, N=6 for all groups. *p<0.05 significantly different compared to control values at the corresponding hour.

of BMY remedy (125, 250, and 500 mg/kg) also significantly attenuated the increase in rectal temperature produced by LPS (p<0.05) with a maximum reduction at 7 hr. The antipyretic effect of increasing doses of BMY was noted at 2, 2, and 3 hr, respectively, and the effect was maintained for the full 7 hr after LPS injection. All doses of the BMY used in this study were found to be as potent as ASA (Table 1 and Figure 1).

DISCUSSION: Fever is an elevation of body temperature above the normal circadian range as the result of a change in the thermoregulatory center located in the anterior hypothalamus. Fever is not a disease itself, but a manifestation of a number of disease processes. Numerous animal models have shown that survival in the face of infection is enhanced by the production of fever (up to a certain temperature). In human *in vitro* and *in vivo* experiments, fever appears to have a beneficial effect on host defenses including enhancing neutrophil migration, increasing the production of antibacterial substances by neutrophils, and increasing T-cell proliferation. Fever is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α) and prostaglandins[3-5]. The cytokine cascade of fever induction starting from initial stimulation of IL-1 and TNF-α by bacterial products that induces secondary synthesis of IL-6 with subsequent induction of prostaglandin (PG) synthesis in the central nervous system (CNS) and fever. Prostaglandin E_2 (PGE_2), thus induced by these cytokines, is considered to be the proximal, final fever mediator in the preoptic-anterior hypothalamus (POAH)[6].

Antipyretics such as ASA and other nonsteroidal anti-inflammatory drugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within CNS thermoregulation sites. They suppress peripheral producing of pyrogenic cytokines including TNF-α and IL-1β while lower the thermoregulatory set point by blocking central COX production of PGE_2[7].

LPS is the most potent stimulus known for TNF-α production and release and also increases circulating levels of another pyrogen, IL-1. This exogenous pyrogen has been shown to produce fever in laboratory animals such as guinea pigs and rabbits by stimulating the production of endogenous TNF-α[3,8]. For characterization the antipyretic activity of BMY, the LPS-induced fever model in rats was employed in this study.

Orally administered ASA, the positive control, significantly attenuated fever in LPS treated rats at all times tested. This could be due to inhibition of COX and therefore interference with the cascade of the synthesis of PGs which induces fever. The oral administration was chosen in order to imitate the normal consumption of 'Ben-Cha-Moon-Yai', the Thai traditional antipyretic herbal medicine. All doses of BMY (125-500 mg/kg) displayed antipyretic activity in the LPS-induced fever model of rats over the period of 2-7 hr after LPS injection, supporting the view that BMY has some influence on PG biosynthesis. Because the synthesis of PG plays a crucial role in the febrile response to endogenous pyrogens (such as the cytokines) or to exogenous pyrogens (such as LPS). The antipyretic effect of BMY (125 and 250 mg/kg) occurred within 2 hr after LPS injection and was sustained for up to 7 hr, similar to that seen with ASA treatment. The antipyretic efficacy of all doses of BMY used was comparable to that of ASA. Additional studies are needed to determine if the antipyretic effect of BMY are due to suppression of TNF-α and inhibition of TNF-α and PG synthesis.

CONCLUSION: The root extract of BMY remedy at all doses tested demonstrated antipyretic effect. Additional studies are required to better understand their potential antipyretic mechanism of action. This is the first study that helps clarifying the pharmacological action of this herbal remedy and provides additional scientific support for this Thai traditional medicine.

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