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สาขาวิชาเภสัชวิทยา (สหสาขาวิชา)

บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

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# ANTIPYRETIC AND ANTINOCICEPTIVE EFFECTS OF FIVE HERBAL ROOT EXTRACTS OF BENCHA-LOGA-WICHIAN REMEDY

Miss Anusara Jongchanapong

A Thesis Submitted in Partial Fulfillment of the Requirements
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| By | Miss Anusara Jongchanapong |
| Field of Study | Pharmacology |
| Advisor | Assistant Professor Flg. Off. Pasarapa Towiwat, Ph.D. |
| Co-Advisor | Associate Professor Nijsiri Ruangrungsi, Ph.D. |

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Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……………………………………………….. Dean of the Graduate School

(Associate Professor Pornpote Piumsomboon, Ph.D.)

THESIS COMMITTEE

……………………………………………….. Chairman

(Assistant Professor Wacharee Limpanasithikul, Ph.D.)

……………………………………………….. Thesis Advisor

(Assistant Professor Flg. Off. Pasarapa Towiwat, Ph.D.)

……………………………………………….. Thesis Co-Advisor

(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

……………………………………………….. Examiner

(Associate Professor Chandhanee Itthipanichapong)

……………………………………………….. External Examiner

(Associate Professor Chongkol Tiangda, Ph.D.)

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ตำรับยาเบญจโลกวิเชียรเป็นตำรับยาไทยแผนโบราณที่มีชื่อเสียงและมีสรรพคุณในการแก้อาการไข้ต่างๆ ประกอบไปด้วยรากของต้นชิงชี่ ย่านาง คนทา ไม้เท้ายายม่อม และมะเดื่อชุมพร การทดลองครั้งนี้เริ่มต้นด้วยการทดสอบฤทธิ์ลดไข้ของสิ่งสกัดจากรากสมุนไพรแต่ละชนิด (CM, HP, TT, CP และ FR) และตำรับยาเบญจโลกวิเชียร (BEN) ด้วยวิธีการเหนี่ยวนำให้หนูแรทเป็นไข้ด้วย LPS โดยสัตว์ทดลองจะถูกเหนี่ยวนำให้เป็นไข้ด้วยการฉีด LPS ขนาด 50 ไมโครกรัม/กก.เข้าทางกล้ามเนื้อ หลังจากป้อน 2% Tween 80, แอสไพรินขนาด 300 มก./กก. หรือ CM, HP, TT, CP, FR และ BEN ขนาด 25, 50, 100, 200 และ 400 มก./กก. ไปแล้ว 1 ชม. โดยจะวัดอุณหภูมิของหนูทางทวารหนักก่อนให้สารและหลังฉีด LPS ทุกชั่วโมงเป็นเวลา 7 ชั่วโมง และพบว่า CM, HP, TT, CP, FR และ BEN ทุกขนาดที่ใช้สามารถลดอุณหภูมิของหนูที่เพิ่มขึ้นได้อย่างมีนัยสำคัญทางสถิติตั้งแต่ชั่วโมงที่ 1 หรือ 2 หลังจากฉีด LPS และ BEN ขนาด 400 มก./กก. น่าจะมีประสิทธิภาพในการลดไข้สูงสุด ต่อจากนั้นจึงทำการศึกษาฤทธิ์ระงับปวดของ CM, HP, TT, CP, FR และ BEN ในขนาดต่างๆ ด้วยวิธี hot-plate โดยจับเวลาที่หนูเม้าส์เพศผู้สายพันธุ์ ICR สามารถทนอยู่บนแผ่นความร้อน (hot-plate latencies) ได้ก่อนให้น้ำเกลือทางช่องท้อง หรือมอร์ฟีนขนาด 10 มก./กก. ทางช่องท้อง 2% Tween 80 โดยการป้อนหรือ CM, HP, TT, CP, FR และ BEN ในขนาด 25, 50, 100, 200 และ 400 มก./กก. โดยการป้อน และจับเวลาที่หนูสามารถทนอยู่บนแผ่นความร้อนได้ที่เวลา 15, 30, 45, 60, 90, 120 และ 240 นาทีหลังได้รับสารทดสอบ นำค่าที่ได้มาคำนวณเปอร์เซ็นต์สูงสุดที่หนูสามารถทนต่อความร้อนได้ (%MPE) แล้วนำมาหาคำนวณหาพื้นที่ใต้กราฟระหว่าง %MPE กับเวลา (area of analgesia) พบว่า เกือบทุกขนาดของ CM, HP, TT และ FR ที่ให้มีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ ส่วน CP และ BEN ในขนาด 400 มก./กก. เท่านั้นที่จะมีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ และพบว่าฤทธิ์ระงับปวดของ CM ขนาด 200 มก./กก. และ HP, TT, CP และ FR ขนาด 400 มก./กก. ถูกยับยั้งได้ด้วยนาลอกโซน แสดงว่ากลไกการออกฤทธิ์ระงับปวดของ CM, HP, TT, CP และ FR น่าจะเกี่ยวข้องกับตัวรับ opioid ในการทดสอบด้วยวิธี tail-flick ทำการจับเวลาที่หนูเม้าส์ทนต่อรังสีความร้อนได้โดยไม่กระดกหางหนี (tail-flick latencies) ก่อนให้น้ำเกลือทางช่องท้อง มอร์ฟีนขนาด 10 มก./กก. ทางช่องท้อง หรือ 2% ทวีน 80 โดยการป้อน หรือ CM, HP, TT, CP, FR และ BEN ในขนาด 25-400 มก./กก. โดยการป้อน และทำการทดสอบหลังได้รับสารทดสอบอีก 7 ครั้งในช่วงเวลา 4 ชั่วโมง พบว่า HP, TT, CP, FR และ BEN มีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ แต่ CM ทุกขนาดที่ให้ไม่มีฤทธิ์ระงับปวดในการทดสอบนี้ ส่วนการทดสอบฤทธิ์ระงับปวดโดยเหนี่ยวนำให้หนูเม้าส์เกิดความเจ็บปวดจนเกิดอาการบิดงอลำตัว (writhing) ด้วยกรดอะซิติก จะทำการฉีดกรดอะซิติก 0.6% ในขนาด 10 มล./กก. เข้าทางช่องท้องของสัตว์ทดลองที่เวลา 30 นาทีหลังจากป้อน 2% Tween 80 อินโดเมทาซิน ขนาด 10 มก./กก. หรือ CM, HP, TT, CP, FR และ BEN ขนาด 25-400 มก./กก. แล้วนับจำนวนครั้งที่หนูเกิดการบิดงอลำตัวเป็นเวลา 30 นาที พบว่าเกือบทุกขนาดของ CM, HP, TT, CP, FR และ BEN สามารถลดจำนวนครั้งของการบิดงอลำตัวของหนูได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม จากผลการทดลองทั้งหมดแสดงว่าสิ่งสกัดจากรากสมุนไพรทั้งห้าชนิดและตำรับยาเบญจโลกวิเชียร มีฤทธิ์ลดไข้ได้ตามสรรพคุณที่ใช้กันมาแต่โบราณ และกลไกการออกฤทธิ์มีส่วนเกี่ยวข้องกับการยับยั้งการสร้างและการปลดปล่อย TNF-$\alpha$ นอกจากนี้ยังแสดงให้เห็นว่า สิ่งสกัดจากรากสมุนไพรทั้งห้าชนิดและตำรับยาเบญจโลกวิเชียรมีฤทธิ์ระงับปวดทั้งในระดับประสาทส่วนกลางและระดับประสาทส่วนปลาย และกลไกการออกฤทธิ์ระงับปวดมีส่วนเกี่ยวข้องกับตัวรับของ opioid ผลการทดลองครั้งนี้จัดเป็นหลักฐานทางวิทยาศาสตร์อีกชิ้นที่สนับสนุนการใช้ตำรับยาแผนโบราณของไทยตำรับนี้


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KEYWORDS : *CAPPARIS MICRACANTHA* / *HARRISONIA PERFORATA* / *TILIACORA TRIANDRA* / *CLERODENDRUM PETASITES* / *FICUS RACEMOSA* / BENCHA-LOGA-WICHIAN REMEDY


ANUSARA JONGCHANAPONG : ANTIPYRETIC AND ANTINOCICEPTIVE EFFECTS OF FIVE HERBAL ROOT EXTRACTS OF BENCHA-LOGA-WICHIAN REMEDY. ADVISOR : ASST. PROF. FLG. OFF. PASARAPA TOWIWAT, PH.D., CO-ADVISOR : ASSOC.PROF. NIJSIRI RUANGRUNGSI, PH.D., 239 pp.


Bencha-loga-wichian remedy is a famous antipyretic drug of 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 property of each herbal root extract (CM, HP, TT, CP and FR) and the extract of Bencha-loga-wichian remedy (BEN) using LPS-induced fever model in rats. The animals were induced with i.m. injection of LPS (50 μg/kg) 1 hr after oral administration of 2%Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) or various doses of CM, HP, TT, CP, FR and BEN (25, 50, 100, 200 and 400 mg/kg). Rectal temperature was measured before the treatment and at 1 hr interval for 7 hr after LPS injection. All doses of CM, HP, TT, CP, FR and BEN significantly ($p<0.05$) reduced the increased rectal temperature at 1 or 2 hr after LPS injection. BEN 400 mg/kg seemed to have the highest antipyretic efficacy. Studies then determined the antinociceptive property of a range of five herbal root extracts and BEN doses in the mouse hot-plate test. Hot-plate latencies were determined in male ICR mice prior to the administration of 0.9% normal saline solution (NSS; 10 ml/kg, i.p.), morphine (MO; 10 mg/kg, i.p.), 2% Tween 80 (10 ml/kg, p.o) or various doses of CM, HP, TT, CP, FR and BEN (25-400 mg/kg, p.o.). Hot-plate latencies were subsequently determined at 15, 30, 45, 60, 90, 120 and 240 min. The percent maximum possible effect (%MPE) was calculated and used in the determination of the area of analgesia (%MPE-min). Most doses of CM, HP, TT, CP, FR tested produced a significant ($p<0.05$) analgesic response, while CP and BEN only at the dose of 400 mg/kg had significant analgesic response. CM 200 mg/kg and HP, TT, CP, FR (400 mg/kg) produced analgesic response that was naloxone-sensitive suggesting opioid-mediated mechanism. In the mouse tail-flick test, tail-flick latencies were determined prior to the administration of NSS (10 ml/kg, i.p), MO (10 mg/kg, i.p.), 2% Tween 80 (10 ml/kg, p.o.) or various doses of CM, HP, TT, CP, FR and BEN (25-400 mg/kg, p.o.) and were subsequently determined at 7 intervals over a 4 hr period. Most doses of HP TT, CP, FR and BEN produced significant analgesic response. All doses of CM failed to produce analgesic response in this method. In the acetic acid-induced writhing in mice, the animals were induced with i.p. injection of 0.6% acetic acid (10 ml/kg) 30 min after the administration of 2% Tween 80, indomethacin (IND; 10 mg/kg, p.o.), or various doses of CM, HP, TT, CP, FR and BEN (25-400 mg/kg, p.o.) and the mean writhing response was determined for 30 min. Most doses of CM, HP, TT, CP, FR and BEN significantly ($p<0.05$) decreased the mean writhing response compared to vehicle controls. Taken together these results demonstrated that all five root extracts and BEN remedy have antipyretic activity and the antipyretic mechanism may involve the inhibition of TNF-α synthesis and release. In addition, all five root extracts and BEN remedy also possess both central and peripheral analgesic activities and mechanism of action seems to be partly related to opioid receptors. These results provide another scientific evidence to support its use in Thai traditional medicine.


Field of Study : Pharmacology ............ Student's Signature ............

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| $\alpha$ | = | alpha |
| $\beta$ | = | beta |
| $\kappa$ | = | kappa |
| μg/kg | = | microgarm per kilogram |
| μl | = | microlitter |
| / | = | per |
| % | = | percent |
| %MPE | = | percentage of the maximum possible effect |
| ºC | = | celcius degree |
| ASA | = | acetylsalicylic acid |
| AUC | = | area under the curves (area of analgesia) |
| BEN | = | Bencha-loga-wichian remedy |
| BW | = | body weight |
| cm | = | centimeter |
| CM | = | the *Capparis micracantha* root extract |
| CNS | = | central nervous system |
| CP | = | the *Clerodendrum petasites* root extract |
| $ED_{50}$ | = | median effective dose |
| *et al*. | = | et alii (and other) |
| FR | = | the *Ficus racemosa* root extract |
| g | = | gram |
| g/kg | = | gram per kilogram |
| sec | = | second |
| HP | = | the *Harrisonia perforata* root extract |
| hr | = | hour |
| IASP | = | International Association for the Study of Pain |
| $IC_{50}$ | = | median inhibition dose |
| i.p. | = | intraperitoneal |
| i.m. | = | intramuscular |

| | | |
|---|---|---|
| IND | = | indomethacin |
| LPS | = | Lipopolysaccharide |
| m | = | meter |
| mg/ear | = | milligram per ear |
| mg/kg | = | milligram per kilogram |
| mg/ml | = | milligram per miliileter |
| min | = | minute |
| ml/kg | = | milliliter per kilogram |
| MO | = | morphine sulphate |
| N | = | sample size |
| NAL | = | naloxone |
| ng/kg | = | nanogram per kilogram |
| NO | = | nitric oxide |
| NOS | = | nitric oxide synthase |
| NSAIDs | = | non-steroidal anti-inflammatory drugs |
| NSS | = | normal saline |
| TT | = | the *Tiliacora triandra* root extract |
| TNF-$\alpha$ | = | tumor necrosis factor-alpha |
| V | = | volt |
| w/w | = | weight by weight |

# CHAPTER I

# INTRODUCTION

## Background and Rationale

Fever and pain are common problems in general population that have impact on lifestyles and health. It may occur as single entity or in combination. Opioids have been most widely used to treat acute and cancer-related pain. It produces both analgesia and side effects by acting on $\mu$-opioid receptors. They also produce respiratory depression, vomiting and constipation. In addition, opioids can cause sedative, confusion, dizziness and euphoria. Opioid use is often associated with the development of tolerance and physical dependence. Nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen (paracetamol) are one of the world most commonly used drugs for the treatment of pain and fever. Although NSAIDs are used for the treatment of pain, fever and inflammation, they are associated with several side effects including nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, and drowsiness. The most serious side effects are renal failure, liver failure, ulcers, gastrointestinal bleeding and prolonged bleeding. Excessive use of acetaminophen can damage multiple organs, especially the liver and kidney. Long term use of these drugs in chronic condition could lead to an expensive cost. Therefore, many researchers are searching for new antipyretic and analgesic drugs with higher efficacy and lower side effects from natural products including herbal plants.

There are several Thai herbal formulas that have been used as antipyretic and analgesic agents including Chan-tha-lee-la, Pra-sa-pai and Bencha-loga-wichian. “Bencha-loga-wichian” is a famous herbal remedy used in Thailand and has been included in “Thailand Herbal Medicine Essential Drug List” as an antipyretic for both children and adults in 2006. The formula is composed of five herbal roots in an equal part by weight including 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). Many researches had been done to investigate various pharmacological effects of the extract of several parts of the plants including root, leaf and stem bark of this five herbal plants.

Even though Bencha-loga-wichian remedy is widely used by many traditional doctors in Thailand as an antipyretic, there is only one scientific data that support its use. The study was done by Konseu et al. in 2008 and proved the antipyretic efficacy of the individual root powder of Bencha-loga-wichian formula and the root powder of the formula using Baker's yeast-induced fever model in rats. This study therefore was designed to investigate the antipyretic effect of each individual root extract of the Bencha-loga-wichian remedy and also the whole root extract of the remedy using lipopolysaccharide-induced fever model in rats in order to provide another scientific evidence to support its use in Thai traditional medicine. In addition, this study was also designed to investigate in various animal models of the antinociceptive effect and the mechanism involved of the individual root extract and the whole root extract of the remedy to look for another useful pharmacological effect of the herbal formula.

## Purpose of the study

1. To investigate the antipyretic effect of each herbal root extract of Bencha-loga-wichian remedy and the whole extract of Bencha-loga-wichian remedy compared with a standard drug.

2. To investigate the antinociceptive effect and the antinociceptive mechanism of each herbal root extract of Bencha-loga-wichian remedy.

## Hypothesis

All five herbal root extracts of Bencha-loga-wichian remedy and the whole extract of Bencha-loga-wichian remedy have antipyretic and antinociceptive effects in various animal models.

## Expected benefit and application

The knowledge obtained from this study may lead to the development of a new analgesic and/or antipyretic agent from natural sources and provide scientific evidence to support the use of Bencha-loga-wichian remedy as an antipyretic agent.

## Research design

Experimental research

## Key words

*Capparis micracantha*

*Harrisonia perforata*

*Tiliacora triandra*

*Clerodendrum petasites*

*Ficus racemosa*

Bencha-loga-wichian remedy

# CHAPTER II

# LITERATURE REVIEWS

## BENCHA-LOGA-WICHIAN REMEDY

Bencha-loga-wichian remedy (BEN) is one of the famous antipyretic herbal remedy in Thai traditional medicine. It is widely used by traditional doctors in Thailand to treat many types of fever without any scientific data support. This remedy has been included in "Thailand Herbal Medicine Essential Drug List" as an antipyretic for both children and adults in the form of powder or tablet. BEN remedy is composed of five herbal roots in an equal part by weight, including roots of *Capparis micracantha* DC. (Ching-chee), *Harrisonia perforata* (Blanco) Merr (Khon-thaa), *Tiliacora triandra* (Colebr.) Diels (Yaa-nang), *Clerodendrum petasites* S. Moore (Mai-tao-yai-mom), and *Ficus racemosa* Linn. (Ma-dueo-chumporn). Many studies have shown that all those plants contain many alkaloids, sterols and other compounds. Recently, Konsue et al., 2008 had investigated the antipyretic effect of the individual root powder and the remedy utilizing yeast-induced fever model in rats. From the study, BEN was found to have antipyretic effect at doses of 100, 200 or 400 mg/kg and BEN 200 mg/kg seemed to have the highest antipyretic efficacy. Each root also seemed to be active as an antipyretic drug, except the root of *Clerodendrum petasites*. This is the only scientific evidence that support its use as an antipyretic drug.

*CAPPARIS MICRACANTHA*

Figure 1 *Capparis micracantha* DC. (Ching-chee) and *Capparis micracantha* root.

***Capparis micracantha* DC.** (Figure 1)

**Synonyms** *Capparis odorata* Blanco (1837), *Capparis myrioneura* Hallier f. (1906).

**Vernacular names** Thailand: chingchee (central), kradaat khaao (central), nuat maeo daeng (northern). Indonesia: balung, kledung (Javanese), sanek (Madurese). Malaysia: kaju tuju. Philippines: salimbagat (Tagalog), tarabtab (Iloko), salimomo (Bisaya). Cambodia: kanchoen bai dach. Laos: say sou. Vietnam: b[uf]ng ch[ef], c[as]p gai nh[or].

**Distribution** From Burma (Myanmar), Indo-China, Thailand and Peninsular Malaysia, to Indonesia and the Philippines.

**Uses** *Root:* carminative; treatment of chronic infected skin diseases. *Stem:* crush with small amount of water and topically apply to relieve sprains and swelling. *Leaf:* used for muscular cramps; boil with water, drink or bathe to relieve fever with chronic vesicular skin diseases; smoke to treat bronchitis. *Root or leaf:* antiasthmatic; treatment of chest pain, fever with vesicular skin diseases, such as measles.

**Observations** A half-erect shrub or small tree with droping branches, 1-6 m tall, rarely a vine 2-4 m tall, young branches zigzag, glabrous; leaves oval to oblong-lanceolate, 9.5-20 cm x 3-11 cm, base rounded, apex variable, rarely acuminate, coriaceous, shining, petiole 0.7-1.5 cm long, thorns patent, straight or slightly curved, 2-7 mm long, on flowering branches often absent; flowers 2-6 in a row, pedicel about 1 cm long; sepals

ovate, 5.5-13 mm long, petals oblong or elliptical, 10-26 mm long, thin, white with yellow base, later turning dark red, stamens 20-45, filaments 2.5-3 cm long, white, gynophore 15-35 mm long, ovary and gynophore sometimes abortive; berry globular or ellipsoid, 2-6 cm in diameter, with 4 longitudinal sutures, yellow, orange or red and strongly smelling when ripe; seeds numerous, in whitish, slimy, sweet pulp. *Capparis micracantha* is found in brushwood, hedges and open forest, also along the seashore and in sandy locations, mostly below 500 m altitude.

## Pharmacological Activities

### Antibacterial activity

The ethanolic extract of *Capparis micracantha* dried root exhibits antibacterial activity against β-streptococcus group A, *Pseudomonas aeruginosa* but is not active against *Staphylococus aureus* and was *Klebsiella pneumoniae* (Laorpaksa et al., 1998).

### Anticancer, anti-tuberculosis and cytotoxicity activities

The hexane extract of the leaves showed inhibition of lung cancer cell with $IC_{50}$ 6.99 µg/ml. It also showed anti-tuberculosis with MIC 200.00 µg/ml. The hexane and dichoromethane extracts of the flowers showed anti-tuberculosis with MIC 100.00 and 200.00 µg/ml, respectively. All crude extracts showed non-cytotoxicity against Vero cells (Khantikaew and Sakulkhaemaruethai, 2007)

### Antipyretic activity

The dried root powder of *Capparis micracantha* was assessed for antipyretic activity utilizing yeast's-induced fever model. The powdered root of *Capparis micracantha* at dose of 40 mg/kg showed significant antipyretic efficacy and no obvious toxic effect could be seen (Konsue et al., 2008).

## *HARRISONIA PERFORATA*

Figure 2 *Harrisonia perforata* (Blanco) Merr (Khon-thaa) and *Harrisonia perforata* root.

***Harrisonia perforata* (Blanco) Merr.** (Figure 2)

**Family** Simarubaceae

**Synonyms** *Harrisonia paucijuga* Oliv. (1868), *Harrisonia bennettii* Benn. (1875).

**Vernacular names** Thailand: khon-thaa (Central), naam chee (Northern). Indonesia: sesepang (Lampung), garut (Sundanese), ri kengkeng (Javanese). Malaysia: kait-kait (Murut, Sabah). Philippines: asimau, mamikil (Tagalog), muntani (Bisaya). Laos: dok kin ta. Vietnam: s[aa]n, da da, h[ar]i s[ow]n.

**Distribution** *Harrisonia perforata* is found in the drier parts from Burma (Myanmar) eastward through Thailand to Indo- China and the Philippines, southward to Peninsular Malaysia (Perlis), South Sumatra, Borneo (Sabah), Sulawesi, Java and the Lesser Sunda Islands.

**Uses** In Indonesia, young shoots are considered a remedy against diarrhoea. In the Philippines, a decoction of the root bark is recommended in the treatment of diarrhoea and dysentery as well as against cholera. In Indo-China, ashes of the roasted leaves mixed with oil or simply crushed leaves are applied to relieve itch. In Thailand, the dried root is considered antipyretic and anti-inflammatory; it is used in wound healing and in the treatment of diarrhoea. The stems are also employed in the treatment of diarrhoea.

**Observations** A scandent to erect prickly shrub up to 4(-6) m tall, leaves imparipinnate up to 20 cm long, with 1-15 pairs of leaflets; petiole 5-30 mm long; stipulate thorns slightly recurved, accrescent to 7 mm; leaflets rhomboid to ovate-lanceolate, 10-20 mm x 5-15 mm, subentire to lobed, rachis narrowly winged; inflorescence 8-20-flowered, flowers (4-)5-merous, pedicellate, calyx small, lobes triangular, petals lanceolate, 6-9 mm x 2-4 mm, red outside, pale red to white inside, stamens (8-) 10, anthers 1.5-4.5 mm long, filaments 7-10 mm long, at base with a ligule which is densely woolly at the margin, disk cup-shaped, ovary slightly lobed, styles 5-8 mm long, pubescent; fruit a berry, 4-9 mm x 11-15 mm, exocarp coriaceous, at least 1 mm thick, endocarp hard, without suture.

**Phytochemicals**

perforatin (Tran V. S. et al.,1994), perforatin C, perforatin D, perforatin E, perforatin F, perforatin G, heteropeucenin-7-methyl ether, heteropeucenig-S-methoxy-7-methyl ether, 2-hydroxymethylallopataeroxylin-5-methyl ether, perforatin A, perforatic acid, perforatic acid methyl, scopoletin, cedrelopsin, xanthoxyletin, phenyl propanoid, and coniferyl aldehyde (Tomoko T. et al., 1995), lectin protein which preferentially agglutinates animal red blood cells (Wongkham S. et al., 1995), haperforine A and haperforine E (Qui Khoung-Huu et al., 2000), haperforine $B_1$ and haperforine D (Chiaroni, A et al., 2000), haperforins C2, haperforins F and haperforins G (Qui Khoung-Huu et al., 2001), perforamone A, perforamone B, perforamone C, perforamone D (Tuntiwachwuttikul P et al., 2006).

**Pharmacological Activities**

**Antihistamimic action**

The 50% ethanolic extract from roots and stem of *Harrisonia perforata* had shown antihistamimic action on guinea pigs ileum at dilution of $10^{-3}$ µl (Mokkhasamit M. et al., 1971).

**Antioxidant activity**

Antioxidative activity of 50% ethyl alcohol extract of *Harrisonia perforata* was determined using DPPH radical scavenging assay. The extract of *Harrisonia perforata*

showed good antioxidative activity with $EC_{50}$ close to those values of standard ascorbic acid and α-tocopherol solutions (Sripanidkulchai et al., 2005).

**Anti-hepatitis B Virus activity**

The 95% ethyl alcohol extract of *Harrisonia perforata* dried root were screened for anti-hepatitis B virus by enzyme-linked immunosorbent assay (ELISA). At the concentration of 2 mg/ml, it could inhibit HbsAg secretion by PLC/PFR/5 cells with % inhibition of 32%. (Sirotamarat et al, 2002).

**Antimalarial and cytotoxicity activities**

The extract of *Harrisonia perforata* leaf was tested for in vitro activity against *Plasmodium falciparum* and assessed for cytotoxicity against the human cancer cell line Hela and the embryonic lung MRC5 cell line. The extract showed interesting antiplasmodial activity with a good selectivity (Nguyen-Pouplin et al., 2007).

**Antipyretic activity**

The dried root powder of *Harrisonia perforata* was assessed for antipyretic activity utilizing yeast's-induced fever model. The powdered root of *Harrisonia perforata* at dose of 40 mg/kg showed significant antipyretic efficacy and no obvious toxic effect could be seen (Konsue et al., 2008).

*TILIACORA TRIANDRA*

Figure 3 *Tiliacora triandra* (Colebr.) Diels (Yaa-nang) and *Tiliacora triandra* root.

***Tiliacora triandra* (Colebr.) Diels** (Figure 3)

**Family** Menispermaceae

**Synonyms** Cocculus triandrus Colebr. (1822), Limacia triandra (Colebr.) Hook.f. & Thomson (1855).

**Vernacular names** Thailand: choi nang (northern), thao wan khieo (central), yat nang (peninsular). Malaysia: akar kunyitkunyit, berkunyit, akar kusin (Peninsular). Vietnam: xanh tam.

**Origin and geographic distribution** *T. triandra* occurs in India (Assam), southern Burma (Myanmar), Indo-China, Thailand and Peninsular Malaysia.

**Uses** In Thailand aerial parts of *T. triandra* are widely used as an antipyretic. In Cambodia the leafy shoots enter into a prescription for the treatment of dysentery. They are also used as a flavoring in cooking in Thailand. In Indo-China the flexible stems are used for rough cordage, thatching and basketry. *T. acuminata (Lamk) Hook.f. & Thomson*, an Indian-Burmese species, appreciated for its ornamental foliage and fragrant flowers and mentioned as a remedy for snakebites, is cultivated in the botanical garden in Bogor, Indonesia.

**Observations** A dioecious liana with puberulous to glabrous and striate stems. Leaves alternate, simple and entire, elliptical, lanceolate or sometimes subovate, 6.5-11(-17) cm x 2-4(-8.5) cm, base cuneate to rounded, apex usually acuminate, with 3-5 basal veins

and 2-6 pairs of lateral veins; petiole 0.5-2 cm long; stipules absent. Inflorescence an axillary or cauliflorous pseudo-raceme, up to 2-8(-17) cm long, composed of 1-few-flowered peduncled cymes. Flowers unisexual, yellowish; sepals 6-12, the outermost smallest, innermost up to 2 mm long; male flowers with 3 or 6 petals c. 1 mm long and 3 stamens; female flowers with 6 petals c. 1 mm long and 8-9 carpels inserted on a gynophore. Fruit consisting of several drupes borne on a branched carpophore; drupes obovoid, 7-10 mm x 6-7 mm, red, glabrous, endocarp transversely and irregularly ridged. In Indo-China *T. triandra* can be found flowering and fruiting throughout the year, but in Thailand from December-July only. As in other Menispermaceae, the pollinators are probably small insects, which are undoubtedly attracted by the scent of the flowers. Tiliacora consists of 19 species in Africa, 2 in tropical Asia and 1 in Australia.

**Properties** More than 15 alkaloids have been identified from *T. triandra* including the bisbenzylisoquinolines tiliacorinine, nortiliacorinine and tiliacorine. These alkaloids act as cardiac and respiratory poisons when injected into frogs. A crude ethanol extract of *T. triandra* leaves showed strong antifeedant activity against the green leafhopper Nephotettix virescens. A methanol extract of roots exhibited antimalarial activity in vitro. An ethanol extract of *T. acuminata* showed strong antibacterial activity. Tiliacorinine isolated from a crude extract showed promising antifungal activity against Alternaria tenuissima, a causal agent of leaf blight in pigeon pea (*Cajanus cajan* (L.) Millsp.).

**Phytochemicals**

**Roots:** alkaloids(1,2), alkaloid G, alkaloid H (Pavanand et al., 1989), aporphine, isoquinolines (Dechatiwaong et al., 1974), lactone(2), tannin(2), nortiliacorinine A, tiliacorine, tiliacorinine, tiliacorinine-2'-N-oxide (Pornsiriprasert et al., 1984)

**Aerial part:** magnoflorine, norisoyanangine, nortiliacorine A, noryanangine (Pachaly and Khosravian, 1988), protoquercitol, (1S, 1R)-tiliageine, tilitriandrene (Pachaly and Khosravian, 1988), Tiliacora triandra oxalate oxidase (Sukontawarin et al., 1985), tiliacorinin-2'-N-oxide (Wiriyachaitra and Phuriyakorn, 1981)

## Pharmacological activities

### Antimalarial activity

The water-insoluble alkaloidal component of the methanol extract of the root of *Tiliacora triandra* was found to have antimalarial activity against *Plasmodium falciparum in vitro* while the water-soluble component was inactive (Pavanand et al., 1989; Dechatiwong et al., 1987)

### Antihistamimic action, Atropine-like action and Papaverine-like action

The 50% ethanolic extract from roots and stem of *Tiliacora triandra* had shown antihistamimic, atropine-like and papaverine-like actions on guinea pigs ileum at dilution of $10^{-3}$ μl (Mokkhasamit M. et al., 1971).

### Analgesic and anti-inflammatory activities

The water extract of *Tiliacora triandra* at 1 g/kg administered orally in Swiss albino male mice was used to test for analgesic activity in the acetic-induced writhing model. The extract of *Tiliacora triandra* elicited significant inhibition of writhing reflex when compared with diclofenac but showed weak analgesic activity in tail-flick test (Tangsucharit et al., 2006).

### Antioxidant activity

The radical scavenging activity of the methanolic extract of *Tiliacora triandra* was determined by DPPH assay. The water soluble fraction and water insoluble fraction showed antioxidant activity with the $IC_{50}$ of 499.79 μg/ml and 772.63 μg/ml, respectively (Wangrug and Piyasuwan, 2006).

### Antipyretic activity

The dried root powder of *Tiliacora triandra* was assessed for antipyretic activity utilizing yeast's-induced fever model. The powdered root of *Tiliacora triandra* at dose of 40 mg/kg showed significant antipyretic efficacy and no obvious toxic effect could be seen (Konsue et al., 2008).

*CLERODENDRUM PETASITES*

Figure 4 *Clerodendrum petasites* S. Moore (Mai-tao-yai-mom) and *Clerodendrum petasites* root.

***Clerodendrum petasites* S. Moore** (Figure 4)

**Family** Verbenaceae

**Synonym** *Clerodendrum indicum* Kuntze.

**Vernacular names** Thailand: Tao-yai-mom, Maitao-rusri.

**Distribution** *C. petasites* is widely grown in India, Malaysia and Thailand.

**Observations** *C. petasites* is erect, shrubs or herbs, 1-2 meter high, strength plant, twings are less, The leaves is 15- 20 cm long and 1.5-2,5 wide, usually opposite and rarely alternate pr whorled, mostly simple. Flowers are long tubes with white color, calyx is cup shaped, typically 5-lobed. The fruit is a drupe (or berry) with a large kernel.

**Uses** Root is used to treat fever decrease body temperature, antiallergic, anti-inflammatory, anticonvulsant.

**Phytochemicals**

hydroquinone, diterpenoid, Clerodendone (Ravindranath et al., 2004), Cleroindicins (Tian et al., 1997), Scutellarein, hispidulin-7-O-glucuronide (Subramanian et al., 1973)

## Pharmacological Activities

### Lipid peroxidation inhibitors

The methanolic extract of *Clerodendrum petasites* bark was evaluated using bovine brain phopholipid liposomes as model membranes. The extract was a highly potent inhibitor of lipid peroxidation with $IC_{50}$ value of 0.93 μg/ml (Kumar and Muller, 1999).

### Bronchodilator activity

The ethanolic extract of *Clerodendrum petasites* dried aerial part was tested to evaluate the spasmolytic activity on isolated guinea-pig tracheal smooth muscle. The crude extract (2.25–9.0 mg/ml) dose-dependently caused relaxation of tracheal smooth muscle which was contracted by exposure to histamine (Hazekamp et al., 2001).

### Anti-inflammatory activity

The methanol extract from *Clerodendrum petasites* S. Moore was assessed for anti-inflammatory and antipyretic activities on the experimental animal models. It was found that the extract possessed moderate inhibitory activity on acute phase of inflammation in a dose-related manner as seen in ethyl phenylpropiolate-induced ear edema ($ED_{50}$ = 2.34 mg/ear) as well as carrageenin-induced hind paw edema ($ED_{30}$ = 420.41 mg/kg) in rats. However, the extract did not elicit any inhibitory effect on arachidonic acid-induced hind paw edema in rats. In subchronic inflammatory model, CP extract provoked a significant reduction of transudation but had no effect on proliferative phase when tested in cotton pellet-induced granuloma model. The extract also reduced the alkaline phosphatase activity in serum of rats in this animal model. Anyhow, the extract did not possess any analgesic activity in acetic acid-induced writhing response in mice. The results obtained show that *C. petasites* has moderate anti-inflammatory activities without ulcerogenic effect (Panthong et al., 2003)

### Antipyretic activity

The methanolic extract possessed an excellent antipyretic effect when tested in yeast-induced hyperthermic rats (Panthong et al., 2003). The dried root powder of *Clerodendrum petasites* at dose of 40 mg/kg showed significant antipyretic activity utilizing yeast's-induced fever model and no obvious toxic effect was observed (Konsue et al., 2008).

*FICUS RACEMOSA*

Figure 5 *Ficus racemosa* Linn. (Ma-dueo-chumporn) and *Ficus racemosa* root.

***Ficus racemosa* Linn.** (Figure 5)

**Family** Moraceae

**Synonyms** *Ficus glomerata* Roxb.

**Vernacular names** Thailand: duea kliang (central, northern), duca nam (peninsular).Cluster fig, red river fig (En). Indonesia: elo (Javanese), loa (Sundanese), arah (Madurese). Singapore: atteeka. Burma: atti, umbar. Cambodia: loviё. Laos: düa kiengz. Vietnam: sung.

**Distribution** North-eastern Africa, India to Indo-China, Malaysia to northern and western Australia. Not in the Philippines. In India also cultivated.

**Uses** The figs, which are rather insipid but sweet, are edible. They are used in various preserves and side-dishes. Leaves eaten as vegetable and are said to be used against diarrhoea. They are also used as animal fodder and they provide valuable mulch. In India the tree is also cultivated as host plant for lac insects, shade tree for coffee and a rootstock for *Ficus carica* L. The latex is used in production of water-resistant paper and as plasticizer for Hevea rubber.

**Observations** Deciduous (in drier areas) cauliflorous tree, 20-30 m tall, buttressed, often with irregular crown. Infructescences in big clusters on branching, leafless twigs on stem and larger branches. Fruit a fig, pyriform to subglobose, 2.5-5 cm in diameter,

rose-red when ripe. In open, deciduous forest, common along river banks in lowlands. Four varieties have been distinguished, mainly based on differences in leaf form and hairiness. is a synonyms names as *Ficus glomerata* Roxb., *Ficus chittagonga* Miq., *Ficus mollis* Miq., *Ficus goolereea* Roxb. *Cavellia glomerata* Miq., *Cavellia mollis* Miq.

**Phytochemicals**

Leaves: steroids, triterpenoids, alkaloids, polyphenolics, courmarins, flavonoids, tannin, glucoside (Rao et al., 2002, Li et al., 2004), tirucallane gluanol acetate, β-sitosterol, β- amyrin, bergapten (M.S.Y. Khan and Kalin Javed, 1998). The stem bark: Two leucoanthacyanins; leucocyanidin-3-0-β-D-glucopyranoside, leucopelargonidin-3-0α-L-rhamnopyranoside. Bark: ceryl behenate, friedelin, β-sitosterol, stigmasterol, α-amyrin acetate. Trunk bark: β-sitosterol. Heart wood, bark and fruits: tirucallane gluanol acetate, 13α, 14β, 17β (H)-20α (H)-lanosai-8, 22-fiene 3B-01, lupeol acetate, taraxasterol. An unusual thermostable aspartic protease from the latex of the plant (Devaraj K. B et al, 2008). Root bark: β-sitosterol, lupeol.

**Pharmacological activity of *Ficus racemosa***

**Anti-inflammatory activity**

The petroleum ether extract of *Ficus racemosa* leaves (0.5 mg/kg) orally administered showed anti-inflammatory activity in carrageenin-induced paw oedema model in rats (Forestieri et al., 1996). The anti-inflammatory activity of the petroleum ether extract of *Ficus racemosa* leaves was evaluated on carrageenin, serotonin, histamine and dextran-induced rat hind paw oedema models. The extract at doses of 200 and 400 mg/kg has been found to possess significant anti-inflammatory activity on the tested experimental models. The extract (400 mg/kg) exhibited maximum anti-inflammatory effect, which is 30.4, 32.2, 33.9 and 32.0% at the end pf 3 h with carrageenin, serotonin, histamine, dextran-induced paw oedema, respectively. In a chronic test, the cotton pellet test, the extract (400 mg/kg) showed 41.5% reduction in granuloma weight. The effect produced by the extract was comparable to that of phenylbutazone, a prototype of non-steroidal anti-inflammatory agents (Mandal et al., 2000).

**Analgesic activity**

The decoction of *Ficus racemosa* leaves (0.5 mg/kg) orally administered showed analgesic activity in both acetic acid-induced writhing and hot-plate models in mice (Forestieri et al., 1996). The dried leaves of *Ficus racemosa* at dose of 0.5 mg/kg showed analgesic activity in hot-plate and acetic acid-induced writhing tests (Almeida et al., 2001). The ethanolic extract of *Ficus racemosa* bark dose 500 mg/kg and leaves at doses of 300 and 500 mg/kg injected intraperitoneally showed analgesic effect in the hot plate model (Malairajan et al., 2006).

**Gastroprotective activity**

The ethanolic extract of *Ficus racemosa* fruits at doses of 50, 100 and 200 mg/kg administered orally showed dose dependent inhibition of ulcer index in pylorus ligation, ethanol and cold restraint stress – induced ulcers. High performance thin layer chromatography (HPTLC) analysis showed the presence of 0.57% and 0.36% w/w of gallic acid and ellagic acid in the extract (Rao et al., 2008).

**Anti-diarrahoeal activity**

The ethanolic extract of *Ficus racemosa* bark were evaluated for anti-diarrhoeal activity against different experimental models of diarrhoea in rats. The ethanolic extract of *Ficus racemosa* bark showed significant inhibitory activity against castor oil induced diarrhoea and $PGE_2$ induced enteropooling in rats. The extract also showed a significant reduction in gastrointestinal motility in charcoal meal tests in rats (Mukherjee et al., 1997).

**Antipyretic activity**

The petroleum ether extract of *Ficus racemosa* leaves at dose 0.5 mg/kg showed antipyretic effect in yeast's-induced fever model (Foresieri et al., 1996).

The antipyretic effect of the methanolic extract of *Ficus racemosa* stem bark was demonstrated in yeast-induced pyrexia in albino rats. The extract at doses of 100, 200, and 300 mg/kg administered orally showed significant dose-dependent reduction in normal body temperature and yeast-provoked elevated temperature. The effect extended up to 5 hr after drug administration. The antipyretic effect of the extract was comparable to that of paracetamol (150 mg/kg, p.o.), a standard antipyretic agent (Rao

et al., 2002). The dried root powder of *Ficus racemosa* was assessed for antipyretic activity utilizing yeast's-induced fever model. The powdered root of *Ficus racemosa* at dose of 40 mg/kg showed significant antipyretic efficacy and no obvious toxic effect could be seen (Konsue et al., 2008).

### Safety and toxicity of five herbal roots of Bencha-loga-wichian remedy

Acute toxicity tests of *Capparis micracantha* (root and stem), *Harrisonia perforata* (root and stem), *Tiliacora triandra* (leaves), *Clerodendrum petasites* (root and stem) and *Ficus racemosa* (root) were performed using Yenken Denken Tokyo Mice (YDT). Mice were received various doses of the 50% alcohol extract of those plants (1000, 3000 and 10000 mg/kg) by oral and subcutaneous administration. Toxicity was not observed after oral and subcutaneous administration of the alcohol extract of *Capparis micracantha* (root and stem), *Harrisonia perforata* (root and stem), *Tiliacora triandra* (leaf), *Clerodendrum petasites* (root and stem) and *Ficus racemosa* (root) at dose of 10 g/kg (Mokhasamit et al., 1975).

## THERMOREGULATION

The human body has the remarkable ability to maintain a relatively constant temperature, despite wide fluctuations in several variables, including ambient temperature, energy expenditure, and energy intake (Goldman and Khine, 2000). Body temperature varies during the day (circadian rhythm) with the peak occurring in the late afternoon (5:00 PM to 7:00 PM) and the trough early in the morning (2:00 AM to 6:00 AM). Normal body temperature varies from an approximate low of 36.48°C (97.68°F) in the morning to a high of 36.98°C (98.58°F) in the late afternoon. This circadian variation can differ significantly between individuals and can be as much as 1.3°C (2.4°F) or as little as 0.1°C (0.2°F). This rhythm is less prominent during the first few months of life, and becomes established by the second year of life. The mechanisms of circadian variation are unclear, but this pattern appears to be a tightly regulated process. Circadian variation in body temperature can persist even during febrile illnesses, although it is absent in patients with hyperthermia. At the heart of thermoregulation is an integrated network of neural connections involving the hypothalamus, limbic system, lower brainstem, the reticular formation, spinal cord, and the sympathetic ganglia. An area in and near the rostral hypothalamus is also important in orchestrating thermoregulation. This region, the "preoptic area," includes the preoptic nuclei of the anterior hypothalamus (POAH) and the septum. In simple terms, the POAH maintains mean body temperature around a set point. This thermoneutral set point temperature is modulated by the balanced activities of temperature-sensitive neurons. These neurons integrate afferent messages regarding core body and peripheral (skin) temperatures and evoke various behavioral and physiologic responses controlling heat production or dissipation (Aronoff and Neilson, 2001 and Goldman and Khine, 2000).

### Fever

Fever is a pathologic elevation of the normal body temperature; it is an active process and resists changes by the external environment (Arman et al., 1985). Fever describes a regulated rise in body temperature after an increase in the hypothalamic set point. Under the influence of the hypothalamus, physiologic and behavioral functions

favoring heat production and heat retention are stimulated until arriving at a newly elevated set point temperature. Typical early behavioral changes prior to fever include seeking a warmer environment or adding clothing. Physiologic alterations include cutaneous vasoconstriction, shivering, and non shivering. Upon reaching the elevated set point of fever, an increase or decrease in core temperature will stimulate thermoregulatory mechanisms similar to those evoked at normal body temperature. In other words, normal thermoregulation modulates at this higher set point (Aronoff and Neilson, 2001).

### Pathology of fever

Many of the mediators underlying pyrexia have been described in recent years (Figure 6). The critical "endogenous pyrogens" involved in producing a highly regulated inflammatory response to tissue injury and infections are polypeptide cytokines. Pyrogenic cytokines, such as interleukin-1$\beta$ (IL-1$\beta$), tumor necrosis factor (TNF-$\alpha$), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to effect a fever response. Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacterial outer membrane lipopolysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1$\beta$. These signals trigger the release of other mediators, most notably prostaglandin $E_2$ ($PGE_2$), in the region of the POAH. $PGE_2$ is believed to be the proximal mediator of the febrile response. Preoptic neurons bearing E-prostanoid receptors alter their intrinsic firing rate in response to $PGE_2$, evoking an elevation in the thermoregulatory set point. There are four known cellular receptors for $PGE_2$: $EP_1$ through $EP_4$. The particular receptor subtype involved in pyrogenesis is unknown. Although mice lacking the neuronal $PGE_2$ receptor subtype $EP_3$ demonstrate an impaired febrile response to both exogenous (endotoxin) and endogenous pyrogens, studies in rats appear to implicate the $EP_4$ receptor. The intracellular events triggering pyrexia after $PGE_2$-EP receptor coupling among species are unclear. Fever is tightly regulated by the immune response. Inflammatory stimuli triggering the generation of propyretic messages provoke the release of endogenous

antipyretic substances. Substances such as arginine vasopressin (AVP), a-melanocyte stimulating hormone, and glucocorticoids act both centrally and peripherally to limit pyrexia. The cytokine interleukin-10 (IL-10) has numerous anti-inflammatory properties, including fever suppression. In addition, a class of lipid compounds known as epoxyeicosanoids generated by certain cytochrome P-450 enzymes plays an important role in limiting the fever and inflammation. Analogous to a biochemical feedback pathway, fever itself appears capable of countering the release of pyrogenic cytokines. For example, febrile temperatures augment early TNF release in endotoxin-challenged mice, yet limit its prolonged (and perhaps detrimental) expression after either lipopolysaccharide injection or bacterial infection (Figure 6) (Aronoff and Neilson, 2001).

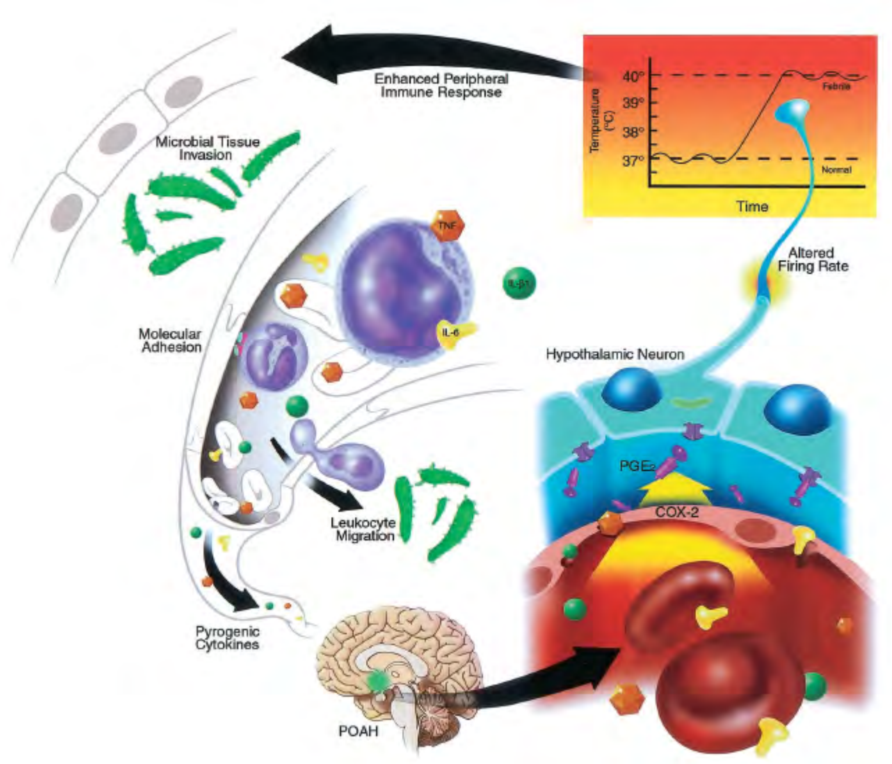


Figure 6 The pathogenesis of fever (Aronoff and Neilson, 2001).

### The role of pyrogenic cytokines

#### Interleukin-1 (IL-1β)

The most convincing experiment that IL-1 was pyrogenic was the induction of a rapid-onset fever in mice, rabbits, rats, guinea pigs and humans following a single injection of the recombinant human or other mammalian IL-1, either IL-1β or IL-1α. Since recombinant cytokines are expressed in Escherichia coli, considerable caution needed to be employed to exclude the involvement of contaminating bacterial endotoxins in the recombinant preparations. Endotoxin evokes fever in rabbits at concentrations as low as 10 ng/kg and in humans even lower doses of 1 ng/kg are pyrogenic (Dinarello, 2009).

#### Interleukin-6 (IL-6)

IL-6 administration in humans causes fever but unlike IL-1, microgram/kilogram doses are needed compared to nanogram/kilogram for IL-1. There have been multiple reports of elevated IL-6 levels in a variety of human diseases and in various human body fluids such as plasma, cerebrospinal fluid and joint fluids. IL-6 gene expression is, in part, under the control of several exogenous pyrogens but also IL-1 and TNF. In fact, IL-1 and particularly IL-1 plus TNF are very potent stimulators of IL-6 gene expression and protein translation. Therefore, IL-6 is often elevated in conditions where IL-1 and TNF-α have been synthesized (Dinarello, 2009).

#### Tumor necrosis factor-alpha (TNF-α)

At 100–200 ng/kg, recombinant human TNF-α produces the typical endogenous pyrogen-fever pattern in rabbits and C3H/HeJ mice. The rapid rise in body temperature that occurs in rabbits after an intravenous injection of TNF-α is indistinguishable from that produced by either form of recombinant IL-1, albeit at 10-fold lower doses. TNF-α evokes a second fever peak 3–4 h after the injection. Plasma taken from rabbits during the second fever peak contains circulating IL-1; this finding formed the basis of the concept that TNF-α induces IL-1 *in vivo* (Dinarello, 2009).

