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ANTINOCICEPTIVE, ANTIPYRETIC AND ANTI-INFLAMMATORY EFFECTS OF THE ETHANOLIC EXTRACT OF *FICUS RACEMOSA* ROOT

Miss Sirakarn Chomchuen

A Thesis Submitted in Partial Fulfillment of the Requirements
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รากของต้นมะเดื่อชุมพรหรือมะเดื่ออุทุมพรถูกนำมาใช้เป็นยาลดไข้ตั้งแต่สมัยโบราณ ในการทดลองครั้งนี้มุ่งศึกษาฤทธิ์ระงับปวด ฤทธิ์ลดไข้และฤทธิ์ต้านการอักเสบของสิ่งสกัดด้วยเอทานอลจากรากมะเดื่อชุมพรในขนาดต่างๆ ในการทดสอบฤทธิ์ระงับปวดในหนูเม้าส์ด้วยวิธี Tail-flick ทำการจับเวลาที่หนูสามารถทนต่อความร้อนได้โดยไม่กระดกหางหนีก่อนให้น้ำเกลือ มอร์ฟีน (10 มก. /กก.) 2% ทวีน 80 หรือสิ่งสกัดจากรากมะเดื่อชุมพรขนาด 50-400 มก. /กก. ทางช่องท้อง และจับเวลาที่หนูทนต่อความร้อนได้ที่เวลา 15, 30, 45, 60, 90, 120 และ 240 นาทีหลังให้สารทดสอบ คำนวณหาเปอร์เซ็นต์สูงสุดที่หนูสามารถทนต่อความร้อนได้ (%MPE) แล้วนำมาคำนวณหาพื้นที่ใต้กราฟระหว่าง %MPEและเวลา (area of analgesia) พบว่าสิ่งสกัดจากรากมะเดื่อชุมพรในขนาด 200 และ 400 มก. /กก. มีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ ส่วนการทดสอบที่เหนี่ยวนำให้หนูเม้าส์เกิดการบิดงอของลำตัว (Writhing) ด้วยกรดอะซิติก ทำโดยการฉีดกรดอะซิติก 0.6% ในขนาด 10 มล. /กก. เข้าทางช่องท้องที่เวลา 30 นาที หลังจากฉีดสารละลาย 2% ทวีน 80, อินโดเมทาซิน (10 มก. /กก.) หรือสิ่งสกัดจากรากมะเดื่อชุมพรขนาด 50-400 มก. /กก. ทางช่องท้องแล้วนับจำนวนครั้งที่หนูเกิดการบิดงอของลำตัวเป็นเวลา 30 นาที พบว่าสิ่งสกัดจากรากมะเดื่อชุมพรทุกขนาดที่ใช้ในการทดสอบสามารถลดการบิดงอของลำตัวของหนูได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม

สำหรับการทดสอบฤทธิ์ลดไข้ของสิ่งสกัดด้วยเอทานอลจากรากมะเดื่อชุมพรในหนูแรทที่เหนี่ยวนำให้เกิดไข้ด้วย LPS ทำการเหนี่ยวนำให้หนูแรทเกิดไข้โดยการฉีด LPS ขนาด 50 ไมโครกรัม/กก. เข้าทางกล้ามเนื้อที่เวลา 1 ชม. หลังจากป้อนสารละลาย 2% ทวีน 80 แอสไพริน (300 มก. /กก.) หรือสิ่งสกัดจากรากมะเดื่อชุมพรขนาด 50-800 มก. /กก. แล้วทำการวัดอุณหภูมิทางทวารหนักของหนูที่เวลา 1-7 ชม.หลังจากฉีด LPS พบว่าสิ่งสกัดจากรากมะเดื่อชุมพรทุกขนาดที่ใช้ในการทดสอบสามารถยับยั้งอุณหภูมิที่เพิ่มขึ้นจากการเหนี่ยวนำด้วย LPS ได้อย่างมีนัยสำคัญทางสถิติและมีฤทธิ์ใกล้เคียงกับแอสไพริน ส่วนการทดสอบฤทธิ์ลดไข้จากการเหนี่ยวนำด้วยยีสต์ ทำการป้อนสารละลาย 2% ทวีน 80 แอสไพริน (300 มก. /กก.) หรือสิ่งสกัดจากรากมะเดื่อชุมพรขนาด 50-400 มก. /กก.แก่หนูแรทหลังจากฉีด 20% ยีสต์ ขนาด 10 มล. /กก. เข้าชั้นใต้ผิวหนังของหนูไปแล้ว 18 ชั่วโมง แล้ววัดอุณหภูมิหนูทางทวารหนักที่เวลา 1-7 ชม. หลังจากป้อนสารทดสอบ พบว่าสิ่งสกัดจากรากมะเดื่อชุมพรทุกขนาดที่ใช้ในการทดสอบสามารถลดไข้จากการเหนี่ยวนำด้วยยีสต์ได้อย่างมีนัยสำคัญทางสถิติและสิ่งสกัดด้วยเอทานอลจากรากมะเดื่อชุมพรในขนาด 200 และ 400 มก. /กก. มีฤทธิ์ใกล้เคียงกับแอสไพริน ในการทดสอบฤทธิ์ต้านการอักเสบของสิ่งสกัดจากรากมะเดื่อชุมพรด้วยการเหนี่ยวนำให้อุ้งเท้าหนูบวมด้วยคาราจีแนนในหนูแรท ทำโดยการฉีดน้ำเกลือ อินโดเมทาซิน (5 มก. /กก.) 2% ทวีน 80 หรือสิ่งสกัดจากรากมะเดื่อชุมพรขนาด 50-800 มก. /กก. เข้าทางช่องท้อง 1 ชม. ก่อนฉีด คาราจีแนน 1% ขนาด 0.1 มล. เข้าทางชั้นใต้ผิวหนังที่อุ้งเท้าหลังขวาของหนูแล้ววัดปริมาตรการบวมของอุ้งเท้าที่เวลา 1-6 ชม. หลังฉีดคาราจีแนน พบว่าสิ่งสกัดจากรากมะเดื่อชุมพรทุกขนาดที่ใช้ในการทดสอบสามารถลดการบวมของอุ้งเท้าหนูได้อย่างมีนัยสำคัญทางสถิติในช่วงเฟสที่สองของการบวม จากผลการทดลองทั้งหมดแสดงว่าสิ่งสกัดด้วยเอทานอลจากรากมะเดื่อชุมพรมีฤทธิ์ระงับปวดทั้งที่ระดับประสาทส่วนกลางและระดับประสาทส่วนปลาย กลไกการออกฤทธิ์ลดไข้และต้านการอักเสบมีส่วนเกี่ยวข้องกับการยับยั้งการสร้างพรอสตาแกลนดิน

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SIRAKARN CHOMCHUEN : ANTINOCICEPTIVE, ANTIPYRETIC AND ANTI-INFLAMMATORY EFFECTS OF THE ETHANOLIC EXTRACT OF *FICUS RACEMOSA* ROOT. THESIS ADVISOR : ASSIST. PROF. LT. PASARAPA TOWIWAT, PH.D., THESIS COADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSI, PH.D., 141 pp.

Ficus racemosa Linn. Is known locally in Thailand as 'Ma-Due-Au-Thum-Porn'. In these studies, we initially determined the antinociceptive property of a range of the ethanolic extract of *F. racemosa* root (EFR) doses in the mouse tail-flick test. Tail-flick latencies were determined in male ICR mice prior to the i.p. administration of 0.9% normal saline solution (NSS; 10 ml/kg), morphine (MO; 10 mg/kg), 2% tween 80 (10 ml/kg) or various doses of EFR (50-400 mg/kg). Tail-flick 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). EFR at doses of 200 and 400 mg/kg produced a significant analgesic response. 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 i.p. administration of NSS, indomethacin (IND; 10 mg/kg), 2% tween 80 or various doses of EFR (50-400 mg/kg) and the mean writhing response was determined for 30 min. All doses of EFR tested significantly ($p<0.01$) decreased the mean writhing response compared to vehicle controls.

Studies then determined the antipyretic property of orally administered EFR using LPS-induced fever model in rats. The animals were induced with intramuscular 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 EFR (50-800 mg/kg). Rectal temperature was measured before the pretreatment and at 1 hr interval for 7 hr after LPS injection. All doses of EFR significantly ($p<0.001$) reduced the increased rectal temperature produced by LPS and were found to be as potent as ASA. In the Brewer's yeast-induced pyrexia model, eighteen hours after s.c. injection of 20% brewer's yeast (10 ml/kg) rats were orally administered with 2% tween 80, ASA or various doses of EFR (50-400 mg/kg). Rectal temperature was measured 1-7 hr after the extract administration. All doses of EFR significantly ($p<0.05$) reduced the pyrexia induced by yeast and EFR doses of 200 and 400 mg/kg appeared to be equally potent as ASA. The anti-inflammatory property of the EFR was then determined using carrageenan-induced paw edema test. Rats were pretreated with i.p. administration of NSS, IND, 2% tween 80 or various doses of EFR (50-800 mg/kg) before inducing inflammatory response with s.c. injection of 1% carrageenan (0.1 ml) into the plantar surface of the right hind paws. The paw volume was measured at 1-6 hr after carrageenan injection. All doses of EFR tested significantly reduced paw volume during the second phase of edema. Taken together these results demonstrated that EFR possesses both central and peripheral analgesic activities. The mechanism of antipyretic and anti-inflammatory effects of EFR may involve in the inhibition of prostaglandin synthesis.

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

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LIST OF ABBREVIATIONS

AUC	area under the curve (area of analgesia)
ASA	Acetylsalicylic acid
BW	body weight
cm	centimeter
CNS	central nervous system
CO_2	carbondioxide
ED_{50}	median effective dose
EFR	the ethanolic extract of *Ficus racemosa* Linn. root
et al.	et alii (and other)
g	gram
hr	hour
IASP	International Association for the Study of pain
IC_{50}	inhibitory concentration 50%
IND	indomethacin
i.p.	intraperitoneal
LPS	lipopolysaccharide
mg/kg	milligram per kilogram
min	minute
ml/kg	milliliter per kilogram
MO	morphine sulphate
N	sample size
NO	nitric oxide
NOS	nitric oxide synthase
NSAIDs	Non-steroidal anti-inflammatory drugs
NSS	normal saline solution
PAF	platelet-activating factor
sec	second
S.E.M.	standard error of mean

TNF–α	tumor necrosis factor–alpha
β	beta
δ	delta
γ	gamma
°C	degree Celsius
µg	microgram (s)
µl	microlitre
α	alpha
/	per
%	percent
%MPE	percentage of the maximum possible effect

CHAPTER I

INTRODUCTION

Background and Rationale

Narcotic analgesic drugs such as opioid agonist are the primary drug class used in the management of moderate to severe pain. Opioids have been most widely used to treat acute and cancer-related pain. It produces both analgesia and side effects by acting on μ-opioid receptors. Through receptors in the medullary respiratory control center, the medullary chemoreceptor zone and the gastrointestinal tract, opioids also produce respiratory depression, vomiting and constipation. In addition, opioids can cause sedative, confusion, dizziness and euphoria. Opioids use is often associated with the development of tolerance and physical dependence.

Non-narcotic analgesic drugs including non-steroidal 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. The most common side effects are nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, and drowsiness. They may also cause fluid retention, leading to edema. The most serious side effects are kidney failure, liver failure, ulcers and prolonged bleeding. Acetaminophen is frequently combined with weak opioids or NSAIDs for the treatment of moderate to severe pain. At recommended doses, acetaminophen does not irritate the lining of the stomach or affect blood coagulation or function of the kidneys as much as NSAIDs. Excessive use of acetaminophen can damage multiple organs, especially the liver and kidney.

Both NSAIDs and steroidal anti-inflammatory drugs can be used to reduce inflammation. Steroidal anti-inflammatory drugs are potent medication which is used to treat many inflammatory conditions including rheumatoid arthritis. It had high efficacy and short duration of action. However, steroidal anti-inflammatory drugs have potent side effects in many organs, for example, pituitary-adrenal suppression, fluid and electrolyte disturbance, carbohydrate, protein and lipid metabolism, cardiovascular and central nervous system effects. At present, analgesic, antipyretic and anti-inflammatory

drugs are widely used for both acute and chronic conditions but are not successful in all the cases and also associated with many adverse effects. Therefore, the investigation of novel analgesic, antipyretic and anti-inflammatory agents without those side effects has been received considerable attention.

