ANTIPYRETIC AND ANTINOCICEPTIVE EFFECTS OF BEN-CHA-LO-KA-WI-CHIAN REMEDY

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ABSTRACT: Ben-Cha-Lo-Ka-Wi-Chian (BLW) herbal remedy is a famous antipyretic drug in Thai traditional medicine which includes roots of Ching-Chee, Khon-Thaa, Yaa-Nang, Mai-Tao-Yai-Mom and Ma-Dueo-Chumporn. We initially determined the antipyretic activity of the root extract of BLW 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 BLW (25-400 mg/kg). Rectal temperature was measured before the pretreatment and at 1 hr intervals for 7 hr after LPS injection. All doses of BLW significantly (p<0.05) attenuated the increased rectal temperature produced by LPS and were found to be as potent as ASA. Studies then determined the antinociceptive property of orally administered BLW (25-400 mg/kg) using hot-plate, tail-flick and acetic acid-induced writhing models in mice. Hot-plate and tail-flick 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.) or various doses of BLW (25-400 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). BLW (400 mg/kg) produced a significant analgesic response in the hot-plate test, while all doses of BLW, except the lowest dose, produced significant analgesic responses in the tail-flick test. In acetic acid-induced writhing, mice were induced with intraperitoneal injection of 0.6% acetic acid 30 min after the oral administration of 2% Tween 80, indomethacin (10 mg/kg) or various doses of BLW (25-400 mg/kg) and the mean writhing response was determined for 30 min. BLW doses of 200 and 400 mg/kg significantly (p<0.05) decreased the mean writhing response compared to vehicle controls. Taken together these results demonstrated that BLW possesses both antipyretic and antinociceptive activities and BLW likely produced both central and peripheral analgesic responses.

Keywords: Ben-Cha-Lo-Ka-Wi-Chian, LPS-induced fever, Hot-plate, Tail-flick, Writhing Test

INTRODUCTION: Fever and pain are common problems found in the general population that have a significant impact on lifestyles and health. They may occur as single entities or in combination. Although many antipyretic and analgesic agents are available for treating these symptoms, they have several adverse effects when used for long term treatment. Therefore, many researchers are searching for new antipyretic and analgesic drugs with higher efficacy and lower levels of side effects, especially from natural products including herbal plants. Thai traditional herbal formulae that have been used as antipyretic agents include Chan-Tha-Lee-la and Ben-Cha-Lo-Ka-Wi-Chian, and Pra-Sa-Plai has been used as an analgesic agent.

BLW, a well-known herbal remedy, has been included in The 2006 Thailand Herbal Medicine Essential Drug List as an antipyretic for both children and adults. The formula is composed of five herbal roots in equal parts by weight and includes roots of *Capparis micracantha* (Ching-Chee), *Tiliacora triandra* (Ya-Nang), *Harrisonia perforata* (Khon-Thaa), *Clerodendrum petasites* (Mai-Tao-Yai-Mom) and *Ficus racemosa* (Ma-Dueo-Chumporn). Although BLW herbal remedy is widely used as an antipyretic by many traditional practitioners in Thailand, there is only one scientific study that supports its use. Konsue *et al.* demonstrated the antipyretic efficacy of the root powder of BLW formula using a Baker's yeast-induced fever model in rats[1]. Thus, the

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present study was designed to investigate the antipyretic effects of the root extract of BLW remedy using a lipopolysaccharide-induced fever model in rats in order to further provide the scientific evidence to support its use in Thai traditional medicine. In addition, this study also investigated the antinociceptive effect of the root extract of BLW remedy in a variety of animal models to determine another useful activity of the herbal formula.

MATERIALS AND METHODS:

Plant material and preparation of plant extract

All five herbal roots of BLW remedy were collected during September, 2008 from Nongkhai Province, Thailand. Voucher specimens were authenticated by one of the authors, N.R. and deposited at 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 macerated with absolute ethanol for 24 hr at room temperature, and filtered. The filtrate was evaporated to dryness under vacuum. Maceration was continued with water for another 24 hr, and the filtrate was lyophilized to dryness. The ethanolic and aqueous extracts of each herbal root was mixed together and the percent yield of each extract was recorded. These extracts were stored at -20°C. The root extract of BLW 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 BLW was suspended in 2% aqueous Tween 80 solution and used for the study.