**Prostaglandins (PGs)**

Interest in these compounds dates back to the early 1970s when it was found that $PGE_1$ was a potent pyretic agent and that antipyretics blocked prostaglandin synthesis in various organs including brain. These two findings implicated a prostaglandin in the genesis of fever. Research in this area developed actively and led to the demonstration that: (a) $PGE_2$, a normal constituent of hypothalamic tissue, is as potent as $PGE_1$ in producing fever moreover, both compounds are like pyrogens in that their action is not influenced by ambient temperature; (b) $PGE_2$ acts upon neurons in the POAH that are also the main target for pyrogens; (c) thermo-sensitive neurons in POAH respond in the same manner to $PGE_2$ and pyrogens; (d) $PGE_2$ fever, unlike pyrogen fever, does not abate following administration of antipyretics; and (e) pyrogen fever is associated with elevated levels in the CSF of a prostaglandin with the biological and immunological properties of $PGE_2$. Collectively, these findings indicate that $PGE_2$ is well suited for being the "central messenger" of fever and specifically of pyrogen fever. According to current knowledge, pyrogens from outside the body (exogenous pyrogen), and foremost among them bacterial endotoxin, as well as pathological conditions causing tissue inflammation and damage (e.g. infarction, malignancy) elicit the formation of a pyrogenic substance (endogenous pyrogen) in neutrophils and in cells of then reticuloendothelial system. The endogenous pyrogen, which is therefore a key intermediate in the sequence of events leading to fever, is then carried to the rostral region of the hypothalamus by the circulation. Because the blood-brain barrier is seemingly impermeable to endogenous pyrogen, and because prostaglandins are rapidly removed from the circulation, one must assume that the vessel wall is the main site where pyrogen action is translated into increased prostaglandin synthesis. Consistent with this hypothesis is the notion that vessels, including cerebral vessels, are endowed with an active prostaglandin-generating system and that hypothalamic blood flow is increased during pyrogen fever. The latter finding implies activation of prostaglandin synthesis in the vessel wall. Alternatively, PG could be released from phagocytosing leukocytes sequestered in the capillary bed of POAH. Any pyrogen crossing the blood-brain barrier may stimulate prostaglandin synthesis in neural tissue.

$PGE_2$, whether formed in the tissue of the POAH or from the vessels, acts at appropriate sites in the thermoregulatory pathways to elevate the "set-point" for temperature regulation, thus causing fever. Once its action is completed, $PGE_2$ is either inactivated enzymatically in situ or enters the extracellular fluid and CSF when it is transported into the circulation. Interference with the latter mechanism results in enhancement of pyrogen effects (Wolf, 1979).

$PGE_2$ is synthesized from arachidonic acid, which is released from cell membrane lipid by phospholipase. Arachidonic acid is metabolized by two isoforms of the COX enzyme, COX-1 and COX-2. COX-1 usually is expressed constitutively and generates prostanoids important to housekeeping functions supporting homeostasis. COX-2, on the other hand, is inducible by inflammatory signals such as the pyrogenic cytokines, IL-1$\beta$, TNF-$\alpha$, and IL-6, and bacterial lipopolysaccharide. Genetically engineered mice that lack either the COX-1 or COX-2 gene demonstrate that the inducible isoform is responsible for hypothalamic $PGE_2$ production during a febrile response. As COX-2 is the key provider of $PGE_2$ during pyrexia, it is not surprising that the selective COX-2 antagonist, is an effective antipyretic in humans. Many cells, including synoviocytes, macrophages, endothelial cells, and chondrocytes, have the capacity to rapidly up-regulate the expression of the COX-2 during inflammation. The most likely cell type in the central nervous system responsible for producing $PGE_2$ is the microvascular endothelial cell, which expresses COX-2 exuberantly after stress.

An effective febrifuge might interrupt pyrexogenesis at any step that connects peripheral inflammation with the central production of $PGE_2$. Stated differently, an antipyretic might blunt peripheral inflammation or depress central pyrogenic signals, or affect both. Inhibiting central production of $PGE_2$ is a well-known mechanism of antipyretic agents, but activated leukocytes and endothelial cells in peripheral areas of inflammation also represent potential drug targets (Aronoff and Neilson, 2001).

**Pharmacological methods of fever management**

**The Antipyretic drugs**

**Acetaminophen**

Acetaminophen is an analgesic that deserves special comment because it is an effective febrifuge but a weak anti-inflammatory drug. Its effects differ considerably from salicylates and other NSAIDs. As opposed to aspirin, acetaminophen is a poor inhibitor of platelet function. Believed to be an inhibitor of cyclooxygenase, acetaminophen's mechanism of action is still poorly understood. Although suprapharmacologic doses of acetaminophen inhibit NF-kB stimulation of inducible nitric oxide synthase, it does not possess the same inhibitory effects on NF-kB–mediated gene transcription that salicylates enjoy. Explanation of the antipyretic and analgesic actions of acetaminophen has been based on tissue-specific COX inhibition not seen with NSAIDs. Acetaminophen penetrates the blood-brain barrier, achieving cerebrospinal fluid levels comparable to those in serum, and may act preferentially within the central nervous system. Central nervous system levels of $PGE_2$ rise during fever and fall to normal levels upon administration of the drug. Acetaminophen reduces the production of prostaglandins in brain preparations more potently than it does from other organs such as spleen (Aronoff and Neilson, 2001).

**Aspirin and NSAIDs**

The antipyretic drug aspirin was in wide clinical use for more than 70 years before Vane demonstrated in 1971 that it exerted its physiologic action by inhibiting the production of prostaglandins. Further work suggests a current model of how aspirin and similar NSAIDs act as antipyretics. Aspirin interferes with the biosynthesis of cyclic prostanoids derived from arachidonic acid, such as thromboxane $A_2$ and prostaglandins. As a nonselective COX inhibitor, aspirin has been widely studied for its anti-inflammatory, antipyretic, and antithrombotic traits. The major mechanism of action of aspirin and other antipyretics involves lowering $PGE_2$ by directly inhibiting COX enzyme activity.

NSAIDs are also capable of reducing $PGE_2$ production by down-regulating the expression of COX enzymes, as opposed to directly inhibiting their enzymatic action.

Sodium salicylate and aspirin also inhibit COX-2 transcription induced by lipopolysaccharide and IL-1β. The clinical effects of sodium salicylate are likely due in part to its actions on COX gene transcription by disabling the transcriptional activator nuclear factor-κB (NF-κB). NF-κB is a heterodimeric protein capable of binding DNA in the 5'-promoter regions of many genes involved in the inflammatory response. Once bound, NF-κB facilitates the transcription of genes encoding pyrogenic cytokines, chemokines, adhesion molecules, and inflammatory enzymes, including inducible nitric oxide synthase and COX-2 in certain cell types. NF-κB resides in an inactive state in the cytoplasm, complexed to another protein, IκB. Upon activation, the IκB silencer is sequentially phosphorylated, ubiquinated, and degraded, releasing NF-κB to translocate into the nucleus. Salicylates reduce the nuclear translocation of NF-κB through stabilization of cytoplasmic IκB by interfering with its phosphorylation. The ability of antipyretics to disable transcription varies among agents and cell type studied. Salicylate and its progenitor aspirin prevent NF-κB translocation in endothelial cells and leukocytes induced by proinflammatory cytokines or lipopolysaccharide. NSAIDs like ibuprofen also block the nuclear trafficking of NF-κB in certain tumor cell lines but fail to do so in activated macrophages. Indomethacin, another COX inhibitor, does not appear to affect NF-κB, and therapeutic doses of acetaminophen also fail to suppress it. The reason for this heterogeneity is unknown (Aronoff and Neilson, 2001).

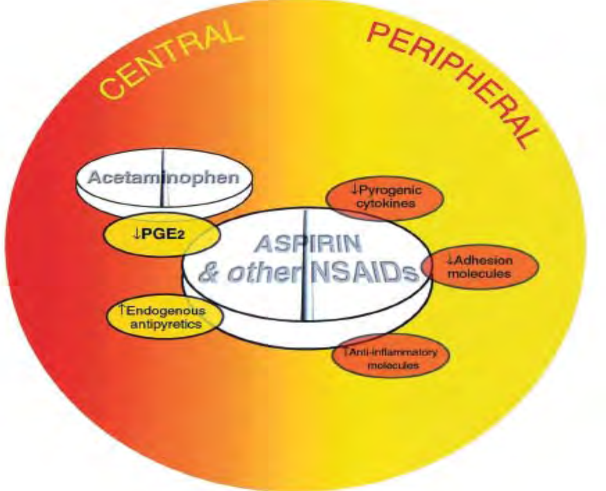


Figure 7 Mechanisms of antipyresis (Aronoff and Neilson, 2001)

## PAIN

The International Association for the Study of pain (IASP) has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage or both." as defined by cause by noxious stimuli including thermal, chemical or mechanical. Pain is both a sensation (conscious awareness of a noxious stimulus) and an emotional experience (intense feeling of displeasure resulting in a pattern of reactive behavior).

Pain can be classified in several different ways. Anatomic (somatic or visceral pain) and temporal (acute or chronic pain) classification schemes have some clinical utility, but do not suggest appropriate analgesic therapy. Mechanistically, pain can also be classified as either inflammatory or neuropathic. As the names suggest, inflammatory pain is associated with tissue trauma and inflammation, while neuropathic pain is associated with nerve injury. Both types of pain can occur as a result of surgical trauma, but inflammatory pain is by far the most common type of pain and its physiology is better understood (Lemke, 2004).

### Physiologic pathways

Specialized receptors provide information to the central nervous system (CNS) about the state of the environment in the vicinity of the organism. Each receptor is specialized to detect a particular type of stimulus (e.g., touch, temperature, pain, etc.) Those receptors in the skin and other tissues that sense pain are free nerve endings, while those for temperature detection can be free nerve endings, bulbs of Krouse or Ruffinigs corpuscles. Receptors are distributed with varying densities in different tissues. Pain receptors may be stimulated by mechanical damage, extremes of temperature, or by irritating chemical substances. While certain pain receptors are responsive to only one of the above stimuli, most can be stimulated by two or more. When the pain receptors in peripheral tissues (such as skin) are stimulated, the nociceptive (pain) impulses are transmitted to the CNS by two distinct types of neurons - the A-delta and C nerve fibers. The A-delta fibers are large-diameter, fast conducting myelinated fibers, which transmit first pain - sharp, prickling, and injurious. The C fibers

are small-diameter, slower conducting unmyelinated fibers that are responsible for second pain - dull, aching and visceral type (Figure 8).

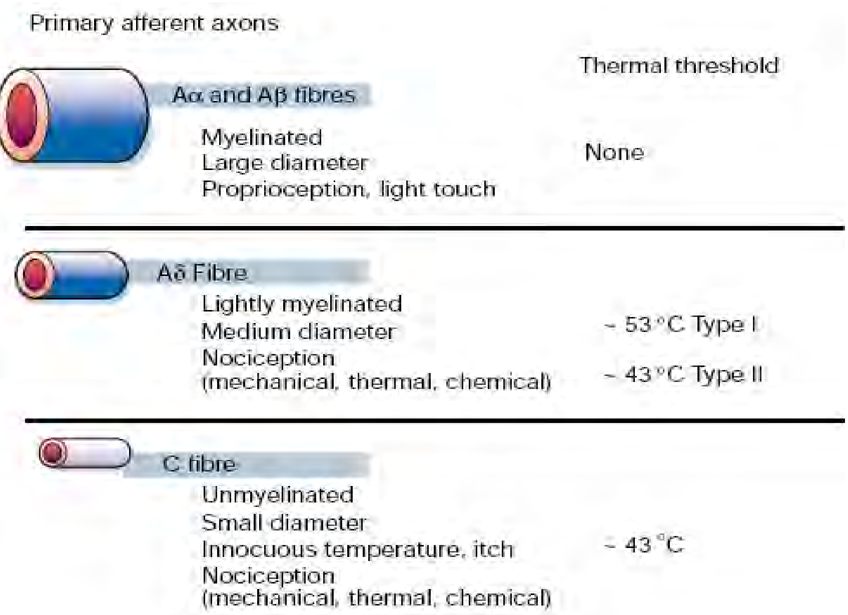


Figure 8 Different nociceptors detect different types of pain (Julius and Basbaum, 2001)

The primary afferent sensory neurons from the periphery then enter the spinal cord and synapse with neurons in the dorsal horn. The second-order neurons, arising from the dorsal horn, have long axons that decussate in the anterior commissure commissure and travel cephalad in the contralateral anterolateral pathway (also known as spinothalamic tract). Some of the long axons that synapsed with type C neurons do not decussate, but pass cranially in the ipsilateral anterolateral spinal pathway. The anterolateral spinal pathway fibers terminate in the thalamus, from which neuronal relays are sent to other CNS centers and the sensory cortex. These higher centers are responsible for the perception of pain and the emotional components that accompany it. There are four distinct processes in the sensory pathway: transduction, transmission, modulation and perception.

### Transduction

Nociceptors, the pain receptors, respond selectively to noxious stimuli and convert chemical, mechanical, or thermal energy at the site of the stimulus into neural impulses, a process known as transduction. The primary afferent nociceptors are the terminal branches of the A-delta and C fibers, whose cell bodies are located in the dorsal root ganglia. Mendell described a functional classification of nociceptive nerve fibers. Wide-dynamic range (WDR) neurons are those that receive input from both noxious and non-noxious stimuli and that exhibit a graded response (Figure 9) (Kelly et al., 2001).



Figure 9 The transduction process (Kelly et al., 2001).

### Transmission

When signal transduction has occurred, impulses are transmitted via A-delta and C fibres to the dorsal horn of the spinal cord. The nerve fibers synapse in the superficial layers of Rexed laminae: the A-delta neurons synapse in laminae I, II and V, and the C fibers in laminae I and II. The borders between these laminae are not distinct. In addition, there is considerable overlap of neuronal cell types between the laminae, with each lamina containing more than one type of neuron. A variety of neurotransmitters are released by the incoming first order nociceptive neurons. One of these is substance P, a neurokinin, which is released from HT fibers. The calcitonin gene-related peptide

(CGRP) is released along with substance P, and extends the spinal cord zone from which substance P is released, thereby contributing to increase excitability. In turn, substance P induces the release of excitatory amino acids (EAAs) such as aspartate and glutamate, which act on the AMPA (2-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) and NMDA (N-methyl-D-aspartate) receptors. Enhanced synaptic transmission due to release of EAAs follows substance P release and the latter can induce a prolonged enhancement of responses by dorsal horn neurons to glutamate or NMDA. This enhanced depolarization causes calcium influx into postsynaptic neurons, which induces persistent changes in the excitability of the cells (Figure 10) (Kelly et al., 2001).

Figure 10 The transmission process (Kelly et al., 2001).

**Modulation**

Modulation is the third process of nociception. The signals are rapidly contract between neuron in brainstem and hypothalamus and interneuron in spinal cord. While the signal contract, neurotransmitters, opioids, serotonin (5-HT), norepinephine (NE) etc are released to blocking the substance P and other mediator of nociceptive in primary afferent fibers.

### Perception

Perception is the final part of the process where there is subjective interpretation by the cortex of the stimulus as pain. This process can be artificially described as involving 2 types of cortical processing. The sensory component of cortical processing is that in which the stimulus can be classified as noxious, its stimulus intensity decoded, and its location identified. However, before such signals represent the true “experience of pain”, something that is only a human experience, the cortex overlays an additional aspect to the neural processing, described as the affective component of pain. Here, the cortex relates the situation and the history of such noxious stimuli to the interpretation of the strict sensory component. Again, the importance of the noxious stimulus in contributing to the experience of pain is “interpreted” in light of the situation and is much worse in pathological states, such as those associated with disease where the patient sees the pain as a signal of progression of the disease (Leon-Casasola, 2007).

### Nociceptive pathway

Nociception is a sequential process that includes transduction of noxious stimuli into electrical signals by peripheral nociceptors, conduction of encoded signals by afferent neurons to the dorsal horn of the spinal cord, and subsequent transmission and modulation of the signals at both supraspinal and spinal levels. In its simplest form, the nociceptive pathway is a 3-neuron chain. The 1$^{st}$ neuron in the chain---the primary afferent neuron---is responsible for transduction of noxious stimuli and conduction of signals from the peripheral tissue to neurons in the dorsal horn of the spinal cord. The 2$^{nd}$ neuron in the chain---the projection neuron---receives input from the primary afferent neurons and projects to neurons in the medulla, pons, midbrain, thalamus and hypothamus. This 3rd order, supraspinal neurons integrate signals from the spinal neurons and project to the subcortical and cortical areas where pain is finally perceived. Primary afferent neurons are bipolar neurons. The cell bodies of these bipolar neurons are located in the dorsal root ganglia and their axons project peripherally to somatic and visceral tissues and centrally to the dorsal horn of the spinal cord. Peripheral

nociceptors respond to mechanical, thermal, and nociceptive stimuli, and primary afferent neurons conduct encoded signals to neuron in the dorsal horn of the spinal cord. Tissue nerve terminals cause the release of the substance P and calcitonin gene-related peptide. Release of these neuropeptides cause mast cell degranulation, vasodilation and edema, and further activation and sensitization of nociceptors (Figure 11).

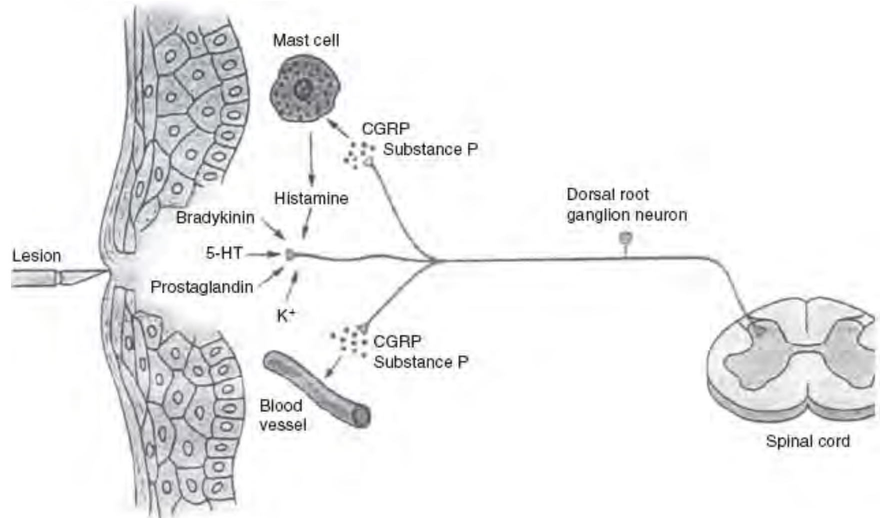


Figure 11 Peripheral nociceptors and primary afferent neurons (Schwartz and Jessell, 2000).

From the nociceptive process; transduction, transmission, modulation and perception, signals are convey to 2 pathways including the ascending pathways and the descending pathways.

## Ascending pathways

The nociceptive information is transmitted from the spinal cord to the thalamus and cerebral cortex along five ascending pathways (Figure 12). The spinothalamic tract is the prominent ascending nociceptive pathway in the spinal cord. It comprises the axons of nociceptive-specific and wide-dynamic-range neurons in laminae I and V–VII of the dorsal horn. These axons project to the contra lateral side of the spinal cord and ascend in the anterolateral white matter, terminating in the thalamus. Electrical stimulation of the spinothalamic tract results in pain, whereas lesions of the tract

(achieved by a procedure called anterolateral cordotomy) result in marked reductions in pain sensation on the side opposite the spinal cord lesion.

The spinoreticular tract comprises the axons of neurons in laminae VII and VIII. It ascends in the anterolateral quadrant of the spinal cord and terminates in both the reticular formation and the thalamus. In contrast to the spinoreticular tract do not cross the midline.

The spinomesencephalic tract comprises the axons of neurons in laminae I and V. It projects in the anterolateral quadrant of the spinal cord to the mesencephalic reticular formation and periaqueductal gray matter, and via the spinoparabrachial tract, it projects to the parabrachial nuclei. In turn, neurons of the parabrachial nuclei project to the amygdale, a major component of the limbic system, the neural system involved in emotion. Thus the spinomesencephalic tract is thought to contribute to the affective component of pain. Many of the axons of the pathway project in the dorsal part of the lateral funiculus rather than in the anterolateral quadrant. Thus, if these fibers are spared in surgical procedures designed to relieve pain, such as anterolateral cordotomy, pain may persist or recur.

The cervicothalamic tract arises from neurons in the lateral cervical nucleus, located in the white matter of the upper two cervical segments of the spinal cord. The lateral cervical nucleus receives input from nociceptive neurons in laminae III and IV. Most axons in the cervicothalamic cross the midline and ascend in the medial lemniscus of the brain stem to nuclei in the midbrain and to the ventroposterior lateral and posteromedial nuclei of the thalamus. Some axons from laminae III and IV project through the dorsal columns of the spinal cord (together with the axons of large-diameter myelinated primary afferent fiber) and terminate in the cuneate and gracile nuclei of the medulla.

The spinohypothalamic tract comprises the axons of neurons in laminae I V and VIII. It projects directly to supraspinal autonomic control centers and is thought to activate complex neuroendocrine and cardiovascular responses (Basbaum and Jessell, 2000).

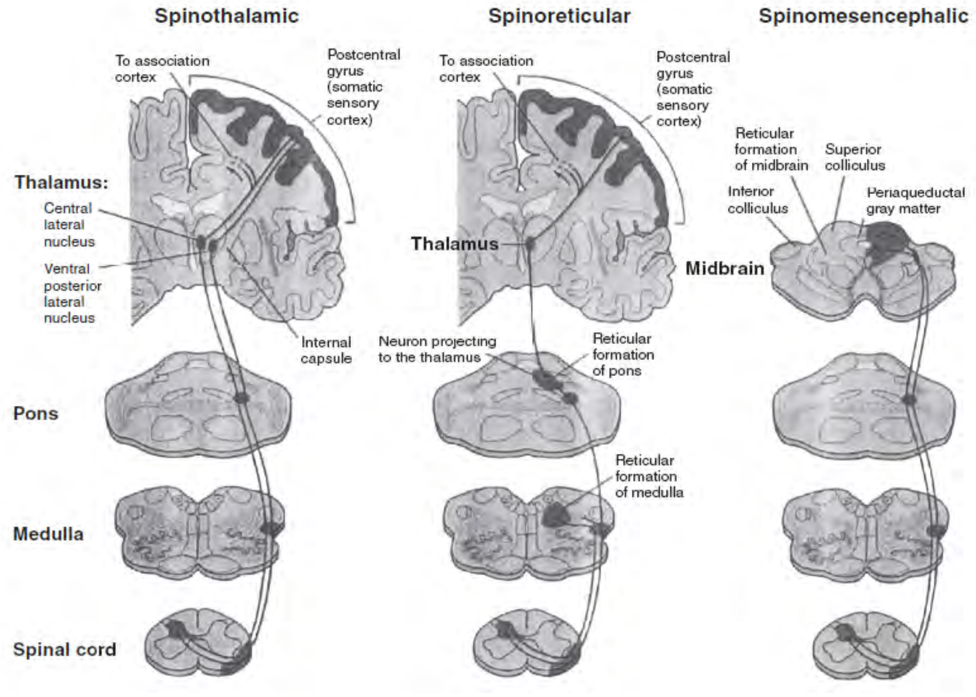


Figure 12 The ascending pathways from spinal cord to thalamus (Schwartz and Jessell, 2000; Lemke, 2004).

Descending antinociceptive pathways

Activation of the descending system by the endorphins occurs through specific receptors called “opioid receptors”. These systems are activated in and around the periaqueductal gray (PAG) region of the midbrain, and such neurons then project to sites in the medulla (eg, nucleus reticularis gigantocellularis, nucleus raphe magnus) and the locus coeruleus (the major source of norepinephrine cells in the brain) through uncertain circuitry where other neurons are activated (probably through disinhibition-that is, inhibition of a tonically active inhibitory interneuron). These descending fibers then project to the dorsal horn of the spinal cord along a tract called the dorsolateral funiculus (located in the dorsolateral portion of the spinal cord) to synapse with either the incoming primary afferent neuron, the second-order pain transmission neuron, or interneurons. Again, the circuitry that occurs at the spinal level is uncertain. In general, however, these descending pain modulatory neurons release nonopioid neurotransmitters in the spinal cord, especially serotonin (5-HT) and norepinephrine (NE) or activate small opioid containing interneurons in the spinal dorsal horn to release

opioid peptides (again through disinhibition). The released NE and 5HT (acting through some types of 5-HT receptors) can act to directly inhibit the release of transmitters from the incoming nociceptive afferent signal, and to inhibit the second–order pain transmission cell. Both of these will produce an inhibition of transmission of the pain signal. NE and 5-HT released from these descending pathways can also activate (indirectly) the release of endogenous opioids from interneurons, again through a process of disinhibition. Activation of the descending pain modulatory system is a good example of why subjects report not feeling pain at all under conditions of stress, or perhaps other situations, where even though the pain is felt, the degree appears to be greatly modulated (Figure 13; Leon-Casasola, 2007)

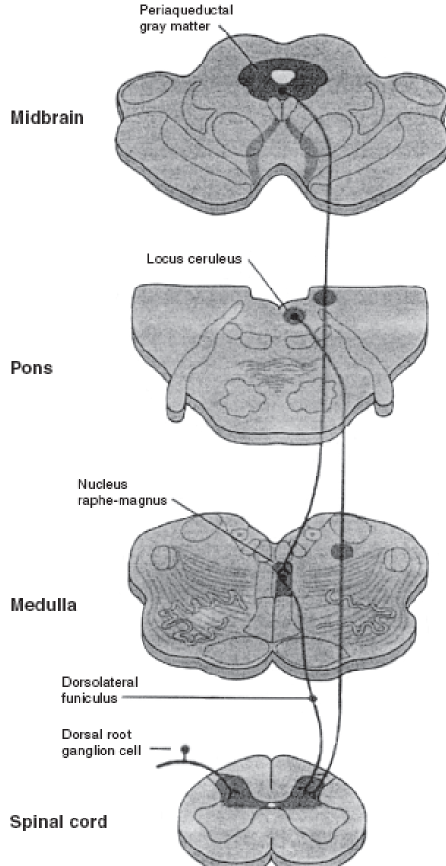


Figure 13 The descending pathways from midbrain to spinal cord (Schwartz and Jessell, 2000; Lemke, 2004).

Pharmacological methods of pain management

1. Nonopioid Drugs

Acetaminophen

Acetaminophen (paracetamol) preferentially reduces central prostaglandin synthesis by unknown mechanism, and as a result produces analgesia and antipyresis but has relatively little anti-inflammatory efficacy. Acetaminophen is frequently combined with weak opioids for the treatment of moderate pain (Griffin and Woof, 2007). For patients who cannot tolerate aspirin, acetaminophen is indicated, administered in doses similar to those used for aspirin. However, some caution is warranted for higher dosing schedules. Acetaminophen tends to be better tolerated than aspirin in individuals who experience GI-related complications with analgesic agents. Acetaminophen has been widely used largely because of its reputation for safety. Its pregnancy category is B, which means that animal studies are negative for fetal abnormalities, or animal studies are positive while human studies are negative. Although its reputation for safety is deserved, acetaminophen is not without risks. Hepatotoxicity is a significant adverse drug reaction associated with the use of acetaminophen. Because many patients with pain take medications on a routine basis, it is the clinician's responsibility to warn of possible liver damage with as little as 2.6 g (some studies say 4.0 g) of acetaminophen daily over extended periods of time. Patients should not take acetaminophen if they have an allergic reaction to it, diminished liver function, alcoholism, fasting, or substantial kidney damage or impaired kidney function (Supernaw, 2002).

Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory agents inhibit the activity of cyclooxygenase enzymes (COX-1 and COX-2) that are necessary for the production of prostaglandins NSAIDs affect pain pathways in at least three different ways. First, prostaglandins reduced the activation threshold at the peripheral terminals of nociceptive primary afferent neurons. Hence, NSAIDs by reducing prostaglandin synthesis reduce inflammatory hyperalgesia and allodynia directly. In addition, NSAIDs reduce the recruitment of leukocytes, which produce other inflammatory mediators. Finally,

prostaglandins act as pain-producing neuromodulators in the spinal cord and dorsal horn, where their production may be prevented by NSAIDs. Because acetaminophen and NSAIDs act through mechanisms different from the opioids. NSAIDs- opioids or acetaminophen- opioids can act synergistically to reduce pain. NSAIDs and COX-2 inhibitors act both peripherally and centrally, whereas acetaminophen acts only centrally (Griffin and Woof, 2007).

Generally, NSAIDs are considered ineffective as sole agents for postoperative pain relief, but have an impressive synergy with co-administered opiates, with most studies showing a 20±30% reduction in opiate use. Although the NSAIDs are a heterogeneous group from a medicinal chemistry viewpoint, being composed of salicylates, acetic and propionic acids, and pyrazolones and anthranilic acids, there seem to be few significant differences between the various individual agents with regard to overall efficacy. The four principal NSAID-associated adverse events are gastrointestinal ulceration, renal dysfunction, impaired haemostasis through platelet inhibition, and aspirin-induced asthma. Toxicity of NSAIDs is increased with age and duration of therapy, as well as by stress, hypovolaemia, decreased renal perfusion and concomitantly administered potentially nephrotoxic drugs such as aminoglycosides, all of which can be found in this group (Macpherson, 2000).

Cyclooxygenase-2 Inhibitors

The mechanism through which NSAIDs provide analgesia and suppress inflammation is the inhibition of the enzyme cyclooxygenase, resulting in decreased prostaglandin synthesis. The suppression of prostaglandin synthesis can also produce gastric and renal toxicity, as well as impair normal platelet function. Cyclooxygenase exists in two isoenzymatic forms, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Cyclooxygenase-1 appears to be expressed in many tissues and produces prostaglandins, which regulate normal cellular functions. However, COX-2 activity is induced by proinflammatory cytokines that mediate the inflammatory response and pain signaling transmission. Traditional nonspecific NSAIDs inhibit both COX-1 and COX-2 activity, and in doing so, not only decrease inflammation and pain, but also promote GI

tract damage and bleeding. The potential clinical benefit of COX-2 inhibitors is significant because of the number of patients chronically treated with NSAIDs have 3- to 10-fold higher risk of GI injury and death associated with traditional NSAIDs. Two medications that predominantly inhibit only COX-2 activity and celecoxib, are currently available by prescription in the United States. Although neither of these medications has been well studied in the postoperative obstetric setting, other studies have supported their use in the postoperative period with similar efficacy as other NSAIDs, but with potentially fewer side effects. Nevertheless, the incidence of cardiovascular events such as stroke and myocardial infarction (MI), which may be associated with the possible inhibitory effect of these medications on vasodilatation and antiaggregatory prostacyclin production, has recently raised some concern about the use of these drugs. Further investigation is needed to warrant the safety of these medications in the obstetric population (Leung, 2004).

### $\alpha_2$ Agonists

Clonidine is a $\alpha_2$ receptor agonist. The $\alpha_2$ receptors are involved in analgesia and are located in the CNS, including the primary afferent terminals, the superficial laminae of the spinal cord, and brainstem nuclei. Clonidine 150 μg injected intrathecally after cesarean section yields analgesia for 4 to 6 hours. Clonidine also has a synergetic effect. The epidural administration of clonidine provides analgesia with a 50% reduction in opioid requirements. An epidural bolus administration of a combination of fentanyl and clonidine will reduce the analgesic dose of each component by approximately 60%. Clonidine will also enhance and prolong the effect of local anesthesia intrathecally. There is also evidence of additional analgesia when clonidine is added to local anesthesia in peripheral nerve blocks. The use of higher doses of clonidine as an analgesic is sometimes limited by its sedative properties (Leung, 2004).

### Anticonvulsants

The effectiveness of the anticonvulsant drugs in the management of neuropathic and central pain states. Anticonvulsants represent a heterogeneous group of agents in

pain management, with considerable variations between members of the class. Carbamazepine, while effective, is associated with a range of adverse effects, such as conduction defects and leukopoenia. Furthermore, its use can result in a number of clinically significant drug interactions, as it is a potent inducer of a variety of enzyme systems, including CYP, epoxide hydrolase, and uridine diphosphate glucuronosyl transferase. The risk of such interactions is important in the chronic pain patient, where multimodal drug therapy is often employed. There have been many studies examining the effectiveness of anticonvulsants in chronic neuropathic pain states, and while the majority of studies have shown positive outcomes, there have been exceptions. This might be explained on the basis that neuropathic pain is not a specific entity, but comprises a variety of pain states with differing sensitivities to varying pharmacological interventions.

Gabapentin (1-(aminomethyl)-cyclohexane acetic acid), a $\gamma$-aminobutyric acid (GABA) analogue, whose mode of action as an anticonvulsant is incompletely understood, has also been widely used in neuropathic pain management. It appears to have effects in addition to those on $Na^{+}$ channels. Studies have shown that gabapentin binds to the $\alpha 2\ \delta$-subunit of the voltage-dependent $Ca^{2+}$ channel, and thus, may also interfere with spinal cord neuronal $Ca^{2+}$ flux, which is known to play an important role in neuropathic pain. Gabapentin has proved effective in the management of neuropathic pain states. It has a favorable side effect profile, but patients still complain of somnolence, dizziness, headache, abnormal thinking or confusion, an ataxia, A further advantage is that hepatic enzyme induction is low, thus minimizing significant drug interactions.

Other anticonvulsants that have been studied include sodium valproate and lamotrigine. Sodium valproate inhibits a number of hepatic enzyme systems, and has a significant degree of protein binding, both of which can lead to significant drug interactions (Anderson, 1998). Lamotrigine blocks both sodium channels and glutamate release; although results have been encouraging, high dosage is required for adequate analgesia and side effects have been prominent (Macpherson, 2000).

### Antidepressants

Tricyclic antidepressants have had a long history of use in neuropathic pain management and act primarily by enhancing adrenergic $\alpha_2$-adrenoreceptor stimulation. Some also possess NMDA receptor-blocking activity. Both secondary and tertiary amines have been used, although most authors suggest that the tertiary amines, such as amitriptyline, imipramine, and doxepin, are more efficacious. The antidepressants have quite a different clinical pharmacological profile when used in pain management, as opposed to endogenous depression. In the case of amitriptyline, for example, the dosage used can be relatively low (10±25 mg initially) and the onset of action is rapid (often a few days). Worrying side effects, especially the well-annotated anticholinergic effects, can limit antidepressant use in the elderly, and sedation, which tends to be dose-dependent, can also be impairment, although single nighttime dosing can help minimize the impact.

The selective 5-HT reuptake inhibitors have been investigated as antinociceptive agents, and would be useful, as they have a favorable side effect profile. Unfortunately, initial studies with selective 5-HT reuptake inhibitors have failed to demonstrate that they have any advantages over conventional treatments (Macpherson, 2000).

### NMDA receptor antagonists

Because of the critical role of NMDA receptors in the induction and maintenance of central sensitization, antagonists of the NMDA receptor are currently under investigation for use in pain treatment. Two currently available drugs act as antagonists at the NMDA receptor, and both of these drugs, the anesthetic ketamine and the antitussive dextromethorphan, effectively reduce chronic pain. Ketamine use is severely limited by its psychomimetic effects. Dextromethorphan, when used at the relatively high doses required for clinically observable analgesia, also produces dizziness, fatigue, confusion, and psychomimetic effects (Griffin and Woof, 2007)

### 2. Opioids

Opioid receptor agonists are the primary drug class used in management of moderate to severe pain. The naturally occurring opioid receptor agonists morphine has

the greatest historical importance and remains in wide use, but synthetic and semisynthetic opioids add pharmacokinetic versatility. Historically, opioids have been most widely used to treat acute and chronic cancer-related pain.

Opioid receptor agonists produce both analgesia and side effects by acting on these molecules act upon different receptors (mu, delta, kappa, sigma) receptors. Sites of analgesic action include the brain, brainstem, spinal cord and primary afferent peripheral terminal. Through recepters in the medullary respiratory control center, the medullary chemorecepter zone and the gastrointestinal tract. Opioids also produce respiratory depression, vomiting and constipation. In addition, opioids can cause sedative, confusion, dizziness and euphoria. Opioids used is often associated with the development of tolerance, in which prolonged used of a constant dose of drugs results in a decreased therapeutic effect. The molecular mechanisms responsible for tolerance remain a matter of debate, and may involve combination of gene regulation and post-translation modification of opioid receptor activity. The development of tolerance requires either a change of analgesic or an increase in the dose of frequency of administration to maintain analgesia. Physical dependence can also occur, such that abrupt cessation of treatment would result in a characteristic withdrawal syndrome. Addition, in which physical dependence is accompanied by substance abuse behavior, is a possible adverse effect of opioid administration (Griffin and Woof, 2007).

## Tramadol

The limitation of opioids has motivated continuous research aimed at discovering drugs that can provide maximum pain relief but with improved tolerability. Tramadol has been shown to be effective in treating cancer pain and was better tolerated than buprenorphine. Tramadol is a synthetic 4-phenyl-piperidine analogue of codeine. Tramadol, a centrally acting analgesic, consists of two enantiomers, both of which contribute to analgesic activity via different mechanisms. Tramadol and the metabolite-O-desmethyltramadol (M1) are agonists of the mu opioid receptor. Tramadol also stimulates presynapticrelease of serotonin and inhibits serotonin reuptake whereas tramadol inhibits norepinephrine reuptake. Thus tramadol enhances inhibitory effects on

pain transmission both by opioid and monoaminergic mechanisms. The complementary and synergistic actions of the two enantiomers improve the analgesic efficacy and tolerability profile of the racemate. Tramadol is available as drops, capsules and sustained-release formulations for oral use, suppositories for rectal use and solution for intramuscular, intravenous and subcutaneous injection (Omoti, 2007).

## ANIMAL MODELS

### Central analgesic activity testing

### 1. Short-Duration Stimuli Tests (Acute Phasic Pain)

Acute tests, such as hot-plate, tail-flick and paw-pressure tests, require a high intensity stimulus (such as thermal, mechanical, or chemical) and do not test a preinjured animal. The response measured (1) is immediate (or within seconds), (2) uses the A$\delta$ and C-fiber input, and (3) is known to activate the spinal dorsal horn, the cells of which are nociceptive-specific and/or wide dynamic range (WDR) neurons. In addition, the response is proportional to the frequency of stimulus and the fiber class of afferent input (Eaton, 2003).

### A. Test based on the use of thermal stimuli

In test involving thermal stimuli, it is always the skin that is stimulated. These tests do not involve visceral or musculoskeletal tissues. In practice, the animal withdraws itself quickly from the stimulus, and therefore only the first part of this scenario takes place. The source of nociceptive stimulation can be distant from its target (e.g., radiant heat from a lamp) or can be in direct contact with the skin (Bars et al., 2001).

### 1) The Tail-flick test

a. The tail-flick test using radiant heat.

The tail-flick test using radiant heat is an extremely simplified version of the method used on human subjects by Hardy et al. (1940). The application of thermal radiation to the tail of an animal provokes the withdrawal of the tail by a brief vigorous movement. It is the reaction time of this movement that is recorded (often referred to as tail-flick latency.). This is achieved by starting a timer at the same time as the application of the heat source. By using a rheostat, the intensity of current through the filament and therefore of radiant heat emission can be controlled in such a way that one can empirically predetermine the time until the withdrawal of the tail. This is usually between 2 and 10 s (most commonly between 2 and 4 s), although it can be much longer. A photoelectric cell stops the timer and switches off the lamp at the moment the tail is

withdrawn. A lengthening of the reaction time is interpreted as an analgesic action. It is advisable not to prolong the exposure to radiant heat beyond 10 to 20 s; otherwise the skin may be burned. The advantages of this method are its simplicity and the small interanimal variability in reaction time measurements under a given set of controlled conditions (Bars et al., 2001). One can demonstrate that the tail-flick is spinal reflex in that, at least in its shorter latency form, it persists after section or cold block of upper parts of the spinal cord. As with all reflexes, it is subject to control by supraspinal structures. Details of the spinal pathways implicated in this reflex can be found elsewhere. It is triggered by C fibers when it is elicited by heat delivered by a CO2 laser (Bars et al., 2001). The tail-flick reflex may not always be purely spinal, notably when the heating slope is slower and there is an increase in the reaction time. Under these conditions, the tail-flick can disappear in the spinal animal. It is possible that the tail-flick is not a purely spinal reflex but is a more complicated one involving higher neural structures (Bars et al., 2001). From a pharmacological point of view, there is a consensus that this test is truly efficient only for revealing the activity of opioid analgesics (but not of opioid partial agonists). In this context, it is adequate for predicting their analgesic effects in humans. For morphine itself, it is not difficult to construct dose-response curves for intravenous doses between 1 and 10 mg/kg (Bars et al., 2001). As far as opioid partial agonists are concerned, some have been shown to increase the tail-flick reaction time when slow rate of heating are applied. It is probable that this pharmacological observation resulted from the aforementioned fact that supraspinal structures are involved when the test is carried out in this fashion (Bars et al., 2001).

b. The tail-flick test using immersion of the tail.

The use of immersion of the tail is apparently a variant of the test described above. The most obvious difference is that the area of stimulation is far greater. Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. This test is actually quite different from the previous one insofar as immersion of the tail in a hot liquid increases its temperature very quickly and in a more or less linear fashion. The main interest in this response-

which arguably has not been exploited sufficiently- lies in the possibility of applying different temperatures. Thus, lower temperatures can be used to seek evidence for the effects of minor analgesics. This also applies to using a bath in which the temperature increases slowly (Bars et al., 2001).

**2) The Paw Withdrawal Test**

In principle, this test is entirely comparable to the test of D'Amour and Smith but offers the advantage that it does not involve the preeminent organ of thermoregulation in rats and mice, i.e., the tail. One can improve the test by minimizing variations in the baseline temperature of the skin. With the aim of studying hyperalgesic phenomena resulting from inflammation, Hargreaves et al. (1988) had an inspired idea for supplementing the model of Randall and Selitto: radiant heat was applied to a paw that had already been inflamed by a subcutaneous injection of carrageenin. For this purpose, inflammation can also be produced by exposure to ultraviolet rays. One advantage in these test is that heat is applied (to the plantar surface of the foot) of a freely moving animal. However, there is a disadvantage in that the position of the leg becomes a factor since the background level of activity in the flexors varies with the position of the animal (Bars et al., 2001).

**3) The Hot Plate test**

This test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or a boiling liquid. A plate heated to a constant temperature produces two behavioral components that can be measured in terms of their reaction times, namely paw-licking and jumping. Both are considered to be supraspinally integrated responses (Bars et al., 2001).

As far as analgesic substances are concerned, the paw-licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol, especially when the temperature of the plate is 50°C or less or if the temperature is increased in a progressive and linear fashion, e.g., from 43 to 52°C at 2.5°C/min. The specificity and

sensitivity of the test can be increased by measuring the reaction time of the first evoked behavior regardless of whether it is paw-licking or jumping, or by lowering the temperature. The behavior is relatively stereotyped in the mouse but is more complex in the rat, which sniffs, licks its forepaws, licks its hind paws, straightens up, and stamps its feet, starts and stops washing itself, among other things. These behaviors have been labeled a chaotic defensive movement (Bars et al., 2001).

4) Test Using Cold Stimuli

Cold is very rarely used to test acute pain. On the other hand, it is more common to test cold allodynia in animal models of neuropathies. The techniques are directly inspired by those that use heat by contact: immersion of the tail or a limb, or placing the animal on a cold surface (Bars et al., 2001).

B. Test based on the use of mechanical stimuli

The preferred sites for applying nociceptive mechanical stimuli are the hind paw and the tail. Tests using constant pressure have been abandoned progressively for those applying gradually increasing pressures. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. When the pressure increases, one can see successively the reflex withdrawal of the paw, a more complex movement whereby the animal tries to release its trapped limb, then a sort of struggle, and finally a vocal reaction. If the first of these reactions is undoubtedly a proper spinal reflex, the last two clearly involve supraspinal structures. This type of mechanical stimulation has a certain number of disadvantages: 1) it is sometimes difficult to measure the intensity of the stimulus with precision; 2) repetition of the mechanical stimulus can produce a diminution or conversely an increase in the sensitivity of the stimulated part of the body-in the latter case, this carries the risk that the tissues may be altered by inflammatory reaction that could call into question the validity of repeated tests; 3) the necessity of applying relatively high pressures-which explains the weak sensitivity of the method and the relatively small number of substances that have been shown to be active by this test; and 4) a non-negligible level of variability of the

responses (Bars et al., 2001). With the aim of improving the sensitivity of the test, Randall and Selitto (1957) proposed comparing thresholds observed with a healthy paw and with an inflamed paw. The inflammation was induced beforehand by a subcutaneous injection into the area to be stimulated of substances such as croton oil, beer yeast, or carrageenin, the last of these being the most commonly used today. Even though it was found that the sensitivity of the method was improved, it was to the detriment of its specificity because, a priori, two different pharmacological effects-analgesic and anti-inflammatory-could be confused (Bars et al., 2001).

2. Long-Duration Stimuli Tests (Tonic Pain)

These tests use an irritant, foreign chemical agent as the nociceptive stimulus. They differ from most other pain tests in that (1) they do not measure a threshold response; (2) they quantitatively measure the resulting behavior after the stimulus, which varies in potency with time; and (3) they are not models of chronic pain, since the duration of the behaviors is short, usually minutes or tens of minutes. Hence, long-duration stimuli tests are considered models of tonic pain. They are usually based on intradermal or intraperitoneal injections of the agent (Eaton, 2003). Intradermal Injection (The formalin test) formalin, a 37 percent solution of formaldehyde, is the most commonly used agent for intradermal paw injection (the formalin test). Other agents less commonly used are hypertonic saline, Freundgs adjuvant, ethylene diamine tetra-acetic acid, capsaicin, or bee sting. A 0.5 to 15 percent solution of formalin (usually about 3.5%) injected into the dorsal or plantar surface of the rat fore- or hind paw produces a biphasic painful response of increasing and decreasing intensity for about 60 min after the injection. Typical responses include the paw being lifted, licked, nibbled, or shaken; these responses are considered nociceptive, since formalin predominantly evokes activity in C fibers, and not in A_ afferents. The initial phase of the response, which lasts 3 to 5 min, is probably due to direct chemical stimulation of nociceptors; this is followed by 10 to 15 min during which animals display little behavior suggestive of nociception. The second phase of this response starts about 15 to 20 min after the formalin injection and lasts 20 to 40 min, initially rising with both number and frequency of nociceptive

behaviors, reaching a peak, then falling off. The intensities of these nociceptive behaviors are dependent on the concentration of formalin used, and the second phase involves a period of sensitization during which inflammatory phenomena occur. These inflammatory phenomena are possibly a result of central processes triggered by the neuronal activation during the first phase (Eaton, 2003).

**Peripheral analgesic activity testing**

**1) Intraperitoneal Injections of Irritants (Writhing Test)**

Intraperitoneal injection of agents (originally phenylbenzoquinone) that are irritating to serous membranes provokes a stereotypical behavior in rodents that is characterized by abdominal constrictions, whole body movements, contortions of the abdominal muscles, and reduced motor activity and incoordination. In this test, commonly called the writhing test, the behaviors are considered reflexive, and are evidence of peritoneovisceral or visceral pain associated with visceral chemoreceptor (Eaton, 2003). The most utilized screening assay for nonnarcotic analgesic agents is the abdominal constriction assay in mice (Lombardino, 1985). Unfortunately, the frequency of cramps decreases spontaneously with time to such an extent, and with such variability, that is difficult to evaluate the effect of an analgesic on the behaviors of any single animal. Even with multiple modifications in the nature of the chemical irritant used, the concentration, temperature, and volume of the injection, and other modifications to simplify the test and measurements of behaviors, the test lacks specificity, because these test work so well for all major and minor analgesics, as well as non-analgesic substances such as muscle relaxants. Even with poor specificity of action, the writhing test can predict effective analgesic doses for agents that can be used in human (Eaton, 2003). In this test both central and peripheral analgesics are detected. The test, therefore, has been used by many investigators and can be recommending as a simple screening method. However, it has to be mentioned that other drugs such as clonidine and haloperidol also show a pronounced activity in this test. Because of the lack of specificity, caution is required in interpreting the results, until other tests have been

performed. Nevertheless, a good relationship exists between the potencies of analgesics in writhing assays and their clinical potencies (Vogel, 2002).

2) Pain in inflamed tissue (RANDALL-SELITTO test)

The method for measuring analgesic activity is based on the principle that inflammation increase the sensitivity to pain and that this sensitivity is susceptible to modification by analgesics. Inflammation decreases the pain reaction threshold and this low pain reaction threshold is readily elevated by non-narcotic analgesics of the salicylate aminopyrine type as well as by the narcotic analgesics. Brewers yeast has been used as an inducer for inflammation which increases pain after pressure.

The mean applied force is determined for each time interval tested. The percentage increase in pain threshold is calculated by subtracting the applied force of the vehicle control from the applied force of the drug group which is divided by the applied force of the vehicle control in order to give the percentage of increase in pain threshold of the drug group (Vogel, 2002). The applied force is continuously monitored by an indicator moving along a linear scale calibrated in grams x 10 with a pointer riveted to slide, e.g., 11.5 = 115 grams. The scale can be multiplied by 2 or 3 by placing on the slide one or two discs, respectively. The application of force is stopped when the rat starts to struggle (vigorous attempt to withdraw the paw) to a noticeable degree (whether or not accompanied by shrill vocalization). The method originally described by RANDALL and SELITTO has been used by many investigators and has been proven to detect central analgesics as well as peripheral analgesics. Peripherally acting analgesics such as the nonsteroidal anti-inflammatory drugs increase only the threshold of the inflamed paw, whereas opiate analgesics increase also the threshold of the intact paw (Vogel, 2002).

# CHAPTER III

# MATERIALS AND METHODS

## ANIMALS

Male Wistar rats weighing 140-180g, male ICR mice weighing 18-25 g from National Laboratory Animal Center, Mahidol University, Salaya, Nakornprathom. They were served as experimental subjects and housed in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under the standard condition of temperature (25 ± 2 ºC), 50-60% of humidity, 12 hr/12 hr light/dark cycles and standard pellet diet of C.P. Company, Thailand and tap water ad libitum. The animals were allowed to acclimate to the facility for 3-5 days before starting the experiments. At the end of each experiment, the animals were sacrificed with carbon dioxide. The number of animals used in each treatment was typically six to ten per group. The study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Appendix A).

## PREPARATION OF FIVE HERBAL ROOT EXTRACTS

Bencha-loga-wichian remedy consists of five herbal roots of *Capparis micracantha* DC. (Ching-chee; CM), *Tiliacora triandra* Diel. (Yaa-nang; TT), *Harrisonia perforata* Merr. (Khon-thaa; HP), *Clerodendrum petasites* S. Moore (Mai-tao-yai-mom; CP) and *Ficus racemosa* Linn. (Ma-deo-chumporn: FR). They were collected from Nongkhai province of Thailand and authenticated by Ruangrungsi. The voucher and number of specimens were deposited at the College of Public Health Sciences, Chulalongkorn University, Thailand. All five roots species were dried under shade and grinded to coarse powders. Each powder of five roots was macerated in absolute ethanol in closed conical flask for 24 hours. The marc after filtration was dried and further macerated with water for 24 hours. The ethanol extracts were evaporated to dryness under vacuum. The water extracts were lyophilized to dryness. The extracts yield were weighed, recorded and stored at -20 ºC. The Bencha-loga-wichian remedy (BEN) was prepared by mixing each extract in the quantity equivalent to the traditional remedy preparation. The extract was prepared by Mr. Chatubhong Singharachai, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Thin-layer chromatographic identification

Methanol extract of the roots of each species in Bencha-loga-wichian remedy that combines with equal part by weight of *Capparis micracantha* DC., *Harrisonia perforate* (Blanco) Merr., *Tiliacora triandra* (Colebr.) Diels,*Clerodendrum petasites* S. Moore and *Ficus racemosa* L. Macerate 1 g of the sample, in powder, with 20 ml of methanol for 12 hours, filter and evaporate to dryness. Dissolve the residue in 0.5 ml of methanol. Apply 10 µl to the thin-layer chromatographic plate, using siliga gel 60 $F_{254}$ as the coating substance.