In the traditional system of medicine, the plant is used for various health problems and disease. There are many researches reporting about new investigated analgesic, antipyretic or anti-inflammatory drugs from natural products, for example, Arul et al. (2005) reported that the extract of the leaves of *Aegle marmelos* Corr. had analgesic activity in acetic acid-induced writhing in mice and caused a significant inhibition of the carrageenin-induced hind paw edema and cotton pellet-induced granuloma in rats. In addition, this plant also had an antipyretic effect in yeast-induced hyperthermia model in rats. In Thailand, there are also several reports about analgesic, antipyretic and anti-inflammatory effects of Thai herbal plants. For example, Panthong et al. (2002) demonstrated that the methanol extract from *Clerodendrum petasites* S. Moore had an anti-inflammatory effect in ethylphenylpropiolate (EPP)-induced ear edema and carrageenin-induced hind paw edema, but did not show any effect on arachidonic acid-induced hind paw edema and cotton pellet-induced granuloma formation in rats. The extract had an antipyretic effect in yeast-induced hyperthermia model in rats, but failed to produce analgesic effect in acetic acid-induced writhing in mice.

In India, Rao et al. (2002a) reported that the methanolic extract of the stem bark of *Ficus racemosa* Linn. showed antipyretic effect in yeast-induced pyrexia in rats and could reduce normal body temperature of rats. Mandal et al. (2000a) presented the anti-inflammatory activity of the petroleum ether extract of *Ficus racemosa* L. leaves in acute and chronic tests. The root of *Ficus racemosa* L. has been used as one of the five herbal roots in Thai traditional herbal medicine “Bencha-Loga-Wichian” remedy for the treatment of fever in children and adults. However, the ethanolic extract of *Ficus racemosa* L. root has never been tested for the analgesic, antipyretic or anti-inflammatory activities. These studies are therefore designed to examine in various

animal models the analgesic, antipyretic and anti-inflammatory properties of the ethanolic extract from *Ficus racemosa* L. root.

Purpose of the study

To investigate the antinociceptive, antipyretic and anti-inflammatory effects of the ethanolic extract of *Ficus racemosa* L. root in comparison with the reference drugs.

Hypothesis

The ethanolic extract of *Ficus racemosa* L. root has antinociceptive, antipyretic and anti-inflammatory effects in various animal models.

Research design

Experimental Research

Expected benefit and application

The knowledge obtained from the study of antinociceptive, antipyretic and anti-inflammatory effects of the ethanolic extract of *Ficus racemosa* L. root may lead to the development of a new analgesic, antipyretic and/or anti-inflammatory agent from natural sources of Thailand.

Key words

Ficus racemosa

Antinociception

Antipyretic

Anti-inflammatory

CHAPTER II

LITERATURE REVIEWS

FICUS RACEMOSA LINN.

Ficus racemosa Linn. belongs to the family Moraceae and known locally in Thailand as Ma-Due-Au-Thum-Porn or Ma-Due-Chum-Porn. It is commonly known as Gular fig, Cluster fig in English, Gular in Hindi and as Udumbara in Sanskrit. The plant is a large deciduous tree distributed all over Thailand, India and Southeast Asia. It is found throughout the year, grows in evergreen forests, moist localities and bank of stream, deciduous forests, to the elevation of 1800 m above sea level, often cultivated in villages for shade and its edible fruits. The tree is up to 18 m high, leaves ovate, ovate-lanceolate or elliptic, subacute, entire and petiolate. Leaves are shed by December and replenished by January and April, when the tree becomes bare for a short period. Figs subglobose or pyriform, red when ripe, borne in large clusters, on short, leafless branches emerging from the trunk and the main branches. The tree is without aerial roots unlike its many family members. It naturally comes up in wasteland and forests in subtropical climate. It is seen dwelling in areas up to 1200 m altitude on hilltop. This requires well-drained medium to heavy soils for its successful cultivation and comes up in all kinds of soil except in water logged and clay soil. The plant is propagated by using cuttings of stem and root suckers. Hardwood cutting 0.5 to 1.5 cm in diameter and about 30 cm long are taken from straight, healthy 1-2 year old shoots and planted in December to February. Seeds can also used for propagation. Natural regeneration is very good from seeds dispersed by animals and birds. Four months old seedlings are transplanted to polythene bags and then planted in field after one month (Cooke, 1967, Chopra et al., 1986, Atal and Kapur, 1982, Paarakh, 2009).

Figure 1 *Ficus racemosa* Linn.

Pharmacognostical characteristics

Macroscopical

A moderate to large sized spreading laticiferous tree without much prominent aerial roots. Leaves dark green, ovate or elliptical; fruits receptacles 2-5 cm in diameter, subglobose or pyriform, smooth or rarely covered with minute soft hairs, when ripe; they are orange, dull reddish or dark crimson and have pleasant smell resembling that of cidar apples. Bark grayish green, soft surface and uneven, 0.5-1.8 cm thick, on rubbing white papery flakes come out from the outer surface, inner surface light brown (Figure 1), fracture fibrous, taste mucilaginous without any characteristics odour The color of the root is light-brown (Figure 2; Cooke, 1967, Duthie, 1960, Kirtikar and Basu, 1988, Paarakh, 2009).

Microscopical

The cork is made up of polygonal or rectangular cells. The phellogen is made up of 1-2 layers of thin walled cells. Phelloderm is well marked compact tissue consisting of mainly parenchymatous cells with isolated or small groups of sclereids, particularly in inner region. Sclereids are lignified with simple pits. Several parenchymatous cells contain single prism of calcium oxalate or some brownish content. The cortex is wide with numerous sclereids and some cortical cells contain resinous mass. Prismatic crystals of calcium oxalate are present in some of the cells. Sclereids are rectangular or

isodiametric and pitted thick walled. Phloem composed of sieve tubes, companion cells, phloem parenchyma, sclereids, phloem fibres and medullary rays. Sclereids have lignified walls with simple pits like those of cortex. Phloem fibers are non-lignified, having narrow lumen without any septa. Prismatic crystals of calcium oxalate and few clustered crystals are also present. Starch grains are ovoid to spherical. Laticiferous vessels with a light brown granular material are present in the phloem region. Cambium when present 2-3 layered of tangentially elongated thin walled cells. Transverse section of *Ficus racemosa* L. root shows periderm, parenchyma of cortex, phloem fiber, vascular cambium, xylem fiber, xylem vessel, phloem tissue (Mitra, 1985, Narayana and Kolammal, 1957, Paarakh, 2009).

Powder

Power is light pink to light brown in colour, faint odour and astringent in taste. Microscopically it shows presence of abundant prismatic crystals of calcium oxalate, either free or in detached parenchymatous cells. Sclereids are separated or more or less in small intact groups. Portions of broken unlignified fibres with narrow lumen are at times associated with sclereids and or with cells containing calcium oxalate crystals. Medullary ray cells have a wavy outline and contain minute starch grains which are spherical or ovoid, simple or 2 to 4 compound. Occasionally parenchymatous cells with brownish contents are seen. Cork cells are polygonal in surface view (Nayar and Bisht, 1959, Paarakh, 2009).

Physical constants

Foreign matter about 2%, total ash 14%, acid soluble ash 1%, alcohol soluble extractive 7% and water soluble extractive 9% (Paarakh, 2009).

Phytochemical properties

Very little phytochemical work has been carried out on this plant *F. racemosa.* The stem bark showed the presence of two leucoanthocyanins: leucocyanidin-3-0-β-

glucopyranoside, leucopelarogonidin-3-0-α-L-rhamnopyranoside, β-sitosterol, unidentified long chain ketone, cerylbehenate, lupeol, its acetate, α-amyrin acetate. From trunk bark, lupeol, β-sitosterol and stigmasterol were isolated. Fruit contains glauanol, hentriacontane, β-sitosterol, gluanol acetate, glucose, tiglic acid, esters of taraxasterol, lupeol acetate, friedelin, higher hydrocarbons and other phytosterol. A new tetracyclic triterpene glauanol acetate which is characterized as 13α, 14β, 17βH, 20αH-lanosta-8, 22-diene-3β-acetate and racemosic acid were isolated from the leaves. An unusual thermostable aspartic protease was isolated from latex of the plant. The stem bark and fruit showed presence of glauanol acetate (Rastogi and Mehrotra, 1993, Agarwal and Misra, 1977, Joshi, 1977, Shrivastava et al. 1977, Agarwal, 1977, Bhatt and Agarwal, 1973, Merchant et al, 1979, Suresh et al. 1979, Li et al. 2004, Devaraj et al. 2008).

Traditional uses

Root is used for the treatments of dysentery, pectoral complaints, diabetes, applied in mumps, other inflammatory glandular enlargements, hydrophobia and pyresis. The bark is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccough, leprosy, dysentery, asthma and piles. The leaves are good wash for wounds and ulcers. They are useful in dysentery and diarrhea. The infusion of bark and leaves is also employed as mouth wash to spongy gums and internally in dysentery, menorrhagia, effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis and haemoptysis. Tender leaves are used in bilious affection and also to improve skin complexion. Tender fruits are astringent, stomachic, refrigerant, dry cough, loss of voice, diseases of kidney and spleen, astringent to bowel, styptic, tonic, useful in the treatment of leucorrhoea, blood disorder, burning sensation, fatigue, urinary discharges, leprosy, menorrhagic, epitasis, intestinal worms and carminative. They are useful in miscarriage, menorrhagia, spermatorrhoea, epididymitis, cancer, myalgia, scabies, haemoptysis, intrinsic haemorrhage, excessive thirst, visceral obstructions. Latex is aphrodisiac and administered in hemorrhoids, diarrhea, diabetes,

boils, alleviates the edema in adenitis, parotitis, orchitis, traumatic swelling, toothache and vaginal disorders. (Chopra et al., 1986,1992, Prabhakar and Suresh, 1990, Vedavathy and Rao, 1995, Konsue et al., 2008, Paarakh, 2009).

Figure 2 *Ficus racemosa* Linn. root

Pharmacological activity of *Ficus racemosa* Linn.

Antipyretic activity

Rao et al., (2002a) reported that the methanolic extract of the stem bark of *Ficus racemosa* L. at doses of 100, 200 and 300 mg/kg, orally administered showed antipyretic effect in yeast-induced pyrexia and reduced normal body temperature in rats. The extract was observed to contain steroid, triterpenoid, alkaloid and tannin. Root powder of *F. racemosa* , one of the five herbal roots in “Bencha-Loga-Wichian” formula, at a dose of 40 mg/kg was tested in yeast-induced fever model in rat and found to significantly reduce rat rectal temperature at 1 to 8 hr after oral administration. In addition, the root powder of “Bencha-Loga-Wichian” formula at doses of 100, 200 and 400 mg/kg showed antipyretic effect in yeast-induced fever model in rats when administered orally especially at the dose of 200 mg/kg (Konsue et al., 2008).

Anti-inflammatory activity

The petroleum ether extract of the leaves of *Ficus racemosa* L. at doses of 200 and 400 mg/kg showed anti-inflammatory activity in carrageenin, serotonin, histamine

and dextran-induced rat hind paw edema models. In chronic tests, the granuloma pouch in rats, the extract at doses of 200 and 400 mg/kg, showed 22.1% and 41.5% reduction in granuloma weights. The extract effectively suppressed the inflammation produced by histamine and serotonin. This suggests that the active principles of the extract are steroid and triterpenoid (Mandal et al., 2000a).

In vitro studies revealed the inhibition of PGE_2 and PGD_2 activities of the ethanolic extract from several parts of *F. racemosa.* The inner bark, stem milky sap and frozen fruit showed % inhibition of PGE_2 and PGD_2 activities of 89.18%, 44.53% and 71.26%, respectively. *F. racemosa* bark exhibited inhibition of COX-1 with the IC_{50} of 100 µg/ml. Aspirin and indomethacin were used as the reference COX-1 inhibitors with IC_{50} of 241 and 1.2 µg/ml, respectively (Li et al., 2003). Racemosic acid isolated from the ethanolic extract of leaves of *F. racemosa* showed potent inhibitory activity against COX-1 and 5-LOX in *in vitro* with IC_{50} values of 90 and 18 µM, respectively. Racemosic acid also demonstrated a strong antioxidant activity to scavenge ABTS free radical cations with an IC_{50} value of 19 µM. In addition, cytotoxic effects of the extracts of *F.* racemosa were investigated *in vitro* using the ATP-based luminescence assay and showed no cytotoxicity on the cell lines skin fibroblasts (1BR3), human caucasian hepatocyte carcinoma (Hep G2) and human caucasian promyelocytic leukaemia (HL-60). Bergenin was also isolated from the same active fraction (Li et al., 2004).

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 ulcer. The extract prevented the oxidative damage of gastric mucosa by blocking lipid peroxidation and by significant decrease in superoxide dismutase, H^+ /K^+-ATPase and increase catalase activity. HPLC analysis showed the presence of phenolic compounds (gallic acid and ellagic acid). The phytochemical results showed the presence of phenolics, flavonoids, sterols and terpenoids in the extract (Rao et al., 2008).

Antitussive activity

The methanol extract of the stem bark of *Ficus racemosa* (MEFR) was tested for antitussive potential against a cough induced by sulphur dioxide gas model in mice. The extract demonstrated antitussive activity which was comparable to that of codeine phosphate (10 mg), a standard antitussive agent. The extract exhibited maximum inhibition of 56.9% at a dose of 200 mg/kg (p.o.) at 90 min after administration (Rao et al., 2003).