Animals

Male Wistar rats weighing 140-180 g and male ICR mice weighing 18-25 g 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), 50-60% of humidity 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) and indomethacin (IND; Sigma Chemical Co., USA) were 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. Morphine sulphate (MO; Thai FDA) and 0.6% acetic acid (Sigma Chemical Co., USA) were dissolved in 0.9% sodium chloride solution (NSS). Acetylsalicylic acid (300 mg/kg) was used as a standard antipyretic drug. Morphine sulphate (10 mg/kg) and indomethacin (10 mg/kg) were used as standard analgesic drugs. The control animals were given with an equivalent volume of vehicle via the same route.

Antipyretic activity test

Lipopolysaccharide-induced fever

The method of Santos and Rao[2] was modified and used for the assessment of the antipyretic activity of BLW. Animals (N=48) 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 BLW (25, 50, 100, 200 and 400 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 rat. 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™ 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.

Antinociceptive activity test

Mouse Hot-Plate

Male ICR mice weighing 18-25 g were used (N=10 per group). Analgesic testing was determined using the hot-plate method. The surface of the hot-plate (Harvard Apparatus) measuring 28x28 cm was set 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 eight treatment groups and underwent 3 pre-drug baseline trials on the hot-plate spaced 5-10 min apart. Only those animals which had a pre-treatment hot-plate latency time of less than 45 sec were utilized in these studies. Mice were then administered various treatments and retested. Each mouse was placed on the hot-plate from an elevation of 5 cm and the latency to the 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 paw-lick latency and returned to its cage. The average of the last two trials served as the baseline pre-drug paw-lick latency.

Immediately, after the third baseline trial on the hot-plate, the drug administration took place with NSS (10 ml/kg) and morphine sulphate (MO; 10 mg/kg) intraperitoneally (i.p.) or 2% Tween 80 (10 ml/kg) and various doses of BLW (25, 50, 100, 200 and 400 mg/kg) orally (p.o.). All animals were placed on the hot-plate for 7 subsequent trials at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of hot-plate latency was expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

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

where the cut-off time was set at 45 sec.

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[3].

Mouse Tail-Flick

These studies employed the tail-flick assay described by D'Amour and Smith in 1941[4], with minor modifications. Male ICR mice weighing 18-25 g were used (N=10 per group). Mice were placed in individual Plexiglas restrainers with an opening to allow the tail to protrude. Each tail rested in a shallow groove housing a light sensitive sensor. A beam of radiant heat (24 V, high amperage 150-watt light bulb situated 8 cm above the tail) was aimed at the middle of a marked dorsal portion of the distal part of each subject's tail that has been blackened length 1 cm with a black ink marker pen in order to absorb the maximum amount of heat and for uniform heat absorption. The device (Harvard Tail-flick Analgesia meter) automatically recorded the latency between the onset of the light beam stimulus and the response to heat, at which point the light beam was terminated. The maximum duration of each test was set at 4.0 sec to minimize the potential for thermal injury. The stimulus intensity was set so that the baseline tail-flick latencies were approximately 1.0-1.5 sec, and the intensity remained constant throughout the experiment. Animals failing to respond within 1.5 sec were excluded from testing. On the day of testing, all animals were tested for 3 pre-drug tail-flick baselines conducted at 10-15 min intervals. The average score of the last two trials served as the baseline measure for each subject.

Immediately after the third baseline trial on the tail-flick test, the drugs were administered: vehicle (NSS; 10 ml/kg), morphine sulphate (MO; 10 mg/kg) i.p., or 2% Tween 80 (10 ml/kg) and various doses of BLW (25, 50, 100, 200 and 400 mg/kg) p.o. Tail-flick latencies were recorded at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of the tail-flick latency was expressed as the mean percent maximum possible effect (%MPE) according to the formula as above, with a cut-off time of 4 sec. Area of analgesia for the tail-flick assays were

calculated using the trapezoidal rule as described above for the hot-plate assays.

Acetic Acid-induced Writhing Test

Male ICR mice weighing 18-25 g were used (N=6 per group). Analgesic testing was determined using the acetic acid-induced writhing method described by Koster *et al.*[5] On the day of testing, animals were randomly assigned to one of seven treatment groups. Mice were then administered 2% Tween 80 (10 ml/kg), indomethacin (10 mg/kg) or various doses of BLW (25-400 mg/kg) p.o. 30 min before i.p. administration of 0.6% acetic acid (10 ml/kg). Each animal was placed in a transparent observational cage. The number of writhing events (abdominal constriction with hind limb extension) was observed and counted at 5 min intervals for 30 min after the acetic acid administration. Antinociceptive activity was reported as percentage of inhibition of 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 writhing response.