The plate was removed and allowed it to dry in air and observed the produced spots in daylight, under short-wave (254 nm) and long-wave (366 nm) ultraviolet light. The plate was spayed with the mixture solution of vanillin-sulfuric acid reagent (vanillin (15 g) in ethanol (250 ml) and concentrated sulfuric acid (2.5 ml). The plate was then placed in the hot air oven at 105°C for 5 min. The thin-layer chromatograms of all five herbal root extracts were done by Mr. Chatubhong Singharachai, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand (Appendix B).

DRUGS

The following drugs were used: 0.9% sodium chloride solution, 2% Tween 80, morphine sulphate (10 mg/kg, Thai FDA), indomethacin (10 mg/kg, Sigma Chemical Co., USA), acetylsalicylic acid (aspirin) (300 mg/kg, Sigma Chemical Co., USA), naloxone (5 mg/kg, Sigma Chemical Co., USA), lipopolysaccharide (LPS) from *E. coli* (50 µg/kg, Sigma Chemical Co., USA), 0.6% acetic acid (10 ml/kg, Merck, Germany), five herbal root extracts of Bencha-loga-wichian remedy: *Capparis micracantha* root extract (CM; 25-400 mg/kg), *Tiliacora triandra* root extract (TT; 25-400 mg/kg), *Harrisonia perforat*a root extract(HP; 25-400 mg/kg), *Clerodendrum petasites* root extract (CP; 25-400 mg/kg) and *Ficus racemosa* root extract (FR; 25-400 mg/kg) and Bencha-loga-wichian remedy (BEN; 25-400 mg/kg).

Morphine, LPS and acetic acid were dissolved in 0.9% sodium chloride solution. Five herbal root extracts of Bencha-loga-wichian remedy, Bencha-loga-wichian remedy, aspirin and indomethacin were suspended in 2% Tween 80. Morphine sulphate and indomethacin were used as the standard analgesic drugs and aspirin was used as the standard antipyretic drug. The control animals were given with equivalent volume of vehicle in the same route.

EXPERIMENTAL METHODS

**Lipopolysaccharide-induced fever in rats**

The method of Santos and Rao in 1998 was modified and used for the assessment of the antipyretic activity of the various doses of five herbal root extracts of Ben-cha-loga-wichien remedy (CM, TT, CP, HP, FR) and Ben-cha-loga-wichien remedy (BEN). The animals were fasted overnight before the experiments. Animals were kept singly in restrainers for 1 hr to acclimatize to their new environment. Fever was induced with 50 µg/kg of LPS injected intramuscularly into the thigh of the rat. The animals were pretreated orally with 2% Tween 80 solution (10 ml/kg), acetylsalicylic acid (ASA; 300 mg/kg), various doses of CM, TT, HP, CP, FR (25, 50, 100, 200 and 400 mg/kg) or BEN (25, 50, 100, 200 and 400 mg/kg) 1 hr before injection of LPS. Rectal temperature was measured 1 hr before the pretreatment of animals and at 1 hr intervals for 7 hr after the administration of the bacterial endotoxin (LPS) with a digital thermometer (Model 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 experiment involved animals treated with 2% Tween 80 plus LPS. All experiments were carried out between 08.00 h and 18.00 h in a quiet laboratory with an ambient temperature of 25± 2°C.

Figure 14 Dgital Thermometer (YSI Precision™ 4000 A)

Hot-plate Analgesic Testing

The 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 was 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 pretreatment 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 for its paw-lick latency and returned to its cage (the maximum time allowed for an animal to remain on the surface of the plate during testing was 45 sec). 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 either intraperitoneal (i.p.) 0.9% sodium chloride solution (NSS; 10 ml/kg), morphine sulphate (MO; 10 mg/kg) or oral administration of 2% Tween 80 (10 ml/kg), various doses of CM, TT, HP, CP, FR (25, 50, 100, 200 and 400 mg/kg) and BEN (25, 50, 100, 200 and 400 mg/kg). All animals were placed on the hot-plate at 15, 30, 45, 60, 92, 120 and 240 min after drug administration. The time-course of hot-plate latency were expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

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

Cut-off time for hot-plate test = 45 sec

Thus, $ED_{50}$ were computed and dose- and time response curve was generated. Dose-effect curves for hot-plate assays were derived by computing the area under the

corresponding 0-240 min time-course-%MPE curves; area was calculated using the trapezoidal rule (Tallarida and Murray, 1987).

**Analysis of the mechanism of antinociceptive action of five herbal root extracts**

The possible participation of the opioid system in the antinociceptive effect of five herbal root extracts was investigated using the model of mouse hot-plate test. Animal were pretreated with naloxone (NAL; 5 mg/kg, i.p.) 10 min before oral administration of five herbal root extracts (CM 200 mg/kg, TT 400 mg/kg, HP 400 mg/kg, CP 400 mg/kg and FR 400 mg/kg) (Miranda et al., 2001).

Figure 15 Hot-Plate Analgesiometer

Tail-flick Analgesic Testing

These studies employed the tail-flick assay described by D'Amour and Smith in 1941, 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 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 (about 4 cm from the tip). The device (Harvard Tail-flick Analgesia meter) automatically recorded (in 0.1 sec) 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 about 1.0-1.5 sec (intensity 3.7 A). The intensity was not changed for any animal within any given 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 subjects.

Immediately, after the third baseline trial, the drug administration took place with vehicle (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg) intraperitoneally (i.p.) or 2% Tween 80 (10 ml/kg), various doses of CM, TT, HP, CP, FR (25, 50, 100, 200 and 400 mg/kg) and BEN (25, 50, 100, 200 and 400 mg/kg) orally (p.o.). Tail-flick latencies were recorded at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of tail-flick latency were expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

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

Cut-off time for hot-plate test = 4 sec

Thus, $ED_{50}$ were computed and dose- and time response curve was generated. Dose-effect curves for the tail-flick assays were derived by computing the area under the corresponding 0-240 min time-course-%MPE curves; area was calculated using the trapezoidal rule (Tallarida and Murray, 1987).

Figure 16 Tail-flick Analgesia Meter

Acetic acid-induced writhing test in mice

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. in 1959. On the day of testing, animals were randomly assigned to one of seven treatment groups. Mice were then administered various doses of treatments 30 min before intraperitoneal administration of 0.6% acetic acid (10 ml/kg, i.p.) which used to induce the constriction response.

The drug administration took place with indomethacin (IND; 10 mg/kg) or various doses of CM, TT, HP, CP, FR (25, 50, 100, 200 and 400 mg/kg) and BEN (25, 50, 100, 200 and 400 mg/kg) orally (p.o.) 30 min before 0.6% acetic acid (10 ml/kg, i.p.). Each animal was placed in transparent observational cage. The number of writhes (abdominal constriction) were observed and counted for 30 min after acetic acid administration (Nguemfo et al., 2007). Antinociceptive activity was reported as percentage of inhibition of writhing response compared with the vehicle control group. The percentage of inhibition of writhing response was calculated using the following formula:

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

Wr = mean writhing response

Figure 17 Writhing response

## DATA TREATMENT AND STATISTICAL ANALYSE

Statistical analyses were performed on the dose-response curves by analysis of variance (ANOVA). Post hoc analyses were performed using Tukey test (SPSS version 16.0 for windows). The minimum level of statistical significance was set at $p<0.05$.

# CHAPTER IV

# RESULTS

## LIPOPOLYSACCHARIDE INDUCED FEVER IN RATS

Lipopolysaccharide (LPS; 50 µg/kg) injected intramuscularly produced a time-dependent increase in rectal temperature of vehicle pretreated rats started from 1 hr and the effect was maintained for 7 hr after LPS injection. The maximum increase in rectal temperature was reached at 3 hr (1.75°C) giving the maximum mean rectal temperature of 38.19 ± 0.09°C after which there was a decrease (Figure 18). At the same period, the maximum mean rectal temperature of normothermic rats was 36.85 ± 0.05°C, thus, LPS significantly ($p<0.05$) increased the rectal temperature (Table 1-6).

Acetylsalicylic acid (ASA; 300 mg/kg) significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 7 hr with a maximum reduction at 7 hr. The mean rectal temperature produced by LPS in the presence of ASA was reduced to 36.73 ± 0.17°C (Table 1-6).

CM at the doses of 25, 100 and 200 mg/kg significantly ($p<0.05$) reduced LPS induced increase in rectal temperature over a period of 2-7 hr with a maximum reduction at 7 hr. CM at the doses of 50 and 400 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 1-7 hr with a maximum reduction at 6 and 3hr, respectively. The mean rectal temperature produced by LPS in the presence of 25, 50, 100, 200, and 400 mg/kg of CM were reduced to 36.61 ± 0.26°C, 36.81 ± 0.23°C, 36.49± 0.14°C, 36.24 ± 0.22°C and 36.44 ± 0.36°C, respectively (Figure 19 & Table 1). CM showed antipyretic effect with all dose tested, especially at the dose of 100 mg/kg (Figure 20).

HP 25 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 2-7 hr with a maximum reduction at 7 hr. HP at the doses of 50, 100 and 400 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 3-7 hr with a maximum reduction at 7

hr. HP 200 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 5-7 hr with a maximum reduction at 7 hr. The maximum reduction of mean rectal temperature produced by LPS in the presence of 25, 50, 100, 200, and 400 mg/kg of HP were 36.57 ± 0.09°C, 36.43 ± 0.07°C, 36.37± 0.12°C, 36.69 ± 0.12°C and 36.49 ± 0.07°C, respectively (Figure 21 & Table 2). HP showed antipyretic effect with all dose tested, especially at the dose of 100 mg/kg (Figure 22).

TT at the doses of 25, 50 and 100 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 2-7 hr with a maximum reduction at 7, 6, 7 hr, respectively. TT at the doses of 200 and 400 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 1-7 hr with a maximum reduction at 6, 4 hr, respectively The maximum reduction of mean rectal temperature produced by LPS in the presence of 25, 50, 100, 200, and 400 mg/kg of TT were 36.29 ± 0.38°C, 36.67 ± 0.25°C, 36.08± 0.26°C, 36.06 ± 0.23°C and 36.36 ± 0.28°C, respectively (Figure 23 & Table 3). TT showed antipyretic effect with all dose tested, especially at the dose of 200 mg/kg (Figure 24).

CP at the doses of 25, 50 and 100 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 2-7 hr with a maximum reduction at 7, 6, 6 hr, respectively. CP at the doses of 200 and 400 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 1-7 hr with a maximum reduction at 7 hr. The maximum reduction of mean rectal temperature produced by LPS in the presence of 25, 50, 100, 200, and 400 mg/kg of CP were 36.51 ± 0.23°C, 36.69 ± 0.05°C, 36.49± 0.08°C, 36.52 ± 0.09°C and 36.45 ± 0.20°C, respectively (Figure 25 & Table 4). CP showed antipyretic effect with all dose tested, especially at the dose of 100 mg/kg (Figure 26).

FR at the doses of 25 and 400 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 1-7 hr with a maximum reduction at 7 hr. FR 50 and 100 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 2-7 hr with a

maximum reduction at 7 hr. FR 200 mg/kg failed to reduce the increased rectal temperature produced by LPS over the entire period The maximum reduction of mean rectal temperature produced by LPS in the presence of 25, 50, 100, 200, and 400 mg/kg of FR were 36.50 ± 0.19°C, 36.34 ± 0.24°C, 36.88± 0.26°C, 36.87 ± 0.21°C and 36.87 ± 0.34°C, respectively (Figure 27 & Table 5). FR showed antipyretic effect with all dose tested, especially at the dose of 50 mg/kg (Figure 28).

BEN at the doses of 25 and 50 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 4-7 hr with a maximum reduction at 7 hr. BEN at the doses of 100 and 200 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 2-7 hr with a maximum reduction at 7 hr. BEN 400 mg/kg significantly ($p<0.05$) reduced the increased rectal temperature produced by LPS over a period of 3-7 hr with a maximum reduction at 7 hr. The maximum reduction of mean rectal temperature produced by LPS in the presence of 25, 50, 100, 200, and 400 mg/kg of BEN were 35.97 ± 0.23°C, 36.46 ± 0.07°C, 36.05± 0.16°C, 36.33 ± 0.10°C and 35.42 ± 0.64°C, respectively (Figure 29 & Table 6). BEN showed antipyretic effect with all dose tested, especially at the dose of 400 mg/kg (Figure 30).

TT 200 mg/kg seemed to have the highest antipyretic potency when compared between the most effective doses of five herbal root extracts. BEN 400 mg/kg caused the maximum reduction in rectal temperature from baseline when compared with any root extracts (Figure 31). All doses of CM, TT, HP, CP, FR and BEN used in this study were found to be as potent as ASA (Table 1-6).

Lipopolysaccharide-induced Fever in Rats

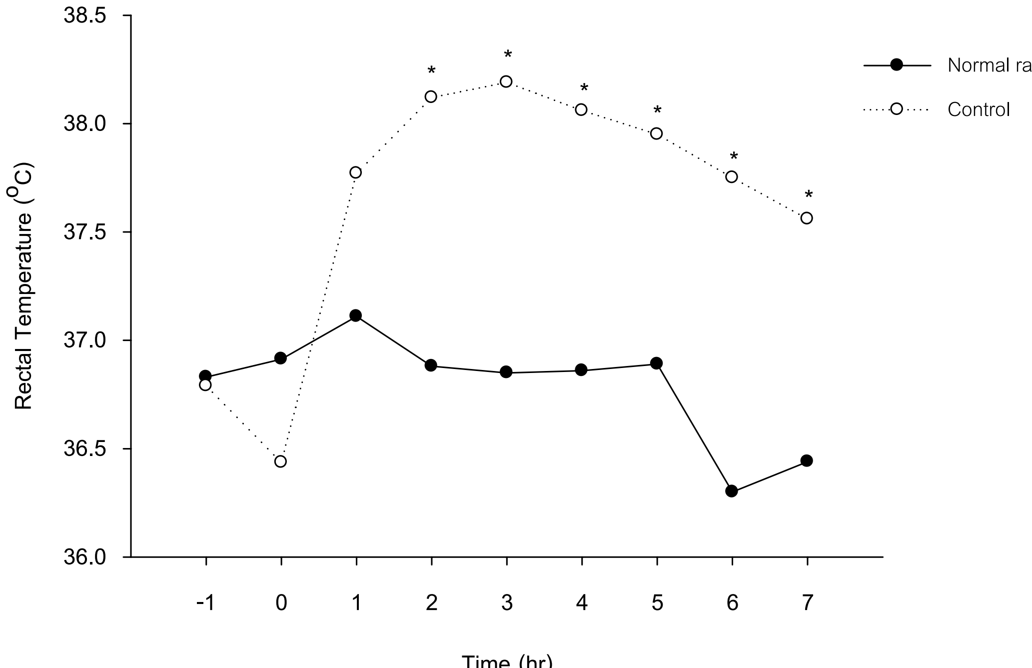


Figure 18 Rectal temperatures after oral administration of 2% Tween 80. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. N=6 for all group. *p<0.05 significantly different compared to control animals.

Lipopolysaccharide-induced Fever in Rats

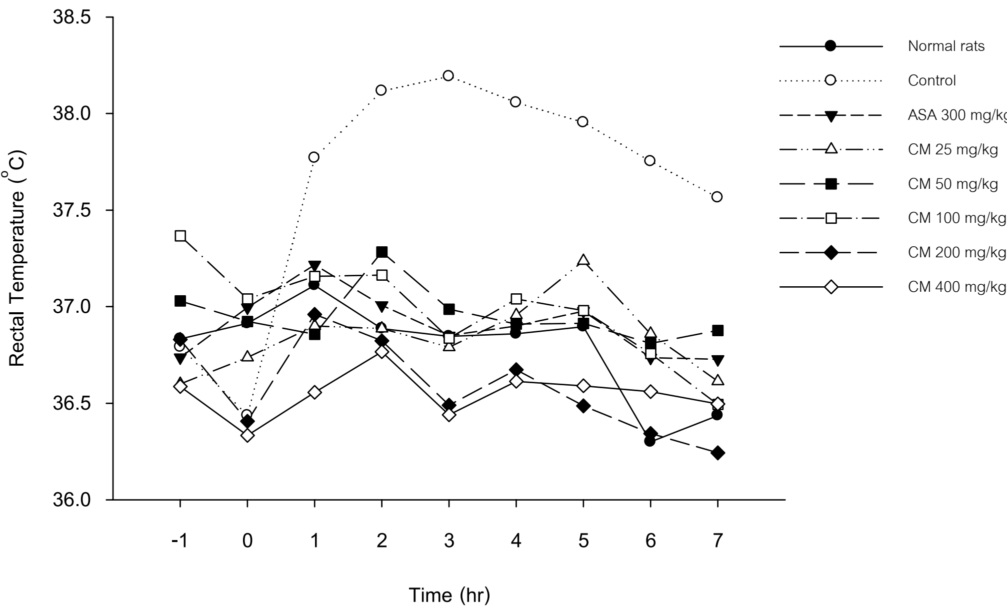


Figure 19 Rectal temperature after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Lipopolysaccharide-induced Fever in Rats

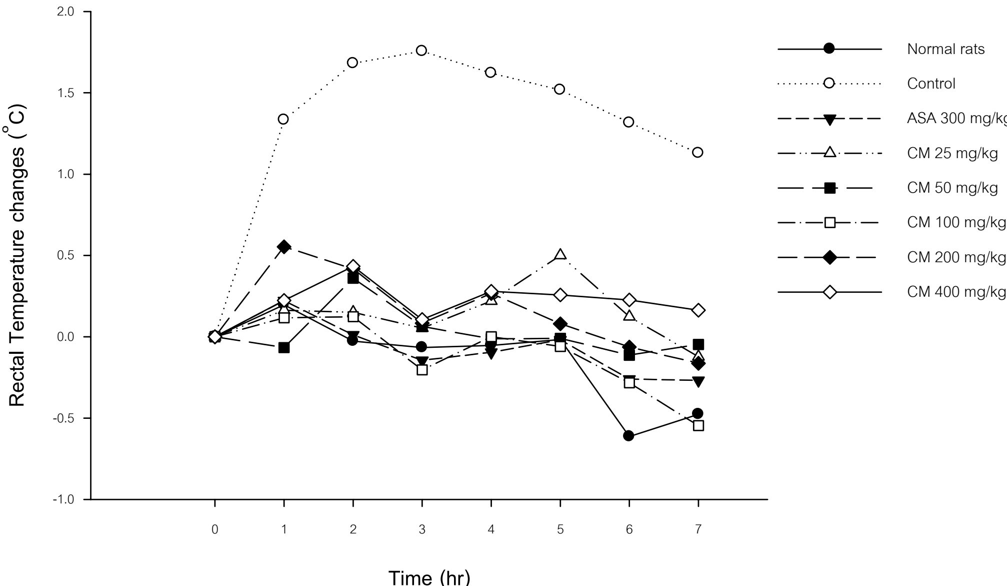


Figure 20 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Table 1 Effect of *Capparis micracantha* root extract (CM; 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 |
| Normal 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[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* |
| CM 25 mg/kg | 36.60 ± 0.45 | 36.47 ± 0.26 | 36.90 ± 0.59 | 36.89 ± 0.20* | 36.79 ± 0.18* | 36.96 ± 0.27* | 37.24 ± 0.13 | 36.86 ± 0.21* | 36.61 ± 0.26* |
| CM 50 mg/kg | 37.03 ± 0.11 | 36.99 ± 0.21 | 36.86 ± 0.33* | 37.28 ± 0.25* | 36.99 ± 0.25* | 36.91 ± 0.25* | 36.91 ± 0.32* | 36.81 ± 0.23* | 36.87 ± 0.22 |
| CM 100 mg/kg | 37.37 ± 0.21 | 37.10 ± 0.18 | 37.16 ± 0.23 | 37.16 ± 0.12* | 36.84 ± 0.21* | 37.04 ± 0.23* | 36.98 ± 0.13* | 36.76 ± 0.24* | 36.49± 0.14* |
| CM 200 mg/kg | 36.83 ± 0.19 | 36.72 ± 0.25 | 36.96 ± 0.22 | 36.82 ± 0.16* | 36.49 ± 0.11* | 36.67 ± 0.22* | 36.49 ± 0.25* | 36.34 ± 0.17* | 36.24 ± 0.22* |
| CM 400 mg/kg | 36.59 ± 0.20 | 36.79 ± 0.15 | 36.56 ± 0.31* | 36.77 ± 0.29* | 36.44 ± 0.36* | 36.61 ± 0.34* | 36.59 ± 0.37* | 36.56 ± 0.19* | 36.49 ± 0.07* |

Each value represents mean± SEM (n=6), [a]Normal rats received 0.9% NSS instead of lioppolysaccharide. [b]Control received 2% Tween 80 solution.

# p<0.001 significantly different compared to normal rat values for the corresponding hour. *p<0.05 significantly different compared to control values for the corresponding hour.

Lipopolysaccharide-induced Fever in Rats

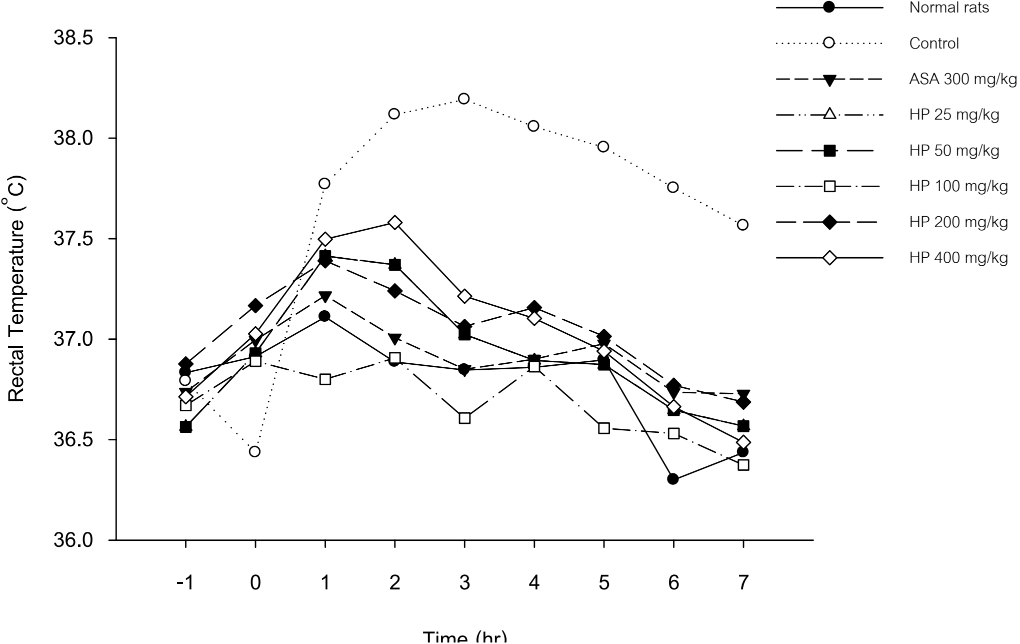


Figure 21 Rectal temperatures after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Lipopolysaccharide-induced Fever in Rats

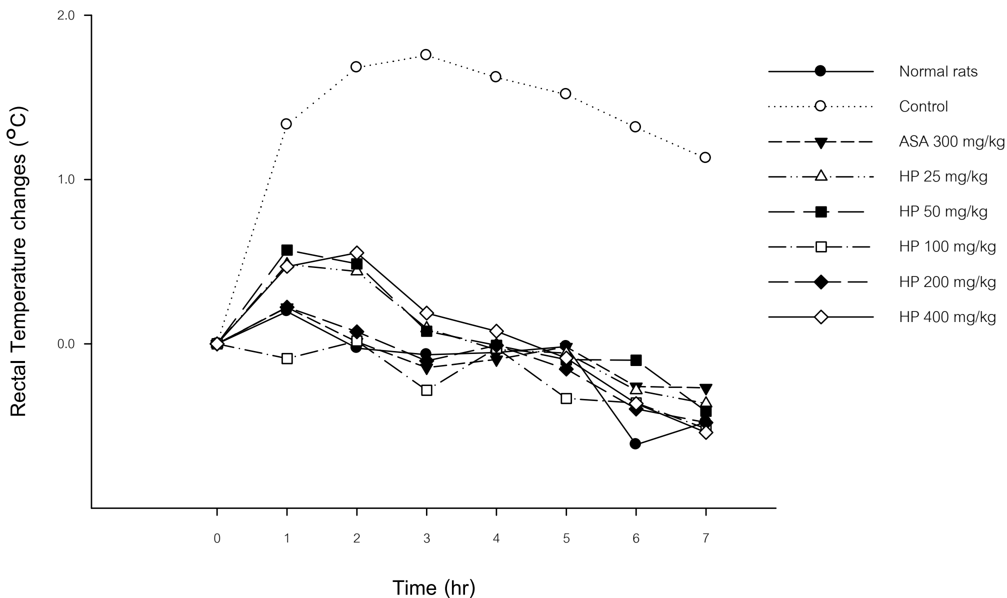


Figure 22 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *$p<0.05$ significantly different compared to control animals.

Table 2 Effect of *Harrisonia perforata* root extract (HP; 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 |
| Normal 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[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* |
| HP 25 mg/kg | 36.56 ± 0.39 | 36.93 ± 0.28 | 37.41 ± 0.10 | 37.37 ± 0.12* | 37.02 ± 0.14* | 36.89 ± 0.21* | 36.87 ± 0.12* | 36.65 ± 0.10* | 36.57 ± 0.09* |
| HP 50 mg/kg | 36.95 ± 0.19 | 36.84 ± 0.26 | 37.41 ± 0.21 | 37.33 ± 0.21 | 36.92 ± 0.09* | 36.83 ± 0.12* | 36.74 ± 0.09* | 36.74 ± 0.13* | 36.43 ± 0.07* |
| HP 100 mg/kg | 36.67 ± 0.28 | 36.89 ± 0.29 | 36.80 ± 0.35 | 36.91 ± 0.33 | 36.61 ± 0.29* | 36.86 ± 0.24* | 36.56 ± 0.16* | 36.53 ± 0.08* | 36.37 ± 0.12* |
| HP 200 mg/kg | 36.88 ± 0.15 | 37.17 ± 0.11 | 37.39 ± 0.21 | 37.24 ± 0.21 | 37.06 ± 0.24 | 37.16 ± 0.21 | 37.01 ± 0.17* | 36.77 ± 0.16* | 36.69 ± 0.12* |
| HP 400 mg/kg | 36.71 ± 0.32 | 37.03 ± 0.25 | 37.50 ± 0.21 | 37.58 ± 0.21 | 37.21 ± 0.18* | 37.10 ± 0.14* | 36.94 ± 0.15* | 36.66 ± 0.15* | 36.49 ± 0.07* |

Each value represents mean± SEM (n=6), [a]Normal rats received 0.9% NSS instead of lioppolysaccharide. [b]Control received 2% Tween 80 solution.

# p<0.001 significantly different compared to normal rat values for the corresponding hour. *p<0.05 significantly different compared to control values for the corresponding hour.

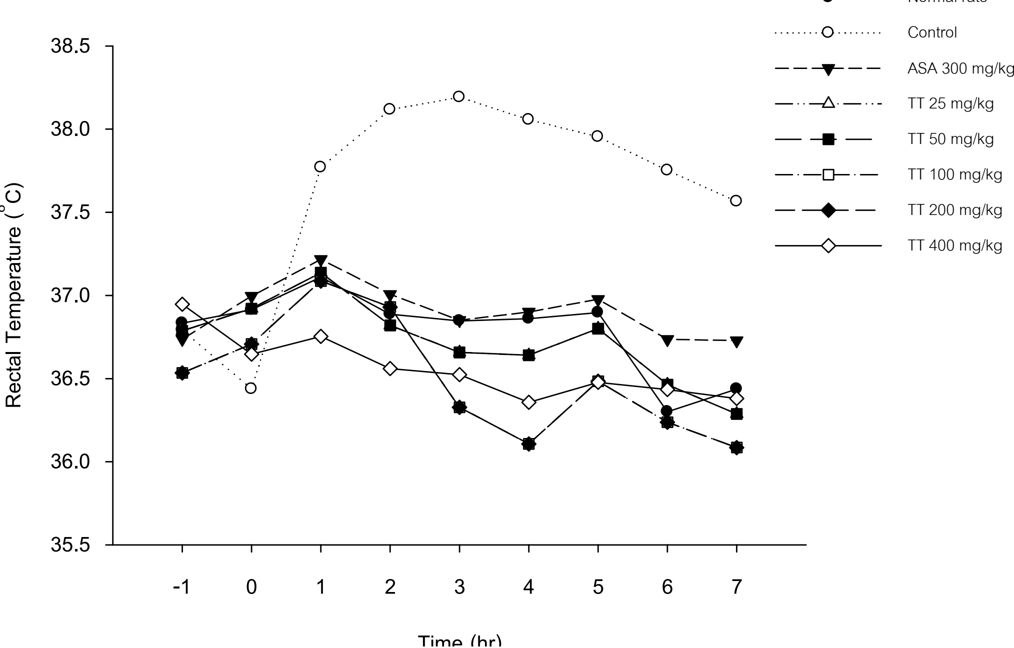


Figure 23 Rectal temperature after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *$p<0.05$ significantly different compared to control animals.

Lipopolysaccharide-induced Fever in Rats

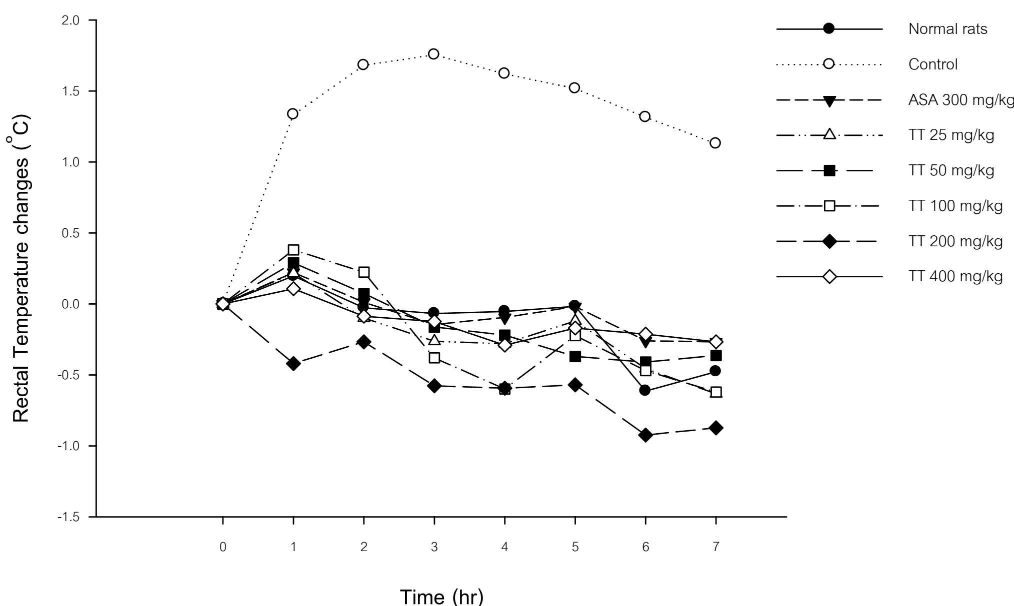


Figure 24 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Table 3 Effect of *Tiliacora triandra* (TT; 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 |
| Normal 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[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* |
| TT 25 mg/kg | 36.79 ± 0.19 | 37.14 ± 0.13 | 37.14 ± 0.20 | 36.82 ± 0.22* | 36.66 ± 0.43* | 36.64 ± 0.34* | 36.80 ± 0.36* | 36.46 ± 0.38* | 36.29 ± 0.38* |
| TT 50 mg/kg | 36.97 ± 0.14 | 37.33 ± 0.16 | 37.32 ± 0.46 | 37.10 ± 0.19* | 36.87 ± 0.23* | 36.81 ± 0.13* | 36.66 ± 0.19* | 36.62 ± 0.23* | 36.67 ± 0.25* |
| TT 100 mg/kg | 36.53 ± 0.15 | 37.21 ± 0.20 | 37.09 ± 0.06 | 36.93 ± 0.19* | 36.33 ± 0.08* | 36.11 ± 0.17* | 36.48 ± 0.19* | 36.24 ± 0.27* | 36.08 ± 0.26* |
| TT 200 mg/kg | 37.79 ± 0.16 | 36.92 ± 0.13 | 36.51 ± 0.40* | 36.66 ± 0.43* | 36.35 ± 0.29* | 36.34 ± 0.31* | 36.36 ± 0.23* | 36.01 ± 0.26* | 36.06 ± 0.23* |
| TT 400 mg/kg | 36.95 ± 0.16 | 36.49 ± 0.39 | 36.75 ± 0.29* | 36.56 ± 0.39* | 36.52 ± 0.27* | 36.36 ± 0.28* | 36.48 ± 0.35* | 36.43 ± 0.31* | 36.38 ± 0.36* |

Each value represents mean± SEM (n=6), [a]Normal rats received 0.9% NSS instead of lioppolysaccharide. [b]Control received 2% Tween 80 solution.

# $p<0.001$ significantly different compared to normal rat values for the corresponding hour. *$p<0.05$ significantly different compared to control values for the corresponding hour.

Lipopolysaccharide-induced Fever in Rats

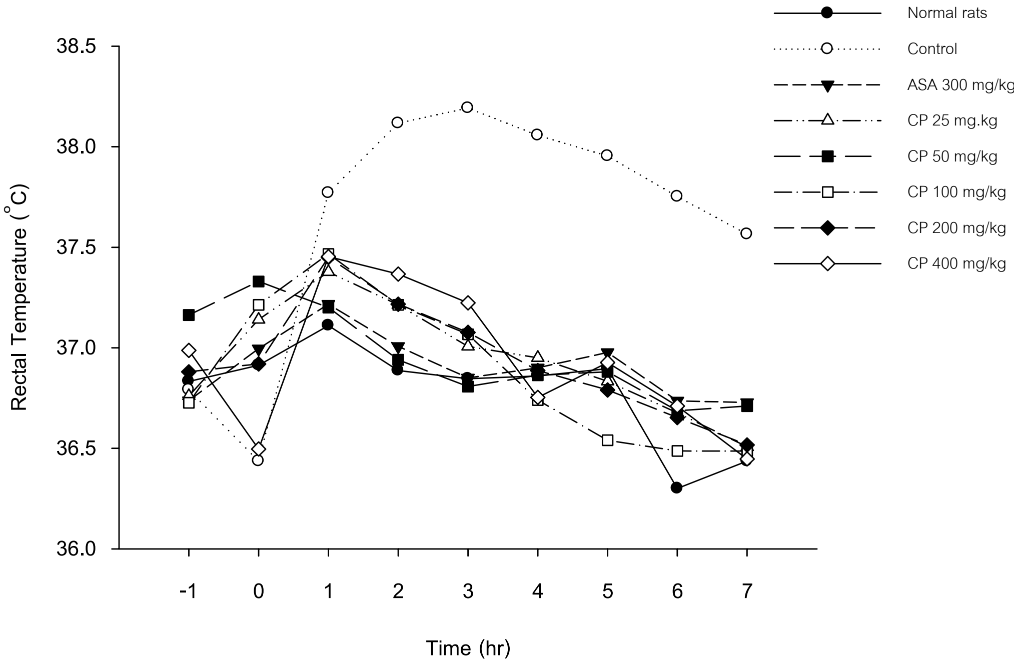


Figure 25 Rectal temperature after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Lipopolysaccharide-induced Fever in Rats

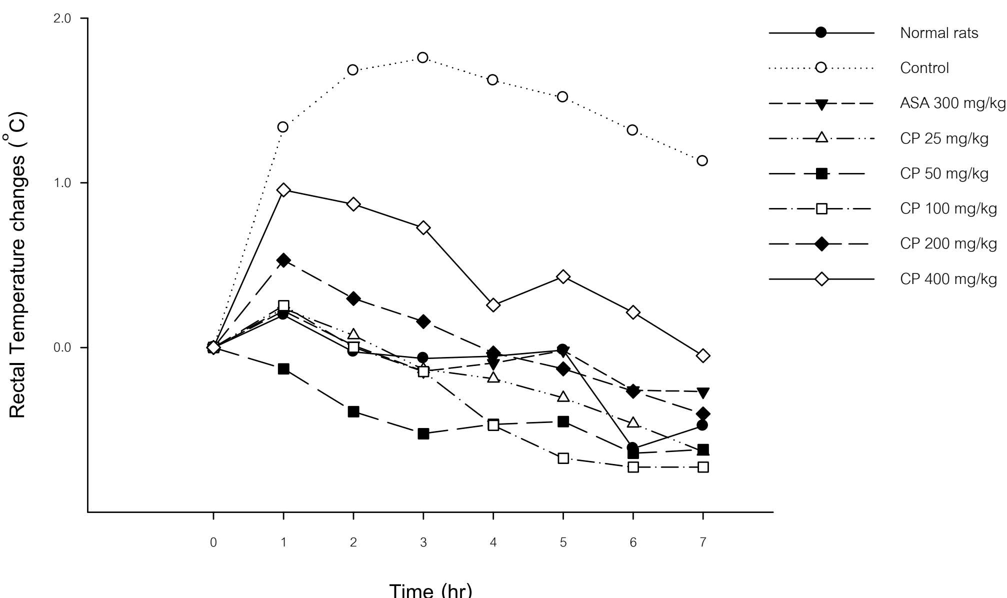


Figure 26 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *$p<0.05$ significantly different compared to control animals.

Table 4 Effect of *Clerodendrum petasites* root extract (CP; 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 |
| Normal 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[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* |
| CP 25 mg/kg | 36.76 ± 0.19 | 37.14 ± 0.13 | 37.38 ± 0.09 | 37.21 ± 0.14* | 37.01 ± 0.16* | 36.95 ± 0.16* | 36.83 ± 0.19* | 36.68 ± 0.19* | 36.51 ± 0.23* |
| CP 50 mg/kg | 37.16 ± 0.52 | 37.33 ± 0.16 | 37.20 ± 0.11 | 36.94 ± 0.19* | 36.81 ± 0.17* | 36.86 ± 0.08* | 36.88 ± 0.05* | 36.69 ± 0.06* | 36.71 ± 0.08* |
| CP 100 mg/kg | 36.73 ± 0.05 | 37.21 ± 0.20 | 37.47 ± 0.11 | 37.21 ± 0.10* | 37.07 ± 0.15* | 36.86 ± 0.19* | 36.54 ± 0.28* | 36.49 ± 0.28 | 36.49 ± 0.08* |
| CP 200 mg/kg | 36.88 ± 0.13 | 36.92 ± 0.13 | 37.45 ± 0.15* | 37.22 ± 0.20* | 37.08 ± 0.15* | 36.89 ± 0.09* | 36.79 ± 0.07* | 36.65 ± 0.07* | 36.52 ± 0.09* |
| CP 400 mg/kg | 36.99 ± 0.10 | 36.49 ± 0.39 | 37.45 ± 0.18* | 37.37 ± 0.26* | 37.22 ± 0.80* | 36.75 ± 0.29* | 36.93 ± 0.19* | 36.71 ± 0.21* | 36.45 ± 0.20* |

Each value represents mean± SEM (n=6), [a]Normal rats received 0.9% NSS instead of lioppolysaccharide. [b]Control received 2% Tween 80 solution.

# p<0.001 significantly different compared to normal rat values for the corresponding hour. *p<0.05 significantly different compared to control values for the corresponding hour.

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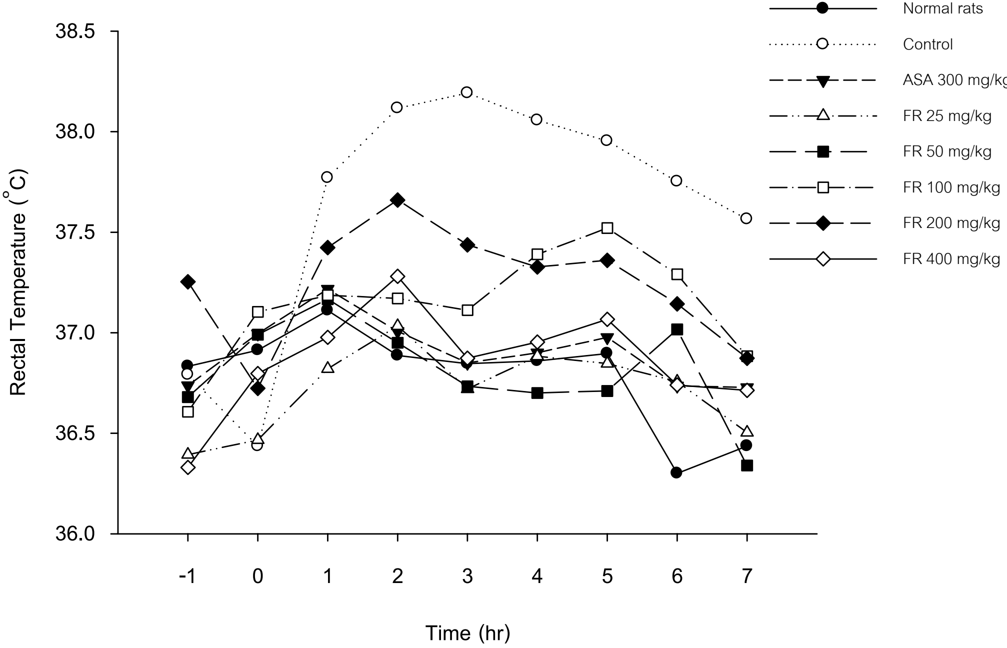



Figure 27 Rectal temperature after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Lipopolysaccharide-induced Fever in Rats

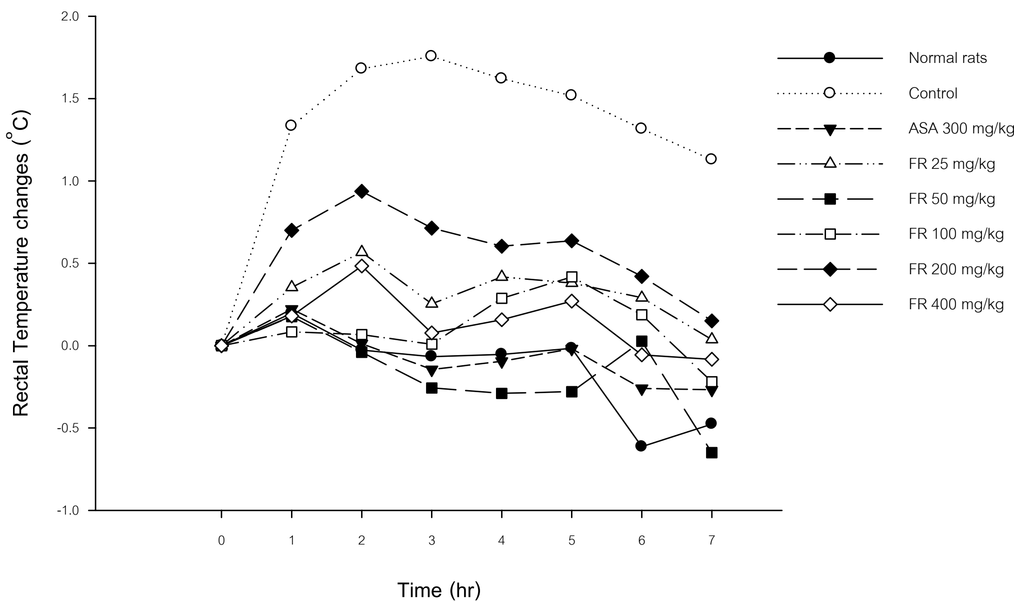



Figure 28 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Table 5 Effect of *Ficus racemosa* root extract (FR; 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 |
| Normal 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[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* |
| FR 25 mg/kg | 36.39 ± 0.16 | 36.47 ± 0.26 | 36.82 ± 0.27* | 37.03 ± 0.24* | 36.72 ± 0.19* | 36.88 ± 0.41* | 36.85 ± 0.29* | 36.75 ± 0.26 | 36.50 ± 0.19* |
| FR 50 mg/kg | 36.68 ± 0.25 | 36.99± 0.21 | 37.17 ± 0.16 | 36.95 ± 0.23* | 36.73 ± 0.29* | 36.70 ± 0.22* | 36.71 ± 0.29* | 37.02 ± 0.62 | 36.34 ± 0.24* |
| FR 100 mg/kg | 36.60 ± 0.20 | 37.10 ± 0.18 | 37.19 ± 0.18 | 37.17 ± 0.27* | 37.11 ± 0.17* | 37.39 ± 0.26 | 37.52 ± 0.15 | 37.29 ± 0.23 | 36.88± 0.26 |
| FR 200 mg/kg | 37.25 ± 0.22 | 36.72 ± 0.25 | 37.42 ± 0.21 | 37.66 ± 0.17 | 37.44 ± 0.13 | 37.33 ± 0.22 | 37.36 ± 0.23 | 37.14 ± 0.19 | 36.87 ± 0.21 |
| FR 400 mg/kg | 36.33 ± 0.17 | 36.79 ± 0.15 | 36.98 ± 0.23* | 37.28 ± 0.31* | 36.87 ± 0.34* | 36.95 ± 0.22* | 37.07 ± 0.15* | 36.74 ± 0.15* | 36.71 ± 0.16 |

Each value represents mean± SEM (n=6), [a]Normal rats received 0.9% NSS instead of lioppolysaccharide. [b]Control received 2% Tween 80 solution.

# p<0.001 significantly different compared to normal rat values for the corresponding hour. *p<0.05 significantly different compared to control values for the corresponding hour.

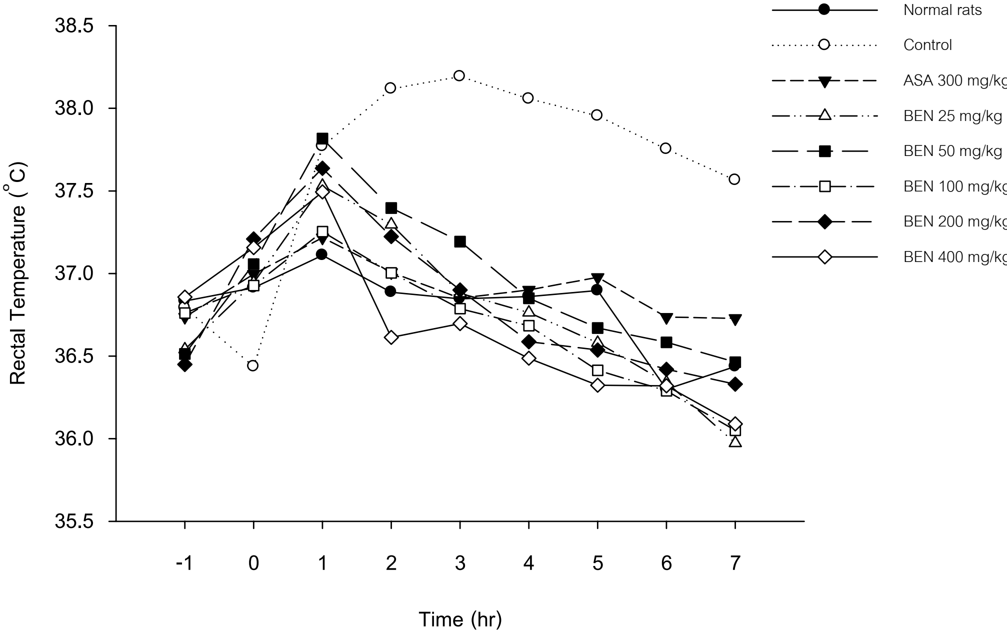



Figure 29 Rectal temperature after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Lipopolysaccharide-induced Fever in Rats

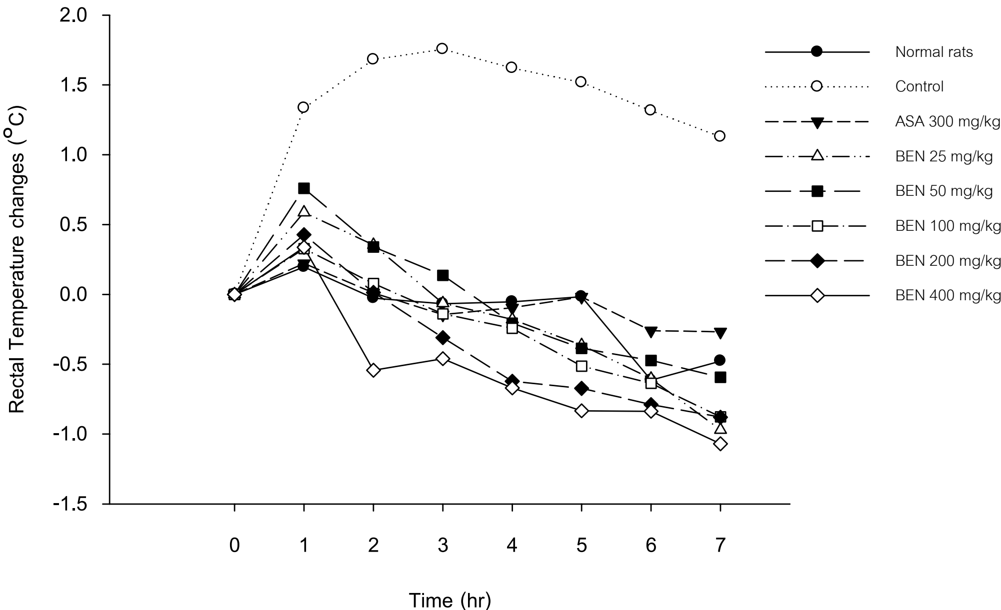



Figure 30 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of 2% Tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of Bencha-loga-wichian remedy (BEN; 25- 400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 μg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

Table 6 Effect of Bencha-loga-wichian remedy (BEN; 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 |
| Normal 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[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* |
| BEN 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* |
| BEN 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* |
| BEN 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* |
| BEN 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* |
| BEN 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± SEM (n=6). [a]Normal rats received 0.9% NSS instead of lioppolysaccharide. [b]Control received 2% Tween 80 solution.

# $p<0.001$ significantly different compared to normal rat values for the corresponding hour. *$p<0.05$ significantly different compared to control values for the corresponding hour.