Antihyperglycemic and hypolipidemic activities

The glucose-lowering efficacy was reported by Rao et al., (2002b). The methanol extract of the stem bark of *Ficus racemosa* was evaluated both in normal and alloxan-induced diabetic in rats. The extract at the doses of 200 and 400 mg/kg orally administered exhibited significant hypoglycaemic activity in both experimental animal models. The activity was also comparable to that of the effect produced by a standard antidiabetic agent, glibenclamide 10 mg/kg.

The antihyperglycemic and hypolipidemic activities of the ethanolic extract of *F. racemosa* bark in alloxan-induced diabetic rats were investigated by Sophia and Manoharan (2007). The extract at dose of 300 mg/kg administered orally to diabetic rats restored the status of blood glucose, lipids and lipoproteins to near normal range. These effects were much comparable to that of the standard reference drug, glibenclamide. Preliminary phytochemical examination of *F. racemosa* revealed the presence of glycosides, 8-sitosterol, lupeol, tannins, and psoralens in the root bark.

The effects of *F. racemosa* fruit extract and fraction were investigated on fasting serum glucose levels of normal, type 1 and type 2 diabetic model in rats. The aqueous 80% EtOH extract and its water soluble fraction of *F. racemosa* fruit showed significant hypoglycaemic effect on the type 1 diabetic model rats but did not show any serum glucose lowering effect on non-diabetic and type 2 diabetic rats at the fasting condition. Both the extract and fraction were consistently active in both non-diabetic and types 1 and 2 diabetic model rats when fed simultaneously with glucose load. On the contrary,

they were ineffective in lowering blood glucose levels when fed 30 min prior to glucose load (Jahan et al., 2009).

Antioxidant activities

In vitro radioprotective potential of the ethanol extract of *Ficus racemosa* (FRE) was study using micronucleus assay in irradiated Chinese hamster lung fibroblast cells (V79). Pretreatment with 20 µg/ml FRE 1 hr prior to 0.5, 1, 2, 3 and 4Gγ γ-radiation resulted in a significantly decrease in the percentage of micronucleated binuclear V79 cells, suggesting its role as radioprotector. The FRE also exhibited potent antioxidant activity against DPPH, ABTS, hydroxyl radical and superoxide radical scavenging and inhibited lipid peroxidation in concentration dependent dose (Veerapur et al., 2007).

The methanol and 70% acetone extracts of *F. racemosa* stem bark showed antioxidant activity. The maximum percentage of total phenolic content of the methanol and 70% acetone extracts showed 59.60% and 54.80%, respectively. Percent DPPH radical scavenging activity of both extracts were dose-dependent (12.5-50 µg). The highest DPPH scavenging activity was shown by the methanol extract of *F. racemosa* (21.50 µg/50% DPPH scavenging activity). Both extracts exhibited antioxidant activity against the linoleic acid emulsion system (34-38%) (Manian et al., 2008).

The 1-BuOH soluble part of the ethanol extract of the *F. racemosa* fruits exhibited significant antioxidant activity in DPPH free radical scavenging assay and 3-O-(E)-Caffeoyl quinate which was isolated for the first time from this plant also showed significant antioxidant activity (Jahan et al., 2009).

Hepatoprotective activity

The extract of the leaves of *Ficus racemosa* was evaluated for hepatoprotective activity in rats by inducing chronic liver damage by subcutaneous injection of 50% v/v carbon tetrachloride in liquid paraffin at a dose of 3 ml/kg on alternate days for a period of 4 weeks. The biochemical parameters SGOT, SGPT, serum bilirubin and alkaline

phosphatase were estimated to assess the liver function. The activity of extract was comparable to a standard liver tonic (Neutrosec) (Mandal et al., 1999).

Nephroprotective activity

Khan and Sultana (2005) had investigated the renal protective activity of *Ficus racemosa* extract in potassium bromate-induced renal oxidative injury in rat model and cell proliferation response. The pretreatment of animals with *F. racemosa* extract at doses of 200 and 400 mg/kg orally resulted in a significant decrease in xanthine oxidase, lipid peroxidation, γ-glutamyl transpeptidase and H_2O_2. There was a significant recovery of renal glutathione content and antioxidant enzymes. There also was reversal in the enhancement of renal ornithine decarboxylase activity, DNA synthesis, blood urea nitrogen and serum creatinine.

Anti-diarrheal activity

The preliminary anti-diarrheal activity was reported by Mukherjee et al., (1998). The ethanol extract of *Ficus racemosa* stem bark at a dose of 400 mg/kg given orally showed significant inhibitory activity against caster oil induced diarrhea and PGE_2 induced enteropooling in rats. The extract also showed significant reduction in gastrointestinal motility in charcoal meal test in rats. The tannin present in the plant extract may be responsible for the anti-diarrheal activity.

Anthelmintic activity

The crude extracts of *Ficus racemosa* bark showed anthelmintic activity in adult earthworms. The ether extract at doses of 5 and 10 mg/ml produced paralysis within 300 and 200 min. Mortality was noted with 50 mg/ml within 180 minutes. The chloroform extract at 5 and 10 mg/ml also produced paralysis within 200 and 180 min. The mortality was also occurred with 50 mg/ml within 180 minutes. Ethanol extract also produced dose-dependent paralysis at concentration of 5 and 10 mg/ml, paralysis was evident at 270 and 220 minutes, while dose 50 mg/ml produced death within 160 min. Aqueous

extracts also produced dose-dependent paralysis at doses of 5 and 10 mg/ml within 300 and 240 min, while higher concentration produced death within 90 min. The high dosage of each crude extract produced paralytic effect much earlier and the time to death was shorter, the effect was comparable with 3% piperazine citrate. *Ficus racemosa* extract showed the presence of tannins, kaempferol, rutin, arabinose, bergapten, psoralenes, flavonoid, ficusin, coumarins, phenolic glycosides lupeol, quercetin and β-sitosterol (Chandrashekhar et al., 2008).

Antifungal activity

The preliminary antifungal activity was reported by Deraniyagala et al., (1998). The 50% methylene chloride in hexane flash column fraction of the extract of the leaves of *Ficus racemosa* was found to have antifungal activity in Cladosporium TLC-bioassay. The extract inhibited the growth of several plant pathogens including *Curvularia* sp., *Colletotrichum gloeosporioides*, *Alternaria* sp., *Corynespora cassiicola* and *Fusarium* sp. and showed maximum % inhibition of 37.68, 32.74, 17.42, 16.37 and 25.53 respectively.

Antibacterial activity

The extracts of *Ficus racemosa* leaves were tested for antibacterial potential against *Escherichia coli* ATCC 10536, *Bacillus pumitis* ATCC 14884, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 25619 and *Staphylococcus aureus* ATCC 29737. The effects produced by the extracts were significant and were compared with chloramphenicol. The petroleum ether extract was the most effective against the tested organisms (Mandal et al., 2000b).

Antidiuretic activity

Ratnasooriya et al., (2003) showed that an aqueous extract of the bark of *Ficus racemosa* had antidiuretic effect in animal model. The extract at doses of 250, 500 and 1000 mg/kg were given orally. The results demonstrated that both the low-and high-

doses significantly impaired the total urine output. The antidiuresis induced by the extract had a rapid onset (within 1 h), peaked at 3 h and lasted throughout the study period (5 h). However, antidiuretic potential of the extract was about 50% lower than that of ADH (the reference drug). The extract caused a reduction in urinary Na^+ level and Na^+/K^+ ratio, and an increase in urinary osmolarity indicating multiple mechanisms of action. The extract was well tolerated even with subchronic administration.

Wound healing activity

Ethanol extract of *Ficus racemosa* stem bark showed significantly wound healing activity in excised and incised wound model in rat. This plant is used in Ayurvedic medicine in India (Biswas and Mukhergee, 2003). The ointment containing *F. racemosa* was proved to be highly efficacious in controlling *Candida albicans* infections and helped in quicker epithelialization in patients of burn. The burns were completely healed in 8 to 26 days of treatment (Bhatt and Kora, 1984).

Antifilarial activity

The preliminary antifilarial activity was reported by Mishra et al., (2005). The alcoholic and the aqueous extracts of *Ficus racemosa* fruit caused inhibition of spontaneous motility of whole worm and nerve muscle preparation of *Setaria cervi* characterized by increase in amplitude and tone of contractions followed by paralysis. The concentrations required to inhibit the movement of the whole worm and nerve muscle preparation for alcoholic extract of fruits of *F. racemosa* were 250 and 50 µg/ml, respectively, whereas aqueous extract caused inhibition of the whole worm and nerve muscle preparation at 350 and 150 µg/ml, respectively. Both alcoholic and aqueous extracts caused death of microfilaria *in vitro*, LC_{50} and LC_{90} for alcoholic extract were 21 and 35 ng/ml, respectively and 27 and 42 ng/ml, respectively for aqueous extract.

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" caused 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 as physiologic, which refers to the body's protective mechanism to avoid tissue injury, or pathologic, which arises from tissue injury and inflammation or damage to a portion of the nervous system. Pathologic pain can be further divided into categories such as **nociceptive** (peripheral tissue injury), **neuropathic** (damage to peripheral nerves or spinal cord), **visceral** (stimulation of pain receptors in the thoracic or abdominal viscera), and **somatic** (injury to tissues other than viscera, such as bones, joints, muscles and skin). It can also be defined temporally as **acute** (arising from a sudden stimulus such as surgery or trauma) or **chronic** (persisting beyond the time normally associated with tissue injury).

Nociception

Nociception refers to the processing of a noxious stimulus resulting in the perception of pain by the brain. The components of nociception include transduction, transmission, modulation and perception.

Transduction

Transduction is the process by which noxious stimuli are converted to electrical signals in the nociceptors. Unlike other sensory receptors, nociceptors are not specialized from a structural point of view, but rather exist as free nerve endings. Nociceptors readily respond to different noxious modalities such as thermal, mechanical or chemical stimuli, but do not respond to non-noxious stimuli. Also in contrast to other types of sensory receptors, nociceptors do not adapt–that is, continued stimulation

results in continuous or repetitive firing of the nociceptor and, in some cases, continued stimulation actually results in a decrease in the threshold at which the nociceptors respond (ie, sensitization of nociceptors). Neurotransmitters that are produced within the cell body–ie, in the dorsal root ganglia (DRG) are the same at both the central and peripheral ends of the nerve fiber and are released at both ends, participating in producing the pain signal centrally, as well as in promoting events that lead to additional pain peripherally. The release of neurotransmitters from the peripheral terminals of the afferent fibers is actually an "efferent" function of these afferent neurons. Peripheral release of neurotransmitter substances lead to the classic "axon reflex", a reflex that does not require the spinal cord–this reflex leads to peripheral changes that are well recognized to contribute to pain (León-Casasola, 2007).

Transmission

Transmission is the second stage of processing of noxious signals, in which information from the periphery is relayed to the thalamus and then to the cortex. Noxious information is relayed mainly via 2 different types of primary afferent nociceptive neurons, which conduct at different velocities.

A-delta fibers are thinly myelinated fibers which conduct in the range of 2 m/s to 20 m/s. All fibers respond to high intensity mechanical stimulation and are therefore termed high threshold mechanoreceptors. Some, but not all fibers also respond to thermal stimuli–the latter are termed mechano–thermal receptors.

C-fibers are non-myelinated fibers that conduct in the range of 0.5 m/s to 2 m/s and transmit noxious information from a variety of modalities including mechanical, thermal, and chemical stimuli–for this reason, they are termed C-polymodal nociceptors (León-Casasola, 2007).

Modulation

Modulation is a third and critically important aspect of the processing of noxious stimuli that occurs–this process represents the changes which occur in the nervous

system in response to a noxious stimulus and allows the noxious signal received at the dorsal horn of the spinal cord to be selectively inhibited, so that the transmission of the signal to higher centers is modified. There is an endogenous pain modulation system, consisting of well-defined descending neural tracts that can inhibit rostral transmission of the pain signal. Activation of this system is thought to involve the release at supraspinal locations of neurotransmitters, including beta-endorphin (eg, β-endorphin) and enkephalins. These peptides represent 2 families of endogenous peptides that are believed to produce pain relief, mainly under situations of stress. Morphine, probably the most clinically important pain-relieving drug, is derived from the poppy plant, but acts by binding to the same opioid receptors that bind the endogenous opioids–for this reason, the endogenous opioids are called "endorphins" or "endogenous morphine" (León-Casasola, 2007).

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 (León-Casasola, 2007).