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:

Antipyretic activity test: *Lipopolysaccharide-induced fever*

Lipopolysaccharide injected intramuscularly significantly ($p<0.001$) produced a time-dependent increase in rectal temperature in vehicle pretreated rats starting from 1 hr, and this effect was maintained for 7 hr after LPS injection. The maximum increase in rectal temperature was reached at 3 hr (1.76°C) giving a maximum observed mean rectal temperature of 38.19±0.09°C after which there was a decrease. During the same period, the maximum mean rectal temperature of normothermic rats was 36.85 ± 0.05°C. Thus, LPS significantly ($p<0.001$) increased the rectal temperature (Table 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 37.21 ± 0.13°C. All doses of the root extract of BLW remedy (25, 50, 100, 200 and 400 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 BLW was noted at 4, 4, 2, 2 and 3 hr, respectively, and the effect was maintained for the full 7 hr after LPS injection. The maximum mean rectal temperature produced by LPS in the presence of 25, 50, 100, 200 and 400 mg/kg of BLW were 37.53 ± 0.19°C, 37.82 ± 0.19°C, 37.25 ± 0.19 °C,37.64±0.13°C and 37.49±0.09°C respectively. BLW 100 and 200 mg/kg were found to be as potent as ASA (Table 1).

Antinociceptive activity test: Mouse Hot-plate

Morphine 10 mg/kg significantly ($p<0.01$) increased the hot-plate latency producing an area of analgesia of 16,992.68 ± 1,940.94 %MPE-min compared with that of normal saline solution (NSS) (-6,908.17± 2,505.75 % MPE-min; Figure 1). BLW 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 2).

Mouse Tail-Flick

Morphine 10 mg/kg significantly ($p<0.01$) increased tail-flick latency producing an area of analgesia of 427.12±117.58 %MPE-min compared with that of normal saline solution (NSS) (-41.84 ± 69.97; Figure 3). All doses of BLW used in this study, except for the lowest dose, significantly ($p<0.05$) increased the tail-flick latencies when compared to the vehicle group (Figure 4).

Acetic Acid-induced Writhing Test

Indomethacin (10 mg/kg) significantly ($p<0.05$) decreased the writhing response by 89% producing a mean number if writhes of 2.5±0.6 compared with that of vehicle control (23.7± 6.0). BLW doses of 200 and 400 mg/kg significantly ($p<0.05$) decreased the number of writhes induced by acetic acid by 63%

Table 1 Effect of the root extract of BLW remedy (25-400 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]	36.83 ± 0.09	36.91 ± 0.26	37.11 ± 0.12	36.88 ± 0.05	36.85 ± 0.05	36.86 ± 0.05	36.89 ± 0.15	36.30 ± 0.24	36.44 ± 0.15
Control LPS[b]	36.79 ± 0.14	36.44 ± 0.24	37.77 ± 0.11[#]	38.12 ± 0.11[#]	38.19 ± 0.09[#]	38.06 ± 0.11[#]	37.95 ± 0.10[#]	37.75 ± 0.09[#]	37.56 ± 0.12[#]
ASA 300 mg/kg	36.74 ± 0.14	36.99 ± 0.11	37.21 ± 0.13	37.01 ± 0.13[*]	36.85 ± 0.13[*]	36.90 ± 0.18[*]	36.97 ± 0.14[*]	36.74 ± 0.16[*]	36.73 ± 0.17[*]
BLW 25 mg/kg	36.54 ± 0.22	36.94 ± 0.14	37.53 ± 0.19	37.30 ± 0.30	36.88 ± 0.25	36.76 ± 0.18[*]	36.58 ± 0.13[*]	36.34 ± 0.18[*]	35.97 ± 0.23[*]
BLW 50 mg/kg	36.51 ± 0.20	37.06 ± 0.15	37.82 ± 0.19	37.40 ± 0.24	37.19 ± 0.23	36.85 ± 0.11[*]	36.67 ± 0.11[*]	36.58 ± 0.11[*]	36.46 ± 0.07[*]
BLW 100 mg/kg	36.76 ± 0.15	36.93 ± 0.11	37.25 ± 0.19	37.00 ± 0.22[*]	36.79 ± 0.13[*]	36.68 ± 0.04[*]	36.41 ± 0.06[*]	36.29 ± 0.15[*]	36.05 ± 0.16[*]
BLW 200 mg/kg	36.45 ± 0.34	37.21 ± 0.14	37.64 ± 0.13	37.22 ± 0.17[*]	36.90 ± 0.13[*]	36.59 ± 0.06[*]	36.54 ± 0.06[*]	36.42 ± 0.15[*]	36.33 ± 0.10[*]
BLW 400 mg/kg	36.86 ± 0.05	37.16 ± 0.17	37.49 ± 0.09	36.61 ± 0.54	36.69 ± 0.10[*]	36.49 ± 0.21[*]	36.32 ± 0.09[*]	36.32 ± 0.10[*]	36.09 ± 0.26[*]