Lipopolysaccharide-induced Fever in Rats

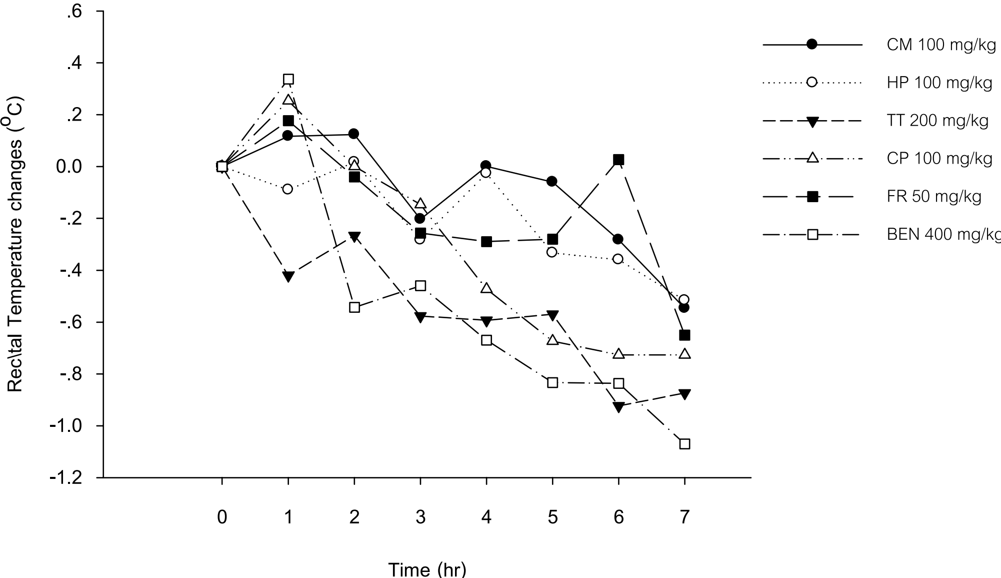



Figure 31 Changes in rectal temperature from baseline on lipopolysaccharide-induced fever after oral administration of CM 100 mg/kg, HP 100 mg/kg, TT 200 mg/kg, CP 100 mg/kg, FR 50 mg/kg and BEN 400 mg/kg to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N=6 for all group. *p<0.05 significantly different compared to control animals.

MOUSE HOT-PLATE TEST

To demonstrate the validity of the hot-plate analgesic testing following drug administration, mice received morphine sulphate (MO; 10 mg/kg) intraperitoneal (i.p.) and were tested during the subsequent 240 min period. As expected MO significantly ($p<0.01$) increased the hot-plate latency producing an area of analgesia of 16992.68± 1940.94 %MPE-min compared with that of normal saline solution (NSS) (-6908.17 ±2505.75 %MPE-min; Figure 32).

Initial studies utilizing the hot-plate test in mice to examine the efficacy of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy in producing analgesia. Mice were administered orally 2% Tween 80 or various doses of five herbal root extracts of Bencha-loga-wichian remedy (25, 50, 100, 200, 400 mg/kg) and Bencha-loga-wichian remedy (25, 50, 100, 200, 400 mg/kg).

All doses of CM tested significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 33). The analgesic peak effects of all doses of CM (25-400 mg/kg) were reached within 240 min after oral administration. Individual time courses of the responses are shown in Figure 34.

HP at the doses of 200 and 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 35). The analgesic peak effects of HP (25, 50, 100, 200 and 400 mg/kg) were reached within 15, 120, 240, 120, and 90 min, respectively after oral administration. Individual time courses of the responses are shown in Figure 36.

TT at the doses of 100, 200 and 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 37). The analgesic peak effects of TT (25, 50, 100, 200 and 400 mg/kg) were reached within 240, 240, 240, 120, and 90 min, respectively after oral administration. Individual time courses of the responses are shown in Figure 38.

CP 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 39). The analgesic peak effects of CP (25, 50, 100, 200 and 400 mg/kg) were reached within 120, 90, 120, 120, and 120 min,

respectively after oral administration. Individual time courses of the responses are shown in Figure 40.

FR at the doses of 100, 200 and 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 41). The analgesic peak effects of FR (25, 50, 100, 200 and 400 mg/kg) were reached within 120, 120, 240, 120, and 240 min, respectively after oral administration. Individual time courses of the responses are shown in Figure 42.

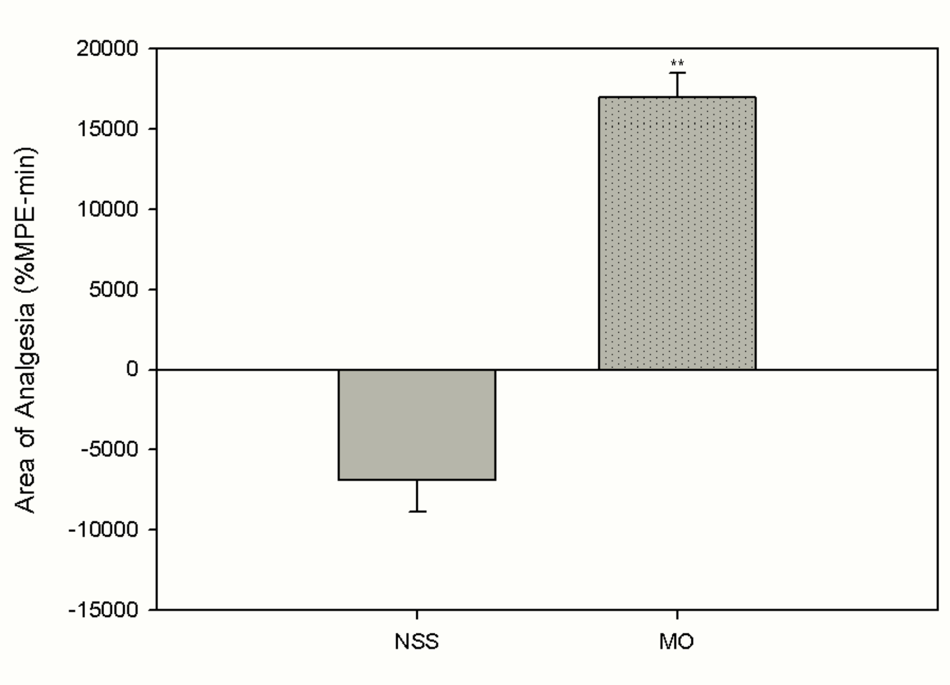
BEN 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 43). The analgesic peak effects of BEN (25, 50, 100, 200 and 400 mg/kg) were reached within 120, 120, 120, 90, 120 min, respectively after oral administration. Individual time courses of the responses are shown in Figure 44.

All five herbal root extracts at dose of 25 mg/kg showed similar analgesic effect when compared to BEN 25 mg/kg (Figure 45). CM and FR at dose of 50 mg/kg showed significant ($p<0.05$) analgesic efficacy when compared to BEN 50 mg/kg (Figure 46). CM, TT and FR at the dose of 100 mg/kg showed significant ($p<0.05$) analgesic efficacy when compared to BEN 100 mg/kg (Figure 47). CM, HP, TT and FR at the dose of 200 mg/kg showed significant ($p<0.05$) analgesic efficacy when compared to BEN 200 mg/kg (Figure 48). CM and FR at the dose of 400 mg/kg showed significant ($p<0.05$) analgesic efficacy when compared to BEN 400 mg/kg (Figure 49).

In order to investigate any role of the opioid receptor in CM, HP, TT, CP, FR, and actions, mice were then administered naloxone (NAL; 5 mg/kg, i.p.), a short-acting opioid receptor antagonist, 2%Tween 80 (10 ml/kg, p.o.), CM (200 mg/kg, p.o.), HP (400 mg/kg, p.o.), TT (400 mg/kg, p.o.), CP (400 mg/kg, p.o.) and FR (400 mg/kg, p.o.) or the combination of naloxone and CM (5/200 mg/kg), the combination of naloxone and HP (5/400 mg/kg), the combination of naloxone and TT (5/400 mg/kg), the combination of naloxone and CP (5/400 mg/kg) and the combination of naloxone and FR (5/400 mg/kg). Naloxone alone failed to produce significant response when compared to vehicle control. CM, HP, TT, CP, FR at the dose tested produced significant ($p<0.05$) response

when compared to vehicle control. The inclusion of naloxone with CM, HP, TT, CP, and FR significantly ($p<0.05$) attenuated the analgesic response due to CM, HP, TT, CP, and FR indicating that opioid receptors are involved in the analgesic response produced by CM, HP, TT, CP, and FR (Figure 50, 52, 54, 56 and 58, respectively). Individual time courses of the responses are shown in Figure 51, 53, 55, 57 and 59, respectively.

Mouse Hot-plate Test




Treatments

Figure 32 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. **p<0.01 significantly different compared to NSS.

Mouse Hot-plate Test

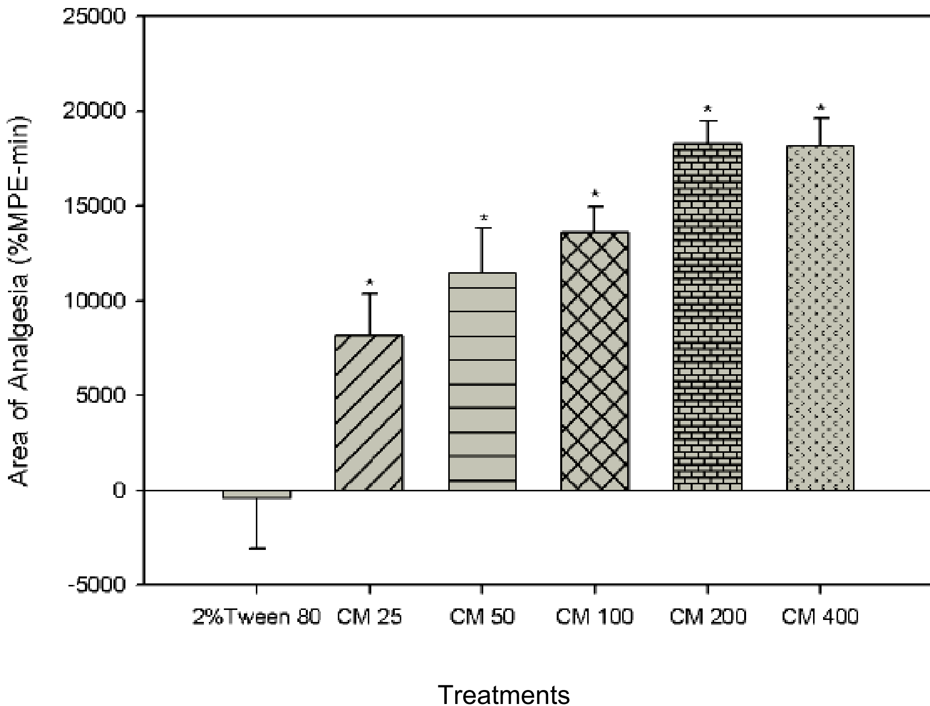



Figure 33 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

## Mouse Hot-plate Test

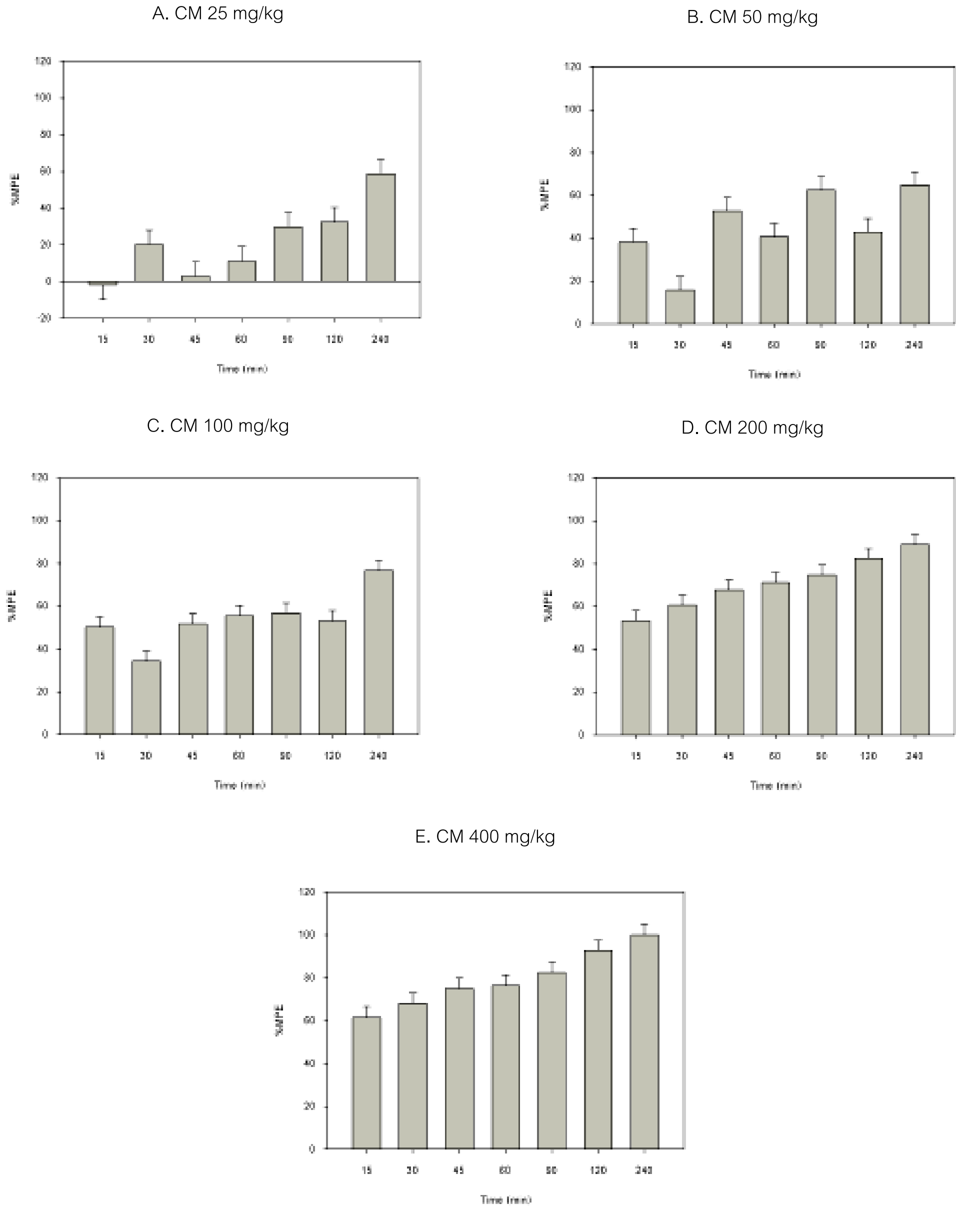



Figure 34 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Capparis micracantha* root extract (CM). A; CM 25 mg/kg, B; CM 50 mg/kg, C; CM 100 mg/kg, D; CM 200 mg/kg and E; CM 400 mg/kg. N=10 for all groups.

Mouse Hot-plate Test

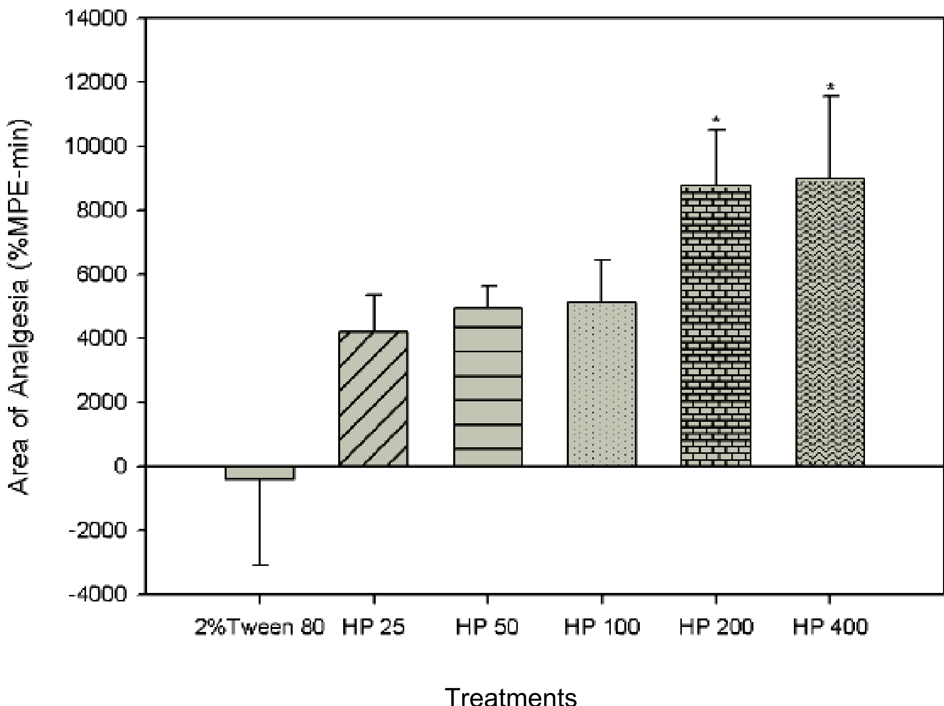



Figure 35 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Harrisonia perforata* root extract (HP; 25- 400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Mouse Hot-plate Test

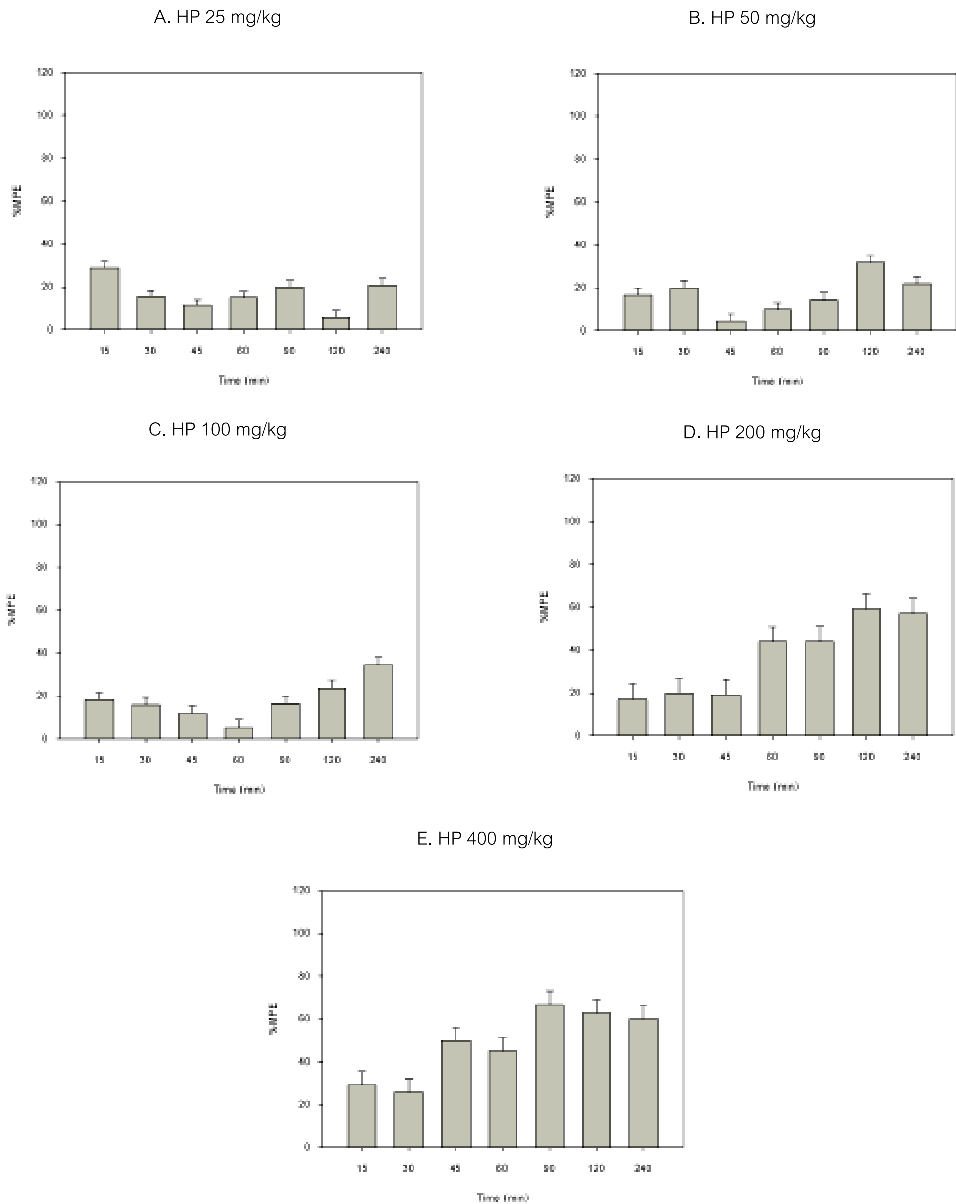



Figure 36 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg). A; HP 25 mg/kg, B; HP 50 mg/kg, C; HP 100 mg/kg, D; HP 200 mg/kg and E; HP 400 mg/kg. N=10 for all groups.

Mouse Hot-plate Test

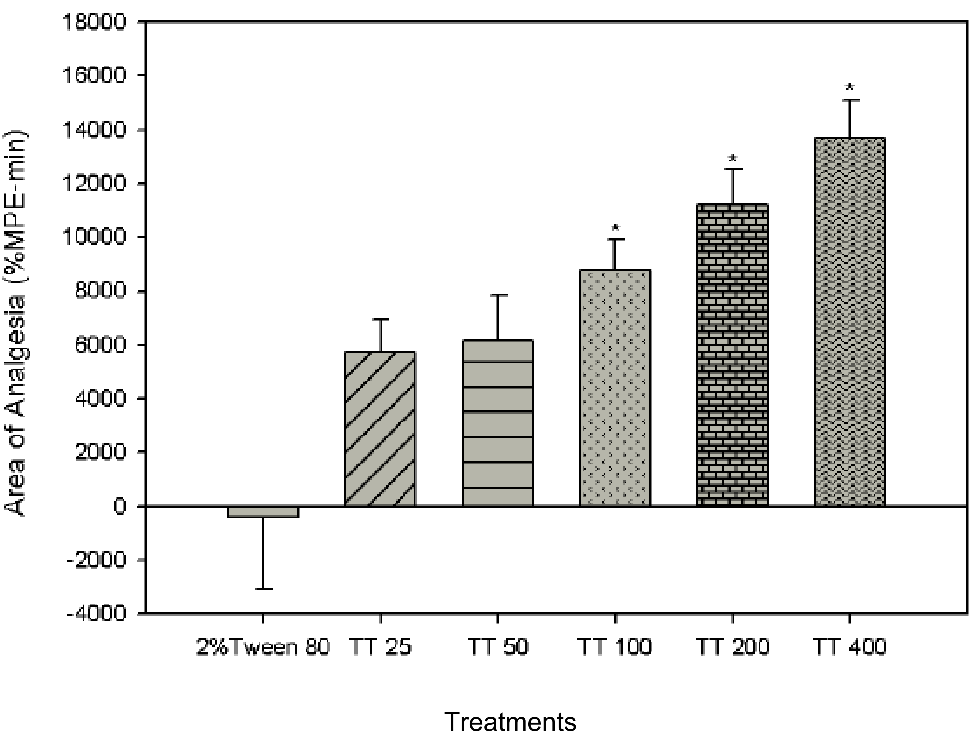



Figure 37 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg). N=10 for all groups. *$p<0.05$ significantly different compared to 2% Tween 80.

Mouse Hot-plate Test

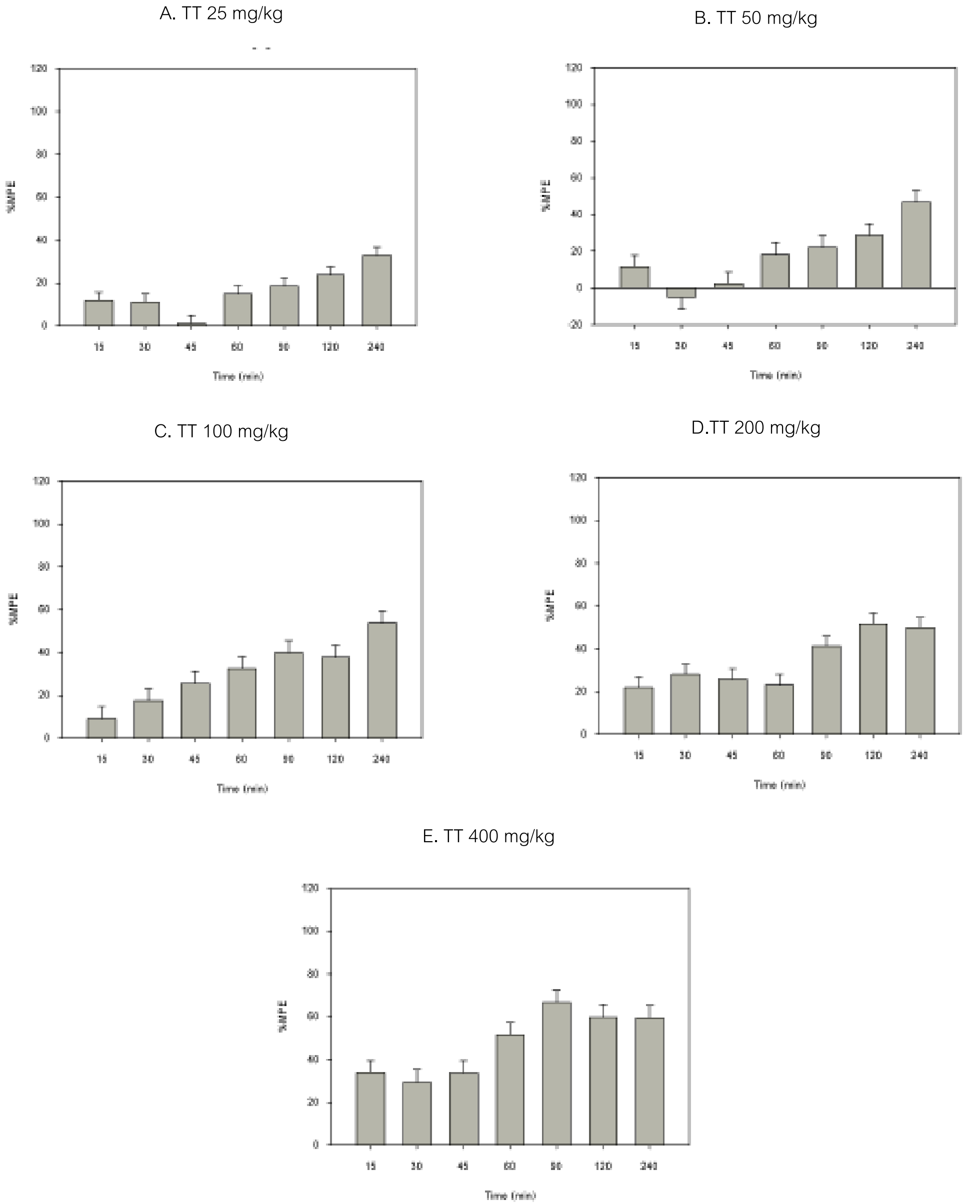
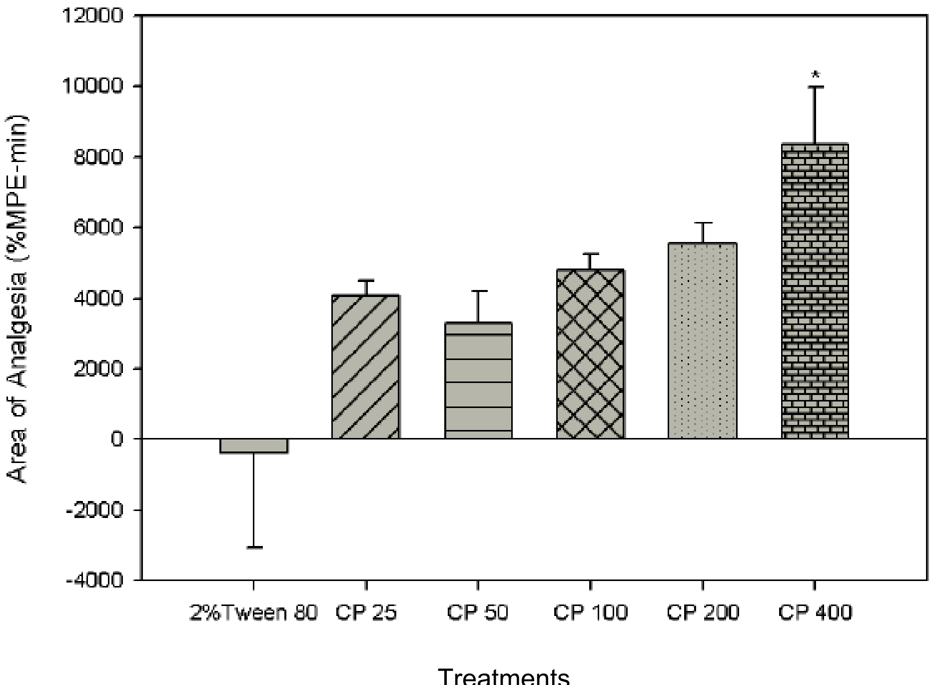



Figure 38 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg). A; TT 25 mg/kg, B; TT 50 mg/kg, C; TT 100 mg/kg, D; TT 200 mg/kg and E; TT 400 mg/kg. N=10 for all groups.

Mouse Hot-plate Test




Treatments

Figure 39 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Clerodendrum petasites* root extract (CP; 25- 400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Mouse Hot-plate Test

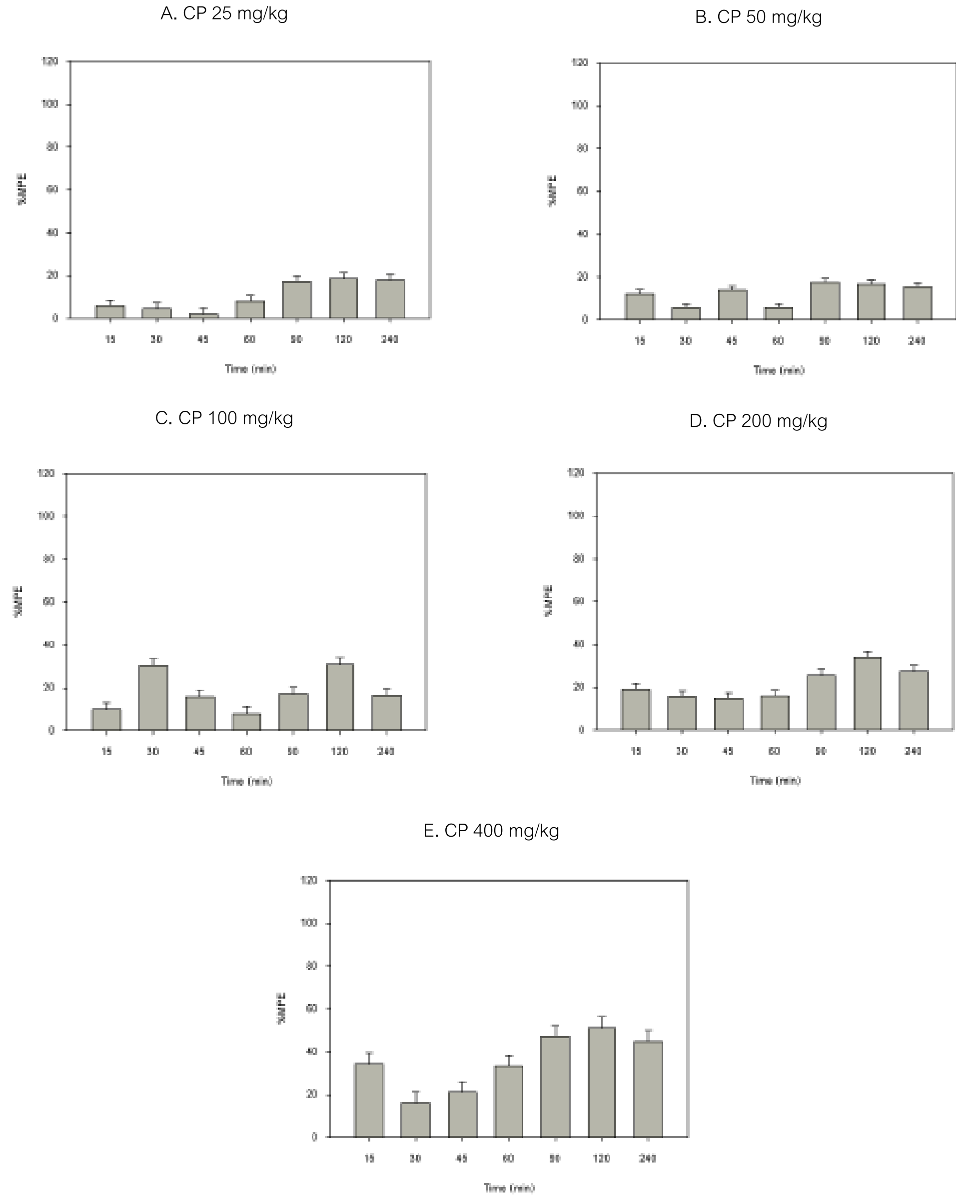



Figure 40 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Clerodendrum petasites* root extract *(*CP; 25- 400 mg/kg). A; CP 25 mg/kg, B; CP 50 mg/kg, C; CP 100 mg/kg, D; CP 200 mg/kg and E; CP 400 mg/kg. N=10 for all groups.

Mouse Hot-plate Test

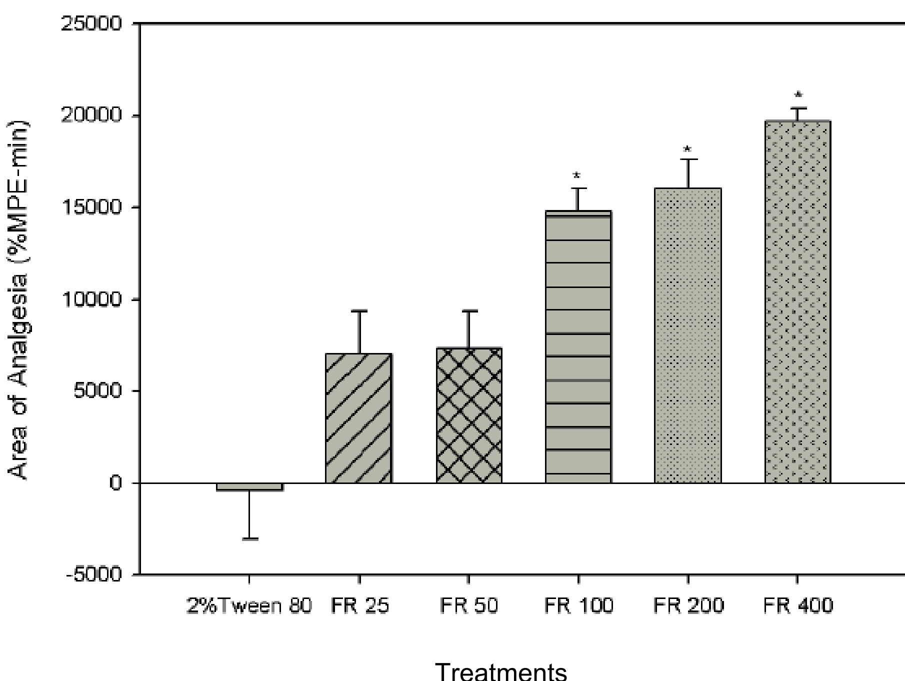



Figure 41 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Ficus racemosa* root extract (FR; 25- 400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Mouse Hot-plate Test

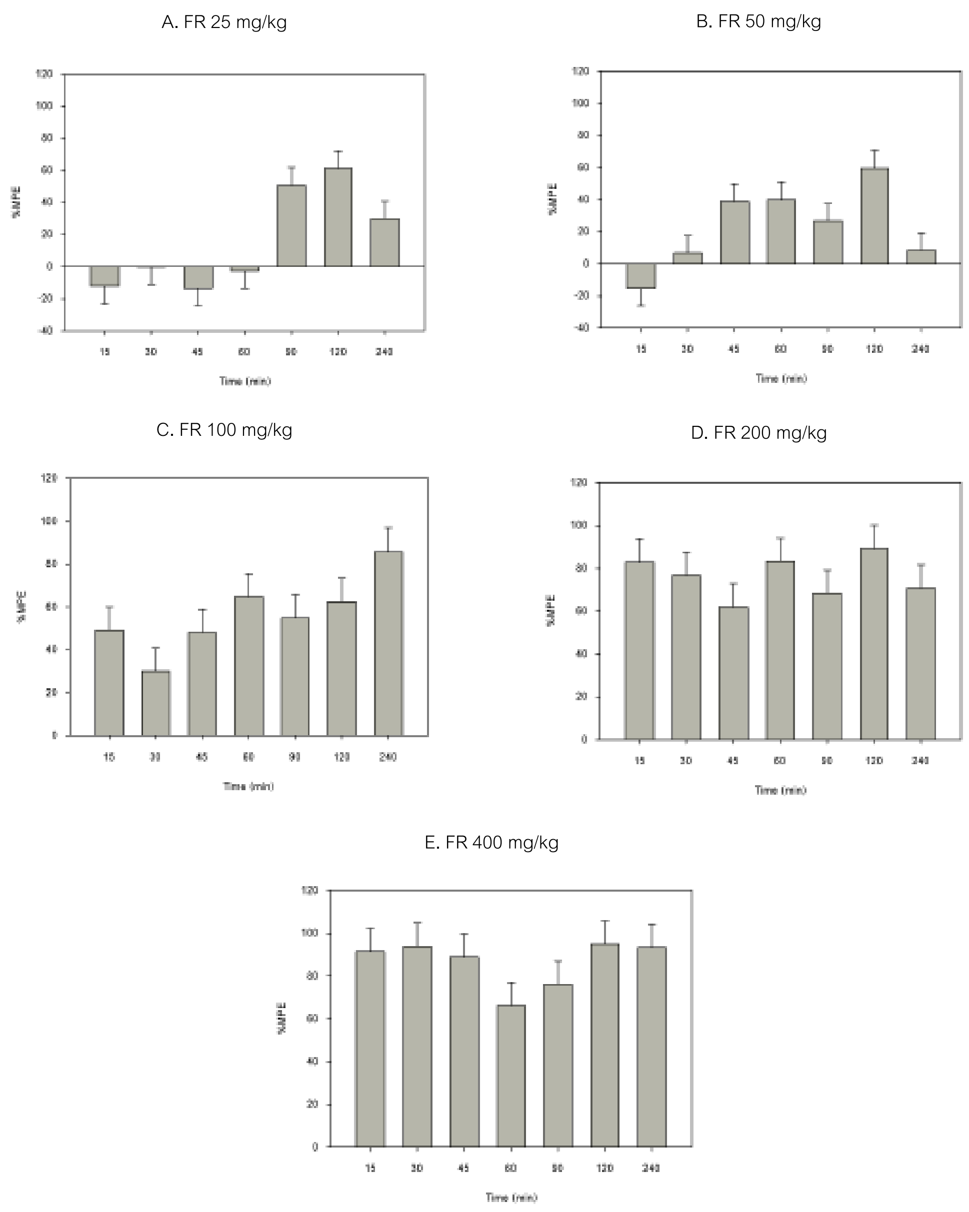



Figure 42 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Ficus racemosa* root extract (FR; 25- 400 mg/kg). A; FR 25 mg/kg, B; FR 50 mg/kg, C; FR 100 mg/kg, D; FR 200 mg/kg and E; FR 400 mg/kg. N=10 for all groups.

Mouse Hot-plate Test

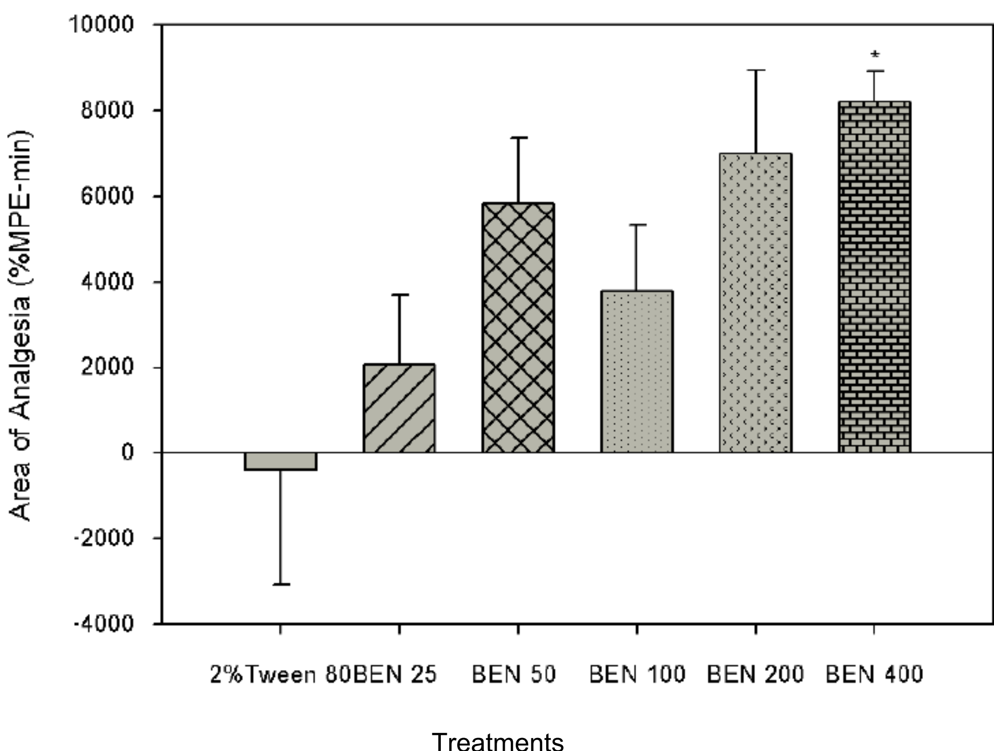



Figure 43 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of Bencha-loga-wichian remedy (BEN; 25- 400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

## Mouse Hot-plate Test

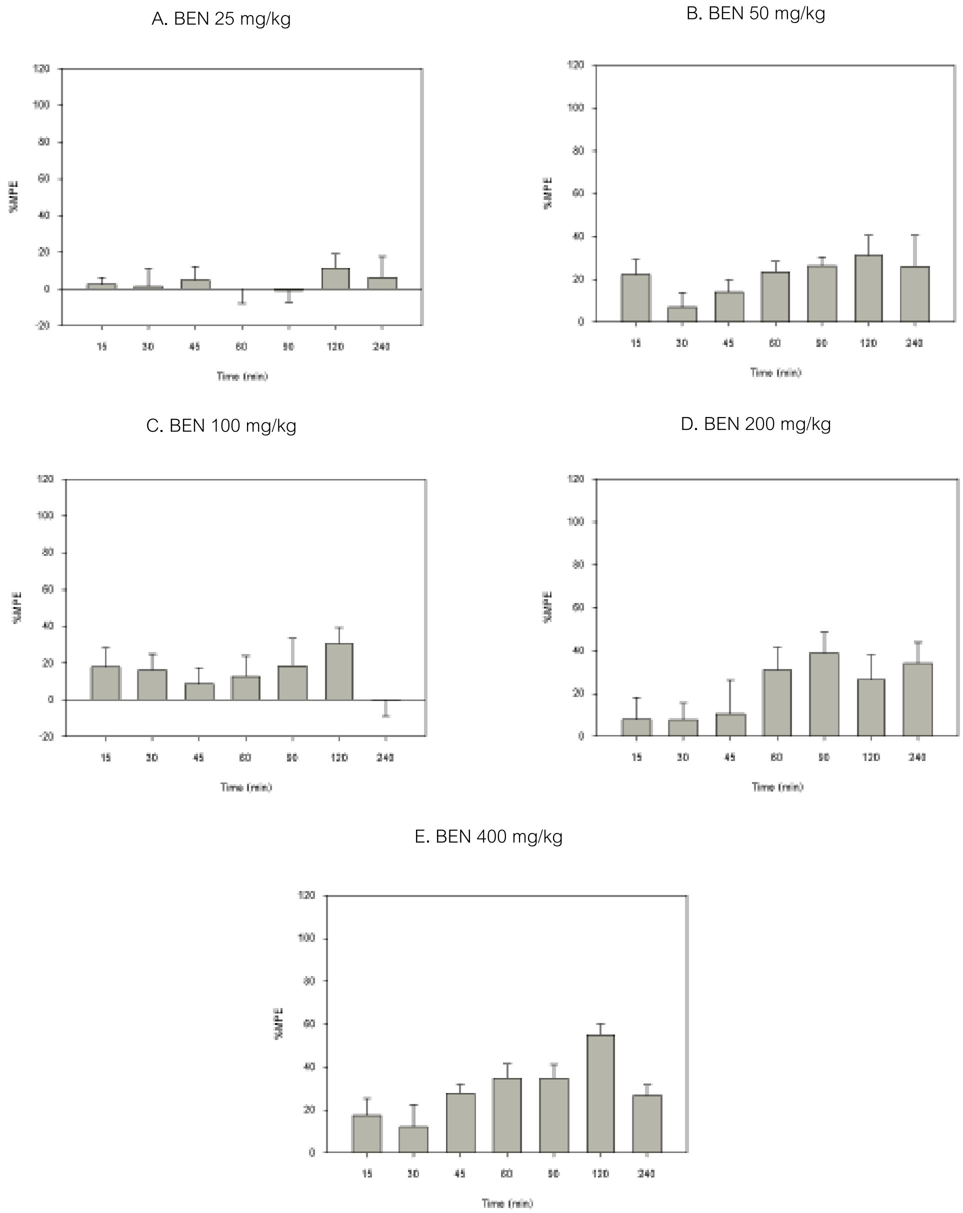



Figure 44 Individual time courses of the response (%MPE versus time (min) after oral administration of various doses of Bencha-loga-wichian remedy (BEN; 25- 400 mg/kg). A; BEN 25 mg/kg, B; BEN 50 mg/kg, C; BEN100 mg/kg, D; BEN 200 mg/kg and E; BEN 400 mg/kg. N=10 for all groups.

Mouse Hot-plate Test

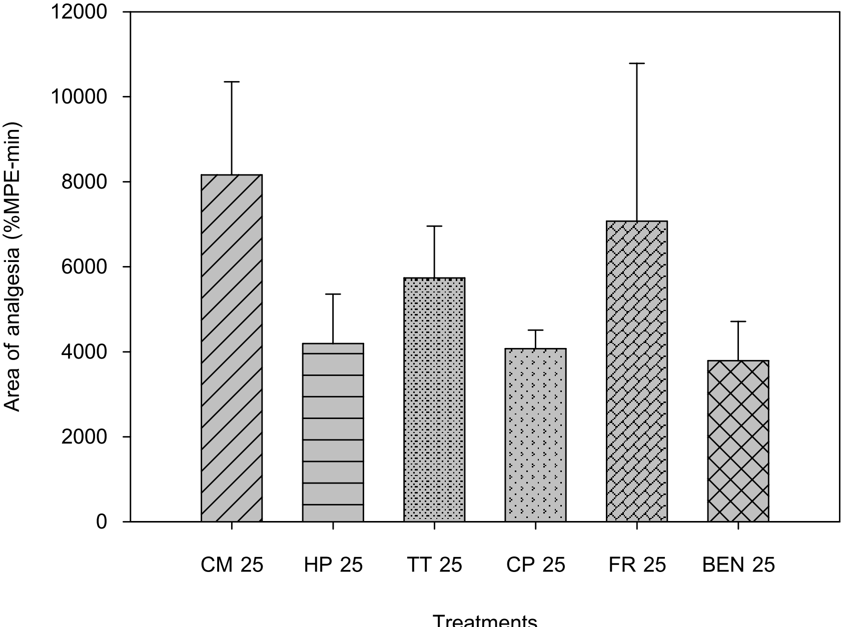



Figure 45 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of five herbal root extracts of Bencha-loga-wichian remedy (CM, HP, TT, CP and FR; 25 mg/kg) and Bencha-loga-wichiain remedy (BEN; 25 mg/kg). N=10 for all groups. *$p<0.05$ significantly different compared to Bencha-loga-wichian remedy (BEN; 25 mg/kg).

Mouse Hot-plate Test

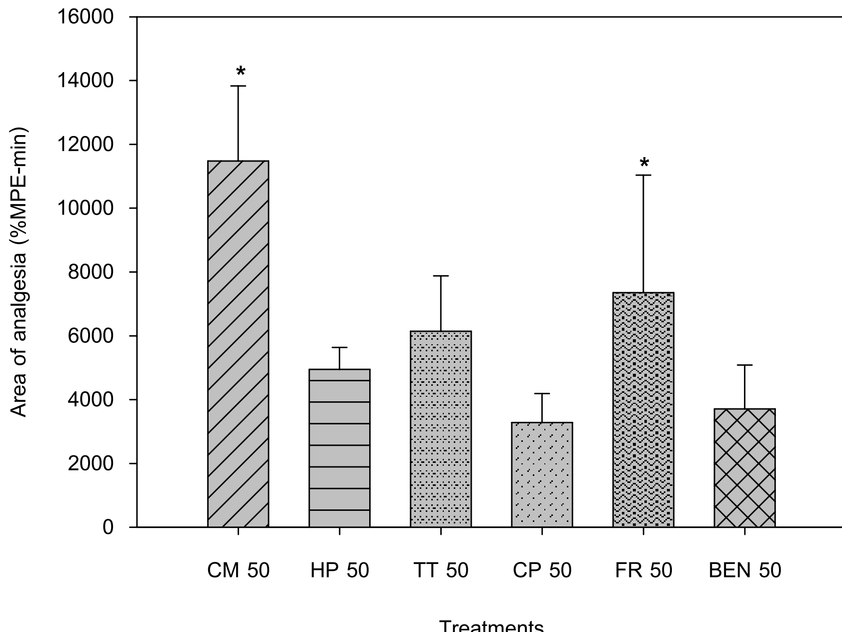



Figure 46 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of five herbal root extracts of Bencha-loga-wichian remedy (CM, HP, TT, CP and FR; 50 mg/kg) and Bencha-loga-wichian remedy (BEN; 50 mg/kg). N=10 for all groups. *$p<0.05$ significantly different compared to Bencha-loga-wichian remedy (BEN; 50 mg/kg).

Mouse Hot-plate Test

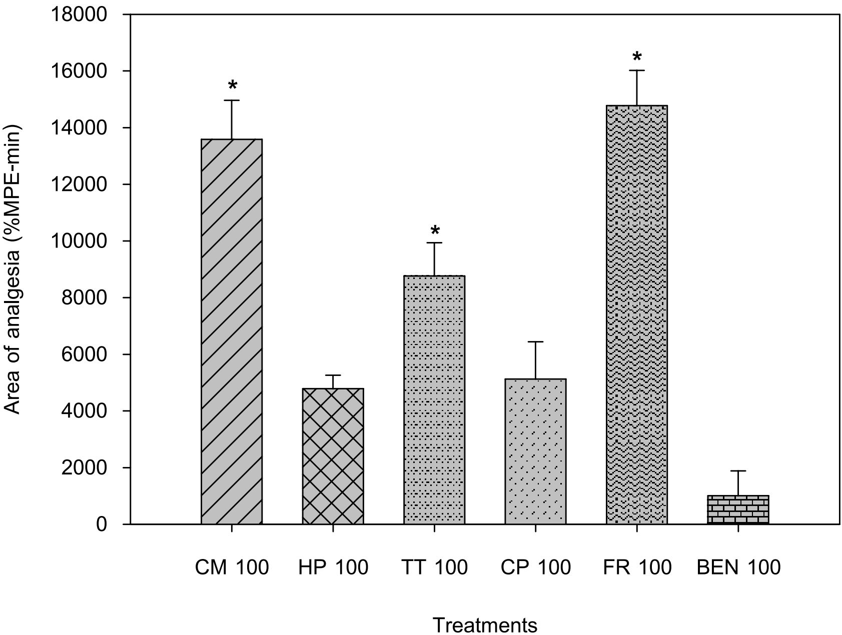



Figure 47 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of five herbal root extracts of Bencha-loga-wichian remedy (CM, HP, TT, CP and FR; 100 mg/kg) and Bencha-loga-wichian remedy (BEN; 100 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to Bencha-loga-wichian remedy (BEN; 100 mg/kg).