Nociceptive Pathways

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 spinal and supraspinal levels. In its simplest form, the nociceptive pathway is a 3-neuron chain. The 1st neuron in the chain- the primary afferent neuron-is responsible for transduction of noxious stimuli and conduction of signals from the peripheral tissues to neurons in the dorsal horn of the spinal cord. The 2nd 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 hypothalamus. These 3rd orders, supraspinal neurons integrate signals from the spinal neurons and project to the subcortical and cortical areas where pain is finally perceived (Lemke, 2004).

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 (Figure 3). Some afferent neurons respond to noxious (high-threshold) stimuli and are called nociceptive neurons. These neurons have free nerve endings or nociceptors that are activated by noxious stimuli, and change or transduce these stimuli into electrical signals. Transduction is mediated by membrane-bound receptors that are activated by noxious mechanical, thermal, and chemical stimuli. Somatic tissues have more nociceptors and smaller receptive fields, while visceral tissues have fewer nociceptors and larger receptive fields. These anatomic differences may account for some of the qualitative differences between somatic (discrete) and visceral (diffuse) pain. Other types of afferent neurons (large, myelinated Aβ fibers) respond to nonnoxious stimuli (touch) but not to noxious stimuli directly. However, they do help to shape (discriminate between mechanical and thermal) and attenuate (gate) nociceptive input at the spinal level.

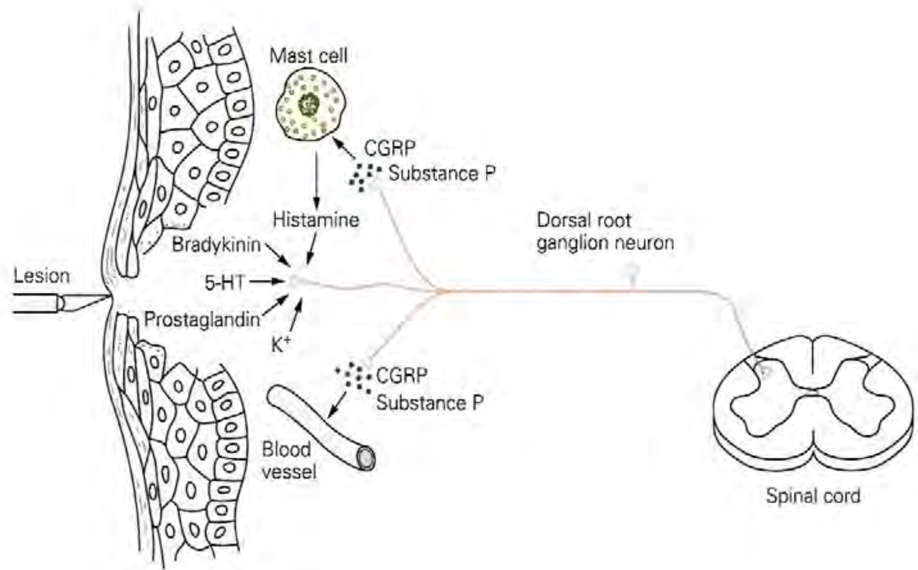

Figure 3 Peripheral nociceptors and primary afferent neurons (Schwartz and Jessell, 2000).

Four classes of nociceptors: mechanical, thermal, polymodal, and silent, have been described. Mechanical nociceptors respond to intense pressure and have small, myelinated Aδ fibers that conduct impulses at a velocity of 3 to 30 m/s. Thermal nociceptors respond to extreme temperatures and also have small, myelinated Aδ fibers that conduct impulses at a velocity of 3 to 30 m/s. Collectively, these 2 types of nociceptors are referred to as Aδ mechano-thermal nociceptors. Polymodal nociceptors respond to noxious mechanical, thermal, and chemical stimuli and have small, unmyelinated C fibers that conduct impulses at a velocity of less than 3 m/s. Small, myelinated Aδ fibers carry the nociceptive input responsible for the fast, sharp pain (first pain) that occurs immediately after injury; and small, unmyelinated C fibers carry the nociceptive input responsible for the prolonged, dull pain (second pain) that occurs several seconds later (Figure 4). Silent nociceptors are activated by chemical stimuli (inflammatory mediators) and respond to mechanical and thermal stimuli only after they have been activated. These nociceptors also have small, unmyelinated C fibers that conduct impulses at a velocity of less than 3 m/s. Sodium channels are responsible for membrane depolarization and impulse conduction in nociceptive and nonnociceptive

afferent fibers. Nociceptive Aδ and C fibers have a type of sodium channel that differs from that found in nonnociceptive Aδ fibers and are potential targets for therapeutic intervention (Lemke, 2004).

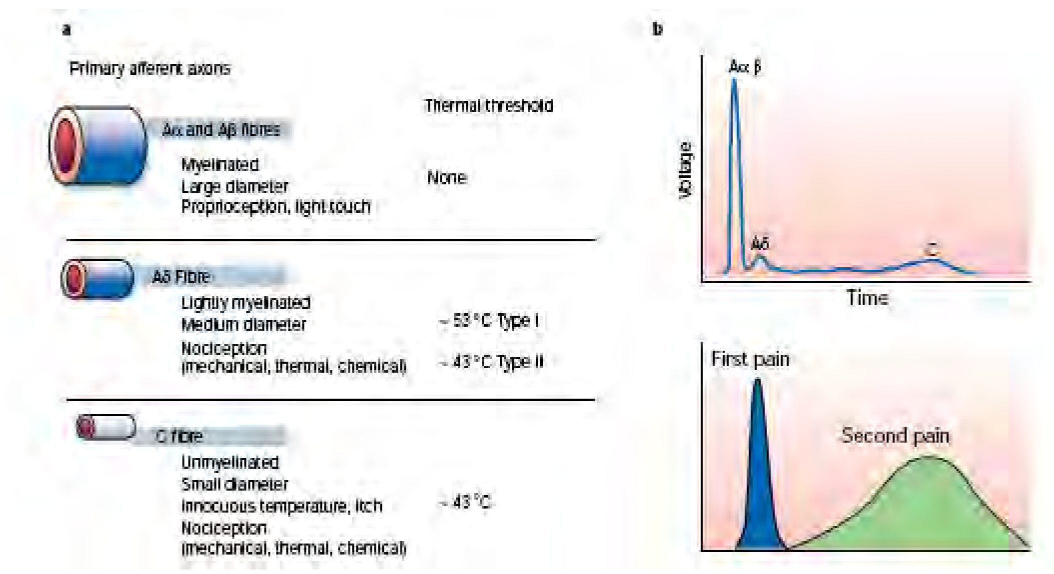

Figure 4 Different nociceptors detect different types of pain (Julius and Basbaum, 2001)

Nociceptive information is transmitted from the spinal cord to the thalamus and cerebral cortex along five ascending pathways (Figure 5).

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 contralateral 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 spinothalamic tract, many of the axons of 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 amygdala, 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).

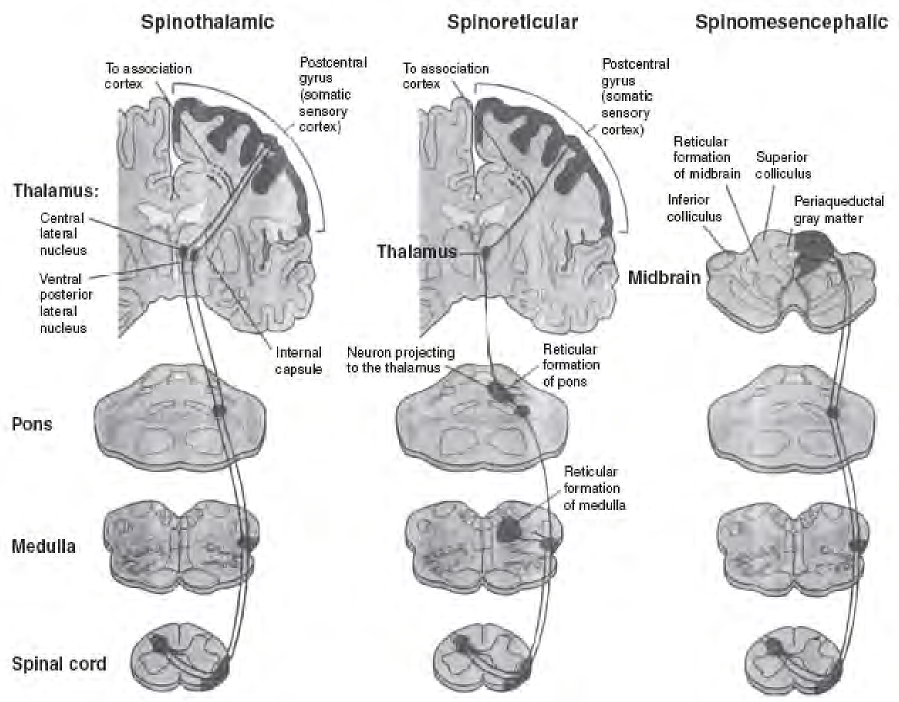

Figure 5 Ascending nociceptive pathways (Schwartz and Jessell, 2000; Lemke, 2004)

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 5-HT (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. As mentioned above, 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 6; León-Casasola, 2007).

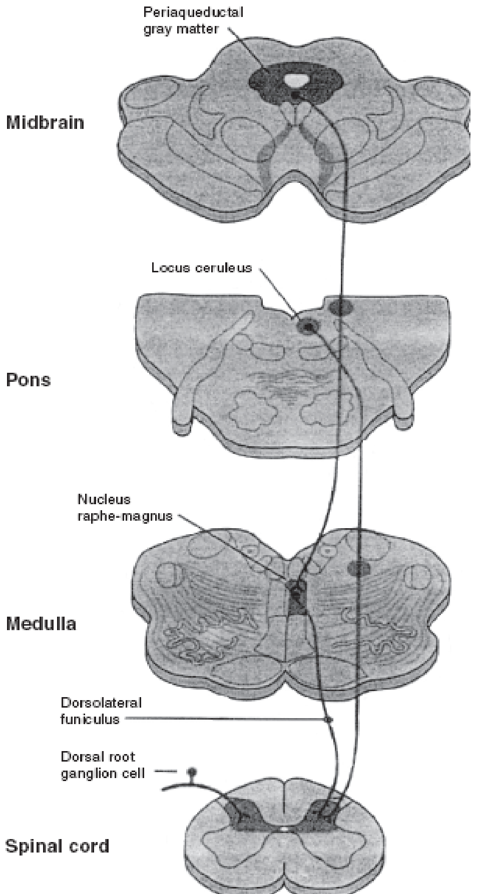

Figure 6 Descending antinociceptive pathways (Schwartz and Jessell, 2000)

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 experienced an allergic reaction to it, have diminished liver function, have alcoholism, are fasting, or have substantial kidney damage or loss of 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 reduced 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 and the 3- to 10-fold higher risk of GI injury and death associated with traditional NSAIDs. Two medications that predominantly inhibit only COX-2 activity, rofecoxib 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).

α_2 Agonists

Clonidine is an α_2 receptor agonist. The α_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 lies in their action as nonselective Na^+-channel-blocking agents. 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 γ-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, and 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 α_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 the 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 μ-opioid receptors. Sites of analgesic action include the brain, brainstem, spinal cord and primary afferent peripheral terminal. Through receptors in the medullary respiratory control center, the medullary chemoreceptor 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 or frequency of administration to maintain analgesia. Physical dependence can also occur, such that abrupt cessation of treatment would result in a characteristic withdrawal syndrome. Addiction, in which physical dependence is accompanied by substance abuse behavior, is a possible adverse effect of opioid administration (Griffin and Woof, 2007).

Tramadol

The limitations of opioids have 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-desmethyl-tramadol (M1) are agonists of the mu opioid receptor. (+) Tramadol also stimulates presynaptic release 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).

NORMAL THERMOREGULATION

Normal body temperature is circadian and 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. 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).

FEVER

The term fever specifically defines the elevation of body core temperature (Tc) that occurs in defensive response to the entry into the body of pathogenic agents (IUPS: Thermal Physiology Commission Glossary, 2002). It is thus distinct from hyperthermia, and the two terms should not be used interchangeably. The distinction is that the Tc rise of fever is the deliberate result of the regulated operation of active thermogenic effectors, whereas that of hyperthermia is the unavoidable consequence of the passive gain of heat in excess of the capability of active thermolytic effectors to dissipate it. Hence, hyperthermia is a pathologic event dependent on the ambient temperature (Ta), whereas fever is a physiological response that can develop at any Ta. A characteristic, thermoregulatory behavior familiar to everyone that differentiates the two conditions is that febrile subjects prefer warm thermal environments, to facilitate heat conservation, while hyperthermic subjects choose cool environments, to enhance heat loss (Blatteis, 2006). Functionally, the onset of fever is manifested by an increase in metabolic heat

production and cutaneous vasocontriction (to reduce heat loss from the skin) and by cessation of sweating, if present. The responses thus evoked are analogous to those caused by acute cold exposure, but, since they occur in a warmer Ta, the consequently narrower gradient between skin temperature (Tsk) and Ta results in less heat flowing from the skin to the environment and more, therefore, being retained in the body; hence, Tc rises. The increased heat production is achieved by the most visible sign of fever production, shivering (‘‘chills’’). In neonates and cold-acclimated rodents, in which shivering thermogenesis is normally replaced by brown adipose tissue (BAT) nonshivering thermogenesis (Cannon & Nedeergaard, 2004), the latter substitutes for shivering in the production of fever (Blatteis, 1976).