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

and 61%, respectively when compared to 2% Tween 80. Indomethacin produced the greatest degree of analgesia compared to all test groups (Figure 5).

DISCUSSION:

These studies have demonstrated the antipyretic and antinociceptive effects of BLW in various animal models. Antipyretic activity was assessed utilizing the LPS-induced fever model. The antinociceptive effect was assessed utilizing thermal (hot-plate and tail-flick tests) and chemical (writhing test) models.

Fever is thought to be produced by several endogenous cytokines. Most prominent among these are tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and interferon-α (IFN-α); the latter is produced predominantly in response to viral infection[6-8]. 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. These cytokines are released into the bloodstream and transported to sites in or close to the preoptic-anterior hypothalamus (POAH), the brain site of the primary thermoregulatory controller that reacts to their stimulation by selectively expressing cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase (mPGES-1). Both isoenzymes are transcriptionally regulated by NF-κB and have been demonstrated to be specifically implicated in the febrile response. Prostaglandin E_2 (PGE_2), thus induced by these cytokines, is considered to be the proximal, final fever mediator in the POAH[9]. Prostaglandin synthesis can be activated by TNF-α or phospholipase A_2[10].

Antipyretics such as ASA and other non-steroidal 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[10].

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[6, 11]. For characterization the antipyretic activity of BLW, the LPS-induced fever model in rats was employed in this study.

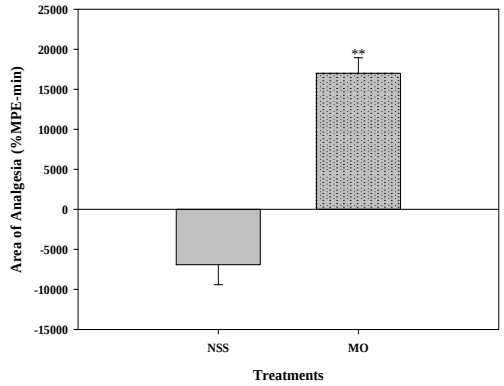

Figure 1 Mouse Hot-Plate Test. Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. ***p*<0.01 significantly different compared to control animals.

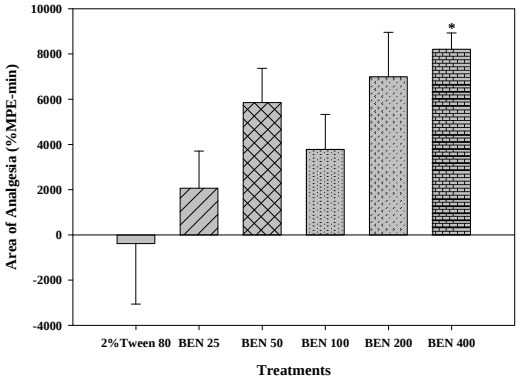

Figure 2 Mouse Hot-Plate Test. Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of the root extract of Bencha-Loga-Wichian remedy (BEN; 25- 400 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. **p*<0.05 significantly different compared to control animals.

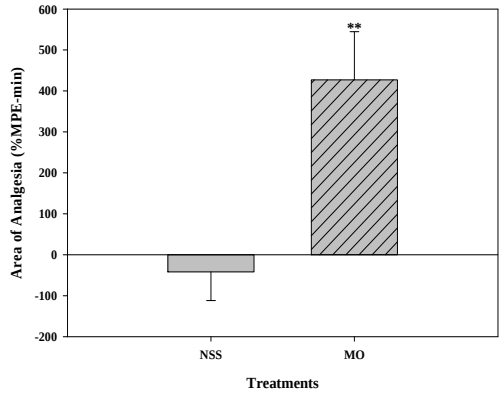

Figure 3 Mouse Tail-Flick Test. Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. ***p*<0.01 significantly different compared to control animals.