Mouse Hot-plate Test

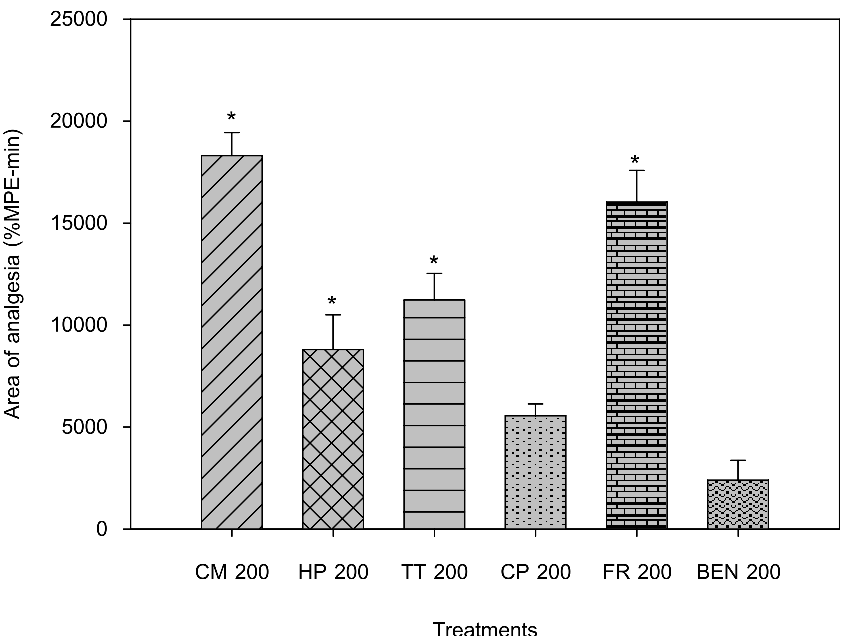



Figure 48 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of five herbal root extracts of Bencha-loga-wichian remedy (CM, HP, TT, CP and FR; 200 mg/kg) and Bencha-loga-wichian remedy (BEN; 200 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to Bencha-loga-wichian remedy (BEN; 200 mg/kg).

Mouse Hot-plate Test

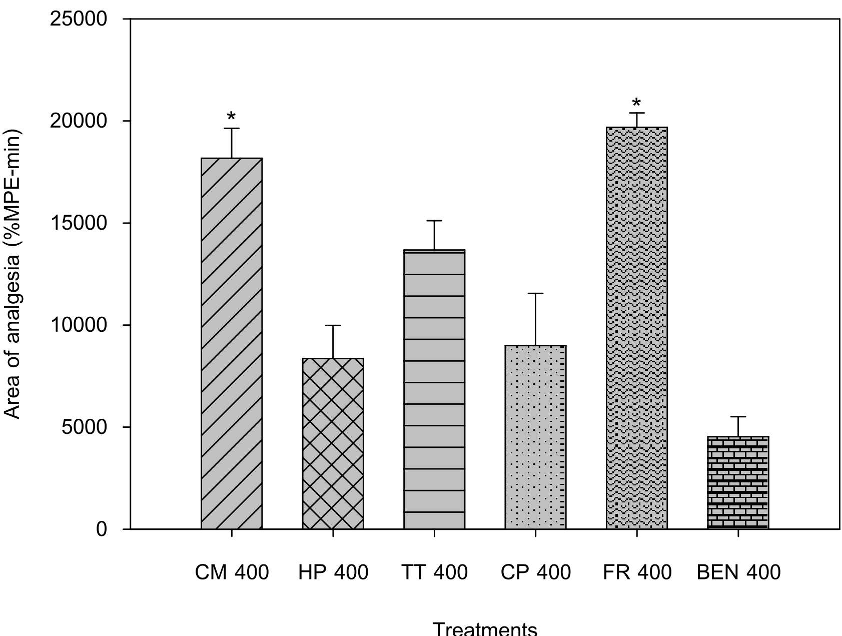



Figure 49 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of five herbal root extracts of Bencha-loga-wichian remedy (CM, HP, TT, CP and FR; 400 mg/kg) and Bencha-loga-wichian remedy (BEN; 400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to Bencha-loga-wichian remedy (BEN; 400 mg/kg).

Mouse Hot-plate Test

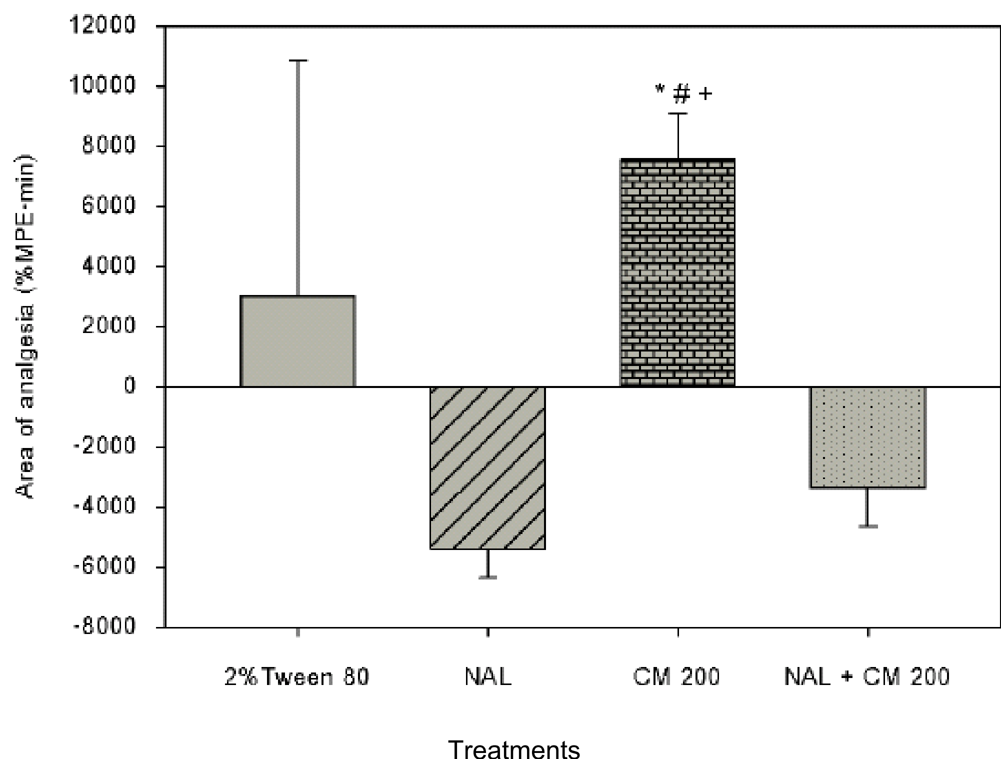



Figure 50 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Capparis micracantha* root extract (CM; 200 mg/kg) and the combination of naloxone and CM (5/200 mg/kg). N=10 for all groups. *p<0.05 significant different compared with 2% Tween 80, #p<0.05 significant different compared with NAL and +p<0.05 significant different compared with NAL+CM.

Mouse Hot-plate Test

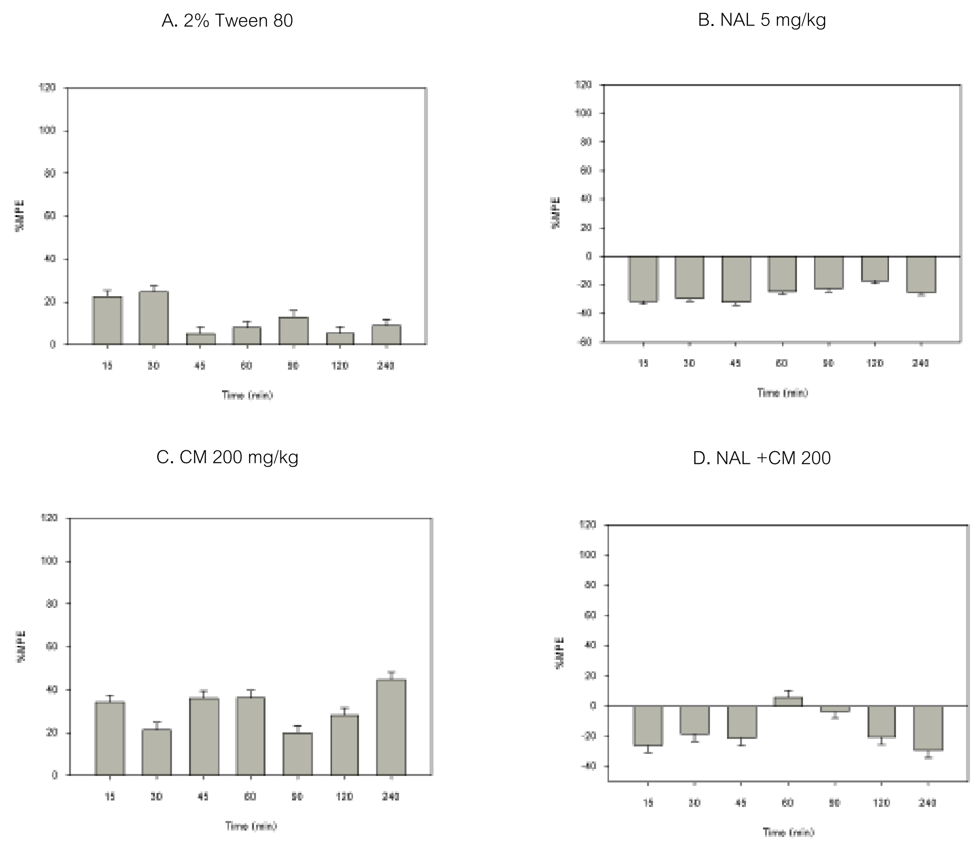
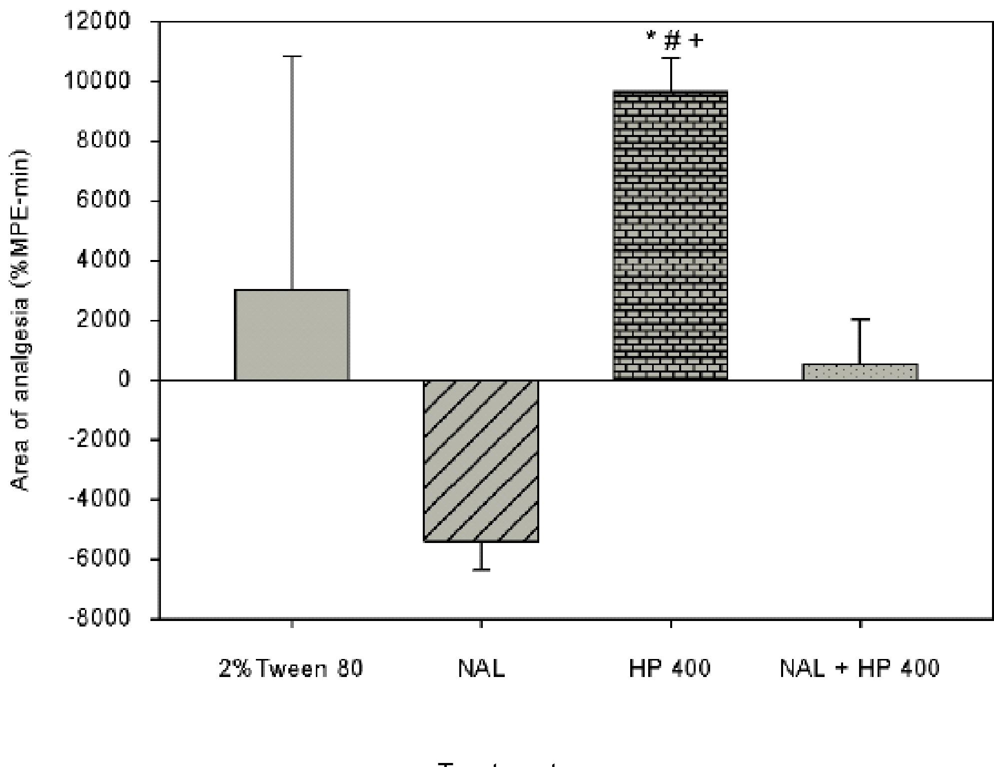



Figure 51 Individual time courses of the response (%MPE versus time (min)) after oral administration of 2% Tween 80 (p.o.), Naloxone (NAL; 5 mg/kg. i.p), *Capparis micracantha* root extract (CM ;200 mg/kg) (p.o) and the combination of naloxone and CM (5/200 mg/kg). A; 2% Tween 80, B; NAL 5 mg/kg, C; CM 200 mg/kg and D; NAL+CM 200. N=10 for all groups.

Mouse Hot-plate Test




Treatments

Figure 52 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Harrisonia perforata* root extract (HP; 400 mg/kg) and the combination of naloxone and HP (5/400 mg/kg). N=10 for all groups. *p<0.05 significant different compared with 2% Tween 80, #p<0.05 significant different compared with NAL and +p<0.05 significant different compared with NAL+HP.

Mouse Hot-plate Test

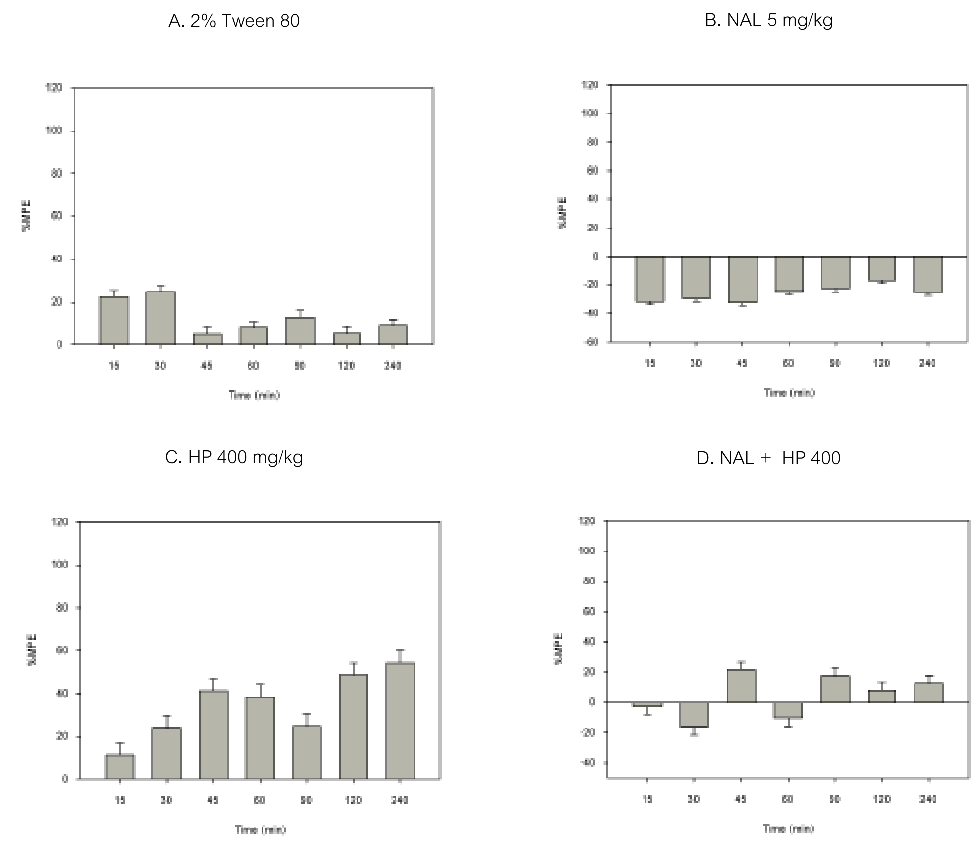



Figure 53 Individual time courses of the response (%MPE versus time (min)) after oral administration of 2% Tween 80 (p.o.), Naloxone (NAL; 5 mg/kg, i.p.), *Harrisonia perforata* root extract (HP; 400 mg/kg) (p.o) and the combination of naloxone and HP (5/400 mg/kg). A; 2% Tween 80, B; NAL 5 mg/kg, C; HP 400 mg/kg and D; NAL+HP 400. N=10 for all groups

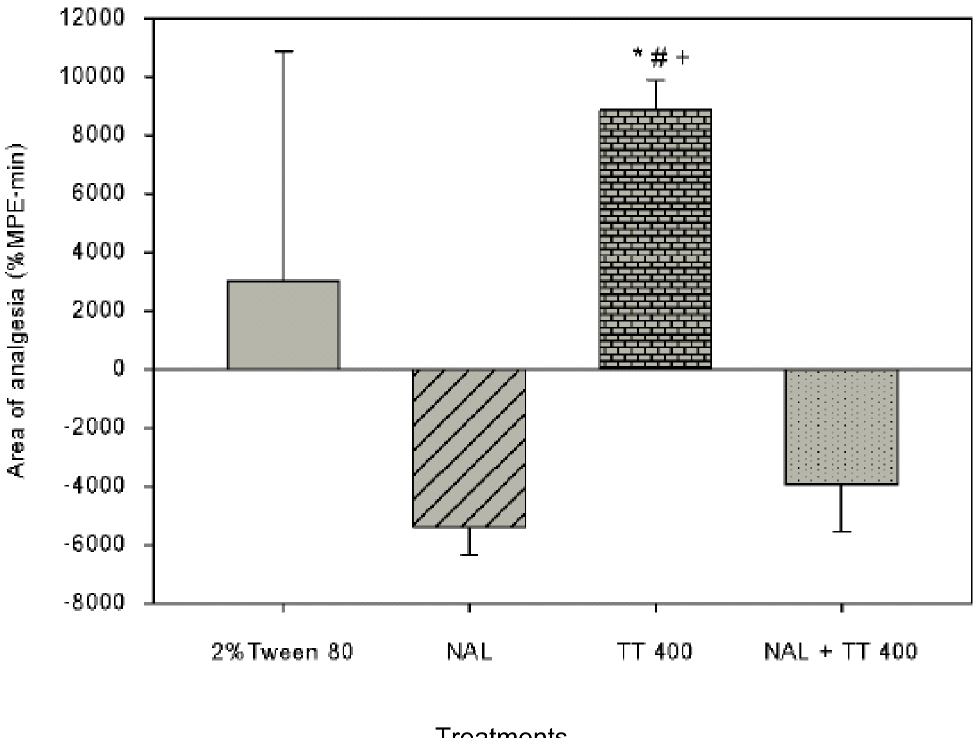



Figure 54 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Tiliacora triandra* root extract (TT; 400 mg/kg) and the combination of naloxone and TT (5/400 mg/kg). N=10 for all groups. *p<0.05 significant different compared with 2% Tween 80, #p<0.05 significant different compared with NAL and +p<0.05 significant different compared with NAL+TT.

Mouse Hot-plate Test

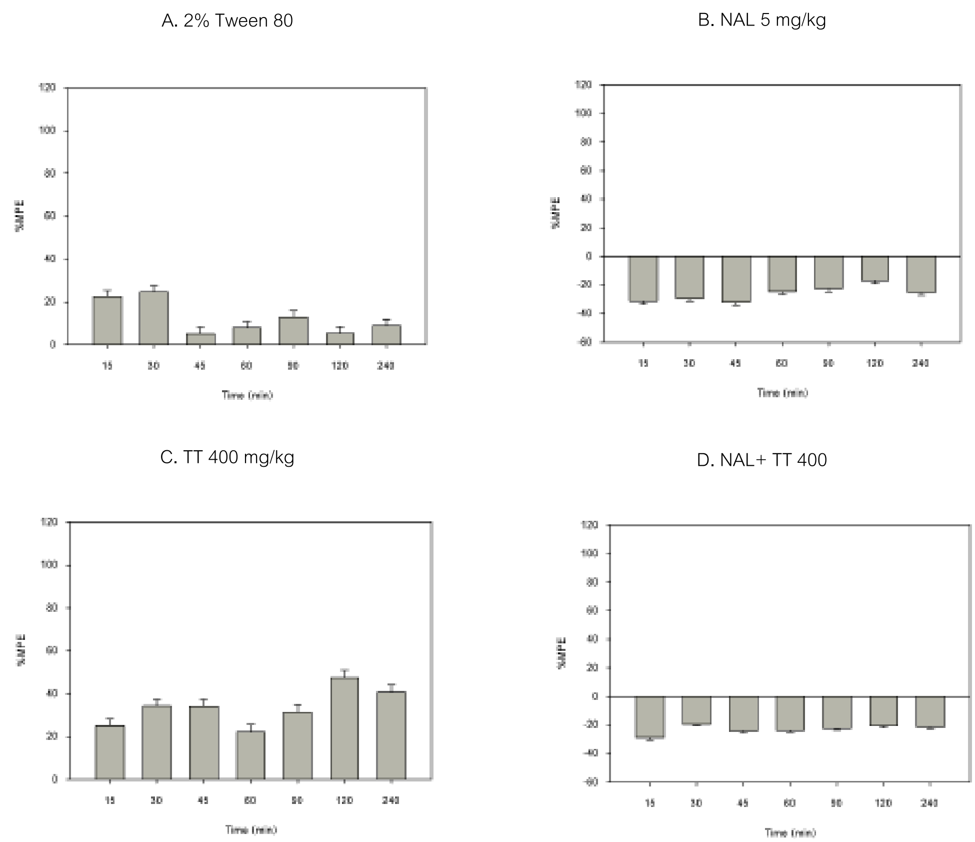



Figure 55 Individual time courses of the response (%MPE versus time (min)) after oral administration of 2% Tween 80 (p.o.), Naloxone (NAL; 5 mg/kg, i.p.), *Tiliacora triandra* root extract (TT; 400 mg/kg) (p.o) and the combination of naloxone and TT (5/400 mg/kg). A; 2% Tween 80, B; NAL 5 mg/kg, C; TT 400 mg/kg and D; NAL+TT 400. N=10 for all groups.

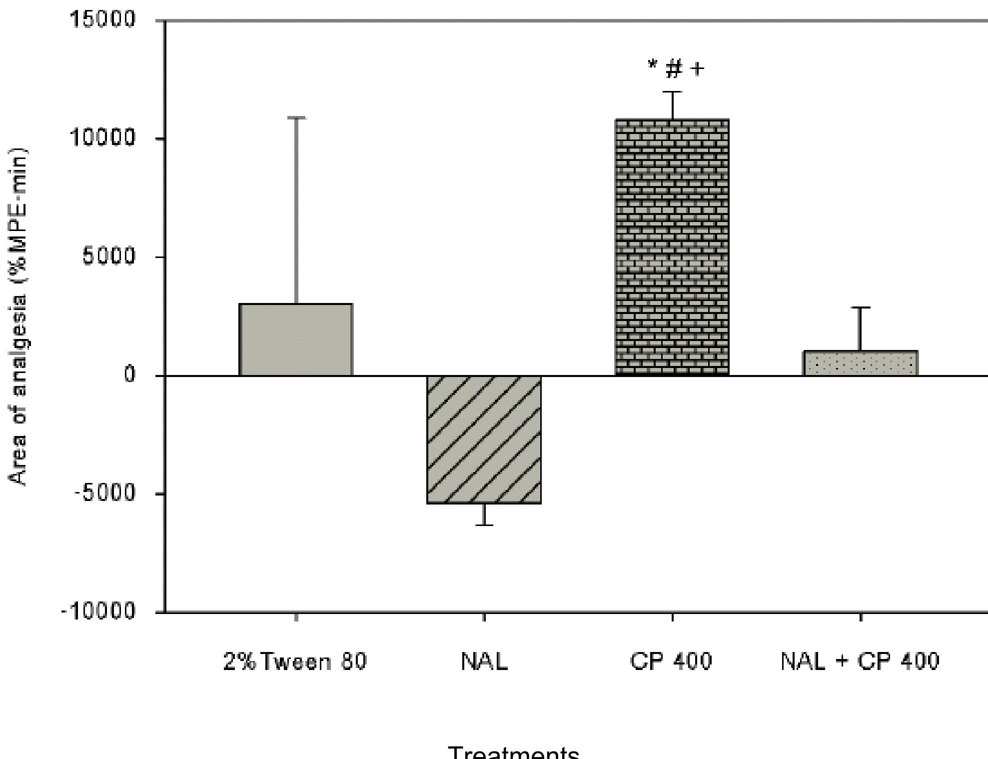



Figure 56 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Clerodendrum petasites* root extract (CP; 400 mg/kg) and the combination of naloxone and CP (5/400 mg/kg). N=10 for all groups. *$p<0.05$ significant different compared with 2% Tween 80, #$p<0.05$ significant different compared with NAL and +$p<0.05$ significant different compared with NAL+CP.

Mouse Hot-plate Test

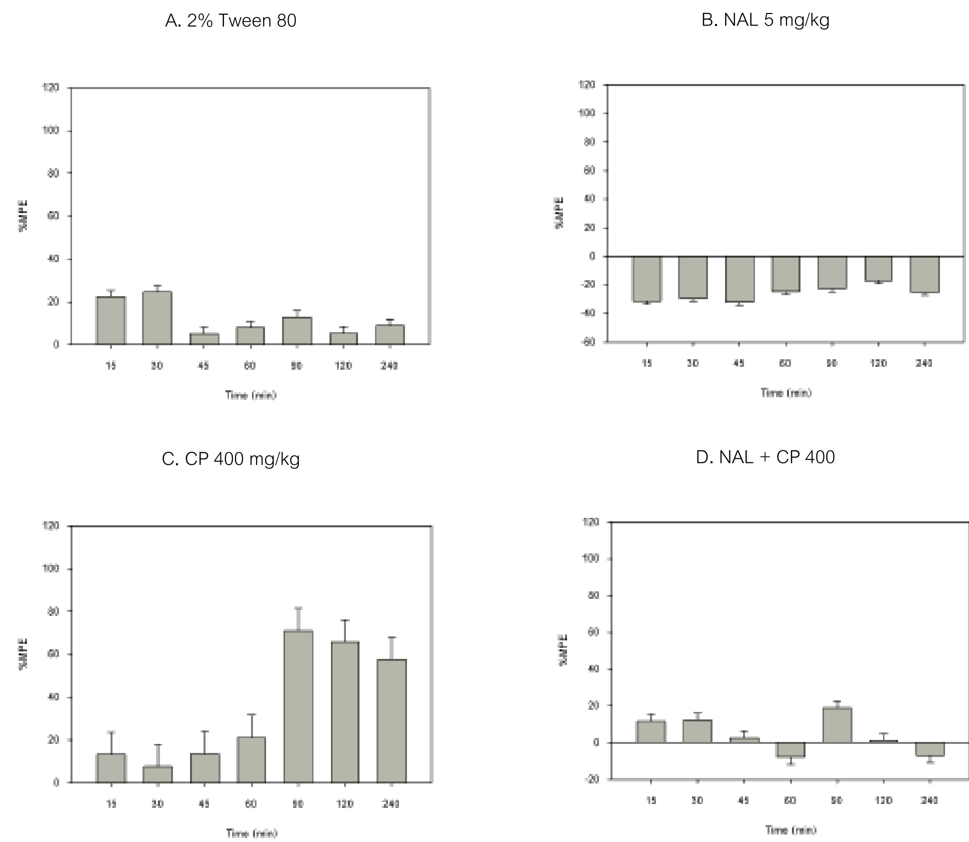



Figure 57 Individual time courses of the response (%MPE versus time (min)) after oral administration of 2% Tween 80 (p.o.), Naloxone (NAL; 5 mg/kg, i.p.), *Clerodendrum petasites* root extract (CP; 400 mg/kg) (p.o) and the combination of naloxone and CP (5/400 mg/kg). A; 2% Tween 80, B; NAL 5 mg/kg, C; FR 400 mg/kg and D; NAL+CP 400. N=10 for all groups.

Mouse Hot-plate Test

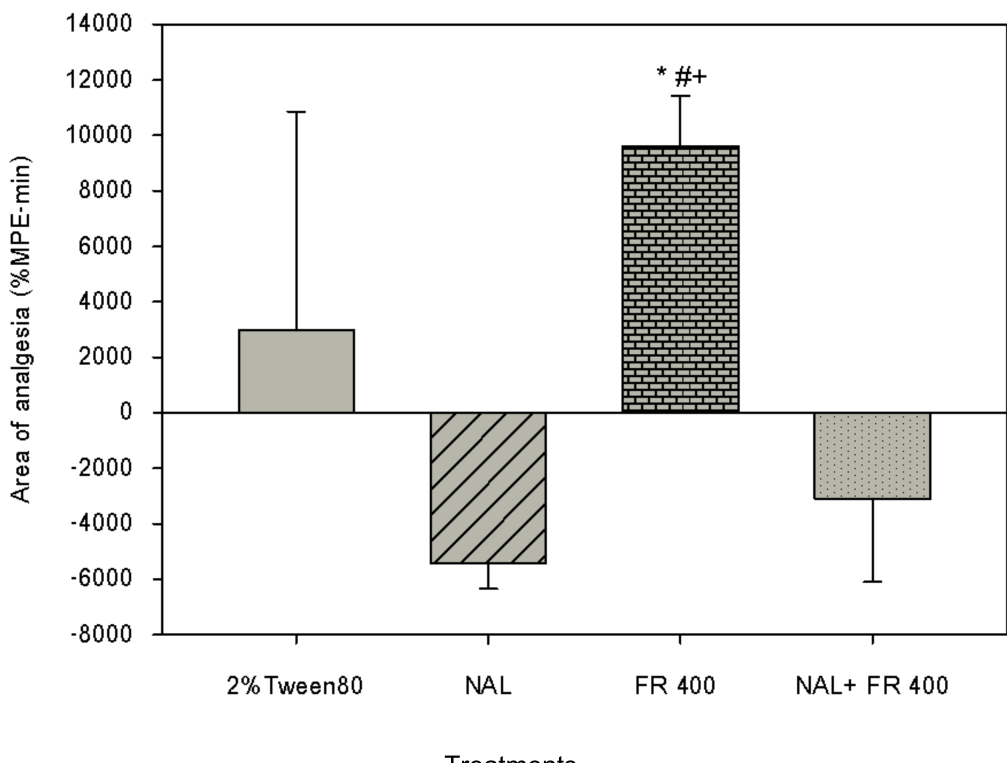



Figure 58 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Ficus racemosa* root extract (FR; 400 mg/kg) and the combination of naloxone and FR (5/400 mg/kg). N=10 for all groups. *p<0.05 significant different compared with 2% Tween 80, #p<0.05 significant different compared with NAL and +p<0.05 significant different compared with NAL+FR.

Mouse Hot-plate Test

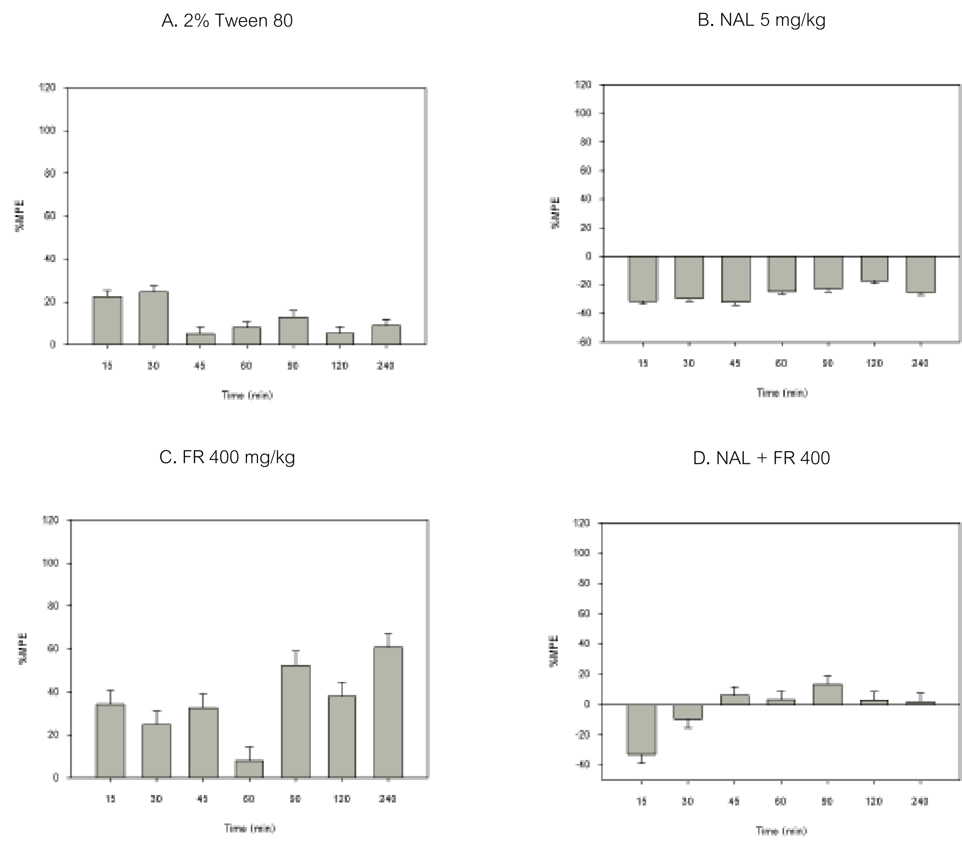



Figure 59 Individual time courses of the response (%MPE versus time (min)) after oral administration of 2% Tween 80 (p.o.), Naloxone (NAL; 5 mg/kg, i.p.), *Ficus racemosa* root extract (FR; 400 mg/kg) (p.o) and the combination of naloxone and FR (5/400 mg/kg). A; 2% Tween 80, B; NAL 5 mg/kg, C; FR 400 mg/kg and D; NAL+FR 400. N=10 for all groups.

MOUSE TAIL-FLICK TEST

To demonstrate the validity of the tail-flick analgesic testing following drug administration, mice received morphine sulfate (MO; 10 mg/kg) i.p. and were tested during the subsequent 240 min period. As expected MO 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 %MPE-min; Figure 60).

Studies then utilized the mouse tail-flick method to examine the efficacy of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy in producing analgesia. Mice were orally administered 2%Tween 80 or various doses of five herbal root extracts of Bencha-loga-wichian remedy (25, 50, 100, 200, 400 mg/kg) and Bencha-loga-wichian remedy (25, 50, 100, 200, 400 mg/kg).

All doses of CM failed to produce analgesic response when compared to the vehicle group (Figure 61). Individual time courses of the responses of CM are shown in Figure 62. All doses of HP significantly ($p<0.05$) increased the tail-flick latency when compared to the vehicle group (Figure 63). The analgesic peak effects of all doses of HP tested were reached within 240 min after oral administration. Individual time courses of the responses of HP are shown in Figure 64.

TT at the doses of 25 and 50 mg/kg significantly ($p<0.05$) increased the tail-flick latency when compared to the vehicle group (Figure 65). The analgesic peak effects of all doses of TT tested were reached within 240 min after oral administration. Individual time courses of the responses of TT are shown in Figure 66.

All doses of CP significantly ($p<0.05$) increased the tail-flick latency when compared to the vehicle group (Figure 67). The analgesic peak effects of CP (25, 50, 100, 200 and 400 mg/kg) were reached within 240, 60, 45, 30, 90 min, respectively after oral administration. Individual time courses of the responses of CP are shown in Figure 68.

FR at the doses of 25, 50, 100 and 200 significantly ($p<0.05$) increased the tail-flick latency when compared to the vehicle group (Figure 69). The analgesic peak

effects of FR (25, 50, 100, 200 and 400 mg/kg) were reached within 240, 120, 90, 240, 240 min, respectively after oral administration. Individual time courses of the responses of FR are shown in Figure 70.

All doses of BEN significantly ($p<0.05$) increased the tail-flick latency when compared to the vehicle group (Figure 71). The analgesic peak effects of BEN (25, 50, 100, 200 and 400 mg/kg) were reached within 90, 90, 120, 240, 90 min after oral administration. Individual time courses of the responses of BEN are shown in Figure 72.

Mouse Tail-flick Test

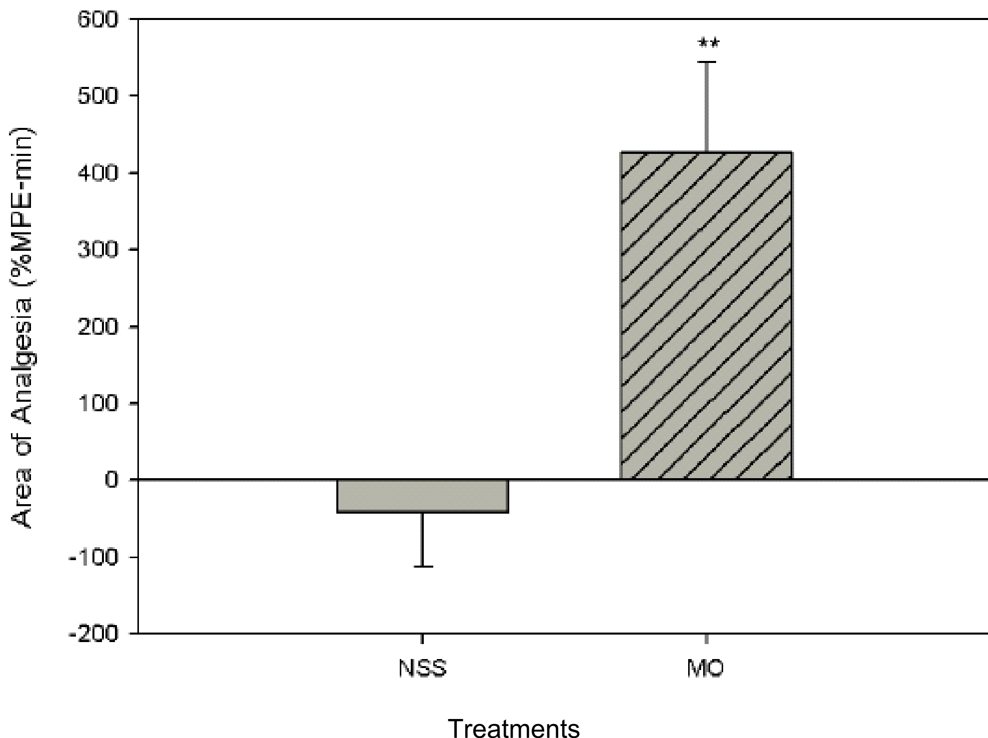



Figure 60 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. **$p<0.01$ significantly different compared to NSS.

Mouse Tail-flick Test

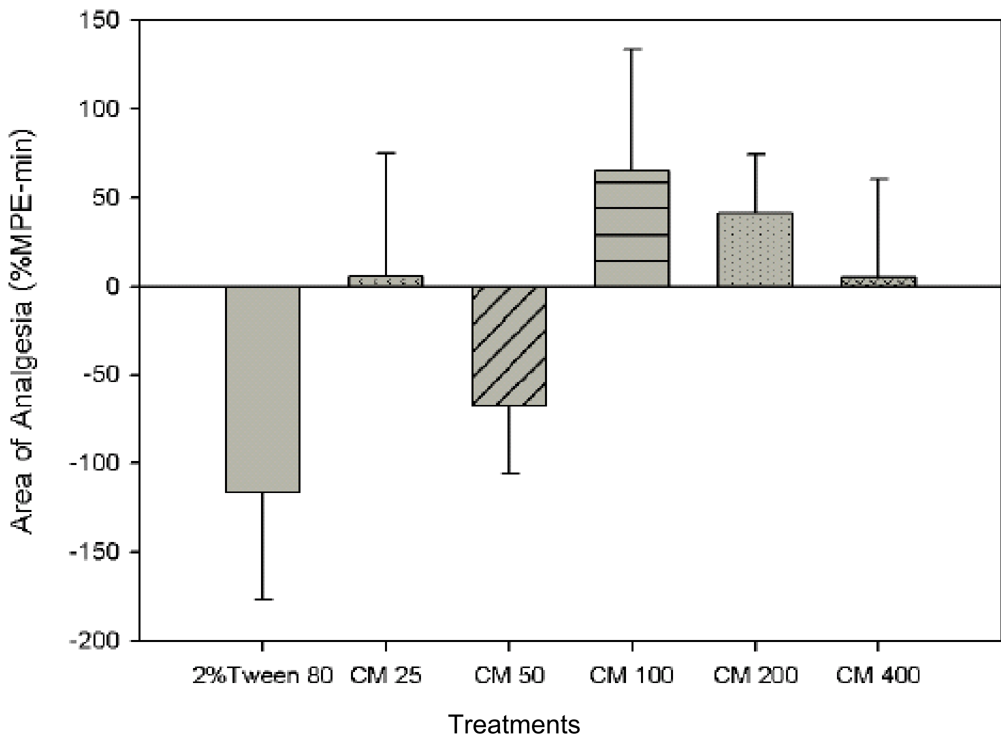



Figure 61 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Mouse Tail-flick Test

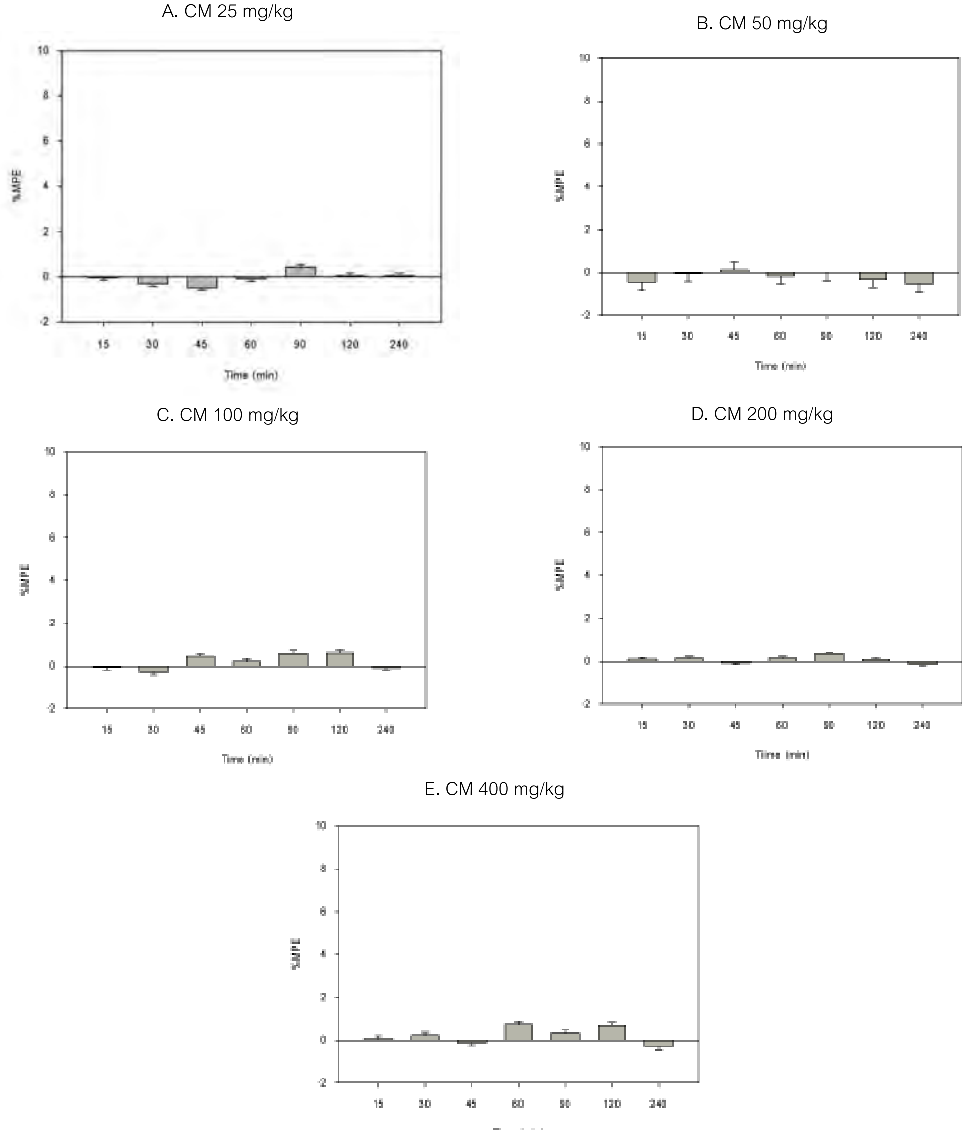



Figure 62 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg). A; CM 25 mg/kg, B; CM 50 mg/kg, C; CM 100 mg/kg, D; CM 200 mg/kg and E; CM 400 mg/kg. N=10 for all groups.

Mouse Tail-flick Test

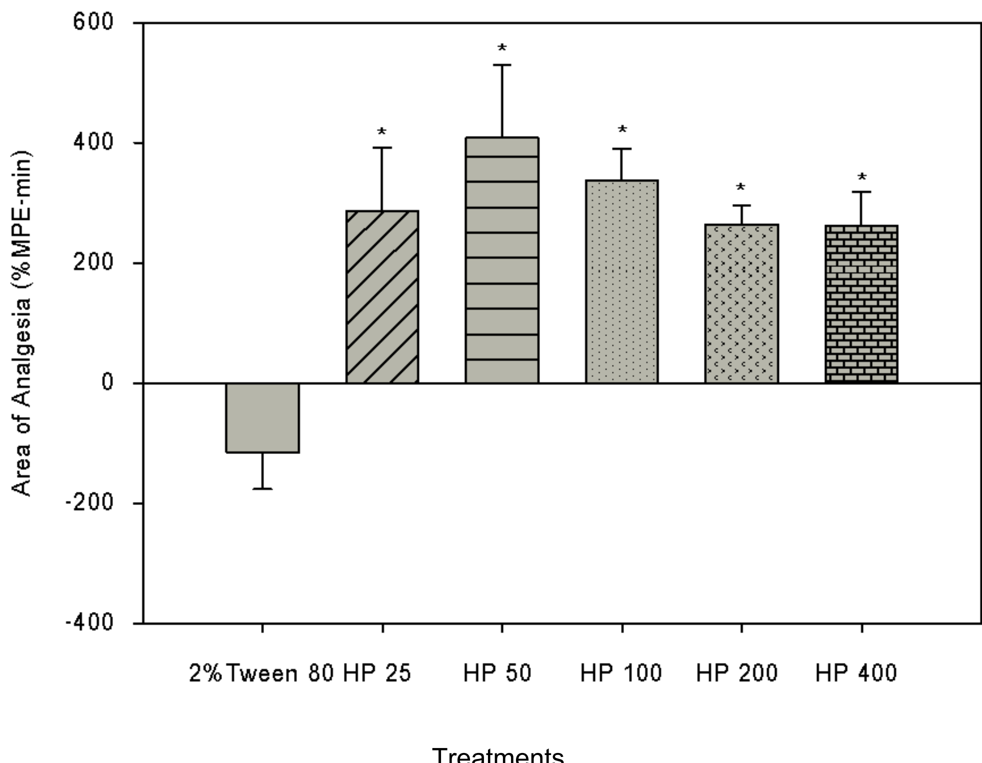



Figure 63 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Harrisonia perforate* root extract (HP; 25- 400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Mouse Tail-flick Test

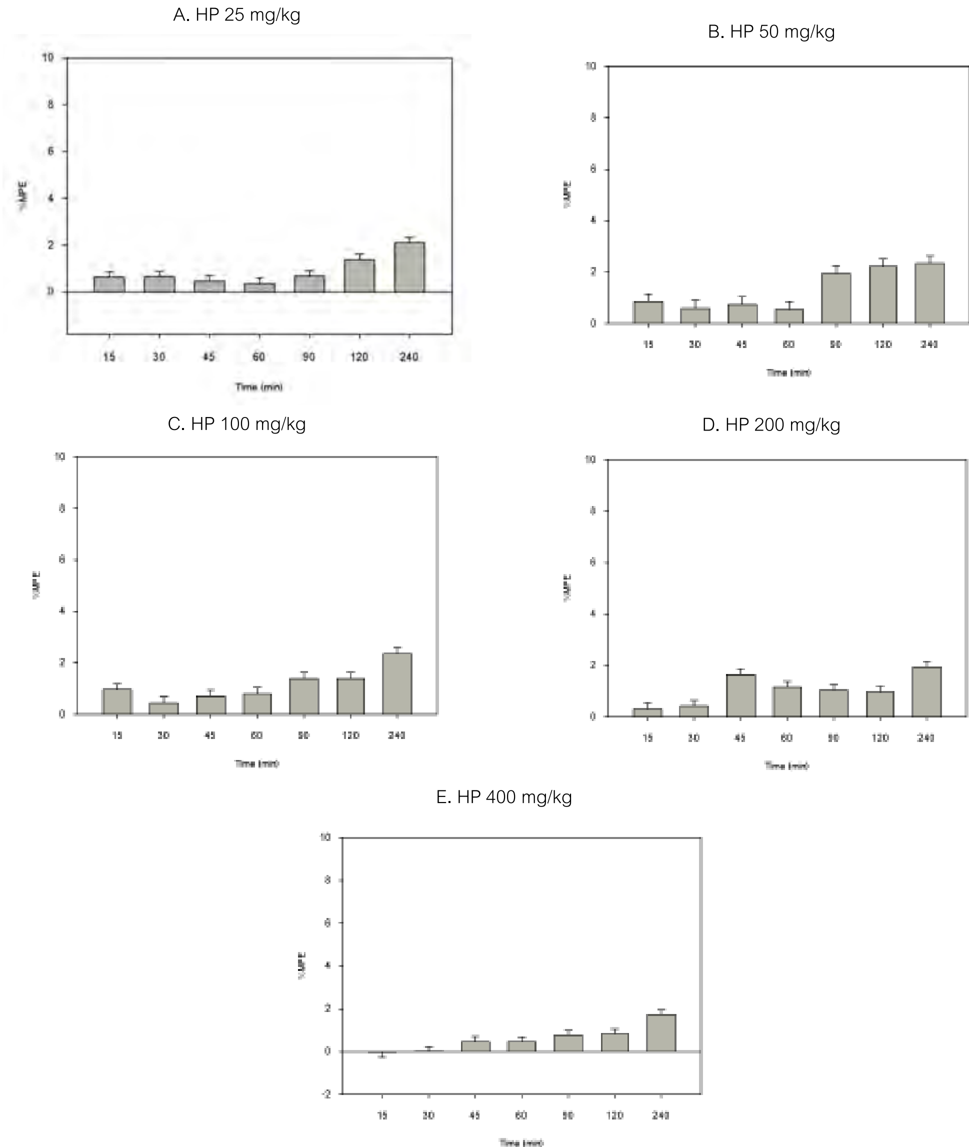



Figure 64 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Harrisonia perforata* root extract (HP; 25- 400 mg/kg). A; HP 25 mg/kg, B; HP 50 mg/kg, C; HP 100 mg/kg, D; HP 200 mg/kg and E; HP 400 mg/kg. N=10 for all groups.