In humans in a thermoneutral environment, febrile rises prototypically range from 0.5 to 3°C. Thus, most infections produce fevers between 38 and 40.5°C; an average fever is 39.5°C (DuBois, 1948, Mackowiak & Boulant, 1996). Although there are no statistically significant gender or racial differences in ‘‘normal’’ Tcs (Mackowiak, 1997a, 1997b), it is uncertain whether febrile Tcs are also not different. Hyperpyrexia, i.e., Tcs above 42°C, is a rare occurrence. More frequently, demonstrably infected patients can remain afebrile or become hypothermic. The latter response particularly prognosticates a poor outcome. It is a shock-associated response, usually manifested in severe sepsis and attributed to a decrease in the threshold Tc at which thermogenic effectors are activated. This results in a widening of the normally narrow intra-threshold zone between heat- and cold-defense mechanisms; Tc, therefore, falls until the threshold for thermogenesis is reached. Interestingly, this is accompanied behaviorally by a marked preference for a cooler Ta, presumably to hasten the Tc fall to its new threshold (Romanovsky, 1996).

The Pathogenesis of Fever

Many of the mediators underlying pyrexia have been described in recent years (Figure 7). 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-1β (IL-1β), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to effect a fever response (Luheshi,1998). 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-1β (Dinarello et al, 1999). These signals trigger the release of other mediators, most notably prostaglandin E_2 (PGE_2), in the region of the POAH (Saper, 1994). 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 (Ushikubi et al, 1998). 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 (Ushikubi et al, 1998), studies in rats appear to implicate the EP_4 receptor (Oka et al, 2000). 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 (Kluger et al, 1998). Substances such as arginine vasopressin (AVP), α-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 (Pajkrt et al, 1997 and Leon et al, 1999) In addition, a class of lipid compounds known as epoxyeicosanoids generated by certain cytochrome P-450 enzymes play an important role in limiting the fever and inflammation (Kozak et al, 2000). Analogous to a biochemical feedback pathway, fever itself appears capable of countering the release of pyrogenic cytokines (Jiang et al, 1999 and 2000) 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 (Jiang et al, 1999, 2000).

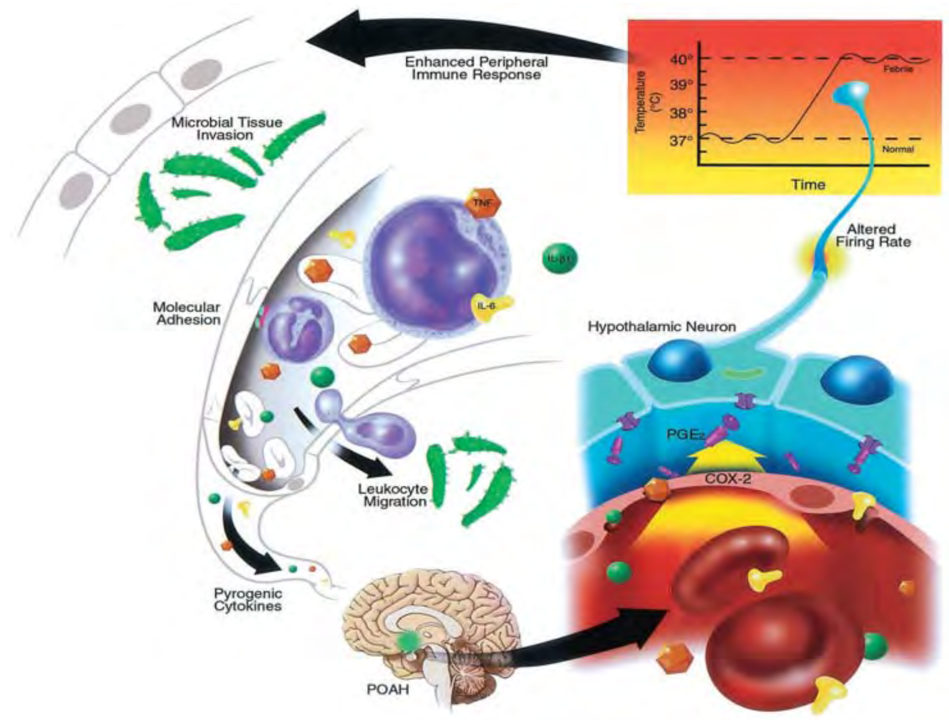

Figure 7 The pathogenesis of fever (Aronoff and Neilson, 2001)

The Role of Prostaglandin E_2

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 (Simon, 1999). COX-2, on the other hand, is inducible by inflammatory signals such as the pyrogenic cytokines, IL-1β, TNF, and IL-6, and bacterial lipopolysaccharide (Simon, 1999). 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 (Li et al, 1999). As COX-2 is the key provider of PGE_2 during

pyrexia, it is not surprising that the selective COX-2 antagonist, rofecoxib, is an effective antipyretic in humans (Schwartz et al, 1999).

Many cells, including synoviocytes, macrophages, endothelial cells, and chondrocytes, have the capacity to rapidly up-regulate the expression of the COX-2 during inflammation (Simon, 1999). 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 (Cao et al, 1996, Matsumura et al, 1998, Li et al, 1999). 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 (Table 1) (Aronoff and Neilson, 2001).

Table 1 Putative Targets and Mechanisms of Action for Antipyretic Medications (Aronoff and Neilson, 2001)
Neutrophils, macrophages, and other immune effector cells:
- Reduced production of inflammatory mediators (such as cytokines, proteases, and reactive oxygen species)
- Enhanced local production of anti-inflammatory molecules (such as adenosine, aspirin-triggered lipoxins, interleukin-10)
Endothelial cells at sites of local infection/inflammation:
- Reduced expression of leukocyte adhesion molecules
Endothelial cells of the central nervous system:
- Reduced prostaglandin E_2 production (cyclooxygenase inhibition)
Endogenous antipyretics:
- Enhanced production or activity of arginine vasopressin, α-melanocyte stimulating hormone, glucocorticoids, and epoxyeicosanoids

Antipyretics

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 (Figure 8). 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).

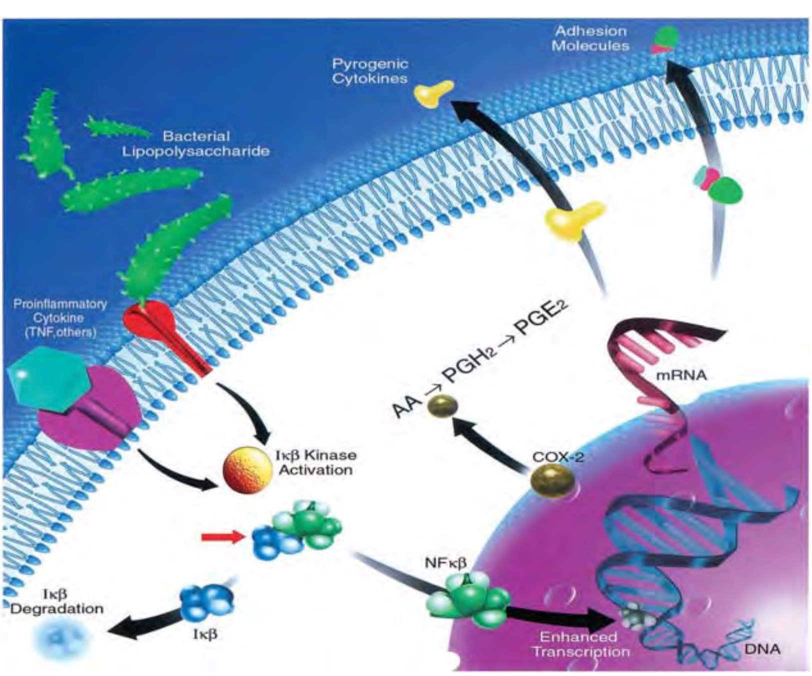

Figure 8 Effects of antipyretics on gene transcription (Aronoff and Neilson, 2001).

Interestingly, clinically useful actions of antipyretics may also be COX independent, and relevant anti-inflammatory effects of aspirin, sodium salicylate, and other NSAIDs are seen only with doses much higher than those required to suppress COX activity. Thus, a variety of noncyclooxygenase-dependent functions have been proposed to explain the full effects of salicylates on the pyrogenic cascade. For example, salicylates and other antipyretics also suppress tissue inflammation through diminished leukocyte-endothelial cell interactions, reduced pyrogenic cytokine production, or enhanced expression of anti-inflammatory molecules. Other mechanisms, such as boosting the activity of endogenous antipyretic messengers, may further contribute (Figure 9).

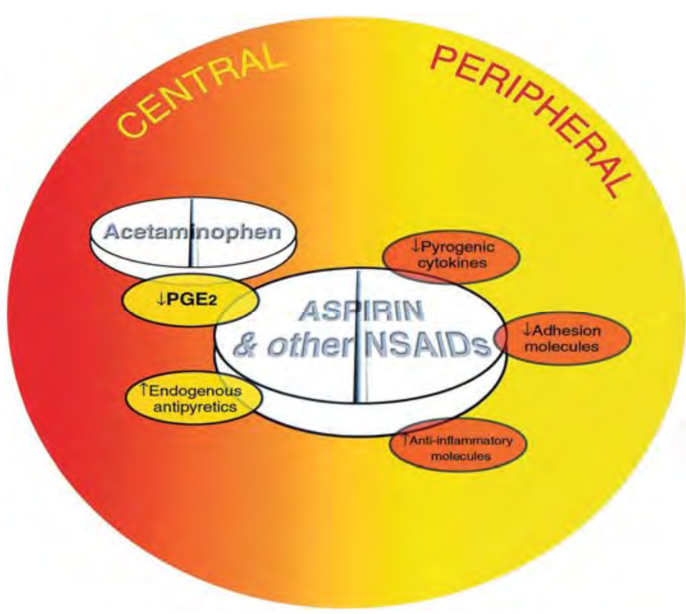

Figure 9 Mechanisms of antipyresis (Aronoff and Neilson, 2001).

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-κB stimulation of inducible nitric oxide synthase, it does not possess the same inhibitory effects on NF-κB–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).

INFLAMMATION

Inflammation is a response to infection, antigen challenge or tissue injury that is designed to eradicate microbes or irritants and to potentiate tissue repair. Excessive inflammation may, however, lead to tissue injury and can, if severe, cause physiological decompensation, organ dysfunction and death. Inflammation can be divided into two major categories—acute and chronic—based on timing and pathological features.

Acute inflammatory is typically of relatively short duration (hours to days) and is characterized by vasodilatation, the exudation of protein-rich fluid (plasma) and a migration of cells (primarily neutrophils) into the site of injury and, in some cases, activation of the coagulation cascade.

Chronic inflammatory diseases include rheumatoid arthritis, systemic lupus erythematosus, silicosis, atherosclerosis and inflammatory bowel disease. These disorders are characterized by a prolonged duration (weeks to months to years) in which active inflammation, tissue destruction and attempts at tissue repair are occurring simultaneously. Infiltration of mononuclear cells and fibrosis are typical histological features of chronic inflammation. Chronic inflammatory diseases commonly pose management challenges to anaesthesiologists. However, disease processes caused by acute inflammation present some of the most intense management problems for anaesthesiologists and critical care practitioners. Sepsis, severe trauma and major surgery all have major acute inflammatory components.

Initiation of the inflammatory response

Vasodilatation, fluid exudation and leukocyte migration

Vasodilatation is a classic feature of acute inflammation and is clinically characterized by redness and warmth at the site of injury. The purpose of the vasodilatory response is to facilitate the local delivery of soluble mediators and inflammatory cells. Inflammation induced vasodilatation is mediated primarily by nitric oxide (NO) and vasodilatory prostaglandins. NO is produced from L-arginine through the action of nitric oxide synthase (NOS) (figure 10). Three isoforms of NOS have been

identified. Endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutively produced, and their expression is increased by calcium flux. Activated leukocytes produce inducible NOS (iNOS) after exposure to microbial products or pro-inflammatory cytokines. The NO produced causes subsequent smooth muscle relaxation through cyclic GMP-dependent mechanisms. The primary vasodilatory prostaglandins are prostacyclin (PGI_2), PGD_2, PGE_2 and $PGF_{2\alpha}$ (figure 11). These lipid mediators are produced from arachidonic acid through the action of cyclooxygenase. Inflammation-induced vasodilatation initially involves arterioles followed by the opening of new microvascular beds. In cases of severe systemic inflammation such as sepsis, widespread vasodilatation can cause systemic hypotension and shock. These cardiovascular alterations are potentiated by sepsis-induced myocardial depression, a condition that is induced by the actions of NO and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) (Sherwood and Toliver-Kinsky, 2004).