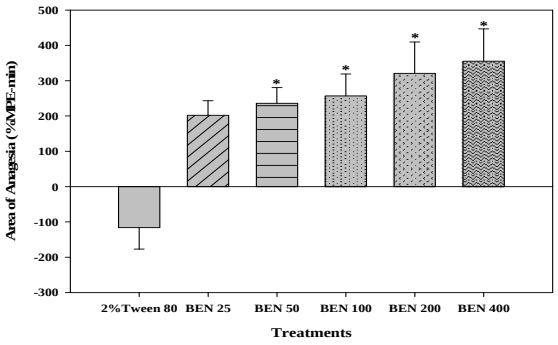

Figure 4 Mouse Tail-Flick Test. Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of the root extract of Bencha-Loga-Wichian remedy (BEN; 25- 400 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. **p*<0.05 significantly different compared to control animals.

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 'BLW', the Thai traditional antipyretic herbal medicine. All doses of BLW (25-400 mg/kg) displayed antipyretic activity in the LPS-induced fever model of rats over the period of 2-7 hr after LPS injection. Additional studies are needed to determine if the antipyretic effect of BLW are due to suppression of TNF-α and inhibition of TNF-α and PG synthesis. The antipyretic effect of BLW (100 and 200 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 BLW used was comparable to that of ASA. These results are consistent with the previous study of Konsue *et al.* in 2008 that investigated the antipyretic effect of dried root powder of BLW herbal drugs.

In order to investigate the antinociceptive properties of BLW, thermal (hot-plate and tail-flick tests) and chemical (writhing test) models was performed in mice. The standard hot-plate test, a central analgesic activity testing model, measures

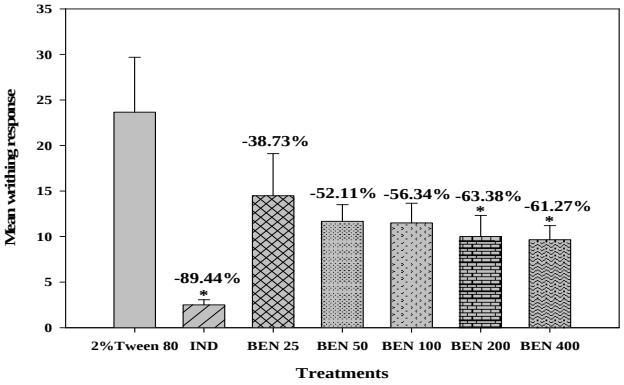

Figure 5 Acetic Acid-induced Writhing Test. Mean writhing response after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of the root extract of Bencha-Loga-Wichian remedy (BEN; 25-400 mg/kg). N=6 for all groups. Values represent the mean±S.E.M. *p<0.05 significantly different compared to control animals. Inhibition is reported as a percentage compared to 2% Tween 80.

two behavioral components including paw licking and jumping which are both considered to be supraspinally integrated responses[12]. This model usually employed morphine (MO) as a reference drug. MO demonstrated potent analgesic effects in this model indicating the sensitivity of this test. The significant analgesic action of BLW (400 mg/kg) was observed during the 240 min test. We also investigated the effectiveness of BLW utilizing the mouse tail-flick technique, another central analgesic activity testing model that is believed to measure spinal reflex. MO administered i.p. produced a significant analgesic response as expected. The significant analgesic effect of most doses of BLW used was observed during the 240 min test. The results obtained from both hot-plate and tail-flick tests indicated that BLW has analgesic activity at both supraspinal and spinal levels.

Additionally, the acetic acid-induced writhing test which is considered a model of visceral inflammation pain was also chosen. This method is commonly used for measuring peripheral analgesic activity. Writhing responses consisted of contraction of the abdomen, twisting and turning of the trunk, and extension of the hind limbs[13]. IND, a non-steroidal anti-inflammatory drug, was used as a reference drug. Oral administration of IND (10 mg/kg) produced a significant analgesic response. Additionally, BLW doses of 200 and 400 mg/kg demonstrated significant analgesic responses, although these were less efficacious compared to IND. Additional studies are required to determine the mechanisms underlying the analgesic properties of BLW.

CONCLUSION: The root extract of BLW remedy possesses both antipyretic and antinociceptive properties in well-established animal models. Additional studies are required to better understand their potential antipyretic and antinociceptive mechanisms of actions. This study helps clarifying the pharmacological action of this herbal remedy and provides additional scientific support for this well-known Thai traditional medicine.

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