Mouse Tail-flick Test

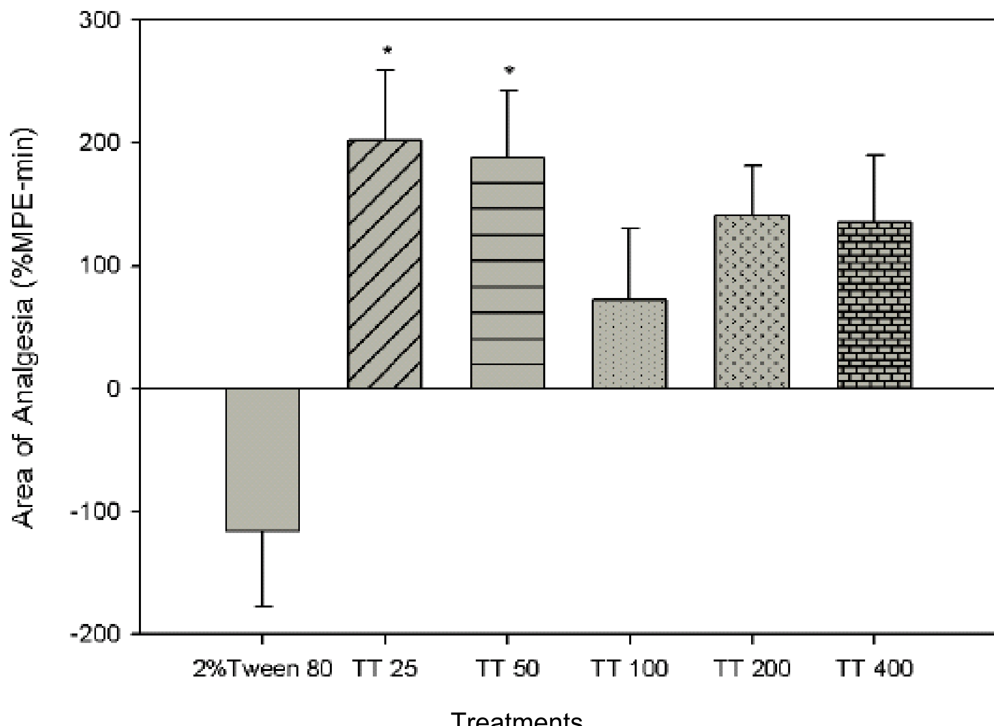



Figure 65 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2%Tween 80 and various doses of *Tiliacora triandra* root extract (TT; 25- 400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2%Tween 80.

Mouse Tail-flick Test

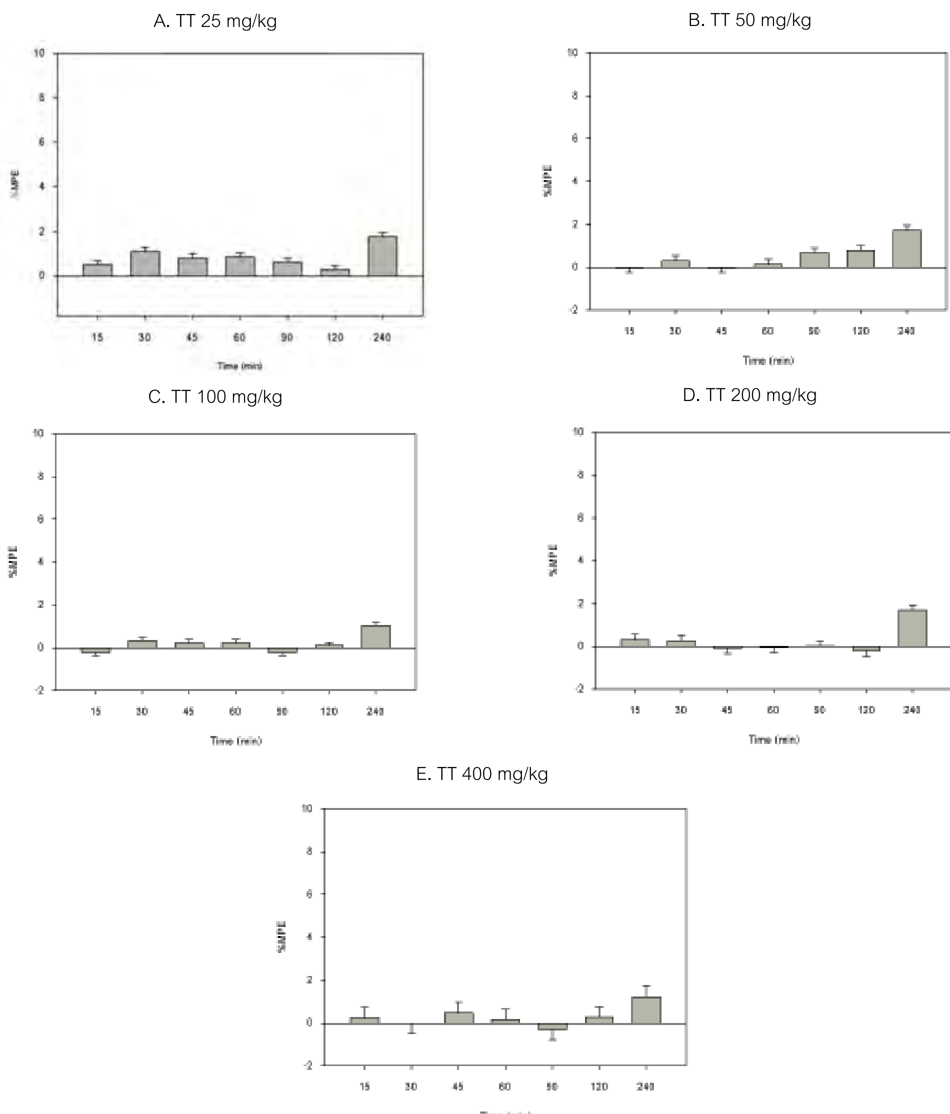



Figure 66 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Tiliacora triandra* root extract (TT; 25- 400 mg/kg). A; TT 25 mg/kg, B; TT 50 mg/kg, C; TT 100 mg/kg, D; TT 200 mg/kg and E; TT 400 mg/kg. N=10 for all groups.

Mouse Tail-flick Test

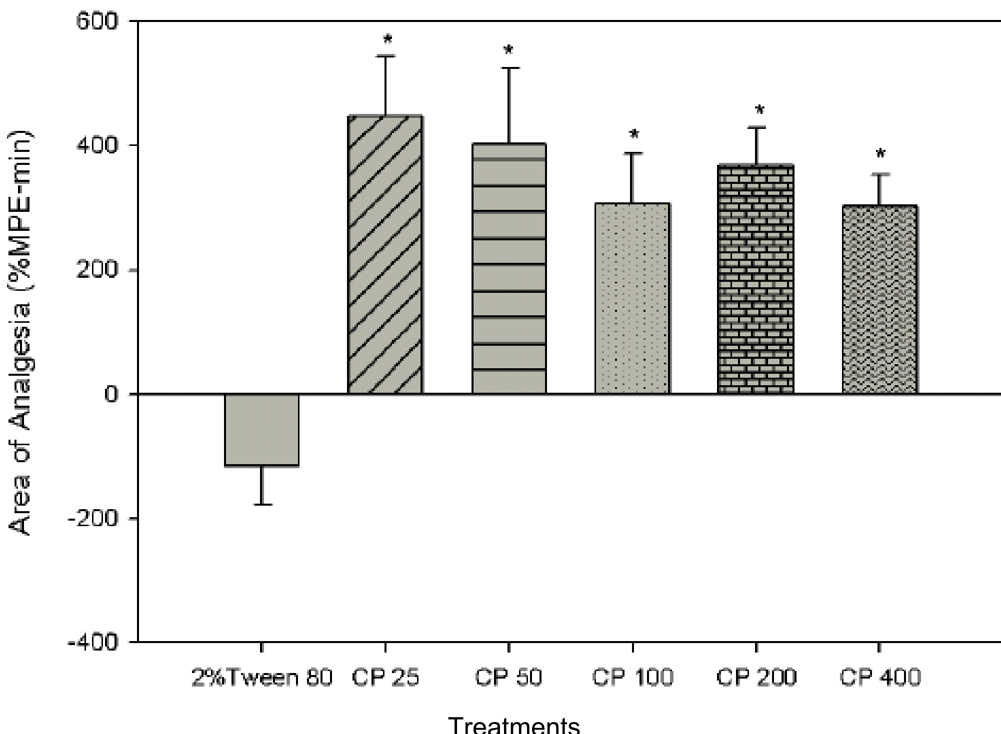



Figure 67 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Mouse Tail-flick Test

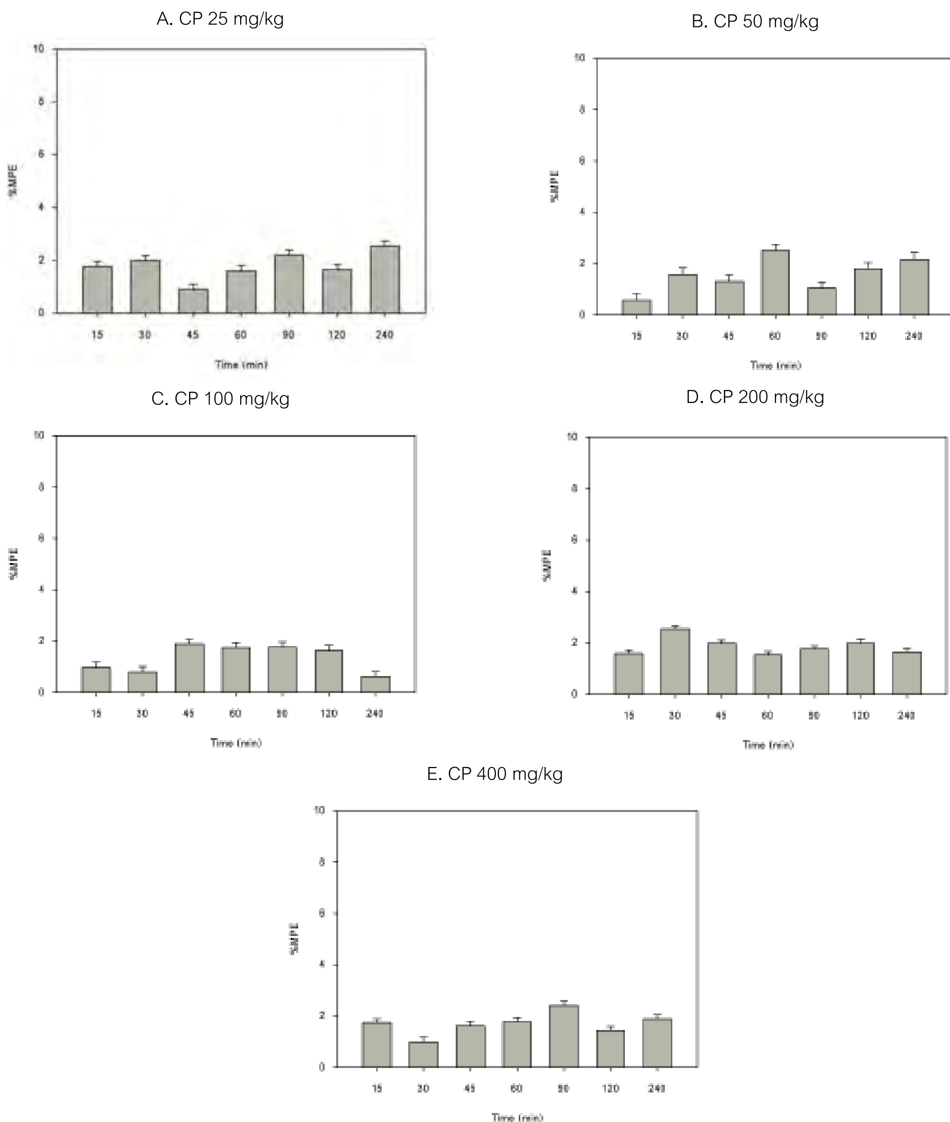



Figure 68 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg). A; CP 25 mg/kg, B; CP 50 mg/kg, C; CP 100 mg/kg, D; CP 200 mg/kg and E; CP 400 mg/kg. N=10 for all groups.

Mouse Tail-flick Test

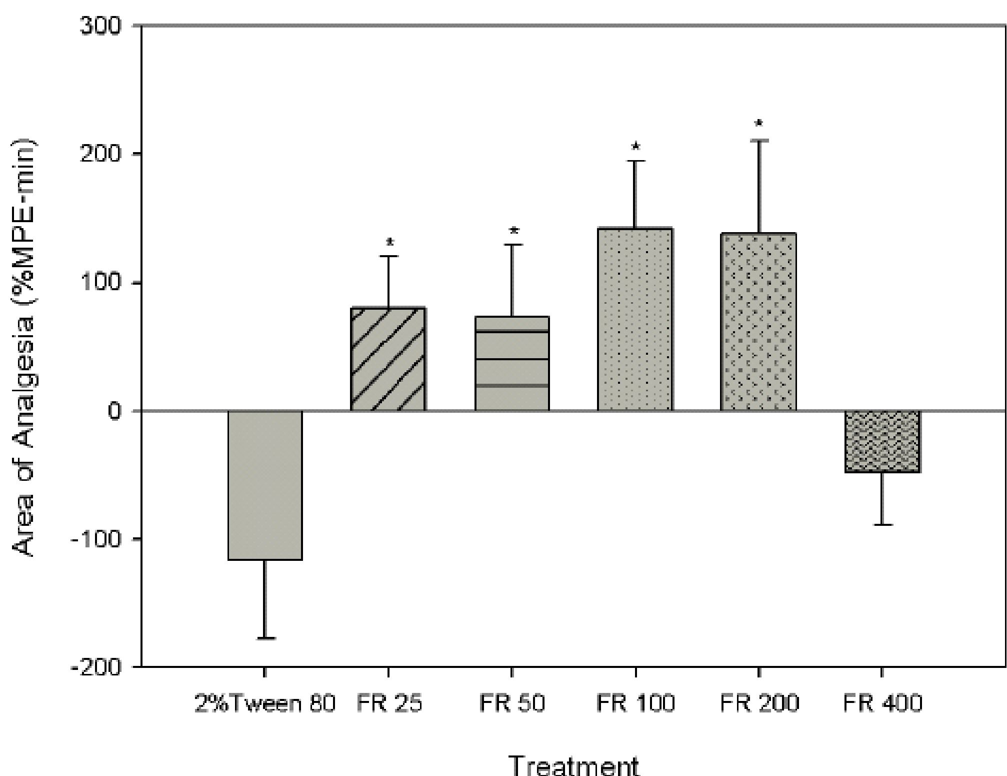



Figure 69 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg). N=10 for all groups. *$p<0.05$ significantly different compared to Tween 80.

## Mouse Tail-flick Test

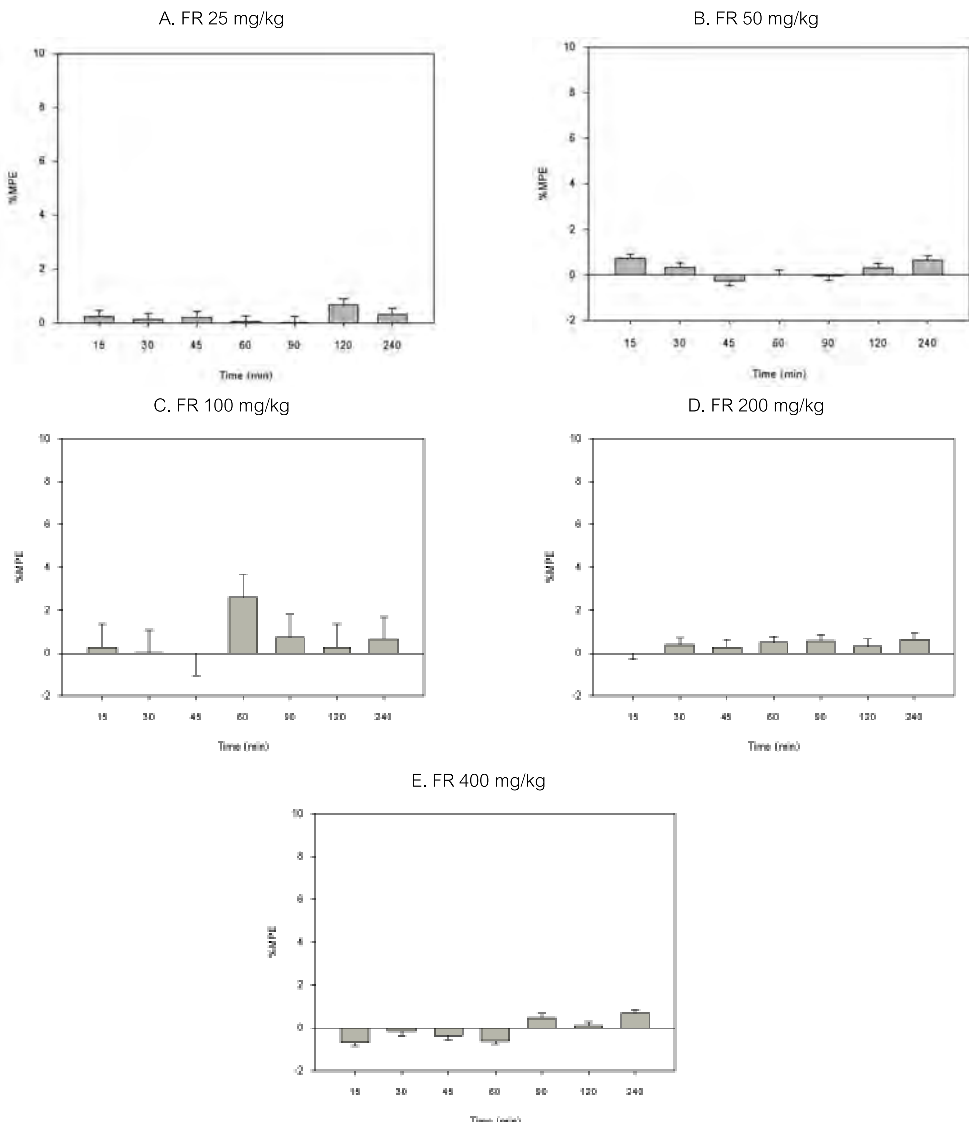



Figure 70 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg). A; FR 25 mg/kg, B; FR 50 mg/kg, C; FR 100 mg/kg, D; FR 200 mg/kg and E; FR 400 mg/kg. N=10 for all groups.

Mouse Tail-flick Test

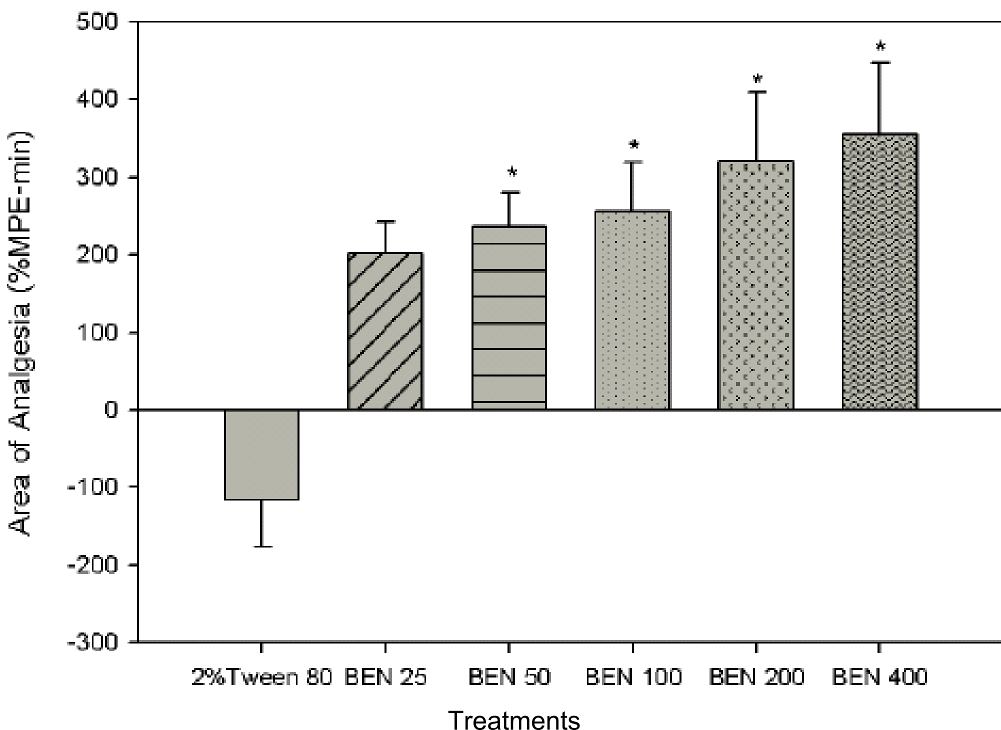



Figure 71 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Mouse Tail-flick Test

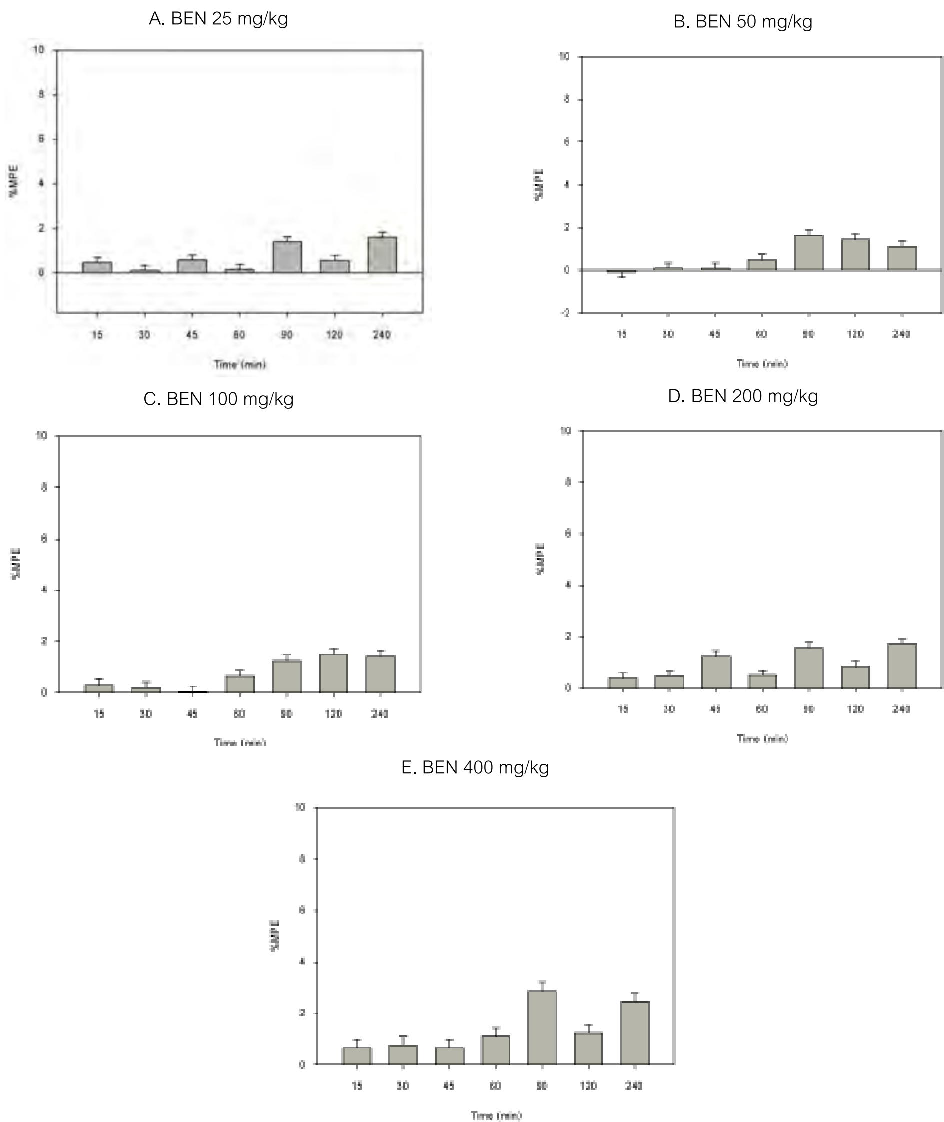



Figure 72 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg). A; BEN 25 mg/kg, B; BEN 50 mg/kg, C; BEN 100 mg/kg, D; BEN 200 mg/kg and E; BEN 400 mg/kg. N=10 for all groups.

ACETIC ACID-INDUCED WRITHING IN MICE

To demonstrate the validity of the acetic aid-induced writhing method following drug administration, mice received indomethacin (IND; 10 mg/kg) orally and were tested during the subsequent 30 min period. As expected IND significantly ($p<0.01$) decreased writhing response by 84.44% producing a mean number of writhes of 2.5±0.56 compared with that of 2%Tween 80 (23.67±6.03; Figure 73).

Studies then utilized the acetic acid-induced writhing method in mice to examine the efficacy of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy in producing analgesia. Mice were administered orally 2%Tween 80 or various doses of five herbal root extracts of Bencha-loga-wichian remedy (25, 50, 100, 200, 400 mg/kg) and Bencha-loga-wichian remedy (25, 50, 100, 200, 400 mg/kg).

CM at the doses of 25, 50, 100, 200, and 400 mg/kg produced a mean number of writhes of 12.00±3.17, 10.50±1.78, 8.50±0.67, 7.17±1.54 and 9.17±2.71, respectively. CM at doses of 50-400 mg/kg significantly ($p<0.05$) decreased the number of writhes induced by acetic cid by 55.63%, 64.04%, 69.72% and 61.27%, respectively, when compared to vehicle control (Figure 74).

HP at the doses of 25, 50, 100, 200, and 400 mg/kg produced a mean number of writhes of 10.67±1.45, 10.33±2.09, 8.83±1.96, 5.50±0.76 and 7.83±2.85, respectively. All doses of HP (25-400 mg/kg) significantly ($p<0.05$) decreased the number of writhes induced by acetic cid by 54.93%, 56.34%, 62.68%, 76.76% and 66.90%, respectively when compared to vehicle control (Figure 75).

TT at the doses of 25, 50, 100, 200, and 400 mg/kg produced a mean number of writhes of 14.83±4.49, 10.50±1.06, 9.00±1.48, 7.50±2.26 and 6.33±1.23, respectively. TT at doses of 100, 200 and 400 mg/kg significantly ($p<0.05$) decreased the number of writhes induced by acetic cid by 61.97%, 68.31% and 72.24%, respectively when compared to vehicle control (Figure 76).

CP at the doses of 25, 50, 100, 200, and 400 mg/kg produced a mean number of writhes of 10.67±1.78, 6.83±0.95, 5.00±1.21, 5.33±1.45 and 4.33±1.09, respectively. All doses of HP (25-400 mg/kg) significantly ($p<0.05$) decreased the number of writhes

induced by acetic cid by 54.93%, 71.13%, 78.87%, 77.46% and 81.69%, respectively when compared to vehicle control (Figure 77).

FR at the doses of 25, 50, 100, 200, and 400 mg/kg produced a mean number of writhes of 11.17±2.52, 9.50±1.43, 8.33±0.80, 13.00±3.86 and 14.00±3.17, respectively. FR at doses of 50 and 100 mg/kg significantly ($p<0.05$) decreased the number of writhes induced by acetic cid by 59.86% and 64.79%, respectively when compared to vehicle control (Figure 78).

BEN at the doses of 25, 50, 100, 200, and 400 mg/kg produced a mean number of writhes of 14.50±4.61, 1.89±1.84, 10.33±2.16, 8.67±2.32 and 9.17±1.54, respectively. BEN at doses of 200 and 400 mg/kg significantly ($p<0.05$) decreased the number of writhes induced by acetic acid by 63.38% and 61.27%, respectively when compared to vehicle control (Figure 79). IND showed the highest analgesia response compared to all test groups (Figure 73, 74, 75, 76, 78 and 79).

Acetic Acid –induced Writhing in Mice

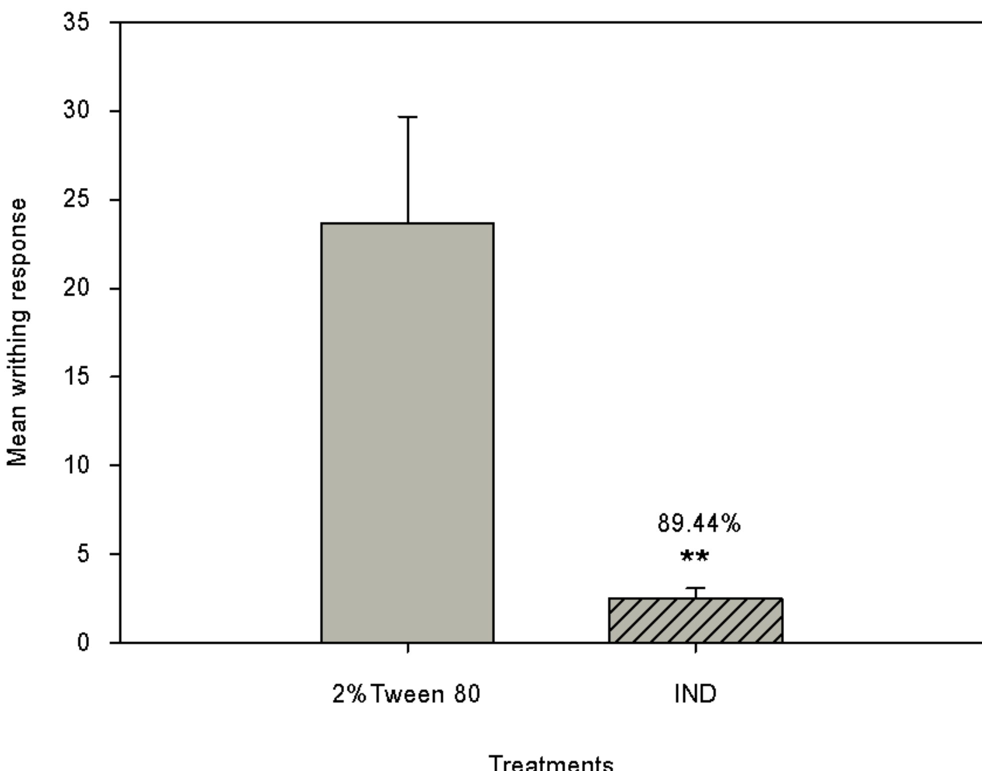



Figure 73 Mean writhing response after oral administration of 2% Tween 80 and indomethacin (IND; 10 mg/kg). N=6 for all groups. **p<0.01 significantly different compared to 2% Tween 80.

Acetic Acid –induced Writhing in Mice

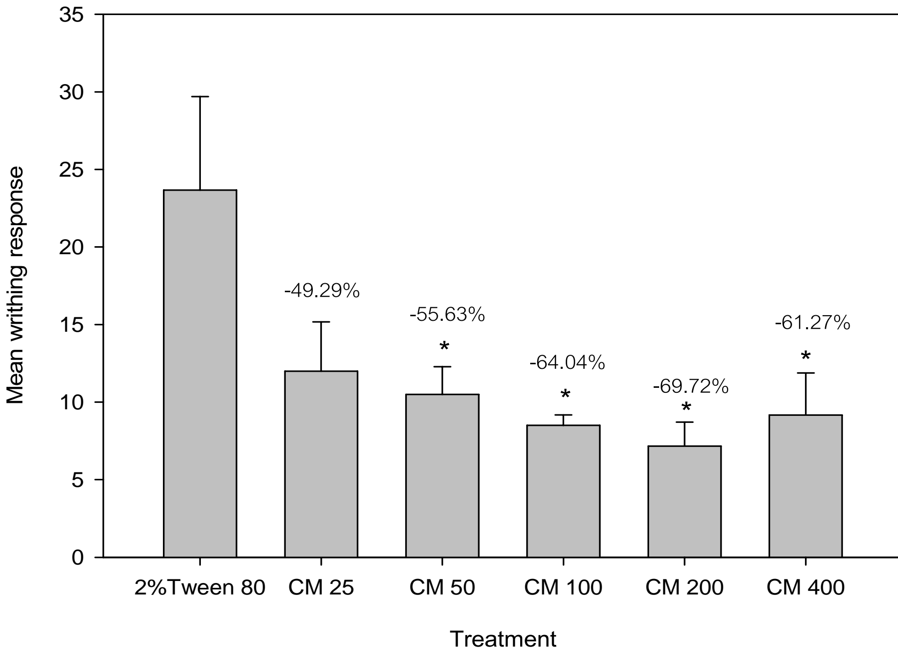



Figure 74 Mean writhing response after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Capparis micracantha* root extract (CM; 25- 400 mg/kg). N=6 for all groups. *$p<0.05$ significantly different compared to 2% Tween 80.

Acetic Acid –induced Writhing in Mice

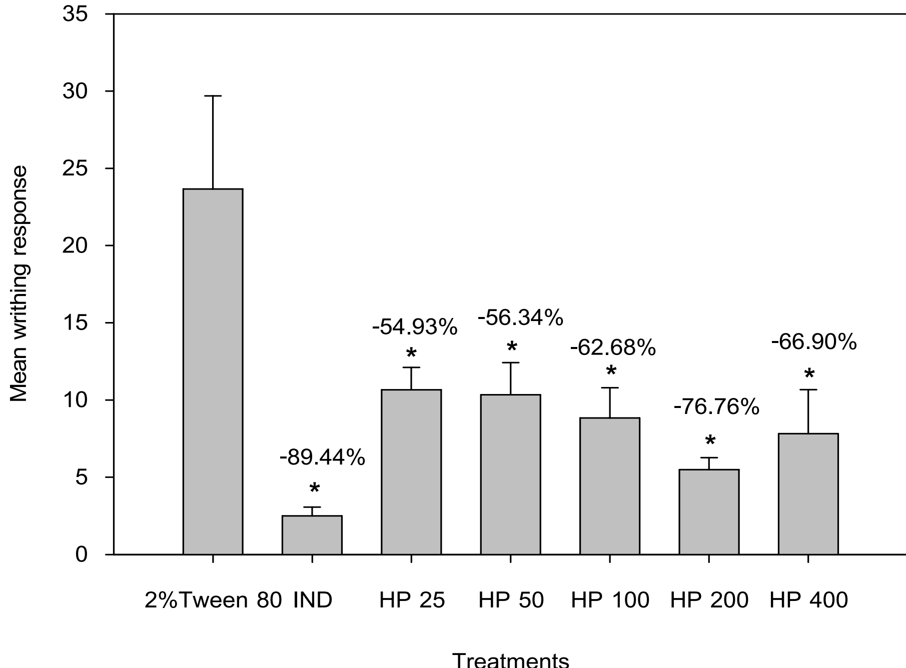



Figure 75 Mean writhing response after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg). N=6 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

Acetic acid –induced writhing in mice

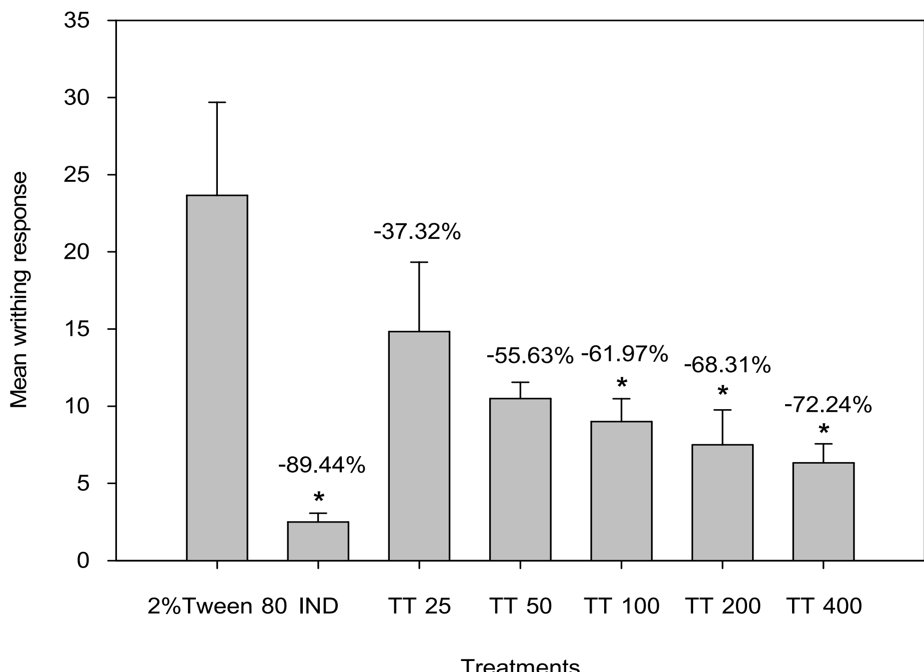



Figure 76 Mean writhing response after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg). N=6 for all groups. *p<0.05 significantly different compared to 2% Tween 80.

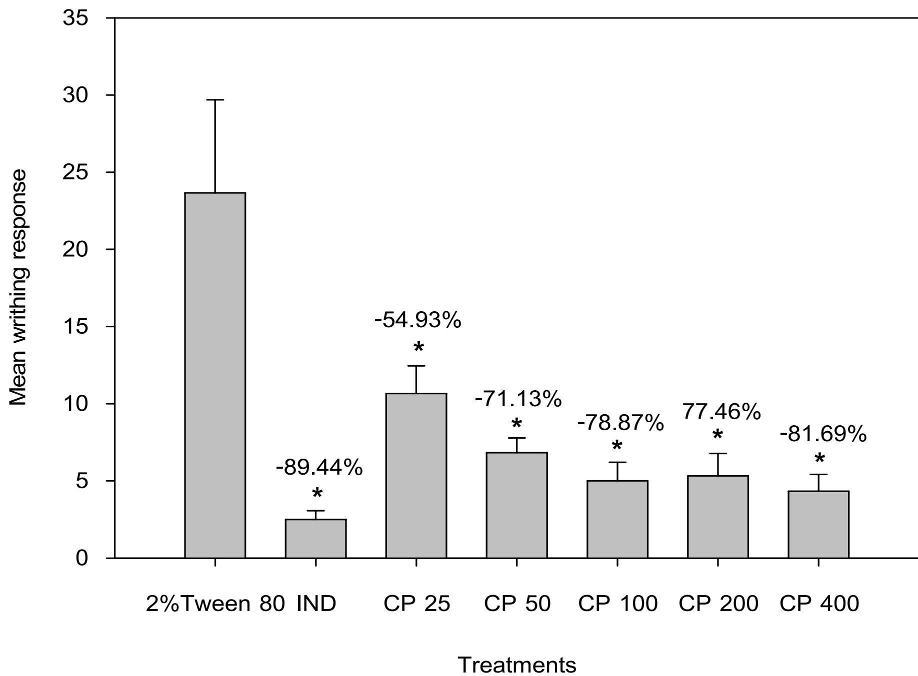



Figure 77 Mean writhing response after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg). N=6 for all groups. *$p<0.05$ significantly different compared to 2% Tween 80.

Acetic Acid –induced Writhing in Mice

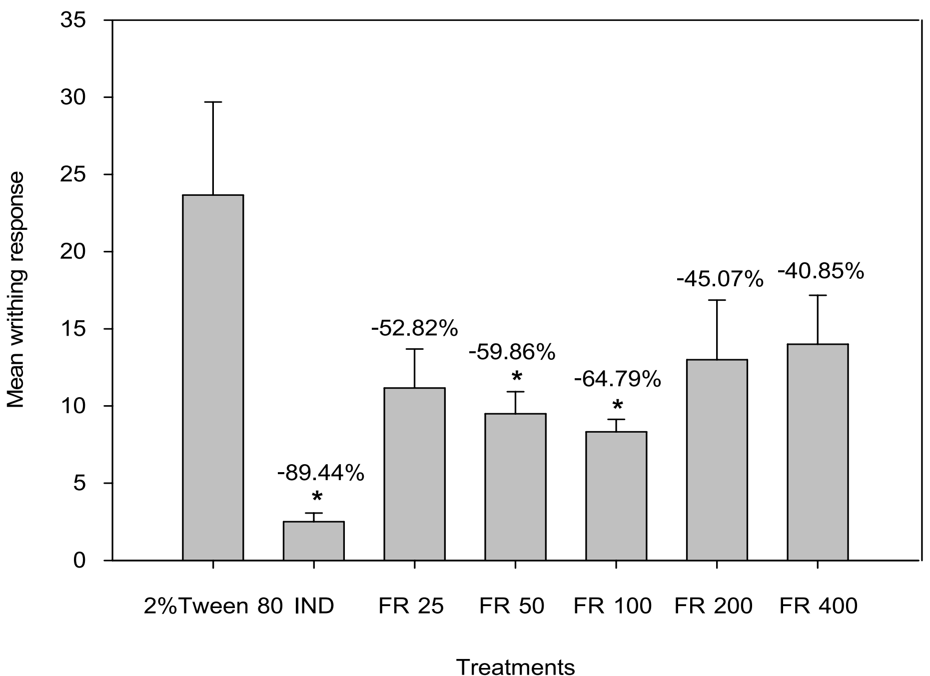



Figure 78 Mean writhing response after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg). N=6 for all groups. *$p<0.05$ significantly different compared to 2% Tween 80.

Acetic Acid –induced Writhing in Mice

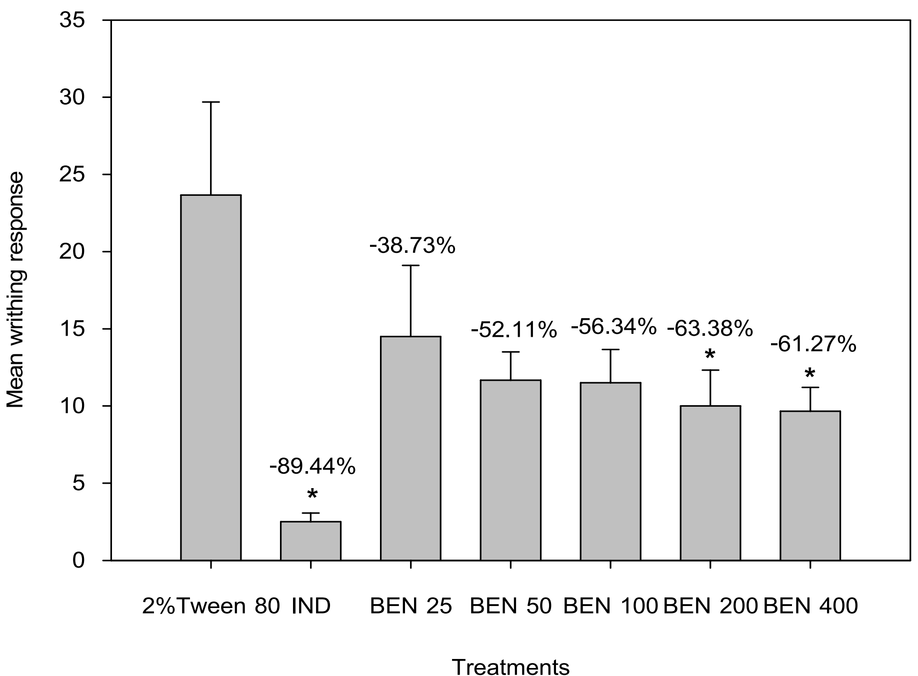



Figure 79 Mean writhing response after oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg). N=6 for all groups. *$p<0.05$ significantly different compared to 2% Tween 80.

# CHAPTER V

# DISCUSSION AND CONCLUSION

These studies have demonstrated the antipyretic and antinociceptive effects of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy in various animal models. Antipyretic activity was assessed utilizing lipopolysaccharide-induced fever model. Antinociceptive activity was assessed utilizing thermal (hot-plate and tail-flick tests) and chemical (acetic acid induced writhing) models.

Fever is thought to be produced by several endogenous cytokines. Most prominent among these are tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1$\beta$ (IL-1$\beta$), interleukin-6 (IL-6), interleukin-8 (IL-8), and interferon (IFN- $\alpha$); the latter is produced predominantly in response to viral infection (Kluger, 1991, Roth and De Souza, 2001, Blatteis et al., 2005). The cytokine cascade of fever induction starting from initial stimulation of IL-1 and TNF-$\alpha$ by bacterial products that induces secondary synthesis of IL-6, with subsequent induction of prostaglandin (PG) synthesis in the CNS and fever. These cytokines are not stored as preformed molecules (except TNF-$\alpha$ and IL-6, present to a small extent in mast cells), but synthesized and secreted after stimulation. They are then released into the bloodstream and transported to sites in or close to the the preoptic-anterior hypothalamus (POAH), the brain site of the primary thermoregulatory controller that react to their stimulation by selectively expressing COX-2 and microsomal prostaglandin E synthase (mPGES-1). Both isoenzymes are transcriptionally regulated by NF-$\kappa$B and have been demonstrated to be specifically implicated in the febrile response. $PGE_2$, thus induced by these cytokines, is considered to be the proximal, final fever mediator in the POAH (Mihai et al., 2000). Prostaglandin synthesis can be activated by TNF-$\alpha$ or phospholipase $A_2$ (Aronoff and Neilson, 2001). Prostaglandin $E_2$ synthesis involves the cleavage of arachidonic acid (AA) released from

membrane phospholipids into the prostaglandin endoperoxides, $PGG_2$ and $PGH_1$; $PGH_2$ is then quickly converted to $PGE_2$ by $PGE_2$ isomerase. The free AA concentration is thus the rate-limiting. In the context of fever production, phospholipase $A_2$ have been considered the key enzyme. It enhanced activation accounts for the release of AA (Blatteis and Šehic, 1997). Lipopolysaccharide (LPS) is the most potent stimulus known for TNF-$\alpha$ production and release and also increases circulating levels of another pyrogen, IL-1. LPS is unquestionably a clinical important pyrogen (Dal Nogare and Sharma, 1997). LPS, exogenous pyrogen has been shown to produce fever in laboratory animals such as guinea pigs and rabbits by stimulating the production of endogenous TNF-$\alpha$ (Kluger 1991 and Roth and Zeisberger, 1995).

Antipyretics such as acetylsalicylic acid (ASA) and other nonsteroidal anti-inflammatory drugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulation sites. They suppress peripheral producing of pyrogenic cytokines such as TNF-$\alpha$ and interleukin-1$\beta$ (IL-1$\beta$) while lower the thermoregulatory set point by blocking central cyclooxygenase production of prostaglandin $E_2$ ($PGE_2$) (Aronoff and Neilson, 2001).

Initial attempts to investigate the antipyretic effect of all five herbal root extracts (CM, HP, TT, CP, FR) and Bencha-loga-wichian remedy (BEN) utilized LPS-induced fever model in rats (Santos and Rao, 1998). This model usually employs ASA as a reference drug. Orally administered ASA, the positive control, significantly attenuated fever in LPS treated rats at all time tested due to inhibition of cyclooxygenase and therefore interfere with the cascade of the synthesis of prostaglandin which induces fever. CM, HP, TT, CP, FR and BEN (25-400 mg/kg) was administered orally by suspending in 2% Tween 80 solution. The oral administration was chosen in order to imitate the normal consumption of “Bencha-loga-wichian”, Thai traditional antipyretic herbal medicine.

From the present study, all doses of CM, HP, TT, CP, FR and BEN (25-400 mg/kg) showed significant antipyretic activity in rats over the period of 2-7 hr after LPS injection indicating that the antipyretic mechanism of all herbal root extracts and BEN

remedy may be due to suppression of TNF-$\alpha$ or inhibition of its synthesis and block prostaglandin synthesis and therefore attenuates fever. The antipyretic effect of all doses of each herbal root extract and BEN remedy occurred within 2 hr after LPS injection and was sustained for up to 7 hr similar to ASA (Figure 19, 21, 23, 25, 27 and 29). The antipyretic efficacy of all doses of CM, HP, TT (except for 100 mg/kg), CP and FR was comparable to that of ASA and was not dose related. BEN at the doses of 100 and 400 mg/kg has higher antipyretic efficacy than aspirin (Table 6). TT 200 mg/kg showed highest antipyretic efficacy when compared between five herbal root extracts (Figure 31). This result is consistent with the previous study of Konsue in 2008 that investigated the antipyretic effects of dried root powder of Bencha-loga-wichian herbal drugs. However, BEN 400 mg/kg seemed to be more potent than any other root extracts include TT (Figure 31). Each herbal root extract was active as an antipyretic drug by itself; however in the BEN remedy it might have some additive and/or antagonistic effects and may contribute other pharmacological effect which will help relieve the symptoms of patients. This study helps clarifying the pharmacological action of this Thai herbal remedy and provides scientific support for the famous traditional medicine.

In order to investigate the analgesic effect of all five herbal root extracts and BEN, the standard mouse hot-plate test (Woolfe and MacDonald, 1944), a central analgesic activity testing model was utilized. This method measures two behavioral components including paw licking and jumping which are both considered to be supraspinally integrated responses. This model usually employs morphine as a reference drug. Morphine (MO) showed potent analgesic effect on the response in this model indicating the sensitivity of this test (Figure 32). All five herbal root extracts and BEN were administered orally by suspending in 2%Tween 80 solution. The significant analgesic action of all doses of CM (25-400 mg/kg) was observed during 240 min period, while HP (200 and 400 mg/kg), TT (100, 200 and 400 mg/kg), CP (400 mg/kg), FR (100, 200 and 400 mg/kg) and BEN (400 mg/kg) showed significant analgesic effect. CM has the most potent analgesic activity. The rank of order of analgesic potencies of the five root extracts and BEN in the mouse hot-plate test is CM > FR > TT > BEN > HP > CP. Individual herbal root extract especially CM (50-400 mg/kg), HP (200 mg/kg), TT

(100-200 mg/kg) and FR (50-400 mg/kg) seemed to be more effective in producing analgesia than BEN remedy at the same dose in this model (Figure 45-49). Considering the dose from 25-400 mg/kg, the antinociceptive peak response of all doses of five herbal root extracts and BEN was observed at different time point after orally administration. This may partly due to the variable absorption of the herbal root extract from the gastrointestinal tract in rodents.

Naloxone, a short acting opioid antagonist, was utilized to investigate the involvement of opioid receptors in the analgesic effects of all five herbal root extracts. The results showed the involvement of opioid receptors in the analgesia produced by all five herbal root extracts (Figure 50, 52, 54, 56 and 58).

Studies were then undertaken to investigate the effectiveness of all five herbal root extracts utilizing the mouse tail-flick technique, another central analgesic activity testing model that measures spinal reflex. MO administered i.p. produced significant analgesic response as expected (Figure 60). All doses of HP, CP and BEN showed significant analgesic effects during 240 min period, while FR only at the doses of 25-200 mg/kg and TT doses of 25 and 50 mg/kg showed significant analgesic activity. All doses of CM tested had no analgesic effect in this model. Again, the antinociceptive peak response of all doses of five herbal root extracts and BEN was observed at different time point after orally administration. The results obtained from hot-plate and tail-flick tests suggested that HP, TT, CP, FR and BEN had analgesic activity at both supraspinal and spinal levels, while CM had analgesic effect only at supraspinal level.

In order to measure an analgesic effect of all five herbal root extracts and BEN against chemical stimuli, an acetic acid-induced writhing test was chosen. This method is commonly used for measuring peripheral analgesic activity and considered as a model of visceral inflammation pain. Writhing responses consist of contraction of the abdomen, twisting and turning of the trunk, and extension of the hind limbs (Svendsen and Hau, 1994). Indomethacin (IND), a non-steroidal anti-inflammatory drug, was used as a reference drug. Oral administration of IND (10 mg/kg) produced significant analgesic response compared to vehicle treated controls (Figure 73). All doses of HP, CP produced significant analgesic response, while CM only at doses of 50-400 mg/kg,

TT doses of 100-400 mg/kg, FR doses of 50 and 100 mg/kg and BEN doses of 200 and 400 mg/kg showed significant analgesic response (Figure 74, 75, 76, 77, 78 and 79). However, all five herbal root extracts and BEN showed less peripheral analgesic efficacy than IND (Figure 74, 75, 76, 77, 78 and 79).