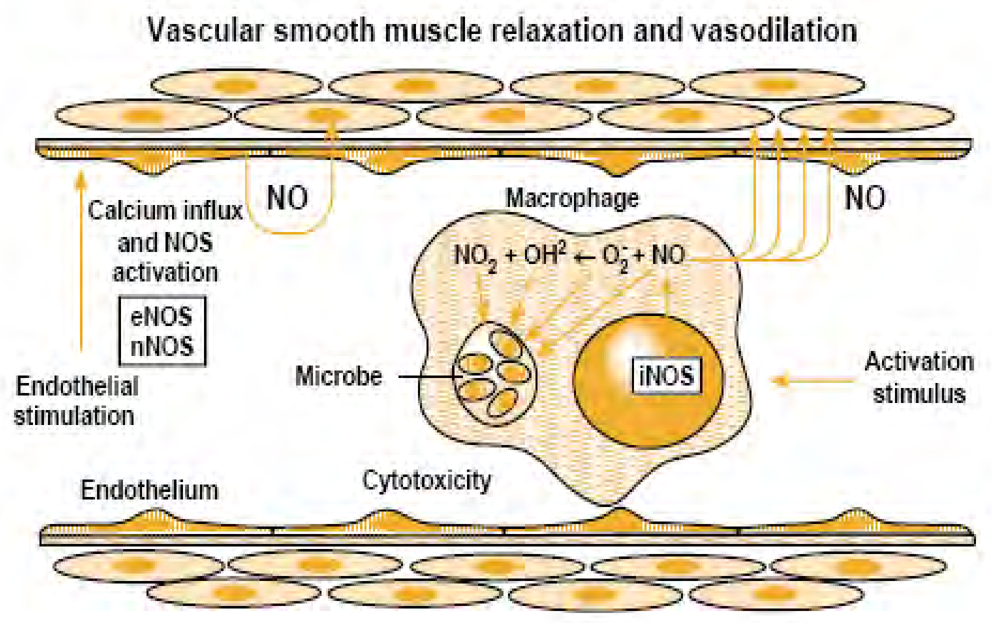

Figure 10 Nitric oxide (NO) in the regulation of vasodilation during inflammation. (Sherwood and Toliver-Kinsky, 2004)

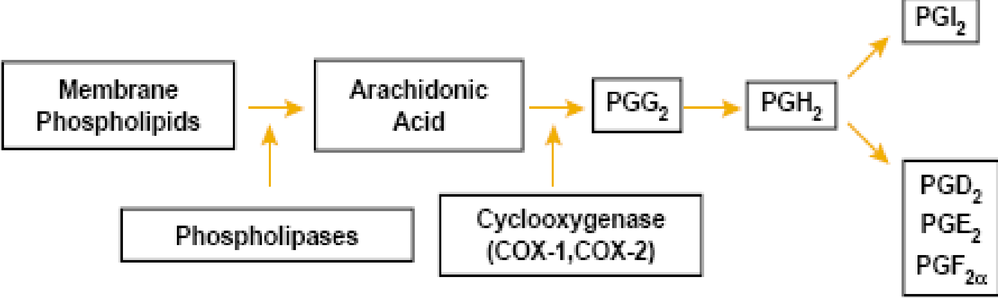

Figure 11 Vasodilatory prostaglandins are produced through the actions of phospholipase and cyclooxygenase. (Sherwood and Toliver-Kinsky, 2004)

Another early sign of inflammation is oedema formation. Oedema is caused by the transvascular flux of protein-rich fluid from the intravascular compartment into the interstitium as a result of the actions of histamine, bradykinin, leukotrienes, complement components, substance P and platelet-activating factor (PAF). These factors markedly alter the barrier functions of small blood vessels and increase the permeability of capillaries and venules for both water and protein. At the same time, capillary hydrostatic pressure is increased at the site of injury early during inflammation or injury as a result of local vasodilatation. The outpouring of protein-rich fluid causes a concentration of erythrocytes in small vessels and increased viscosity of the blood. This transvascular fluid flux eventually returns intravascular pressures at the site of inflammation to normal. At the same time, the loss of plasma proteins decreases the intravascular oncotic pressure. Together, the increase in vascular permeability, transient augmentation of capillary hydrostatic pressure and fall in plasma oncotic pressure act to induce a transvascular flux of fluid and protein into the inflamed interstitium.

Vasodilatation and fluid exudation are accompanied by leukocyte margination, adhesion and migration. Neutrophils are the first and most abundant leukocytes to be delivered to a site of infection or inflammation. The process of neutrophil migration from the intravascular space into the inflamed interstitium occurs primarily in postcapillary

venules in the systemic circulation and in pulmonary capillaries in the lung. The transmigration phenomenon is divided into several distinct steps: margination, rolling, adhesion, diapedesis and chemotaxis (Figure 12). Margination is the process of neutrophil movement from the central bloodstream to the periphery of the vessel. This phenomenon is facilitated by stasis following fluid exudation at the site of inflammation and physical interactions between erythrocytes and neutrophils. After margination, a weak adhesive interaction develops between neutrophils and vascular endothelial cells, causing neutrophils to remain in close proximity to the vascular endothelium. Neutrophil rolling is facilitated by the shear stress of passing erythrocytes, rolling velocity being proportional to red cell velocity. (Sherwood and Toliver-Kinsky, 2004)

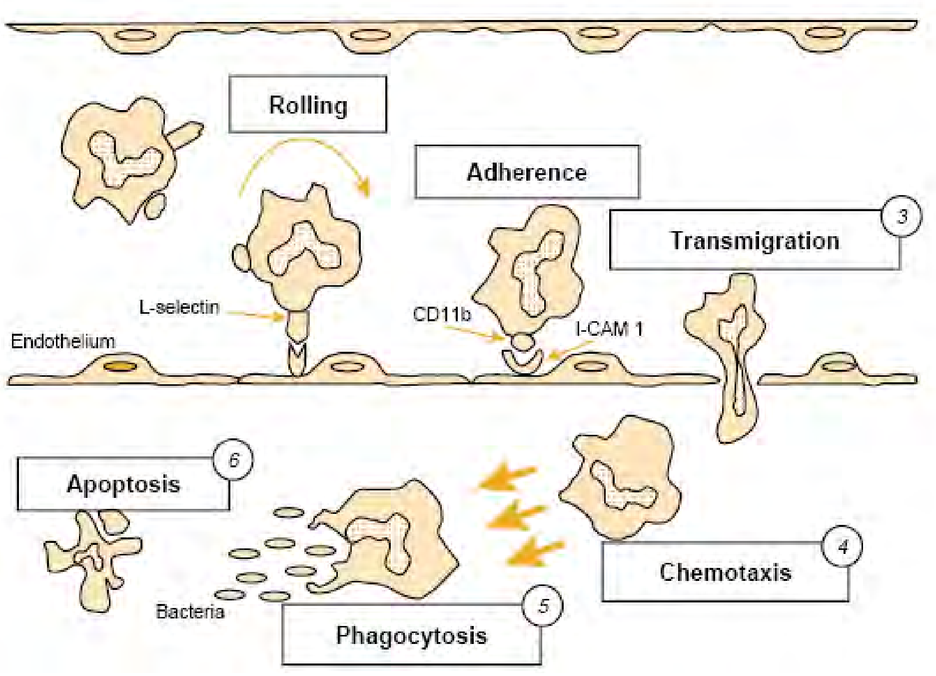

Figure 12 Mechanisms of neutrophil rolling, adherence, diapedesis and chemotaxis. (Sherwood and Toiver-Kinsky, 2004)

Chemical Mediators of Inflammation

The list of endogenous molecules that regulate the inflammatory process is shown in Table 2, and all of these signaling systems have been explored as potential pharmacologic targets. Only those most crucial to inflammation and those for which therapies already exist are discussed in detail (Armstrong et al, 2005).

Table 2 Chemical Mediators of the Inflammatory Response

RESPONSE	MEDIATORS
Vasodilation	Prostaglandins (PG) PGI_2,PGE_1,PGE_2,PGD_2
	Nitric oxide (NO)
Increased vascular permeability	Histamine
	C3a, C5a (complement components)
	Bradykinin
	Leukotrienes (LT), especially LTC_4, LTD_4, LTE_4
	Platelet-activating factor
	Substance P
	Calcitonin gene-related peptide (CGRP)
Chemotaxis and leukocyte activation	C5a
	LTB_4, lipoxins (LX) LXA_4, LXB_4
	Bacterial products
Tissue damage	Neutrophil and macrophage lysosomal products
	Oxygen radicals
	NO
Fever	IL-1, IL-6, TNF
	LTB_4, LXA_4, LXB_4
Pain	PGE_2, PGI_2
	Bradykinin
	CGRP

Prostaglandins

Prostaglandins are a large family of structurally similar compounds. The name of the family derives from their initial identification in the genitourinary system of male sheep. Prostaglandins all share a chemical structure, called a prostanoid that is a 20-carbon carboxylic acid containing a cyclopentane ring and a 15-hydroxyl group. Prostaglandins are divided into three major subseries PG_1, PG_2, and PG_3. The subscript

numeral indicates the number of double bonds in the molecule. The PG_2 series is the most prevalent because these are direct derivatives of arachidonic acid, an eicosatetraenoic acid. The PG_1 series derive from the arachidonic acid precursor dihomo-γ-linolenic acid (DHGLA), an eicosatrienoic acid, while the PG_3 series derive from an eicosapentaenoic acid (EPA).

The prostaglandin PGH_2 represents the critical juncture of the cyclooxygenase pathway because it is the precursor to PGD_2, PGE_2, $PGF_{2\alpha}$, thromboxane A_2 (TxA_2), and prostacyclin (PGI_2). The distribution of these eicosanoids in various tissues is determined by the expression pattern of the different enzymes of prostaglandin synthesis in the tissues.

The prostaglandins are important in many physiologic processes, most of which are not directly related to inflammation. Note especially the important housekeeping function of PGE_2, broadly referred to as cytoprotective roles, in which such organs as gastric mucosa, myocardium, and renal parenchyma are shielded from the effects of ischemia by PGE_2-mediated vasodilation and regulation of blood flow. PGE_2 may also be involved in inflammatory cell activation, and PGE_2 that is synthesized by COX-2 and PGE_2 isomerase in cells near the hypothalamus may have a role in fever (Dudzinski and Serhan, 2005).

Histamine

Histamine is one of the initiators of the inflammatory response. It is constitutively synthesized and stored in the granules of mast cells and basophils. These cells migrate through tissue on continual basis. Any injury, from physical trauma to microbial invasion, stimulates mast cells to release histamine granules into the interstitium. Histamine is referred to as a “vasoactive amine” because its inflammatory effects occur mainly on the vasculature, histamine release stimulates dilation of arterioles and postcapillary venules, constriction of veins, and contraction of endothelial cells. These effects are responsible for the early changes in hemodynamics and vascular permeability (Armstrong et. al. 2005)

Serotonin

5-HT is synthesized by enterochromaffin cells (EC) of the gastrointestinal (GI) tract, released under stimulation and taken up by circulating platelets. At inflammatory sites, activation of platelets by factors such as platelet-activating factor complements anaphylatoxin C5a and IgE-containing immune complexes and leads to their aggregation and a rapid release of micromolar concentrations of 5-HT in proximity of blood cells (Mossner and Lesch,1998). The C5a also triggers mast cells to release 5-HT, and this aspect is particularly important, as it is believed to favor an immune resistance to primary tumors and metastases. The autonomic innervations of lymphoid tissues represent a local source of 5-HT: 5-HT colocalizes with noradrenaline in nerve terminals, and it can be released under nerve stimulation. Accordingly, activation of parasympathetic neurons has been shown to enhance plasmatic concentrations of free 5-HT. Finally, 5-HT concentrations can also be regulated by its transport into monocytes, macrophages, dendritic cells (DC), and lymphocytes through activation of 5-HT uptake systems {5-HT transporter (5-HTT)}. (Tayarani and Changeux, 2006)

Leukotrienes

Leukotriene (LT) C_4 and its products, LTD_4 and LTE_4, make up the biologic mixture previously known as the slow-reacting substance of anaphylaxis. Leukotrienes are generated by most cell types that participate in inflammatory reactions including mast cells, basophils, eosinophils, neutrophils, and monocytes. As chemical mediators of inflammation, they have biologic activity similar to that of histamine. Studies of the effects of H_1-receptor antagonists on leukotriene release suggest that the mechanism may involve blocking the activity of receptor-coupled G proteins.

Leukotrienes are derived from arachidonic acid, which is made available from cell membrane phospholipids by the action of phospholipase A_2 or by the sequential action of phospholipase C and diacylglycerol lipase. After being released from cell membranes by the action of phospholipase A_2, arachidonic acid is converted by 5-lipoxygenase to 5*S*-hydroperoxy-6,8-*trans*-11,14-*cis*eicosatetraenoic acid. The same 5-

lipoxygenase pathway catalyzes the conversion of 5*S*-hydroperoxy-6,8-*trans*-11,14-*cis*-eicosatetraenoic acid to LT_4 (LTA_4). LTA_4 can then be converted to LTB_4 or conjugated with reduced glutathione to form LTC_4. In the later phases of the allergic reaction, after transport into the extracellular environment, LTC_4 can undergo further metabolism to LTD_4 and LTE_4 (White, 1999).

Kinins

Kinins are potent peptide hormones formed de novo in body fluids and tissues during inflammation. They are derived from $\alpha 2$ globulins (high and low molecular weight kininogens) through proteolytic cleavage by a variety of enzymes, the most important of which are plasma and tissue kallikreins. Three distinct kinins have been identified in humans: bradykinin, kallidin (lys-bradykinin), and met-lys-bradykinin. Although some quantitative differences exist, all of the kinins possess the same basic pharmacologic properties including the induction of smooth muscle contractility, vasodilation, and increased capillary blood flow (Keele, 1969, White, 1999).

Platelet activating factor

Platelet activating factor (PAF) is an ether-linked phospholipid, designated as such because of its discovery as a basophil-derived mediator of rabbit platelet activation. PAF may be produced by several of the cells that participate in the inflammatory response including mast cells, macrophages, neutrophils, and eosinophils (Barnes, 1993). The synthesis of PAF occurs as a 2-step pathway in which phospholipase A_2 hydrolyzes a 1-O-alkyl-2-acyl-glyceryl-3-phosphorylcholine to produce 1-O-alkyl-2-acyl-glyceryl-3-phosphocholine (lyso-PAF), which is then converted by an acetyltransferase enzyme to PAF. The biologic activities of PAF include platelet activation, activation of neutrophils, and smooth muscle contraction (Schwartz and Austen, 1984). Because PAF is rapidly inactivated *in vivo*, it is likely that it triggers a chain of inflammatory events. PAF stimulates the accumulation of eosinophils to endothelial surfaces, which may be the first step in the recruitment of eosinophils into

tissues. Eosinophils also are a source of PAF and can attract additional eosinophils, which can potentiate the inflammatory reaction. PAF stimulates eosinophils to release basic proteins, leading to epithelial cell damage, and causes an increased expression of low-affinity IgE receptors on eosinophils and monocytes (White, 1999).

Cytokines

Cytokines are proteins that act in a paracine manner to regulate leukocyte activity. Interleukins are cytokines secreted by cells of the hematopoietic lineage. Interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) are two cytokines elaborated as part of an acute inflammatory response. Other notable cytokine include the hematopoietic growth factors granulocyte-monocyte colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF). Because cytokines affect the proliferation and function of cells that mediate innate and adaptive immune response, selective inhibition or stimulation of cytokines action has the potential to modulate immune and inflammatory response (Armstrong et. al. 2005).

Nitric oxide

Reactive oxygen species such as nitric oxide and superoxide play important roles in inflammatory and immune responses, including defense mechanisms against invading microbes. They are released by a number of cell types, including neutrophils and macrophages as well as astrocytes and microglia. Nitric oxide is a diffusible free radical that is synthesized by three distinct nitric oxide synthases (NOS), of which the neuronal and endothelial forms (nNOS and eNOS) are constitutive, while the inducible form (iNOS) is upregulated in immune cells. Once released, nitric oxide can react with superoxide radicals to form peroxynitrite, which is toxic and may cause tissue damage (Moalem and Tracey, 2006).

Anti-inflammatory Drugs

Phospholipase inhibitors

Inhibition of phospholipase A_2 prevents the generation of arachidonic acid, the rate-limiting step in eicosanoid synthesis. In the absence of proinflammatory mediators derived from arachidonic acid, inflammatory limited.

Glucocorticoids

Prednisones, and other glucocorticoids administered at pharmacologic doses, inhibit the action of COX-2 by several mechanisms: (1) repressing COX-2 gene expression; (2) repressing the expression of cytokines that activate COX-2 substrate (arachidonic acid) by inhibiting phospholipase A_2 In combination, these mechanisms create a powerful anti-inflammatory effect because virtually all eicosanoid pathways are inhibited. Because of this profound and global suppression of immune and inflammatory responses, glucocorticoids are indicated in the treatment of a number of autoimmune conditions (Dudzinski and Serhan, 2005).

Cyclooxygenase inhibitor

A number of cyclooxygenase pathway inhibitors exist; these agents are some of the most frequently prescribed drugs in medicine. The non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen are the most commonly used agents in this class.

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are important because of the combined anti-inflammatory, antipyretic and analgesic activity. The ultimate goal of most NSAIDs therapies is to inhibit COX-2 competitively and thereby, to prevent the generation of pro-inflammatory eicosanoids and to limit the extent of inflammation, fever and pain. The drugs' antipyretic activity may be related to their decreasing the levels of PGE_2 particularly in the region of the brain surrounding the hypothalamus. Despite the benefits of NSAIDs, these drugs only suppress the signs of the underlying inflammatory response. Class of the NSAIDs,

mechanism of action and side effects of the NSAIDs are discussed in Pharmacological methods of pain management (Dudzinski and Serhan, 2005).

COX-2 Inhibitors

Because of the severe gastrointestinal side effects associated with long-term NSAIDs therapy, recent strategies for inhibition of cyclooxygenase pathway have focused on selective inhibition of COX-2. This approach should have the theoretical advantage of inhibiting the chemical mediators responsible for inflammation, while maintaining the cytoprotective effects of the products of COX-1 activity (Dudzinski and Serhan, 2005).

Cytokine Inhibitors

The proinflammatory cytokines TNF-α and IL-1 both upregulate COX-2. Novel molecular technologies have provided the ability to inhibit the action of these cytokines and, thus, to inhibit the process whereby an injurious stimulus activates COX-2 and initiates the inflammatory response. Three TNF-α antagonists, etanercept, infliximab, and adalimumab are currently used in the treatment of rheumatoid arthritis. Etanercept consists of the extracellular domain of the TNF-α receptor coupled to human IgG1; infliximab is a humanized mouse monoclonal antibody directed against TNF-α. With few side effects, these drugs halt joint destruction and bone erosion, decrease pain, improve swollen and tender joints, and limit overall disease progression in rheumatoid arthritis. Infliximab has also been approved for the treatment of Crohn's disease. Anakinra is a recombinant form of the human IL-1 receptor produced in *E.coli*; this drug is approved for use in rheumatoid arthritis. Additional IL-1 antagonists are being developed for use in inflammatory and autoimmune diseases (Dudzinski and Serhan, 2005).

CHAPTER III
MATERIALS AND METHODS

Experimental Animals

Male ICR mice weighing 18-25 g, male Wistar rats weighing 140-220 g (National Laboratory Animal Center, Mahidol University, Salaya, Nakornprathom) were served as experimental subjects. The animals were housed in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under the standard condition of temperature (25±2°C), 12 hr/12 hr light/dark cycles and had accessed to standard pellet diet (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 G).

Preparation of the Ethanolic Extract of *Ficus racemosa* Linn. Root

The *Ficus racemosa* L. root was collected during September, 2007 from Karnchanaburi, Thailand. Roots were washed, air-dried, and powderized. The dried-root powder was successively extracted with 100% ethanol for 24 hr at room temperature. The filtrate was evaporated to dryness by evaporator. The paste appeared dark-brown color (yield 4.27% w/w). The ethanolic extract of *Ficus racemosa* L. root (EFR) was kept in tight container at 2-8°C until the time of treatment. A weighed amount of the EFR was suspended in 2% (w/v) aqueous tween 80 solution and used for the study. The EFR was obtained from Chatubhong Singharachai, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Thin-layer chromatographic identification

Extract 1 g of the ground sample with 20 ml methanol, filtered and evaporated to dryness. Dissolved the residue in 0.5 ml of methanol, applied 10 µl to the thin-layer plastic plate coat with silica gel 60 F_{254} (Polygram® SIL G/UV254, 0.25 mm thickness, 20

cm X 20 cm). The chromatogram was developed in the chamber with chloroform. 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 (365 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 120°C for 10 min.

Drugs

The following drugs were used: 0.9% sodium chloride solution, 2% tween 80 solution (Srichanosoth, Thailand), morphine sulphate (10 mg/kg, Thai FDA), indomethacin (5 mg/kg, Sigma Chemical Co., USA), acetylsalicylic acid (300 mg/kg, Sigma Chemical Co., USA), 0.6% acetic acid (10 ml/kg, Merck, Germany) lipopolysaccharide (LPS; bacterial endotoxin) from *Escherichia coli* (50 µg/kg, Sigma Chemical Co., USA), 20% brewer's yeast (10 ml/kg, Sigma Chemical Co., USA), 1% carrageenan (0.1 ml/rat, Merck, Germany) and the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-800 mg/kg).

Morphine, indomethacin, acetic acid, carrageenan, lipopolysaccharide and brewer's yeast were dissolved in 0.9% sodium chloride solution. EFR and acetylsalicylic acid were suspended in 2% tween 80 solution. Morphine sulphate, acetylsalicylic acid, indomethacin were used as standard drugs. The control animals were given with an equivalent volume of vehicle in the same route.

Experimental Method

Antinociceptive Activity test

Tail-flick Analgesic Testing

These studies employed the tail-flick assay described by D'Amour and Smith in 1941, with minor modifications. The 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 the marked dorsal portion of the distal part of each subject's tail that has been blackened length 1 cm with a black ink maker 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 approximately 1.0-1.5 sec (intensity 3.7 A). The intensity was not changed for any animals within any given experiments. 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 baseline trials 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 on the tail-flick test, the drug administration took place with either intraperitoneal (i.p.) vehicle (10 ml/kg), morphine sulphate (10 mg/kg), 2% tween 80 (10 ml/kg) or various doses of EFR (50-400 mg/kg). 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:

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

Note: cut-off time for tail-flick test = 4 sec

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

Figure 13 Tail Flick Analgesia Meter

Acetic Acid-induced Writhing Test in Mice

Male ICR mice weighing 18-25 g were used (N=10 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 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) which used to induce the constriction response.

The drug administration took place with intraperitoneal (i.p.) vehicle (10 ml/kg), indomethacin (10 mg/kg), 2% tween 80 or various doses of EFR (50-400 mg/kg) 30 min before the 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:

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

Note: Wr = mean writhing response

Figure 14 Writhing response

Antipyretic activity test

Lipopolysaccharide-induced Fever

The method of Santos and Rao in 1998 was modified and used for the assessment of the antipyretic activity of the EFR. The animals were fasted overnight before the experiments. Animals were kept singly in restrainers for 30 min 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) or the EFR doses of 50, 100, 200, 400 and 800 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 endotoxin 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.

Yeast-induced Pyrexia

Fifty-six male Wistar rats (140-180 g) were randomly divided into 7 groups and fasted overnight before the experiment with free access to water. The normal body temperature of each rat was measured rectally at predetermined interval and recorded. Fever was induced according to the method described by Smith and Hambourger in 1935. The animals were trained to remain quiet in a broom-style restrainer for 30 min before treatment. A thermometer probe was inserted 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. Temperature was measured on digital thermometer (Model YSI Precision ™ 4000A, USA). After measuring the basal rectal temperature, animals were injected subcutaneously with 10 ml/kg of 20% w/v brewer's yeast suspension in the dorsum of the rats. Rats were then returned to their housing cages.

Eighteen hours after brewer's yeast injection, the animals were again restrained for rectal temperature recording, as described previously. Only rats that showed an

increase in temperature of at least 1°C were used for this study. The EFR at the doses of 50, 100, 200 and 400 mg/kg were then administered orally to four groups of animals. The control group received an equivalent volume of vehicle (2% tween 80 solution) and the positive-control group received acetylsalicylic acid (ASA; 300 mg/kg) orally. Rectal temperature was measured at 1, 2, 3, 4, 5, 6 and 7 hr after the drug administration. The rectal temperature of normal rats (normothermic) was also measured at 1 hr intervals for 7 hr. The results were expressed as percentage of the pre-drug temperature recorded for same animals as following formula (Makonnan et al., 2003):

$$\% \text{ Reduction} = \frac{\text{yeast-induced pyrexia} - \text{post treatment temperature}}{\text{yeast-induced pyrexia}} \times 100$$

Figure 15 Digital thermometer

Anti-inflammatory Activity Test

Carrageenan-induced Paw Edema Test

The anti-inflammatory activity of the EFR was determined using carrageenan-induced paw edema test in hind paws of rats as described by Winter et al. in 1962. Male Wistar rats (N=6 per group), 180-220 g, were fasted overnight before the experiment with free access to water. The animals were treated intraperitoneally with indomethacin (5 mg/kg), vehicle control (0.9% NSS or 2% tween 80 10 ml/kg) or various doses of EFR (50-800 mg/kg). One hour later, the rats were challenged by subcutaneous injection of 0.1 ml of 1% solution of carrageenan into the plantar surface of the right hind paw. The rat's paw was marked with black ink at the level of the lateral malleolus. The paw volume was measured at 1 hr prior to the injection of carrageenan and at 1, 2, 3, 4, 5 and 6 hr after injection using plethysmometer. Edema was expressed as a mean increase in paw volume in relation to control. The percentage of inhibition of edema was calculated using the following formula:

$$\% \text{ Inhibition of edema} = 100\,(1 - V_t/V_c)$$

Note: V_c = edema volume in control group; V_t = edema volume in tested group

Figure 16 Plethysmometer

DATA TREATMENT AND STATISTICAL ANALYSE

Data are expressed as means ± SEM. The obtained data were evaluated by the one-way analysis of variance (ANOVA) followed by Tukey HSD' or Dunnett T3' post hoc testing (SPSS version 13.0 for windows). P-values less than 0.05 were considered statistically significance.