In conclusion, these studies has demonstrated that all five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy have antipyretic activity when assessed with LPS-induced fever model in rats. The antipyretic mechanism is possibly due to inhibition of TNF-$\alpha$ and $PGE_2$ synthesis by cyclooxygenase pathway. In addition, all five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy also has pronounced antinociception in hot-plate when assessed with thermal (hot-plate and tail-flick test) and chemical (writhing test) models of nociception in rodents. All five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy has both central and peripheral analgesic activities. The analgesic mechanism is partly involved with opioid pathway.

## FUTURE RESEARCH

The future researches may consist of several objectives as listed below

1. To investigate the antipyretic effect of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy in other models.
2. To investigate the antinociceptive effect of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy in other models.
3. To investigate the anti-inflammatory effect of various doses of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy compared with non-steroidal anti-inflammatory drugs.
4. To investigate other routes of administration of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy that might enhance the analgesic antipyretic and anti-inflammatory effects.
5. To investigate the anti-inflammatory mechanism of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy.
6. To better understand the mechanism of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy that is involved in producing analgesic and antipyretic effects.
7. To test side effect s and toxicity of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy at high doses.

The other studies may provide important clues to help understand the mechanism underlying the antipyretic, analgesic and anti-inflammatory effects of five herbal root extracts of Bencha-loga-wichian remedy and Bencha-loga-wichian remedy and further support the use of Thai traditional medicine in a clinical setting.

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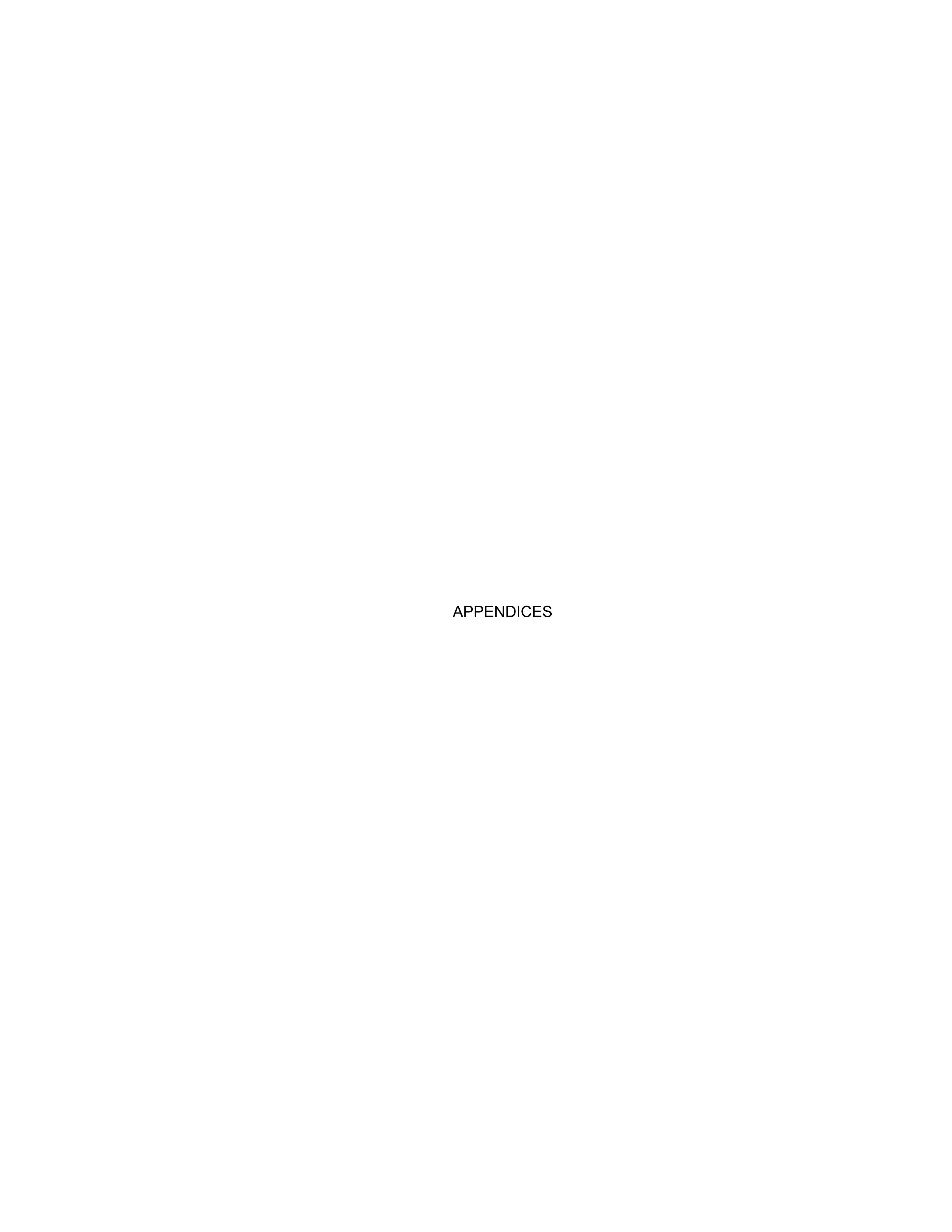


APPENDICES

# Appendix A
Study Protocol Approval by the Institutional Animal Care and Use Committee,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

ศธ 0512.14 กสว 021/ 2552

## Chulalongkorn University Animal Care and Use Committee

| Certificate of Project Approval | ☐ Original ☐ Renew |
|---|---|
| **Animal Use Protocol No. 09-33-007** | **Approval No. 09-33-007** |

**Protocol Title**

Antipyretic, antinociceptive and anti-inflammatory effects of bencha-loga-wichien herbal drug in animal models

**Principal Investigator**

Pasarapa Towiwat, Ph.D.

**Certification of Institutional Animal Care and Use Committee (IACUC)**

This project has been reviewed and approved by the IACUC in accordance with university regulations and policies governing the care and use of laboratory animals. The review has followed guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand.

| **Date of Approval** | **Date of Expiration** |
|---|---|
| January 30, 2009 | January 30, 2010 |

**Applicant Faculty/Institution**

**Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Rd., Pathumwan BKK-THAILAND. 10330**

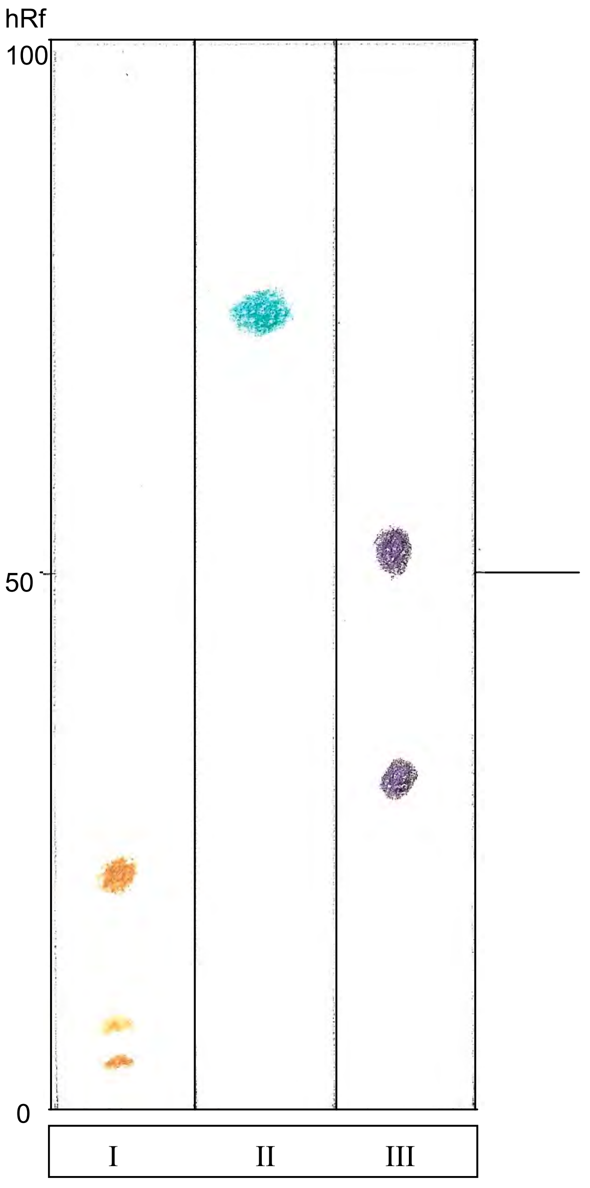
| **Signature of Chairperson** | **Signature of Authorized Official** |
|---|---|
| Withaya Janthasoot | |
| **Name and Title** | **Name and Title** |
| **WITHAYA JANTHASOOT**<br>**Chairman** | **RUNGPETCH SAKULBUMRUNGSIL, Ph.D.**<br>**Associate Dean (Research and Academic Service)** |

*The official signing above certifies that the information provided on this form is correct. The institution assumes that investigators will take responsibility, and follow university regulations and policies for the care and use of animals.*

*This approval is subjected to assurance given in the animal use protocol and may be required for future investigations and reviews.*

# Appendix B

## Thin-Layer Chromatogram of the Methanolic Extract of Five Herbal Roots of Bencha-loga-wichian Remedy




**Solvent system**; Toluene: Ethyl acetate 75:25 **Stationary phase;** $SiO_2$: $GF_{254}$

**Detection**

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with vanillin-sulfuric acid

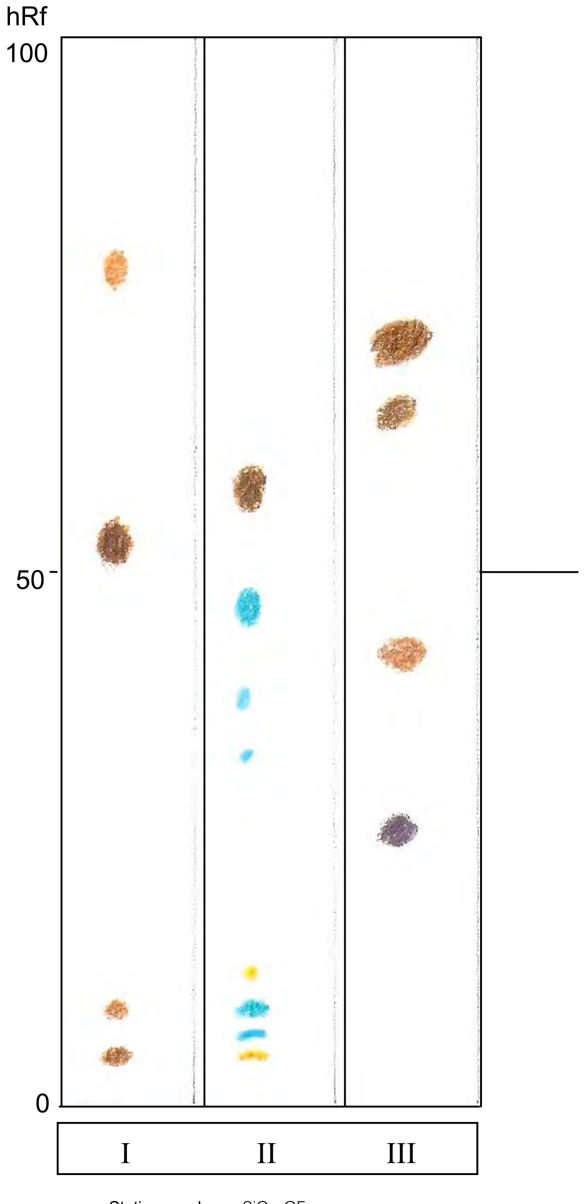
*Vanillin-sulfuric acid regent

Preparation: vanillin (15 g) in ethanol (250 ml) and conc. sulfuric acid (2.5 ml)

**Spot color Development

Heat the plate after sprayed at 120 $^{o}$C for 10 minutes.

Figure 80 Thin-layer chromatogram of the methanolic extract of *Capparis micracantha* root.




**Solvent system;** Chloroform **Stationary phase;** $SiO_2$: $GF_{254}$

**Detection**

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with vanillin-sulfuric acid

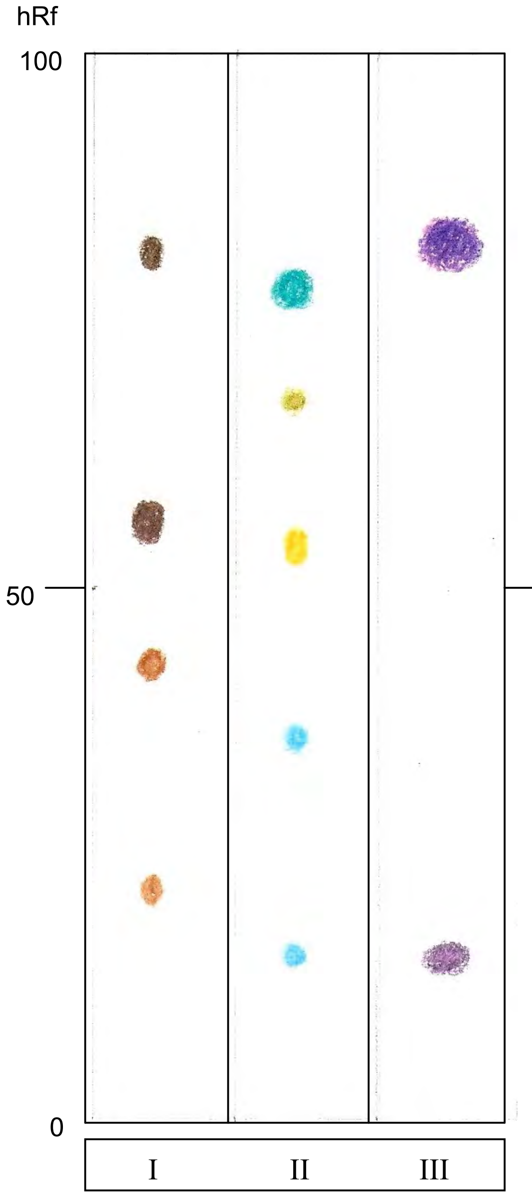
*Vanillin-sulfuric acid regent

Preparation: vanillin (15 g) in ethanol (250 ml) and conc. sulfuric acid (2.5 ml)

**Spot color Development

Heat the plate after sprayed at 120 °C for 10 minutes.

Figure 81 Thin-layer chromatogram of the methanolic extract of *Harrisonia perforata* root.




**Solvent system;** Chlorofrom:Methanol 9:1 **Stationary phase;** $SiO_2$: $GF_{254}$

**Detection**

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with vanillin-sulfuric acid regent*

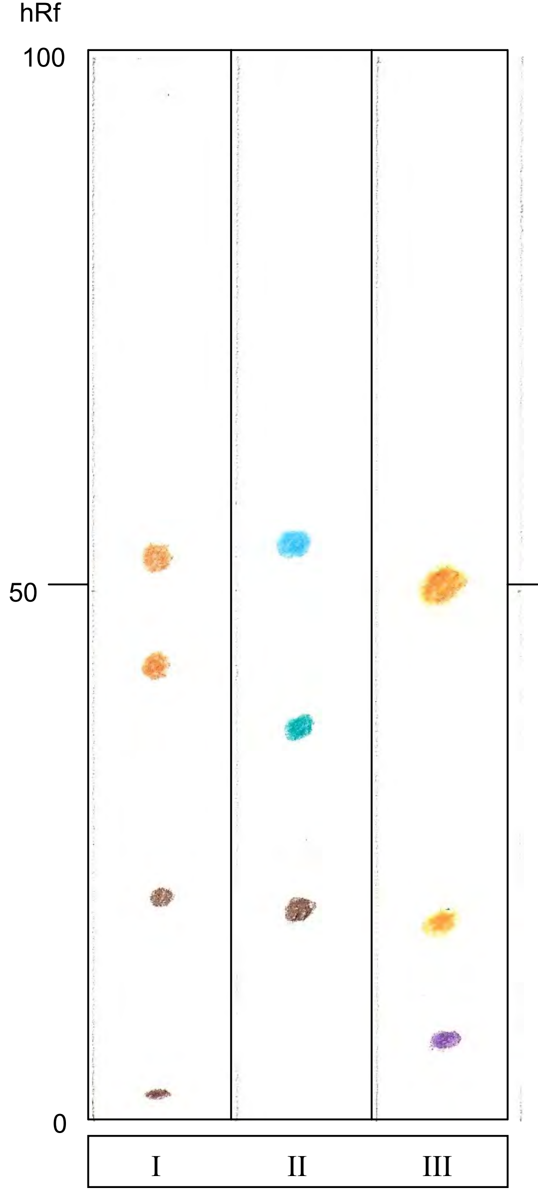
*Vanillin-sulfuric acid regent

Preparation: vanillin (15 g) in ethanol (250 ml) and conc. sulfuric acid (2.5 ml)

**Spot color Development

Heat the plate after sprayed at 120 °C for 10 minutes.

Figure 82 Thin-layer chromatogram of the methanolic extract of *Tiliacora triandra* root.




**Solvent system;** Chloroform **Stationary phase;** $SiO_2$: $GF_{254}$

**Detection**

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with vanillin-sulfuric acid

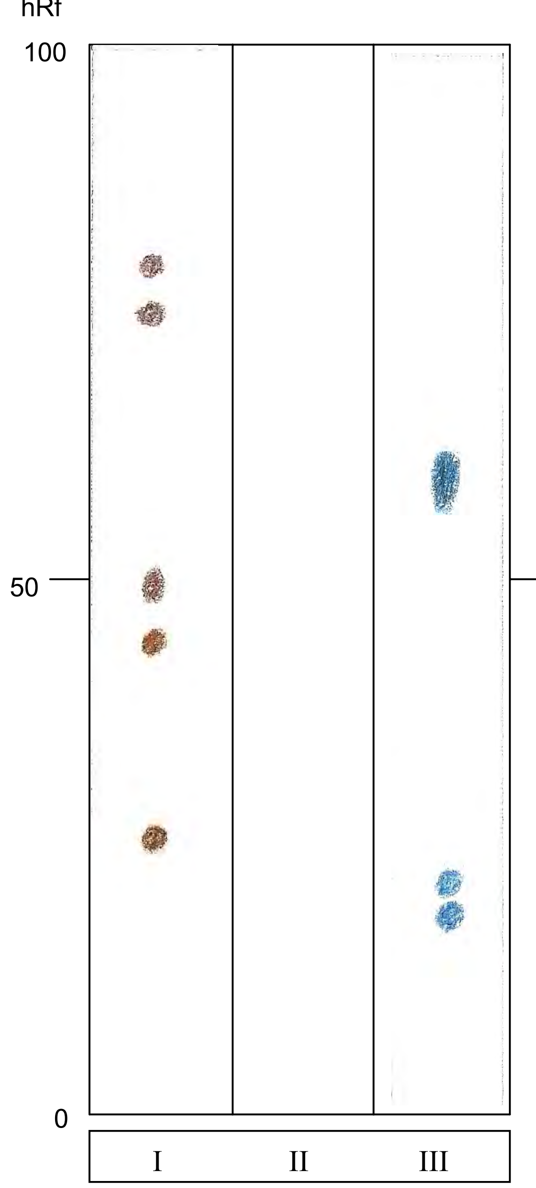
*Vanillin-sulfuric acid regent

Preparation: vanillin (15 g) in ethanol (250 ml) and conc. sulfuric acid (2.5 ml)

**Spot color Development

Heat the plate after sprayed at 120 °C for 10 minutes.

Figure 83 Thin-layer chromatogram of the methanolic extract of *Clerodendrum petasites* root.




**Solvent system;** Toluene: Ethyl acetate 75:25 **Stationary phase;** $SiO_2$: $GF_{254}$

**Detection**

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with vanillin-sulfuric acid

*Vanillin-sulfuric acid regent

Preparation: vanillin (15 g) in ethanol (250 ml) and conc. sulfuric acid (2.5 ml)

**Spot color Development

Heat the plate after sprayed at 120 °C for 10 minutes.

Figure 84 Thin-layer chromatogram of the methanolic extract of *Ficus racemosa* root.

# Appendix C

## Data of Lipopolysaccharide-induced Fever in Rats

Table 7 Effect of NSS (10 ml/kg; i.m.) on lipopolysaccharide-induced fever in rats (normal rats).

| No. | Baseline | Rectal temperature (°C) before and after treatment (NSS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.98 | 36.92 | 37.32 | 36.82 | 36.94 | 36.76 | 37.02 | 36.48 | 36.32 |
| 2 | 36.96 | 36.94 | 36.72 | 36.84 | 36.76 | 36.98 | 37.00 | 36.60 | 36.18 |
| 3 | 36.96 | 36.82 | 37.20 | 37.08 | 36.96 | 36.82 | 36.38 | 35.26 | 36.70 |
| 4 | 36.66 | 37.00 | 37.42 | 36.98 | 36.94 | 36.90 | 37.46 | 36.96 | 37.04 |
| 5 | 36.44 | 36.94 | 36.78 | 36.74 | 36.62 | 36.98 | 36.62 | 36.02 | 36.32 |
| 6 | 37.00 | 36.86 | 37.22 | 36.86 | 36.86 | 36.72 | 36.90 | 36.48 | 36.06 |
| average | 36.83 | 36.91 | 37.11 | 36.89 | 36.85 | 36.86 | 36.90 | 36.30 | 36.44 |

Table 8 Effect of 2% Tween 80 (10 ml/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.98 | 36.68 | 38.34 | 38.18 | 38.50 | 38.40 | 37.98 | 37.94 | 37.76 |
| 2 | 37.12 | 36.84 | 37.68 | 38.00 | 38.42 | 38.62 | 38.10 | 37.82 | 37.40 |
| 3 | 37.02 | 36.96 | 38.06 | 38.02 | 38.24 | 38.44 | 38.42 | 38.12 | 37.96 |
| 4 | 36.96 | 36.74 | 37.90 | 38.94 | 38.68 | 37.38 | 38.16 | 37.56 | 37.42 |
| 5 | 37.10 | 36.98 | 38.00 | 38.40 | 38.32 | 38.28 | 38.32 | 38.04 | 37.82 |
| 6 | 37.12 | 36.54 | 38.02 | 38.00 | 38.46 | 38.08 | 37.72 | 37.62 | 37.92 |
| 7 | 36.50 | 35.36 | 37.74 | 37.92 | 38.02 | 38.14 | 38.00 | 37.98 | 37.82 |
| 8 | 36.94 | 36.92 | 37.94 | 38.40 | 38.10 | 38.00 | 37.76 | 37.62 | 37.56 |
| 9 | 36.82 | 35.00 | 37.12 | 38.38 | 38.10 | 38.30 | 38.38 | 38.18 | 38.00 |
| 10 | 35.34 | 35.08 | 37.30 | 37.56 | 37.64 | 37.94 | 37.86 | 37.32 | 36.86 |
| 11 | 36.68 | 36.68 | 37.90 | 37.90 | 37.98 | 37.60 | 37.30 | 37.28 | 36.70 |
| 12 | 36.92 | 37.46 | 37.24 | 37.70 | 37.84 | 37.50 | 37.44 | 37.54 | 37.56 |
| average | 36.79 | 36.44 | 37.77 | 38.12 | 38.19 | 38.06 | 37.95 | 37.75 | 37.57 |

Table 9 Effect of ASA (300 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.64 | 37.34 | 36.70 | 36.58 | 36.74 | 36.90 | 37.08 | 36.62 | 36.54 |
| 2 | 36.98 | 36.86 | 36.86 | 36.12 | 36.74 | 37.14 | 36.92 | 36.80 | 36.48 |
| 3 | 36.42 | 37.30 | 37.56 | 36.94 | 37.56 | 37.58 | 37.64 | 37.18 | 37.42 |
| 4 | 36.14 | 37.58 | 38.08 | 37.46 | 37.38 | 37.88 | 38.16 | 37.50 | 36.92 |
| 5 | 35.68 | 36.42 | 36.38 | 36.80 | 36.52 | 36.14 | 36.50 | 35.90 | 36.86 |
| 6 | 36.98 | 37.32 | 36.90 | 36.66 | 36.94 | 36.20 | 36.94 | 36.66 | 37.68 |
| 7 | 36.82 | 36.90 | 37.50 | 37.68 | 37.06 | 37.52 | 37.84 | 37.28 | 36.62 |
| 8 | 37.00 | 37.12 | 37.34 | 37.72 | 37.18 | 38.08 | 37.32 | 37.40 | 37.70 |
| 9 | 38.20 | 37.10 | 38.16 | 37.58 | 37.66 | 37.70 | 37.16 | 37.38 | 37.66 |
| 10 | 36.88 | 37.44 | 36.26 | 36.18 | 35.66 | 36.28 | 36.78 | 36.20 | 36.38 |

Table 9 Effect of ASA (300 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats (Continue).

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 11 | 37.52 | 36.38 | 37.18 | 37.30 | 37.30 | 37.18 | 37.16 | 37.20 | 37.22 |
| 12 | 36.82 | 37.58 | 36.80 | 36.40 | 36.00 | 35.20 | 36.06 | 35.66 | 35.46 |
| 13 | 36.76 | 37.28 | 37.72 | 37.24 | 36.48 | 36.08 | 36.30 | 35.66 | 35.54 |
| 14 | 36.60 | 36.48 | 37.50 | 37.22 | 36.90 | 37.24 | 36.98 | 37.50 | 36.80 |
| 15 | 36.98 | 35.86 | 37.02 | 36.76 | 36.54 | 36.60 | 36.10 | 36.20 | 36.24 |
| 16 | 36.32 | 37.36 | 37.64 | 37.72 | 37.76 | 37.50 | 37.42 | 37.50 | 37.64 |
| 17 | 36.82 | 36.90 | 37.62 | 37.50 | 36.36 | 36.44 | 36.72 | 36.48 | 35.84 |
| 18 | 35.70 | 36.70 | 36.68 | 36.26 | 36.52 | 36.54 | 36.50 | 36.12 | 36.10 |
| average | 36.74 | 37.00 | 37.22 | 37.01 | 36.85 | 36.90 | 36.98 | 36.74 | 36.73 |

Table 10 Effect of *Capparis micracantha* root extract (25 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 35.38 | 36.20 | 36.88 | 37.18 | 37.18 | 38.02 | 37.70 | 37.14 | 36.68 |
| 2 | 36.66 | 37.26 | 35.80 | 35.88 | 35.96 | 36.24 | 36.88 | 35.98 | 35.58 |
| 3 | 36.88 | 37.62 | 37.42 | 37.18 | 37.06 | 37.34 | 37.50 | 37.16 | 37.10 |
| 4 | 37.62 | 37.10 | 37.02 | 37.08 | 36.64 | 36.42 | 36.98 | 36.64 | 36.18 |
| 5 | 37.86 | 37.12 | 36.88 | 36.96 | 36.86 | 36.62 | 37.02 | 36.84 | 36.86 |
| 6 | 35.20 | 35.12 | 37.40 | 37.04 | 37.04 | 37.10 | 37.34 | 37.40 | 37.28 |
| average | 36.60 | 36.74 | 36.90 | 36.89 | 36.79 | 36.96 | 37.24 | 36.86 | 36.61 |

Table 11 Effect of *Capparis micracantha* root extract (50 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 h | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.70 | 37.52 | 38.00 | 37.52 | 37.18 | 37.48 | 37.18 | 37.28 | 37.42 |
| 2 | 36.80 | 36.06 | 35.58 | 38.22 | 38.02 | 37.84 | 38.30 | 37.68 | 37.48 |
| 3 | 36.88 | 37.28 | 37.08 | 36.48 | 36.44 | 36.34 | 36.14 | 36.44 | 37.08 |
| 4 | 37.14 | 36.92 | 36.60 | 36.74 | 36.66 | 36.70 | 36.76 | 36.52 | 36.26 |
| 5 | 37.30 | 36.72 | 36.66 | 37.24 | 36.40 | 36.42 | 36.24 | 36.16 | 36.34 |
| 6 | 37.36 | 37.04 | 37.22 | 37.50 | 37.22 | 36.68 | 36.86 | 36.78 | 36.68 |
| average | 37.03 | 36.92 | 36.86 | 37.28 | 36.99 | 36.91 | 36.91 | 36.81 | 36.88 |

Table 12 Effect of *Capparis micracantha* root extract (100 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 37.40 | 36.68 | 36.46 | 37.16 | 36.24 | 37.84 | 36.84 | 35.88 | 35.80 |
| 2 | 37.62 | 37.54 | 37.60 | 36.90 | 36.80 | 36.94 | 37.58 | 37.64 | 36.64 |
| 3 | 36.42 | 37.40 | 37.22 | 36.92 | 37.00 | 36.88 | 36.82 | 36.90 | 36.60 |
| 4 | 37.92 | 36.86 | 37.50 | 37.24 | 36.68 | 36.96 | 36.98 | 36.96 | 36.58 |
| 5 | 37.46 | 37.22 | 36.48 | 37.04 | 36.56 | 36.18 | 36.70 | 36.50 | 36.82 |
| 6 | 37.38 | 36.54 | 37.68 | 37.72 | 37.74 | 37.44 | 36.96 | 36.66 | 36.52 |
| average | 37.37 | 37.04 | 37.16 | 37.16 | 36.84 | 37.04 | 36.98 | 36.76 | 36.49 |

Table 13 Effect of *Capparis micracantha* root extract (200 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.84 | 35.98 | 36.72 | 36.80 | 36.28 | 36.64 | 35.82 | 36.34 | 35.90 |
| 2 | 36.62 | 36.96 | 36.16 | 36.66 | 36.14 | 36.14 | 36.56 | 35.72 | 35.62 |
| 3 | 37.20 | 36.42 | 37.32 | 36.64 | 36.74 | 37.64 | 37.58 | 36.86 | 37.16 |
| 4 | 36.02 | 35.40 | 37.32 | 36.78 | 36.62 | 36.26 | 36.26 | 36.30 | 36.10 |
| 5 | 37.24 | 37.14 | 37.60 | 37.60 | 36.80 | 36.86 | 36.62 | 36.70 | 36.56 |
| 6 | 37.06 | 36.54 | 36.64 | 36.46 | 36.36 | 36.50 | 36.08 | 36.14 | 36.12 |
| average | 36.83 | 36.41 | 36.96 | 36.82 | 36.49 | 36.67 | 36.49 | 36.34 | 36.24 |

Table 14 Effect of *Capparis micracantha* root extract (400 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.98 | 37.96 | 37.88 | 37.76 | 38.16 | 38.12 | 37.38 | 36.72 | 36.98 |
| 2 | 36.24 | 35.88 | 35.92 | 35.82 | 36.24 | 35.70 | 36.64 | 36.30 | 36.24 |
| 3 | 36.34 | 36.46 | 36.08 | 35.34 | 35.72 | 35.74 | 35.92 | 36.60 | 36.34 |
| 4 | 36.14 | 36.20 | 36.92 | 36.06 | 36.28 | 36.36 | 36.36 | 36.28 | 36.14 |
| 5 | 35.08 | 36.90 | 36.94 | 36.70 | 36.56 | 36.90 | 36.60 | 36.62 | 35.08 |
| 6 | 37.22 | 35.94 | 36.86 | 36.96 | 36.72 | 36.72 | 36.46 | 36.46 | 37.22 |
| average | 36.33 | 36.56 | 36.77 | 36.44 | 36.61 | 36.59 | 36.56 | 36.50 | 36.33 |

Table 15 Effect of *Harrisonia perforata* root extract (25 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 35.08 | 36.42 | 37.08 | 37.16 | 36.52 | 36.04 | 36.50 | 36.66 | 36.66 |
| 2 | 37.28 | 37.44 | 37.32 | 37.24 | 37.24 | 37.50 | 37.30 | 37.02 | 36.88 |
| 3 | 36.98 | 37.02 | 37.32 | 37.08 | 37.04 | 36.92 | 36.94 | 36.50 | 36.42 |
| 4 | 36.96 | 37.70 | 37.48 | 37.90 | 37.54 | 37.18 | 36.94 | 36.50 | 36.54 |
| 5 | 35.66 | 35.84 | 37.44 | 37.40 | 36.92 | 37.10 | 37.02 | 36.86 | 36.68 |
| 6 | 37.42 | 37.16 | 37.84 | 37.44 | 36.88 | 36.62 | 36.54 | 36.34 | 36.22 |
| average | 36.56 | 36.93 | 37.41 | 37.37 | 37.02 | 36.89 | 36.87 | 36.65 | 36.57 |

Table 16 Effect of *Harrisonia perforata* root extract (50 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.34 | 36.22 | 37.86 | 37.18 | 36.64 | 36.44 | 36.96 | 36.86 | 36.52 |
| 2 | 37.20 | 37.34 | 36.78 | 36.86 | 37.04 | 36.94 | 36.76 | 37.16 | 36.66 |
| 3 | 36.84 | 37.74 | 38.04 | 38.08 | 37.10 | 36.98 | 36.78 | 36.58 | 36.36 |
| 4 | 36.96 | 37.00 | 37.70 | 37.88 | 37.22 | 37.04 | 36.88 | 36.60 | 36.50 |
| 5 | 37.70 | 36.10 | 37.02 | 37.00 | 36.78 | 37.08 | 36.80 | 36.26 | 36.20 |
| 6 | 36.68 | 36.64 | 37.06 | 36.96 | 36.72 | 36.50 | 36.28 | 36.98 | 36.34 |
| average | 36.95 | 36.84 | 37.41 | 37.33 | 36.92 | 36.83 | 36.74 | 36.74 | 36.43 |

Table 17 Effect of *Harrisonia perforata* root extract (100 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.32 | 36.40 | 36.46 | 36.34 | 36.26 | 36.74 | 36.12 | 36.44 | 36.80 |
| 2 | 37.08 | 37.02 | 38.24 | 37.94 | 37.54 | 37.72 | 37.00 | 36.88 | 36.64 |
| 3 | 36.62 | 37.36 | 36.88 | 37.10 | 36.90 | 36.78 | 36.52 | 36.34 | 36.12 |
| 4 | 36.96 | 37.08 | 36.80 | 37.46 | 36.76 | 36.82 | 36.58 | 36.56 | 36.32 |
| 5 | 35.52 | 35.72 | 35.60 | 35.68 | 35.44 | 35.96 | 36.12 | 36.38 | 36.10 |
| 6 | 37.52 | 37.76 | 36.82 | 36.92 | 36.74 | 37.16 | 37.00 | 36.58 | 36.26 |
| average | 36.67 | 36.89 | 36.80 | 36.91 | 36.61 | 36.86 | 36.56 | 36.53 | 36.37 |

Table 18 Effect of *Harrisonia perforata* root extract (200 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.68 | 36.98 | 37.16 | 36.60 | 36.38 | 36.90 | 37.06 | 36.98 | 36.70 |
| 2 | 37.20 | 37.64 | 37.74 | 37.44 | 37.10 | 37.50 | 37.38 | 37.00 | 36.88 |
| 3 | 36.26 | 36.88 | 37.84 | 38.04 | 38.08 | 37.96 | 37.52 | 37.20 | 37.00 |
| 4 | 36.90 | 37.22 | 37.08 | 36.90 | 36.70 | 36.42 | 36.32 | 36.12 | 36.88 |
| 5 | 36.92 | 37.12 | 36.62 | 36.94 | 36.88 | 37.12 | 36.94 | 36.74 | 36.46 |
| 6 | 37.30 | 37.16 | 37.90 | 37.52 | 37.24 | 37.04 | 36.86 | 36.58 | 36.20 |
| average | 36.88 | 37.17 | 37.39 | 37.24 | 37.06 | 37.16 | 37.01 | 36.77 | 36.69 |

Table 19 Effect of *Harrisonia perforata* root extract (400 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.00 | 36.80 | 37.72 | 37.72 | 37.32 | 37.04 | 36.96 | 36.56 | 36.46 |
| 2 | 37.12 | 37.90 | 38.12 | 38.12 | 37.90 | 37.66 | 37.50 | 37.28 | 36.86 |
| 3 | 37.00 | 37.42 | 36.94 | 37.40 | 37.48 | 37.18 | 36.96 | 36.60 | 36.42 |
| 4 | 37.60 | 37.22 | 37.94 | 38.12 | 37.02 | 36.96 | 36.84 | 36.62 | 36.14 |
| 5 | 35.52 | 36.16 | 36.84 | 36.80 | 36.68 | 37.14 | 37.02 | 36.74 | 36.62 |
| 6 | 37.04 | 36.66 | 37.42 | 37.32 | 36.88 | 36.64 | 36.36 | 36.18 | 36.42 |
| average | 36.71 | 37.03 | 37.50 | 37.58 | 37.21 | 37.10 | 36.94 | 36.66 | 36.49 |

Table 20 Effect of *Tiliacora triandra* root extract (25 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.82 | 36.92 | 37.60 | 37.28 | 36.78 | 36.52 | 37.04 | 36.42 | 36.46 |
| 2 | 37.32 | 37.50 | 37.26 | 37.20 | 37.94 | 37.60 | 37.48 | 36.96 | 36.64 |
| 3 | 36.08 | 37.10 | 36.80 | 36.34 | 35.96 | 35.86 | 35.58 | 35.10 | 34.78 |
| 4 | 36.86 | 35.22 | 36.32 | 35.98 | 35.06 | 35.78 | 35.94 | 35.78 | 36.10 |
| 5 | 36.44 | 37.38 | 37.22 | 37.12 | 37.52 | 37.68 | 37.90 | 37.70 | 37.64 |
| 6 | 37.20 | 37.40 | 37.62 | 37.00 | 36.68 | 36.40 | 36.86 | 36.82 | 36.10 |
| average | 36.79 | 36.92 | 37.14 | 36.82 | 36.66 | 36.64 | 36.80 | 36.46 | 36.29 |

Table 21 Effect of *Tiliacora triandra* root extract (50 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 h |
| 1 | 37.36 | 37.12 | 37.96 | 37.48 | 37.18 | 36.64 | 36.58 | 36.60 | 36.62 |
| 2 | 37.30 | 37.26 | 36.80 | 36.38 | 35.90 | 36.54 | 36.14 | 36.16 | 36.00 |
| 3 | 37.10 | 37.30 | 37.56 | 37.06 | 37.36 | 37.18 | 36.56 | 36.56 | 36.58 |
| 4 | 36.84 | 36.78 | 37.40 | 37.42 | 37.22 | 37.16 | 37.52 | 37.56 | 37.64 |
| 5 | 36.78 | 36.76 | 36.78 | 36.78 | 36.44 | 36.46 | 36.74 | 36.84 | 37.06 |
| 6 | 36.46 | 36.96 | 37.42 | 37.50 | 37.10 | 36.88 | 36.42 | 36.00 | 36.10 |
| average | 36.97 | 37.03 | 37.32 | 37.10 | 36.87 | 36.81 | 36.66 | 36.62 | 36.67 |

Table 22 Effect of *Tiliacora triandra* root extract (100 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.16 | 36.96 | 37.32 | 36.58 | 36.18 | 35.78 | 35.66 | 35.02 | 35.06 |
| 2 | 36.72 | 36.34 | 37.16 | 36.94 | 36.38 | 35.94 | 36.30 | 36.00 | 35.83 |
| 3 | 37.18 | 37.24 | 37.04 | 37.54 | 36.56 | 36.76 | 37.06 | 36.56 | 35.86 |
| 4 | 36.50 | 35.68 | 37.10 | 36.90 | 36.56 | 36.30 | 36.64 | 36.40 | 36.84 |
| 5 | 36.24 | 37.42 | 37.00 | 36.30 | 36.18 | 35.62 | 36.68 | 36.56 | 36.36 |
| 6 | 36.40 | 36.60 | 36.90 | 37.32 | 36.10 | 36.24 | 36.56 | 36.88 | 36.56 |
| average | 36.53 | 36.71 | 37.09 | 36.93 | 36.33 | 36.11 | 36.48 | 36.24 | 36.09 |

Table 23 Effect of *Tiliacora triandra* root extract (200 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.16 | 35.84 | 36.14 | 36.14 | 36.04 | 35.78 | 35.88 | 35.16 | 35.44 |
| 2 | 37.32 | 37.68 | 37.80 | 37.84 | 37.14 | 37.44 | 37.38 | 37.10 | 36.98 |
| 3 | 37.02 | 37.50 | 37.34 | 37.66 | 36.90 | 36.42 | 36.56 | 36.10 | 36.00 |
| 4 | 36.64 | 36.74 | 35.54 | 35.68 | 36.08 | 36.22 | 36.18 | 36.12 | 35.86 |
| 5 | 36.76 | 36.82 | 35.38 | 35.40 | 35.22 | 35.30 | 35.86 | 35.74 | 35.68 |
| 6 | 36.84 | 37.00 | 36.86 | 37.26 | 36.74 | 36.86 | 36.30 | 35.82 | 36.38 |
| average | 36.79 | 36.93 | 36.51 | 36.66 | 36.35 | 36.34 | 36.36 | 36.01 | 36.06 |

Table 24 Effect of *Tiliacora triandra* root extract (400 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 h |
| 1 | 37.28 | 37.20 | 38.10 | 37.72 | 36.94 | 36.50 | 37.12 | 36.54 | 36.16 |
| 2 | 37.46 | 36.58 | 36.22 | 36.24 | 35.46 | 35.44 | 36.28 | 35.68 | 35.84 |
| 3 | 36.82 | 36.56 | 36.90 | 36.98 | 36.78 | 36.64 | 35.42 | 36.28 | 36.60 |
| 4 | 36.86 | 36.08 | 36.22 | 35.02 | 36.62 | 36.74 | 37.08 | 37.14 | 37.10 |
| 5 | 36.96 | 36.72 | 36.64 | 37.16 | 37.28 | 37.20 | 37.42 | 37.42 | 37.50 |
| 6 | 36.30 | 36.74 | 36.44 | 36.24 | 36.06 | 35.62 | 35.54 | 35.54 | 35.08 |
| average | 36.95 | 36.65 | 36.75 | 36.56 | 36.52 | 36.36 | 36.48 | 36.43 | 36.38 |

Table 25 Effect of *Clerodendrum petasites* root extract (25 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.84 | 37.06 | 37.20 | 37.38 | 36.98 | 36.68 | 36.24 | 36.08 | 35.80 |
| 2 | 36.20 | 37.76 | 37.76 | 37.76 | 37.52 | 37.48 | 37.62 | 37.34 | 37.26 |
| 3 | 36.24 | 37.02 | 37.20 | 36.96 | 36.30 | 36.56 | 36.64 | 36.68 | 36.52 |
| 4 | 37.44 | 37.00 | 37.46 | 37.32 | 37.14 | 37.40 | 37.10 | 36.76 | 36.88 |
| 5 | 36.94 | 36.90 | 37.46 | 36.78 | 37.02 | 36.86 | 36.66 | 36.26 | 35.94 |
| 6 | 36.90 | 37.10 | 37.18 | 37.08 | 37.08 | 36.72 | 36.74 | 36.94 | 36.64 |
| average | 36.76 | 37.14 | 37.38 | 37.21 | 37.01 | 36.95 | 36.83 | 36.68 | 36.51 |

Table 26 Effect of *Clerodendrum petasites* root extract (50 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.82 | 37.68 | 37.38 | 36.98 | 37.00 | 37.06 | 36.88 | 36.88 | 36.92 |
| 2 | 36.52 | 37.96 | 37.12 | 36.96 | 36.88 | 36.84 | 36.82 | 36.56 | 36.56 |
| 3 | 36.32 | 37.08 | 36.90 | 36.40 | 36.64 | 37.08 | 37.12 | 36.82 | 36.90 |
| 4 | 36.68 | 37.04 | 37.14 | 36.74 | 36.26 | 36.68 | 36.82 | 36.50 | 36.84 |
| 5 | 36.92 | 37.18 | 37.66 | 37.80 | 37.46 | 36.92 | 36.86 | 36.74 | 36.52 |
| 6 | 39.72 | 37.04 | 37.00 | 36.76 | 36.60 | 36.60 | 36.78 | 36.62 | 36.52 |
| average | 37.16 | 37.33 | 37.20 | 36.94 | 36.81 | 36.86 | 36.88 | 36.69 | 36.71 |

Table 27 Effect of *Clerodendrum petasites* root extract (100 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.62 | 37.16 | 37.38 | 36.98 | 37.02 | 36.96 | 36.66 | 36.82 | 36.36 |
| 2 | 36.82 | 37.76 | 37.44 | 37.34 | 37.16 | 37.24 | 36.98 | 36.92 | 36.80 |
| 3 | 36.80 | 37.86 | 37.26 | 37.38 | 36.82 | 35.92 | 35.26 | 35.16 | 36.56 |
| 4 | 36.74 | 36.76 | 37.68 | 37.22 | 36.88 | 37.00 | 37.20 | 36.98 | 36.48 |
| 5 | 36.56 | 36.72 | 37.88 | 37.50 | 37.76 | 36.76 | 36.40 | 36.34 | 36.20 |
| 6 | 36.82 | 37.02 | 37.16 | 36.86 | 36.76 | 36.56 | 36.74 | 36.70 | 36.52 |
| average | 36.73 | 37.21 | 37.47 | 37.21 | 37.07 | 36.74 | 36.54 | 36.49 | 36.49 |

Table 28 Effect of *Clerodendrum petasites* root extract (200 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 h |
| 1 | 37.34 | 36.36 | 37.58 | 36.94 | 37.24 | 37.18 | 36.96 | 36.92 | 36.80 |
| 2 | 37.10 | 36.76 | 37.42 | 37.84 | 37.64 | 36.82 | 36.78 | 36.52 | 36.72 |
| 3 | 36.78 | 37.04 | 37.70 | 37.30 | 37.18 | 37.18 | 36.90 | 36.68 | 36.48 |
| 4 | 36.46 | 37.06 | 36.74 | 36.50 | 36.58 | 36.70 | 36.90 | 36.64 | 36.28 |
| 5 | 36.62 | 37.14 | 37.54 | 37.06 | 36.84 | 36.70 | 36.70 | 36.74 | 36.54 |
| 6 | 36.98 | 37.16 | 37.72 | 37.66 | 36.98 | 36.74 | 36.50 | 36.42 | 36.28 |
| average | 36.88 | 36.92 | 37.45 | 37.22 | 37.08 | 36.89 | 36.79 | 36.65 | 36.52 |

Table 29 Effect of *Clerodendrum petasites* root extract (400 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.98 | 35.48 | 37.32 | 37.08 | 37.04 | 36.70 | 36.76 | 36.44 | 36.44 |
| 2 | 37.46 | 35.98 | 37.96 | 38.12 | 37.92 | 37.86 | 37.80 | 37.76 | 37.34 |
| 3 | 36.76 | 35.52 | 36.98 | 36.48 | 36.42 | 36.32 | 36.50 | 36.30 | 35.82 |
| 4 | 36.86 | 37.68 | 37.76 | 37.82 | 37.32 | 35.84 | 36.92 | 36.50 | 36.26 |
| 5 | 36.90 | 37.08 | 36.90 | 36.90 | 37.14 | 36.60 | 36.60 | 36.54 | 36.40 |
| 6 | 36.96 | 37.24 | 37.80 | 37.80 | 37.50 | 37.20 | 36.98 | 36.72 | 36.42 |
| average | 36.99 | 36.50 | 37.45 | 37.37 | 37.22 | 36.75 | 36.93 | 36.71 | 36.45 |

Table 30 Effect of *Ficus racemosa* root extract (25 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.14 | 37.14 | 36.78 | 36.54 | 36.52 | 36.78 | 36.76 | 36.98 | 36.42 |
| 2 | 36.86 | 36.50 | 37.62 | 37.78 | 37.56 | 38.22 | 37.92 | 37.80 | 37.36 |
| 3 | 36.54 | 36.84 | 36.48 | 36.96 | 36.48 | 36.62 | 36.80 | 36.96 | 36.52 |
| 4 | 35.74 | 35.48 | 36.60 | 36.90 | 36.66 | 35.90 | 36.04 | 36.38 | 36.34 |
| 5 | 36.68 | 36.90 | 37.56 | 37.72 | 36.92 | 37.92 | 37.40 | 36.50 | 36.44 |
| 6 | 36.40 | 35.94 | 35.88 | 36.30 | 36.18 | 35.86 | 36.16 | 35.92 | 35.94 |
| average | 36.39 | 36.47 | 36.82 | 37.03 | 36.72 | 36.88 | 36.85 | 36.76 | 36.50 |

Table 31 Effect of *Ficus racemosa* root extract (50 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 35.68 | 36.82 | 37.08 | 36.88 | 37.46 | 37.36 | 36.94 | 39.86 | 36.80 |
| 2 | 36.96 | 36.46 | 37.64 | 37.36 | 37.50 | 37.14 | 37.48 | 37.10 | 37.00 |
| 3 | 36.50 | 36.76 | 37.06 | 37.66 | 37.02 | 36.64 | 36.62 | 36.76 | 36.42 |
| 4 | 36.40 | 36.92 | 37.10 | 37.08 | 36.64 | 36.92 | 37.44 | 36.74 | 36.18 |
| 5 | 37.34 | 37.02 | 36.56 | 36.64 | 35.94 | 36.18 | 35.60 | 35.30 | 35.26 |
| 6 | 37.20 | 37.96 | 37.56 | 36.08 | 35.84 | 35.96 | 36.18 | 36.34 | 36.38 |
| average | 36.68 | 36.99 | 37.17 | 36.95 | 36.73 | 36.70 | 36.71 | 37.02 | 36.34 |

Table 32 Effect of *Ficus racemosa* root extract (100 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 h |
| 1 | 36.12 | 37.10 | 36.90 | 37.06 | 36.95 | 36.96 | 37.26 | 37.50 | 37.38 |
| 2 | 37.12 | 37.68 | 36.82 | 36.90 | 36.82 | 37.90 | 37.74 | 37.96 | 37.60 |
| 3 | 36.58 | 36.60 | 37.14 | 36.86 | 37.28 | 37.16 | 37.48 | 37.52 | 36.86 |
| 4 | 37.18 | 36.96 | 37.88 | 37.74 | 37.14 | 37.62 | 37.84 | 36.84 | 36.24 |
| 5 | 36.66 | 37.58 | 37.56 | 38.16 | 37.84 | 38.22 | 37.88 | 37.52 | 37.20 |
| 6 | 35.98 | 36.70 | 36.82 | 36.30 | 36.64 | 36.48 | 36.92 | 36.40 | 36.02 |
| average | 36.61 | 37.10 | 37.19 | 37.17 | 37.11 | 37.39 | 37.52 | 37.29 | 36.88 |

Table 33 Effect of *Ficus racemosa* root extract (200 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.88 | 36.20 | 37.42 | 37.46 | 36.96 | 36.82 | 37.14 | 36.94 | 36.84 |
| 2 | 37.06 | 37.28 | 36.94 | 37.50 | 37.66 | 36.64 | 36.40 | 36.42 | 36.10 |
| 3 | 36.76 | 37.54 | 36.78 | 38.26 | 37.68 | 37.98 | 37.90 | 37.36 | 37.50 |
| 4 | 36.92 | 36.02 | 37.76 | 37.68 | 37.50 | 37.46 | 37.46 | 37.60 | 36.54 |
| 5 | 37.78 | 36.34 | 38.24 | 37.96 | 37.72 | 37.84 | 37.94 | 37.58 | 37.34 |
| 6 | 38.12 | 36.96 | 37.40 | 37.10 | 37.10 | 37.22 | 37.32 | 36.96 | 36.92 |
| average | 37.25 | 36.72 | 37.42 | 37.66 | 37.44 | 37.33 | 37.36 | 37.14 | 36.87 |

Table 34 Effect of *Ficus racemosa* root extract (400 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 h |
| 1 | 36.56 | 37.06 | 36.90 | 37.34 | 37.58 | 37.14 | 36.98 | 36.94 | 36.64 |
| 2 | 36.92 | 37.20 | 37.50 | 38.04 | 37.96 | 37.84 | 37.70 | 37.38 | 37.44 |
| 3 | 35.80 | 36.16 | 36.62 | 36.62 | 36.44 | 36.42 | 37.00 | 36.56 | 36.76 |
| 4 | 36.62 | 36.88 | 37.56 | 37.56 | 36.76 | 36.90 | 37.26 | 36.58 | 36.34 |
| 5 | 36.16 | 36.72 | 37.24 | 38.00 | 36.90 | 37.08 | 36.86 | 36.46 | 36.40 |
| 6 | 35.92 | 36.76 | 36.04 | 36.12 | 35.60 | 36.34 | 36.60 | 36.52 | 36.70 |
| average | 36.33 | 36.80 | 36.98 | 37.28 | 36.87 | 36.95 | 37.07 | 36.74 | 36.71 |

Table 35 Effect of Bencha-loga-wichian remedy (25 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.14 | 37.12 | 36.74 | 36.30 | 35.98 | 36.20 | 36.64 | 36.28 | 36.20 |
| 2 | 35.60 | 37.26 | 37.60 | 38.00 | 37.76 | 37.70 | 37.20 | 37.04 | 36.72 |
| 3 | 36.90 | 37.16 | 37.90 | 37.96 | 36.84 | 36.62 | 36.38 | 35.78 | 35.20 |
| 4 | 36.78 | 37.10 | 38.02 | 37.86 | 37.28 | 36.80 | 36.46 | 36.02 | 35.44 |
| 5 | 36.96 | 36.62 | 37.60 | 36.96 | 36.74 | 36.68 | 36.44 | 36.48 | 36.16 |
| 6 | 36.86 | 36.40 | 37.32 | 36.70 | 36.68 | 36.58 | 36.36 | 36.44 | 36.12 |
| average | 36.54 | 36.94 | 37.53 | 37.30 | 36.88 | 36.76 | 36.58 | 36.34 | 35.97 |

Table 36 Effect of Bencha-loga-wichian remedy (50 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 35.66 | 37.10 | 38.08 | 37.96 | 37.94 | 37.66 | 37.14 | 36.88 | 36.72 |
| 2 | 36.24 | 36.96 | 38.04 | 36.74 | 36.60 | 36.50 | 36.80 | 36.42 | 36.32 |
| 3 | 36.88 | 37.10 | 38.34 | 38.08 | 37.66 | 36.94 | 36.54 | 36.28 | 36.34 |
| 4 | 36.84 | 37.70 | 37.90 | 37.74 | 37.40 | 36.86 | 36.50 | 36.36 | 36.38 |
| 5 | 36.94 | 36.94 | 37.02 | 36.88 | 36.64 | 36.34 | 36.40 | 36.76 | 36.60 |
| 6 | 36.52 | 36.54 | 37.52 | 36.98 | 36.92 | 36.80 | 36.64 | 36.80 | 36.42 |
| average | 36.51 | 37.06 | 37.82 | 37.40 | 37.19 | 36.85 | 36.67 | 36.58 | 36.46 |

Table 37 Effect of Bencha-loga-wichian remedy (100 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 37.28 | 37.14 | 37.26 | 37.12 | 36.90 | 36.74 | 36.42 | 36.22 | 36.16 |
| 2 | 37.04 | 36.46 | 37.40 | 36.70 | 36.32 | 36.70 | 36.42 | 36.40 | 36.36 |
| 3 | 36.88 | 36.82 | 36.40 | 36.20 | 36.56 | 36.52 | 36.16 | 35.78 | 35.34 |
| 4 | 36.46 | 36.88 | 37.86 | 37.80 | 37.28 | 36.78 | 36.58 | 36.00 | 35.84 |
| 5 | 36.48 | 37.00 | 37.24 | 37.14 | 36.88 | 36.76 | 36.56 | 36.66 | 36.36 |
| 6 | 36.42 | 37.26 | 37.36 | 37.06 | 36.78 | 36.60 | 36.34 | 36.68 | 36.24 |
| average | 36.76 | 36.93 | 37.25 | 37.00 | 36.79 | 36.68 | 36.41 | 36.29 | 36.05 |

Table 38 Effect of Bencha-loga-wichian remedy (200 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 35.02 | 36.98 | 37.32 | 36.98 | 36.68 | 36.46 | 36.72 | 36.54 | 36.42 |
| 2 | 37.10 | 36.98 | 37.78 | 37.16 | 36.84 | 36.38 | 36.66 | 36.62 | 36.22 |
| 3 | 36.96 | 37.56 | 37.80 | 37.56 | 37.06 | 36.68 | 36.46 | 35.86 | 36.00 |
| 4 | 36.82 | 37.46 | 38.10 | 37.88 | 37.48 | 36.76 | 36.32 | 36.08 | 36.14 |
| 5 | 36.96 | 37.50 | 37.34 | 36.84 | 36.70 | 36.60 | 36.48 | 36.72 | 36.52 |
| 6 | 35.84 | 36.78 | 37.48 | 36.92 | 36.64 | 36.64 | 36.58 | 36.70 | 36.68 |
| average | 36.45 | 37.21 | 37.64 | 37.22 | 36.90 | 36.59 | 36.54 | 36.42 | 36.33 |

Table 39 Effect of Bencha-loga-wichian remedy (400 mg/kg; p.o.) on lipopolysaccharide-induced fever in rats.

| No. | Baseline | Rectal temperature (°C) before and after treatment (LPS injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr |
| 1 | 36.88 | 36.78 | 37.40 | 36.62 | 36.24 | 36.90 | 36.04 | 36.06 | 35.82 |
| 2 | 36.72 | 36.60 | 37.44 | 37.54 | 36.64 | 36.84 | 36.42 | 36.34 | 36.00 |
| 3 | 36.76 | 37.10 | 37.88 | 37.64 | 36.82 | 36.44 | 36.22 | 36.04 | 35.76 |
| 4 | 37.06 | 37.66 | 37.42 | 34.04 | 36.74 | 35.52 | 36.32 | 36.28 | 32.24 |
| 5 | 36.80 | 37.56 | 37.60 | 36.72 | 36.76 | 36.48 | 36.26 | 36.54 | 36.36 |
| 6 | 36.92 | 37.24 | 37.22 | 37.12 | 36.98 | 36.74 | 36.68 | 36.66 | 36.34 |
| average | 36.86 | 37.16 | 37.49 | 36.61 | 36.70 | 36.49 | 36.32 | 36.32 | 35.42 |

# Appendix D

# Data of Mouse Hot-plate Test

Table 40 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of various doses of *Capparis micarcantha* root extract (CM; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CM (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 26.28±1.95 | 29.53±1.19 | 27.58±2.18 | 28.89±1.22 | 31.31±2.41 | 32.82±1.76 | 38.4±1.69 |
| 50 | 30.93±2.68 | 25.8±2.46 | 34.83±2.14 | 31.14±2.32 | 36.18±2.01 | 32.34±2.09 | 37.34±2.07 |
| 100 | 32.29±2.76 | 29.68±2.57 | 33.75±1.36 | 34.28±1.98 | 36.15±2.13 | 33.99±2.68 | 39.05±1.93 |
| 200 | 32.98±3.16 | 35.1±2.63 | 39.59±1.91 | 38.00±2.20 | 39.3±1.37 | 39.64±2.01 | 42.94±1.28 |
| 400 | 36.59±1.89 | 37.53±1.64 | 36.79±1.63 | 38.56±1.80 | 35.77±4.14 | 41.4±1.06 | 44.57±0.43 |

Table 41 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| HP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 25.56±3.68 | 24.23±3.03 | 22.60±2.69 | 23.77±3.23 | 24.62±2.68 | 21.65±1.25 | 25.10±1.91 |
| 50 | 20.25±1.69 | 21.60±1.69 | 17.01±0.76 | 18.73±1.12 | 19.79±1.70 | 24.61±1.97 | 22.13±1.24 |
| 100 | 21.92±2.15 | 21.26±2.33 | 20.16±2.47 | 18.51±1.76 | 22.03±2.89 | 23.50±2.46 | 26.01±2.68 |
| 200 | 21.84±2.12 | 22.34±1.91 | 22.71±2.30 | 25.96±2.45 | 26.67±2.57 | 29.62±2.59 | 30.08±2.75 |
| 400 | 27.88±2.36 | 26.75±2.21 | 27.79±3.59 | 27.78±2.65 | 32.74±2.77 | 31.11±3.01 | 31.33±2.61 |

Table 42 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| TT (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 20.76±1.66 | 20.52±0.84 | 17.78±0.74 | 21.61±1.47 | 22.68±1.52 | 24.11±1.34 | 27.02±2.52 |
| 50 | 24.99±2.04 | 21.43±1.69 | 23.29±2.10 | 25.45±1.55 | 26.53±1.14 | 28.35±1.49 | 32.16±2.24 |
| 100 | 20.80±1.48 | 23.30±2.31 | 25.09±2.01 | 26.67±2.19 | 29.63±2.15 | 28.08±1.96 | 32.46±2.77 |
| 200 | 24.18±2.02 | 25.85±1.93 | 27.44±1.31 | 27.76±2.55 | 30.23±1.63 | 33.07±1.80 | 35.15±1.90 |
| 400 | 28.88±1.87 | 27.94±1.85 | 28.24±2.08 | 31.35±2.18 | 37.50±1.82 | 36.46±2.36 | 37.26±1.95 |

Table 43 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 17.04±1.23 | 16.59±1.29 | 16.09±0.59 | 17.83±1.07 | 20.32±1.35 | 21.02±0.55 | 20.61±1.37 |
| 50 | 20.09±1.28 | 18.19±1.34 | 20.37±1.58 | 18.34±1.49 | 21.66±1.61 | 21.39±0.78 | 20.71±1.66 |
| 100 | 17.74±0.89 | 23.21±2.99 | 19.79±1.45 | 17.40±1.21 | 19.85±0.99 | 23.74±1.58 | 19.91±0.67 |
| 200 | 20.02±1.44 | 18.94±1.35 | 18.82±1.06 | 19.25±0.76 | 21.27±1.26 | 23.17±0.93 | 22.55±1.49 |
| 400 | 25.74±2.66 | 21.34±1.51 | 23.3±1.24 | 27.33±2.06 | 27.03±2.74 | 28.50±2.27 | 27.72±2.48 |

Table 44 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| FR (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 24.15±2.19 | 25.89±1.94 | 24.61±2.03 | 28.79±2.25 | 33.25±2.18 | 36.94±2.14 | 31.72±2.26 |
| 50 | 27.23±2.22 | 25.98±2.65 | 30.63±2.85 | 32.58±2.20 | 30.93±2.63 | 35.96±1.75 | 31.5±0.98 |
| 100 | 33.9±3.14 | 31.15±2.14 | 34.56±2.94 | 37.62±2.08 | 36.43±2.34 | 37.56±2.18 | 41.53±2.12 |
| 200 | 35.06±3.23 | 34.21±3.05 | 35.82±2.41 | 37.24±2.34 | 38.95±2.24 | 38.98±2.12 | 37.9±2.58 |
| 400 | 39.47±1.85 | 39.87±2.02 | 39.86±1.47 | 39.37±1.94 | 39.74±2.21 | 42.27±1.58 | 42.51±1.44 |

Table 45 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| BEN (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 20.65±1.53 | 21.12±1.59 | 21.65±1.51 | 20.44±1.69 | 20.53±0.51 | 23.51±1.38 | 22.75±2.09 |
| 50 | 22.41±6.57 | 18.21±4.93 | 20.02±4.19 | 22.35±5.98 | 23.58±2.85 | 25.29±8.07 | 24.26±11.99 |
| 100 | 24.36±2.31 | 22.97±2.63 | 21.67±2.12 | 23.10±2.32 | 26.71±2.75 | 27.36±1.89 | 18.89±2.85 |
| 200 | 21.41±2.67 | 21.50±2.21 | 25.28±3.72 | 27.94±2.65 | 29.82±2.41 | 28.75±2.58 | 26.65±2.53 |
| 400 | 22.36±2.63 | 22.69±2.15 | 24.32±1.74 | 29.55±1.57 | 27.73±1.66 | 32.18±1.52 | 25.53±1.41 |

Table 46 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CM (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | -1.80±17.66 | 20.30±6.41 | 3.03±17.67 | 11.14 ±12.24 | 29.89±10.54 | 32.58±12.42 | 58.63±12.14 | 8159.37±2193.70 |
| 50 | 38.13±11.62 | 15.89±12.68 | 52.75±12.71 | 40.64±9.96 | 62.57±8.39 | 42.79±12.62 | 64.55±11.99 | 11475.66±2360.57 |
| 100 | 50.15±10.08 | 34.32±12.50 | 51.83±7.25 | 55.59±7.96 | 56.67±8.34 | 53.14±10.44 | 76.72±11.99 | 13584.04±1380.13 |
| 200 | 53.44±11.88 | 60.60±10.47 | 67.65±7.43 | 71.27±7.90 | 74.69±5.73 | 82.37±8.54 | 88.92±5.12 | 18303.52±1134.09 |
| 400 | 61.47±8.56 | 68.01±6.86 | 74.98±6.62 | 76.39±7.50 | 82.46±29.77 | 92.57±5.55 | 100.00±1.65 | 18175.59±1460.05 |

Table 47 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| HP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | 28.84±12.94 | 15.31±13.76 | 11.23±11.62 | 14.89±12.21 | 19.95±9.82 | 5.99±6.65 | 20.87±6.49 | 4193.84±1163.55 |
| 50 | 16.58±4.75 | 19.99±6.32 | 4.32±4.56 | 9.82±5.96 | 14.25±5.98 | 31.69±6.66 | 21.82±5.29 | 4948.31±684.19 |
| 100 | 18.06±7.13 | 15.83±7.41 | 11.82±8.81 | 5.31±6.89 | 16.30±10.61 | 23.57±9.07 | 34.47±8.82 | 5129.97±1309.66 |
| 200 | 16.91±7.23 | 19.95±5.73 | 19.01±8.29 | 43.99±8.58 | 44.14±8.53 | 59.29±9.55 | 57.16±9.59 | 8793.58±1710.23 |
| 400 | 29.15±10.49 | 25.72±9.39 | 49.80±15.11 | 45.11±12.83 | 66.65±13.20 | 62.75±13.77 | 60.16±11.36 | 8991.86±2563.81 |

Table 48 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| TT (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | 11.86±6.58 | 11.17±3.95 | 1.08±4.96 | 14.99±6.09 | 18.45±5.84 | 23.79±5.14 | 32.87±9.83 | 5736.97±1219.76 |
| 50 | 11.43±13.06 | -5.14±15.15 | 2.28±8.67 | 18.25±8.67 | 22.07±6.88 | 28.53±8.40 | 46.72±11.06 | 6144.08±1733.65 |
| 100 | 8.98±4.45 | 17.46±10.22 | 25.64±6.27 | 32.36±7.34 | 39.86±6.99 | 37.74±5.90 | 53.79±9.25 | 8762.85±1174.41 |
| 200 | 21.86±8.76 | 27.95±7.69 | 25.87±5.81 | 23.12±10.04 | 41.03±6.09 | 51.60±7.26 | 49.81±8.02 | 11227.57±1306.75 |
| 400 | 33.80±9.02 | 29.49±9.48 | 33.74±10.37 | 51.44±7.91 | 66.62±8.27 | 59.74±9.47 | 59.47±8.86 | 13679.46±1432.75 |

Table 49 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | 5.78±3.76 | 4.44±3.64 | 2.02±4.29 | 7.99±4.20 | 17.11±3.12 | 18.61±2.12 | 17.89±3.30 | 4074.49±436.22 |
| 50 | 12.07±5.38 | 5.52±5.52 | 13.76±5.54 | 5.54±6.93 | 17.45±6.81 | 16.59±4.17 | 15.31±5.09 | 3280.93±912.09 |
| 100 | 9.65±3.13 | 30.32±9.03 | 15.60±6.44 | 7.68±6.19 | 16.99±3.29 | 30.62±3.68 | 16.26±4.01 | 4788.47±472.01 |
| 200 | 18.88±4.26 | 15.57±3.76 | 14.68±4.11 | 15.87±3.05 | 25.62±4.15 | 33.91±3.26 | 27.25±5.17 | 5550.33±586.79 |
| 400 | 34.38±7.53 | 16.00±5.24 | 20.93±5.66 | 33.24±8.79 | 46.98±10.30 | 51.14±9.23 | 44.85±9.53 | 8354.51±1628.98 |

Table 50 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| FR (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | -12.13±15.82 | -0.14±11.16 | -13.45±14.44 | -2.60±16.39 | 50.83±11.01 | 61.11±14.29 | 29.85±12.84 | 7074.25±2285.75 |
| 50 | -15.27±20.05 | 6.58±11.54 | 38.92±14.69 | 40.00±9.07 | 26.53±12.66 | 59.52±9.40 | 8.48±17.40 | 7353.23±1976.46 |
| 100 | 49.19±14.29 | 29.88±10.57 | 48.02±15.23 | 64.74±9.29 | 54.78±13.42 | 62.39±11.67 | 85.89±8.54 | 14839.99±1223.01 |
| 200 | 82.77±12.65 | 76.79±11.65 | 61.88±10.72 | 83.19±9.14 | 68.24±10.54 | 89.08±8.79 | 70.66±12.26 | 16030.41±1552.24 |
| 400 | 91.53±6.98 | 93.76±7.56 | 88.79±5.77 | 66.17±10.24 | 76.15±8.78 | 94.89±6.33 | 93.24±5.24 | 19682.32±708.32 |

Table 51 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| BEN (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | 2.69±3.49 | 1.47±9.88 | 4.67±7.78 | -0.04±7.80 | -0.72±6.70 | 11.47±7.60 | 6.13±11.70 | 2066.04±1640.06 |
| 50 | 22.32±7.03 | 7.09±6.72 | 14.05±5.50 | 23.43±4.86 | 26.13±4.33 | 31.11±9.37 | 25.91±14.57 | 5850.36±1516.25 |
| 100 | 17.94±10.36 | 16.12±8.62 | 8.86±8.46 | 12.65±11.51 | 18.53±14.80 | 30.71±8.58 | -0.13±8.67 | 3779.60±1548.00 |
| 200 | 7.86±10.21 | 7.70±7.93 | 10.34±15.86 | 30.93±10.69 | 38.98±9.54 | 26.42±11.56 | 34.17±9.68 | 6989.44±1963.43 |
| 400 | 17.48±7.83 | 12.14±10.35 | 27.72±4.25 | 34.84±6.54 | 34.80±6.41 | 54.97±5.21 | 26.75±4.82 | 8205.52±724.12 |

# Appendix E

# Data of Mouse Tail-flick Test

Table 52 Latency (sec) in mouse tail-flick test from 0-240 min after oral administration of various doses of *Capparis micarcantha* root extract (CM; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CM (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 1.53±0.06 | 1.41±0.05 | 1.34±0.07 | 1.51±0.10 | 1.74±0.27 | 1.57±0.16 | 1.57±0.14 |
| 50 | 1.27±0.06 | 1.45±0.03 | 1.51±0.12 | 1.40±0.07 | 1.47±0.13 | 1.33±0.08 | 1.23±0.09 |
| 100 | 1.49±0.13 | 1.38±0.05 | 1.70±0.13 | 1.60±0.22 | 1.78±0.13 | 1.78±0.17 | 1.48±0.08 |
| 200 | 1.43±0.09 | 1.45±0.10 | 1.42±0.08 | 1.57±0.13 | 1.60±0.07 | 1.45±0.09 | 1.39±0.08 |
| 400 | 1.59±0.15 | 1.66±0.19 | 1.50±0.06 | 1.76±0.22 | 1.64±0.10 | 1.63±0.18 | 1.42±0.08 |

Table 53 Latency (sec) in mouse tail-flick test from 0-240 min after oral administration of various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| HP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 1.28±0.19 | 1.29±0.29 | 1.21±0.15 | 1.16±0.19 | 1.30±0.12 | 1.61±0.30 | 1.93±0.30 |
| 50 | 1.47±0.29 | 1.36±0.18 | 1.42±0.15 | 1.34±0.14 | 1.95±0.29 | 2.07±0.37 | 2.13±0.23 |
| 100 | 1.34±0.19 | 1.11±0.03 | 1.23±0.11 | 1.27±0.16 | 1.44±0.18 | 1.53±0.19 | 1.96±0.25 |
| 200 | 1.03±0.02 | 1.08±0.03 | 1.43±0.29 | 1.56±0.19 | 1.31±0.12 | 1.38±0.13 | 1.58±0.11 |
| 400 | 1.00±0.04 | 1.02±0.02 | 1.44±0.17 | 1.48±0.16 | 1.32±0.13 | 1.55±0.12 | 1.82±0.16 |

Table 54 Latency (sec) in mouse tail-flick test from 0-240 min after oral administration of various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| TT (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 1.47±0.06 | 1.73±0.12 | 1.60±0.09 | 1.62±0.09 | 1.52±0.16 | 1.37±0.09 | 2.02±0.23 |
| 50 | 1.30±0.06 | 1.44±0.07 | 1.30±0.09 | 1.37±0.11 | 1.60±0.14 | 1.65±0.21 | 2.06±0.23 |
| 100 | 1.3 ±0.06 | 1.53±0.07 | 1.48±0.15 | 1.48±0.11 | 1.25±0.11 | 1.43±0.13 | 1.84±0.12 |
| 200 | 1.44±0.12 | 1.42±0.13 | 1.49±0.18 | 1.30±0.06 | 1.37±0.09 | 1.46±0.15 | 2.00±0.12 |
| 400 | 1.40±0.10 | 1.30±0.06 | 1.60±0.23 | 1.45±0.07 | 1.31±0.05 | 1.49±0.10 | 1.89±0.12 |

Table 55 Latency (sec) in mouse tail-flick test from 0-240 min after oral administration of various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 2.15±0.19 | 2.25±0.24 | 1.77±0.09 | 2.08±0.22 | 2.34±0.23 | 2.10±0.19 | 2.49±0.34 |
| 50 | 1.64±0.11 | 2.08±0.17 | 1.96±0.12 | 2.48±0.22 | 1.84±0.12 | 2.17±0.26 | 2.34±0.36 |
| 100 | 1.77±0.09 | 1.70±0.12 | 2.17±0.09 | 2.11±0.19 | 2.12±0.21 | 2.06±0.25 | 1.62±0.09 |
| 200 | 2.06±0.11 | 2.48±0.28 | 2.23±0.08 | 1.94±0.11 | 1.99±0.15 | 2.16±0.20 | 1.87±0.15 |
| 400 | 2.11±0.16 | 1.79±0.05 | 2.09±0.18 | 2.06±0.10 | 2.17±0.13 | 1.78±0.09 | 1.88±0.14 |

Table 56 Latency (sec) in mouse tail-flick test from 0-240 min after oral administration of various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| FR (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 1.72±0.12 | 1.66±0.22 | 1.7±0.15 | 1.63±0.15 | 1.62±0.12 | 1.90±0.29 | 1.75±0.16 |
| 50 | 1.76±0.17 | 1.59±0.19 | 1.33±0.04 | 1.45±0.08 | 1.43±0.13 | 1.58±0.13 | 1.73±0.17 |
| 100 | 1.53±0.11 | 1.42±0.09 | 1.41±0.08 | 2.54±0.14 | 1.74±0.15 | 1.53±0.09 | 1.69±0.13 |
| 200 | 1.87±0.27 | 1.95±0.25 | 1.71±0.11 | 1.52±0.16 | 1.69±0.13 | 1.90±0.29 | 1.85±0.23 |
| 400 | 1.38±0.09 | 1.43±0.07 | 1.4±0.06 | 1.25±0.04 | 1.60±0.12 | 1.51±0.07 | 1.61±0.13 |

Table 57 Latency (sec) in mouse tail-flick test from 0-240 min after oral administration of various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| BEN (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 25 | 1.40±0.16 | 1.25±0.06 | 1.45±0.16 | 1.27±0.19 | 1.81±0.23 | 1.44±0.09 | 1.90±0.25 |
| 50 | 1.09±0.03 | 1.16±0.07 | 1.16±0.09 | 1.33±0.13 | 1.84±0.26 | 1.75±0.14 | 1.61±0.16 |
| 100 | 1.19±0.09 | 1.14±0.07 | 1.07±0.04 | 1.34±0.13 | 1.62±0.16 | 1.71±0.16 | 1.68±0.27 |
| 200 | 1.25±0.06 | 1.29±0.11 | 1.44±0.19 | 1.30±0.13 | 1.73±0.25 | 1.75±0.29 | 2.00±0.31 |
| 400 | 1.34±0.29 | 1.39±0.21 | 1.22±0.12 | 1.47±0.21 | 1.99±0.34 | 1.75±0.16 | 1.95±0.28 |

Table 58 %MPE-Time in mouse tail-flick from 0-240 min after oral administration of various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CM (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | -3.17±0.24 | -3.45±0.31 | -3.61±0.33 | -3.22±0.41 | -2.69±0.65 | -3.08±0.37 | -3.08±0.47 | 5.92±69.07 |
| 50 | -0.46±0.19 | -0.05±0.17 | 0.09±0.21 | -0.16±0.27 | -0.003±0.35 | -0.32±0.25 | -0.55±0.17 | -67.52±38.11 |
| 100 | -0.05±0.21 | -0.30±0.26 | 0.43±0.29 | 0.20±0.55 | 0.59±0.48 | 0.62±0.42 | -0.07±0.32 | 65.22±68.05 |
| 200 | 0.10±0.23 | 0.15±0.31 | -0.08±0.18 | 0.15±0.29 | 0.34±0.20 | 0.08±0.19 | -0.15±0.19 | 40.95±33.43 |
| 400 | 0.05±0.32 | 0.21±0.42 | -0.12±0.28 | 0.73±0.51 | 0.30±0.25 | 0.69±0.41 | -0.31±0.31 | 5.19±55.28 |

Table 59 %MPE-Time in mouse tail-flick test from 0-240 min after oral administration of various doses of *Harrisonia perforata* root extract (HP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| HP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | -2.49±0.47 | -2.47±0.64 | -2.66±0.38 | -2.77±0.47 | -2.45±0.37 | -1.75±0.73 | -1.03±0.79 | 285.03±106.09 |
| 50 | 0.84±0.68 | 0.59±0.38 | 0.73±0.34 | 0.54±0.47 | 1.93±0.66 | 2.21±0.84 | 2.34±0.59 | 408.48±120.78 |
| 100 | 0.95±0.43 | 0.43±0.15 | 0.70±0.22 | 0.79±0.36 | 1.39±0.32 | 1.38±0.42 | 2.36±0.51 | 336.17±52.93 |
| 200 | 0.29±0.09 | 0.41±0.06 | 1.64±0.65 | 1.15±0.39 | 1.04±0.30 | 0.96±0.26 | 1.94±0.29 | 264.25±31.26 |
| 400 | -0.02±0.18 | 0.02±0.12 | 0.45±0.10 | 0.45±0.39 | 0.76±0.27 | 0.83±0.31 | 1.74±0.42 | 262.08±56.32 |

Table 60 %MPE-Time in mouse tail-flick test from 0-240 min after oral administration of various doses of *Tiliacora triandra* root extract (TT; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| TT (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | -2.62±0.15 | -2.03±0.34 | -2.32±0.21 | -2.28±0.26 | -2.51±0.45 | -2.85±0.27 | -1.36±0.54 | 201.89±57.52 |
| 50 | -0.02±0.15 | 0.29±0.20 | -0.02±0.20 | 0.14±0.25 | 0.66±0.30 | 0.78±0.43 | 1.72±0.48 | 188.33±54.55 |
| 100 | -0.21±0.19 | 0.32±0.28 | 0.21±0.36 | 0.21±0.26 | -0.21±0.27 | 0.09±0.33 | 1.03±0.32 | 71.86±59.06 |
| 200 | 0.31±0.26 | 0.26±0.29 | -0.09±0.39 | -0.06±0.12 | 0.02±0.21 | -0.21±0.31 | 1.69±0.29 | 141.21±40.45 |
| 400 | 0.22±0.35 | -0.003±0.25 | 0.46±0.31 | 0.15±0.25 | -0.31±0.21 | 0.26±0.32 | 1.22±0.36 | 135.01±63.32 |

Table 61 %MPE-Time in mouse tail-flick test from 0-240 min after oral administration of various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| CP (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | -1.37±0.45 | -1.14±0.52 | -2.24±0.24 | -1.53±0.55 | -0.93±0.49 | -1.48±0.46 | -0.59±0.79 | 448.06±94.82 |
| 50 | 0.57±0.31 | 1.58±0.43 | 1.31±0.31 | 2.49±0.54 | 1.03±0.31 | 1.78±0.68 | 2.17±0.89 | 402.99±121.96 |
| 100 | 0.96±0.29 | 0.80±0.31 | 1.88±0.23 | 1.74±0.49 | 1.76±0.55 | 1.62±0.60 | 0.62±0.29 | 305.34±81.01 |
| 200 | 1.58±0.27 | 2.54±0.63 | 1.98±0.22 | 1.53±0.32 | 1.76±0.34 | 1.99±0.48 | 1.64±0.38 | 367.72±61.09 |
| 400 | 1.73±0.37 | 0.99±0.17 | 1.62±0.47 | 1.77±0.31 | 2.42±0.31 | 1.43±0.25 | 1.89±0.34 | 303.32±49.32 |

Table 62 %MPE-Time in mouse tail-flick test from 0-240 min after oral administration of various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| FR (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | 0.25±0.33 | 0.12±0.32 | -0.19±0.43 | -0.31±0.35 | 0.29±0.34 | 0.68±0.46 | 0.84±0.33 | 80.01±39.97 |
| 50 | -2.41±0.32 | -2.79±0.42 | -3.39±0.18 | -3.12±0.24 | -3.17±0.29 | -2.83±0.39 | -2.48±0.45 | 73.00±56.47 |
| 100 | 0.26±0.39 | 0.01±0.31 | -0.01±0.19 | 0.29±0.22 | 0.74±0.33 | 0.26±0.19 | 0.63±0.35 | 89.97±35.89 |
| 200 | 0.73±0.53 | 0.91±0.53 | 0.36±0.25 | -0.09±0.45 | 0.31±0.35 | 0.79±0.58 | 0.68±0.46 | 137.82±72.33 |
| 400 | -0.48±0.22 | -0.37±0.17 | -0.44±0.15 | -0.78±0.13 | 0.02±0.28 | -0.19±0.18 | 0.04±0.33 | -47.56±40.71 |

Table 63 %MPE-Time in mouse tail-flick test from 0-240 min after oral administration of various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg). N=10 for all groups and data were expressed as mean ± S.E.M.

| BEN (mg/kg) | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 25 | -2.66±0.37 | -3.01±0.18 | -2.55±0.34 | -2.96±0.43 | -1.73±0.57 | -2.57±0.26 | -1.52±0.55 | 202.00±41.07 |
| 50 | -0.07±0.15 | 0.09±0.16 | 0.09±0.27 | 0.48±0.36 | 1.64±0.65 | 1.43±0.35 | 1.12±0.35 | 236.12±44.21 |
| 100 | 0.31±0.20 | 0.19±0.17 | 0.03±0.15 | 0.65±0.34 | 1.24±0.39 | 1.49±0.38 | 1.42±0.63 | 256.65±62.16 |
| 200 | 0.39±0.22 | 0.48±0.28 | 1.25±0.43 | 0.49±0.32 | 1.56±0.56 | 0.83±0.66 | 1.71±0.74 | 320.77±88.81 |
| 400 | 0.66±0.70 | 0.77±0.48 | 0.66±0.30 | 1.12±0.49 | 2.86±0.79 | 1.23±0.36 | 2.44±0.64 | 355.09±91.66 |

# Appendix F

## Data of Acetic Acid-induced Writhing Test in Mice

Table 64 The writhing response of various doses of *Capparis micracantha* root extract (CM; 25-400 mg/kg) from 0-30 min on acetic acid-induced writhing in mice. N=6 for all groups. Data were expressed as mean ± S.E.M.

| Treatments | Time (5 min interval) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 | |
| 2%Tween 80 | 0.50±0.22 | 2.50±1.34 | 3.50±1.31 | 5.50±1.50 | 4.50±1.38 | 7.17±2.19 | 23.67±6.03 |
| 25 mg/kg | 0.00±0.00 | 1.67±0.49 | 3.33±1.38 | 2.83±0.70 | 1.83±0.40 | 2.33±0.71 | 12.00±3.17 |
| 50 mg/kg | 0.50±0.34 | 1.17±0.17 | 2.83±0.70 | 2.83±0.75 | 1.83±0.60 | 1.33±0.49 | 10.50±1.78 |
| 100 mg/kg | 0.67±0.21 | 1.83±0.40 | 2.50±0.50 | 1.33±0.33 | 1.50±0.43 | 0.67±0.33 | 8.50±0.67 |
| 200 mg/kg | 0.17±0.17 | 1.29±0.33 | 1.00±0.45 | 2.00±0.52 | 1.67±0.49 | 1.00±0.37 | 7.17±1.54 |
| 400 mg/kg | 0.17±0.17 | 1.50±0.34 | 2.67±0.92 | 2.00±0.73 | 1.67±0.67 | 1.17±0.31 | 9.17±2.71 |

Table 65 The writhing response of various doses of *Harrisonia perforata* root extract (HP 25-400 mg/kg) from 0-30 min on acetic acid-induced writhing in mice. N=6 for all groups. Data were expressed as mean ± S.E.M.

| Treatments | Time (5 min interval) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 | |
| 2%Tween 80 | 0.50±0.22 | 2.50±1.34 | 3.50±1.31 | 5.50±1.50 | 4.50±1.38 | 7.17±2.19 | 23.67±6.03 |
| 25 mg/kg | 0.33±0.21 | 1.50±0.22 | 2.00±0.37 | 2.83±0.87 | 1.33±0.21 | 2.67±0.67 | 10.67±1.45 |
| 50 mg/kg | 0.17±0.17 | 1.67±0.33 | 2.67±0.80 | 3.17±0.54 | 1.17±0.31 | 1.50±0.76 | 10.33±2.09 |
| 100 mg/kg | 0.50±0.34 | 1.50±0.22 | 1.83±0.48 | 2.33±0.80 | 1.67±0.71 | 1.00±0.45 | 8.83±1.96 |
| 200 mg/kg | 0.00±0.00 | 1.33±0.21 | 1.33±0.21 | 1.00±0.26 | 1.33±0.42 | 0.50±0.34 | 5.50±0.76 |
| 400 mg/kg | 0.17±0.17 | 2.00±0.82 | 2.33±0.95 | 1.83±0.40 | 1.00±0.82 | 0.50±0.34 | 7.83±2.85 |

Table 66 The writhing response of various doses of *Tiilacora triandra* root extract (TT; 25-400 mg/kg) from 0-30 min on acetic acid-induced writhing in mice. N=6 for all groups. Data were expressed as mean ± S.E.M.

| Treatments | Time (5 min interval) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 | |
| 2%Tween 80 | 0.50±0.22 | 2.50±1.34 | 3.50±1.31 | 5.50±1.50 | 4.50±1.38 | 7.17±2.19 | 23.67±6.03 |
| 25 mg/kg | 0.50±0.34 | 3.17±1.45 | 4.00±1.51 | 2.50±0.56 | 2.17±0.48 | 2.50±1.06 | 14.83±4.49 |
| 50 mg/kg | 0.50±0.22 | 1.33±0.21 | 2.67±0.49 | 2.00±0.37 | 2.50±0.56 | 1.50±0.56 | 10.50±1.06 |
| 100 mg/kg | 0.67±0.33 | 2.00±0.68 | 2.33±0.71 | 1.67±0.49 | 1.33±0.42 | 1.00±0.26 | 9.00±1.48 |
| 200 mg/kg | 0.67±0.33 | 2.50±0.72 | 1.50±0.56 | 1.17±0.60 | 1.17±0.40 | 0.50±0.34 | 7.50±2.26 |
| 400 mg/kg | 0.33±0.21 | 1.33±0.21 | 1.67±0.56 | 1.00±0.26 | 1.50±0.22 | 0.50±0.34 | 6.33±1.23 |

Table 67 The writhing response of various doses of *Clerodendrum petasites* root extract (CP; 25-400 mg/kg) from 0-30 min on acetic acid-induced writhing in mice. N=6 for all groups. Data were expressed as mean ± S.E.M.

| Treatments | Time (5 min interval) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 | |
| 2%Tween 80 | 0.50±0.22 | 2.50±1.34 | 3.50±1.31 | 5.50±1.50 | 4.50±1.38 | 7.17±2.19 | 23.67±6.03 |
| 25 mg/kg | 0.17±0.17 | 2.00±0.63 | 2.00±0.37 | 2.33±0.61 | 2.00±0.58 | 2.17±0.70 | 10.67±1.78 |
| 50 mg/kg | 0.17±0.17 | 1.33±0.21 | 1.17±0.17 | 1.17±0.31 | 1.50±0.50 | 1.50±0.56 | 6.83±0.95 |
| 100 mg/kg | 0.17±0.17 | 1.00±0.00 | 1.33±0.21 | 1.00±0.37 | 0.50±0.22 | 1.00±0.52 | 5.00±1.21 |
| 200 mg/kg | 0.17±0.17 | 1.50±0.43 | 1.50±0.43 | 1.00±0.45 | 0.50±0.34 | 0.67±0.42 | 5.33±1.45 |
| 400 mg/kg | 0.17±0.17 | 1.33±0.21 | 1.67±0.42 | 0.50±0.22 | 0.33±0.21 | 0.33±0.33 | 4.33±1.09 |

Table 68 The writhing response of various doses of *Ficus racemosa* root extract (FR; 25-400 mg/kg) from 0-30 min on acetic acid-induced writhing in mice. N=6 for all groups. Data were expressed as mean ± S.E.M.

| Treatments | Time (5 min interval) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 | |
| 2%Tween 80 | 0.50±0.22 | 2.50±1.34 | 3.50±1.31 | 5.50±1.50 | 4.50±1.38 | 7.17±2.19 | 23.67±6.03 |
| 25 mg/kg | 0.17±0.17 | 1.67±0.67 | 3.83±1.25 | 2.17±0.70 | 1.83±0.48 | 1.50±0.22 | 11.17± 2.52 |
| 50 mg/kg | 0.17±0.17 | 1.17±0.31 | 2.33±0.42 | 2.50±0.56 | 1.67±0.49 | 1.67±0.33 | 9.50± 1.43 |
| 100 mg/kg | 0.17±0.17 | 2.17±0.60 | 1.83±0.48 | 1.00±0.50 | 1.67±0.37 | 8.33±0.33 | 8.33± 0.80 |
| 200 mg/kg | 0.33±0.21 | 3.00±1.00 | 3.17±1.40 | 2.83±0.95 | 2.33±0.92 | 1.33±0.80 | 13.00±3.86 |
| 400 mg/kg | 0.83±0.31 | 2.67±0.67 | 4.17±1.08 | 3.17±0.91 | 2.00±0.58 | 1.17±0.48 | 14.00±3.17 |

Table 69 The writhing response of various doses of Bencha-loga-wichian remedy (BEN; 25-400 mg/kg) from 0-30 min on acetic acid-induced writhing in mice. N=6 for all groups. Data were expressed as mean ± S.E.M.

| Treatments | Time (5 min interval) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 0-5 | 5-10 | 10-15 | 15-20 | 20-25 | 25-30 | |
| 2%Tween 80 | 0.50± 0.22 | 2.50±1.34 | 3.50±1.31 | 5.50±1.50 | 4.50±1.38 | 7.17±2.19 | 23.67±6.03 |
| 25 mg/kg | 0.83±0.65 | 2.50±0.67 | 2.50±0.76 | 3.17±1.40 | 3.00±1.24 | 2.50±1.18 | 14.50±4.61 |
| 50 mg/kg | 0.17±0.17 | 1.33±0.21 | 2.83±0.40 | 2.00±0.37 | 2.33±0.71 | 2.67±0.80 | 1.89±1.84 |
| 100 mg/kg | 0.50±0.34 | 1.50±0.22 | 2.67±0.71 | 2.17±0.60 | 1.67±0.61 | 1.83±0.40 | 10.33±2.16 |
| 200 mg/kg | 0.17±0.17 | 1.17±0.17 | 1.67±0.56 | 2.00±0.63 | 1.83±0.40 | 1.83±0.70 | 8.67±2.32 |
| 400 mg/kg | 0.17±0.17 | 1.33±0.21 | 2.33±0.61 | 1.67±0.33 | 1.83±0.48 | 1.83±0.75 | 9.17±1.54 |

# Appendix G

## Data of the Study of Mechanism of Analgesic Action of Five Herbal Root Extracts of Bencha-loga-wichian Remedy in the Mouse Hot-plate Test

Table 70 Latency (sec) in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Capparis micracantha* (CM; 200 mg/kg, p.o.) and the combination of naloxone and CM (5/200 mg/kg). N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 24.98±1.05 | 25.63±1.77 | 20.82±1.28 | 21.38±1.14 | 22.72±2.23 | 20.56±1.71 | 21.69±1.17 |
| Naloxone 5 mg/kg | 10.18±0.50 | 10.65±1.02 | 9.89±1.24 | 11.84±1.23 | 12.51±1.10 | 13.80±1.01 | 11.90±0.99 |
| CM 200 mg/kg | 27.02±2.45 | 23.96±1.70 | 28.19±2.36 | 28.26±2.70 | 24.15±1.2 | 26.76±2.25 | 31.09±2.00 |
| Naloxone + CM | 15.86±1.29 | 17.63±1.11 | 18.00±1.15 | 21.44±1.99 | 21.47±2.17 | 19.49±1.58 | 15.81±0.45 |

Table 71 Latency (sec) in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Harrisonia perforata* (HP; 400 mg/kg, p.o.) and the combination of naloxone and HP (5/400 mg/kg). N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 24.98±1.05 | 25.63±1.77 | 20.82±1.28 | 21.38±1.14 | 22.72±2.23 | 20.56±1.71 | 21.69±1.17 |
| Naloxone 5 mg/kg | 10.18±0.50 | 10.65±1.02 | 9.89±1.24 | 11.84±1.23 | 12.51±1.10 | 13.80±1.01 | 11.90±0.99 |
| HP 400 mg/kg | 19.53±1.33 | 23.08±2.19 | 27.79±3.15 | 26.72±2.37 | 25.42±1.79 | 30.12±1.99 | 31.60±2.24 |
| Naloxone + HP | 16.74±2.37 | 13.65±1.19 | 21.65±3.45 | 14.22±1.32 | 18.90±2.38 | 19.69±1.40 | 19.71±2.32 |

Table 72 Latency (sec) in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Tiliacora triandra* (TT; 400 mg/kg, p.o.) and the combination of naloxone and TT (5/400 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 24.98±1.05 | 25.63±1.77 | 20.82±1.28 | 21.38±1.14 | 22.72±2.23 | 20.56±1.71 | 21.69±1.17 |
| Naloxone 5 mg/kg | 10.18±0.50 | 10.65±1.02 | 9.89±1.24 | 11.84±1.23 | 12.51±1.10 | 13.80±1.01 | 11.90±0.99 |
| TT 400 mg/kg | 24.55±2.15 | 27.18±1.21 | 26.97±2.01 | 24.24±1.51 | 28.35±1.61 | 30.75±1.35 | 28.66±2.89 |
| Naloxone + TT | 9.87±0.54 | 12.30±1.06 | 13.90±2.08 | 12.10±1.34 | 12.34±0.9 | 13.83±1.11 | 13.69±0.88 |

Table 73 Latency (sec) in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Clerodendrum petasites* (CP; 400 mg/kg, p.o.) and the combination of naloxone and CP (5/400 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 24.98±1.05 | 25.63±1.77 | 20.82±1.28 | 21.38±1.14 | 22.72±2.23 | 20.56±1.71 | 21.69±1.17 |
| Naloxone 5 mg/kg | 10.18±0.50 | 10.65±1.02 | 9.89±1.24 | 11.84±1.23 | 12.51±1.10 | 13.80±1.01 | 11.90±0.99 |
| CP 400 mg/kg | 17.68±2.11 | 15.99±1.28 | 17.80±1.32 | 19.95±3.21 | 34.11±2.29 | 34.19±1.43 | 31.66±1.87 |
| Naloxone + CP | 18.75±1.81 | 19.73±1.20 | 19.02±1.91 | 17.45±2.34 | 22.22±1.40 | 17.16±1.59 | 14.88±1.18 |

Table 74 Latency (sec) in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Ficus racemosa* (FR; 400 mg/kg, p.o.) and the combination of naloxone and FR (5/400 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min |
|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 24.98±1.05 | 25.63±1.77 | 20.82±1.28 | 21.38±1.14 | 22.72±2.23 | 20.56±1.71 | 21.69±1.17 |
| Naloxone 5 mg/kg | 10.18±0.50 | 10.65±1.02 | 9.89±1.24 | 11.84±1.23 | 12.51±1.10 | 13.80±1.01 | 11.90±0.99 |
| FR 400 mg/kg | 26.35±3.44 | 24.02±2.62 | 26.61±1.58 | 19.81±2.12 | 31.05±2.60 | 28.68±1.93 | 34.41±2.63 |
| Naloxone + FR | 12.64±1.64 | 15.60±3.38 | 14.74±1.79 | 16.09±2.43 | 17.27±1.79 | 15.55±2.89 | 14.14±1.81 |

Table 75 %MPE-Time in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Capparis micracantha* (CM; 200 mg/kg, p.o.) and the combination of naloxone and CM (5/200 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 22.40±3.30 | 24.53±6.66 | 5.01±8.01 | 7.81±6.36 | 12.72±9.38 | 5.30±7.22 | 8.80±6.75 | 2990.14±1205.52 |
| Naloxone 5 mg/kg | -31.46±4.73 | -29.33±5.03 | -32.02±5.30 | -24.37±4.07 | -22.59±5.64 | -17.09±6.58 | -25.14±6.03 | -5408.04±940 |
| CM 200 mg/kg | 34.22±7.68 | 21.21±4.56 | 35.93±8.85 | 36.15±10.33 | 19.95±6.29 | 27.98±11.25 | 44.69±9.53 | 7561.53±1535.53 |
| Naloxone+CM | -25.86±6.66 | -18.36±6.23 | -21.33±7.19 | 5.89±8.90 | -3.41±9.00 | -20.44±7.66 | -29.09±6.82 | -3371.07±1265.87 |

Table 76 %MPE-Time in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Harrisonia perforata* (HP; 400 mg/kg, p.o.) and the combination of naloxone and HP (5/400 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 22.40±3.30 | 24.53±6.66 | 5.01±8.01 | 7.81±6.36 | 12.72±9.38 | 5.30±7.22 | 8.80±6.75 | 2990.14±1205.52 |
| Naloxone 5 g/kg | -31.46±4.73 | -29.33±5.03 | -32.02±5.30 | -24.37±4.07 | -22.59±5.64 | -17.09±6.58 | -25.14±6.03 | -5408.04±940 |
| HP 400 mg/kg | 11.28±3.78 | 23.95±7.20 | 41.20±10.78 | 38.39±7.81 | 24.71±6.61 | 48.59±6.63 | 54.43±7.53 | 9661.99±1079.25 |
| Naloxone+HP | -2.57±6.13 | -16.03±4.19 | 21.64±10.41 | -10.36±7.57 | 17.65±9.09 | 8.015±7.37 | 12.37±7.65 | 533.32±1073.72 |

Table 77 %MPE-Time in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Tiliacora triandra* (TT; 400 mg/kg, p.o.) and the combination of naloxone and TT (5/400 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 22.40±3.30 | 24.53±6.66 | 5.01±8.01 | 7.81±6.36 | 12.72±9.38 | 5.30±7.22 | 8.80±6.75 | 2990.14±1205.52 |
| Naloxone 5 mg/kg | -31.46±4.73 | -29.33±5.03 | -32.02±5.30 | -24.37±4.07 | -22.59±5.64 | -17.09±6.58 | -25.14±6.03 | -5408.04±940 |
| TT 400 mg/kg | 25.04±6.81 | 34.01±3.77 | 33.96±7.25 | 22.22±5.93 | 31.45±5.35 | 47.51±4.64 | 40.72±10.74 | 8844.85±1035 |
| Naloxone+TT | -28.88±8.34 | -19.24±6.47 | -24.21±11.20 | -23.76±8.49 | -22.32±4.66 | -20.36±8.66 | -21.43±7.14 | -3925.36±1646.10 |

Table 78 %MPE-Time in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Clerodendrum petasites* (CP; 400 mg/kg, p.o.) and the combination of naloxone and CP (5/400 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 22.40±3.30 | 24.53±6.66 | 5.01±8.01 | 7.81±6.36 | 12.72±9.38 | 5.30±7.22 | 8.80±6.75 | 2990.14±1205.52 |
| Naloxone 5 mg/kg | -31.46±4.73 | -29.33±5.03 | -32.02±5.30 | -24.37±4.07 | -22.59±5.64 | -17.09±6.58 | -25.14±6.03 | -5408.04±940 |
| CP 400 mg/kg | 13.03±7.91 | 7.61±6.32 | 13.44±6.27 | 21.03±9.97 | 71.03±8.03 | 65.72±4.95 | 57.58±6.27 | 11499.00±1153.68 |
| Naloxone+CP | 11.57±3.21 | 12.37±6.21 | 2.52±7.83 | -7.89±10.65 | 18.87±9.97 | 1.00±11.67 | -7.03±8.46 | 1008.90±1874.23 |

Table 79 %MPE-Time in mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg, i.p.), 2%Tween 80 (10 ml/kg, p.o.), *Ficus racemosa* (FR; 400 mg/kg, p.o.) and the combination of naloxone and FR (5/400 mg/kg), N=10 for all groups. Data were expressed as mean±S.E.M.

| Treatments | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | 240 min | Area of analgesia |
|---|---|---|---|---|---|---|---|---|
| 2%Tween 80 | 22.40±3.30 | 24.53±6.66 | 5.01±8.01 | 7.81±6.36 | 12.72±9.38 | 5.30±7.22 | 8.80±6.75 | 2990.14±1205.52 |
| Naloxone 5 mg/kg | -31.46±4.73 | -29.33±5.03 | -32.02±5.30 | -24.37±4.07 | -22.59±5.64 | -17.09±6.58 | -25.14±6.03 | -5408.04±940 |
| FR 400 mg/kg | 34.11±11.77 | 24.68±8.31 | 32.42±6.16 | 7.87±8.38 | 52.45±12.25 | 37.95±8.12 | 60.78±10.16 | 9611.94±1321.00 |
| Naloxone+FR | -32.98±27.18 | -9.53±13.77 | 6.17±24.42 | 3.22±7.65 | 13.37±16.47 | 2.72±5.48 | 1.93±23.65 | -3119.75±2972.40 |

# VITAE

Miss Anusara Jongchanapong was born on December 24, 1983 at Siriraj Hospital, Bangkok, Thailand. She graduated with Bachelor of Science in Industrial Physics and Medical Instrumentations (Medical Instrumentations) in 2006 from King Mongkut's Institute of Technology North Bangkok. After graduation, she had worked as an employee of the Medical Equipment Center of Klang Hospital, Bangkok, Thailand for 6 months.