CHAPTER IV
RESULTS

MOUSE TAIL-FLICK TEST

To demonstrate the validity of the mouse tail-flick analgesic testing following intraperitoneal (i.p.) drug administration, mice received morphine sulphate (MO; 10 mg/kg) i.p. and were tested during the subsequent 240 min period. As expected MO significantly ($p<0.001$) increased tail-flick latency producing an area of analgesia of 13808.34±2294.40 %MPE-min compared with that of normal saline solution (NSS) (-900.31±480.12 %MPE-min; Figure 17)

Initial studies utilizing the mouse tail-flick method to examine the efficacy of the ethanolic extract of *Ficus racemosa* L. root (EFR) in producing analgesia. Mice were then administered 2%tween 80 or various doses of EFR (50, 100, 200 and 400 mg/kg) i.p. The EFR doses of 200 and 400 mg/kg produced significant ($p<0.01$, $p<0.05$, respectively) analgesic responses compared with that of 2%tween 80 (Figure 18). MO showed the highest analgesic response compared to all test groups (Figure 19).

When the log of the EFR dose was plotted versus the area of analgesia, a linear correlation (r^2) equal to 0.8222 was observed (Figure 20). The linear correlation coefficient (r^2) of the log of EFR dose versus %MPE values at the time point at which peak antinociceptive response were observed after i.p. administration of the EFR; 50–400 mg/kg was equal to 0.8327 (Figure 21). The analgesic peak effects of EFR (50, 100, 200 and 400 mg/kg) were reached within 15, 30, 60, 30 min after i.p administration, respectively. Individual time courses of the responses are shown in Figure 22.

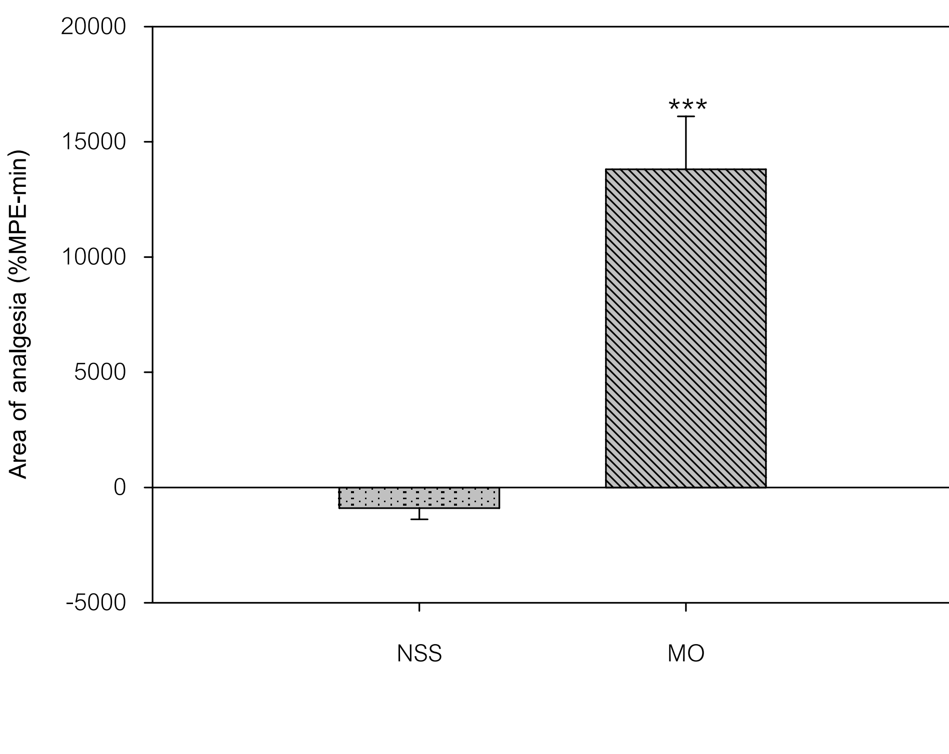

Figure 17 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. ***p<0.001 significantly different compared to NSS.

Mouse Tail-flick Test

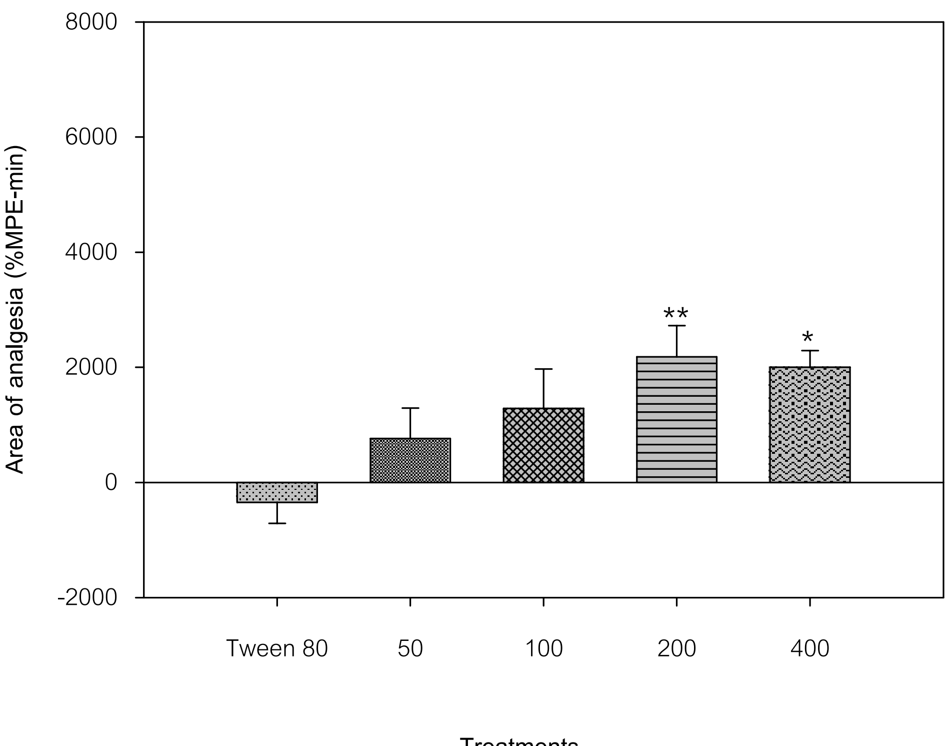

Figure 18 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 2% tween 80 and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50–400 mg/kg). N=10 for all groups. *p<0.05, **p<0.01 significantly different compared to 2% tween 80.

Mouse Tail-flick Test

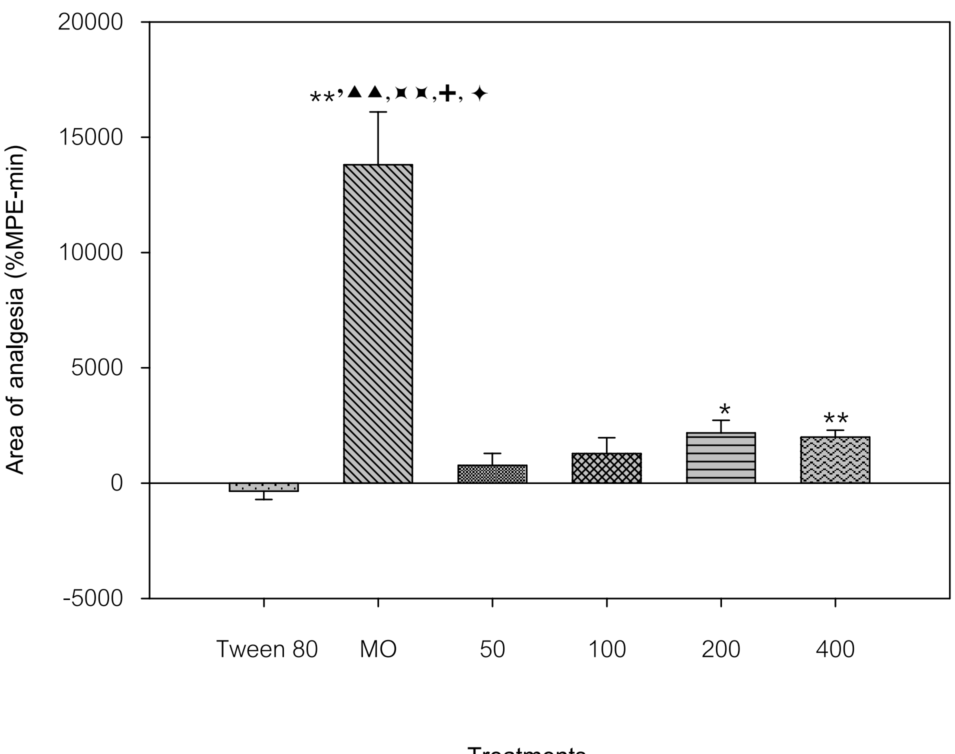

Figure 19 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 2% tween 80, morphine sulphate (MO; 10 mg/kg) and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50–400 mg/kg). N=10 for all groups. *p<0.05, **p<0.01 significantly different compared to 2% tween 80. ▲▲p<0.01 significantly different compared to EFR 50. ⌘⌘p<0.01 significantly different compared to EFR 100. +p<0.05 significantly different compared to EFR 200. ✦p<0.05 significantly different compared to EFR 400.

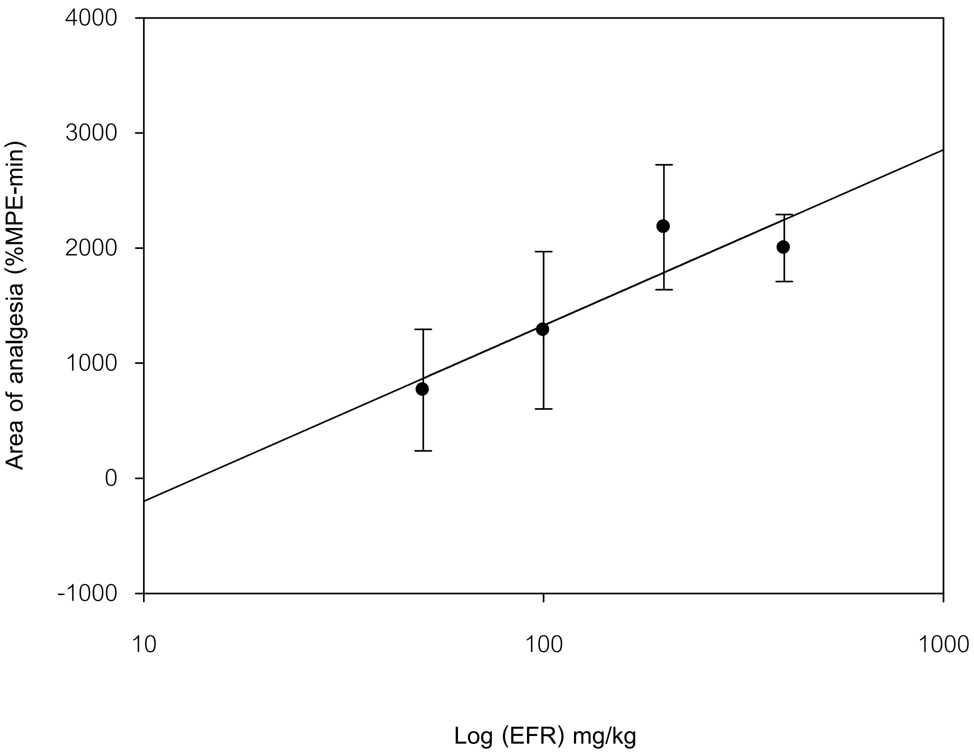

Figure 20 Linear regression of area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50–400 mg/kg). N=10 for all groups. The regression equation was Y= 663.1075*Ln(x) - 1725.41, r^2 = 0.8222.

Mouse Tail-flick Test

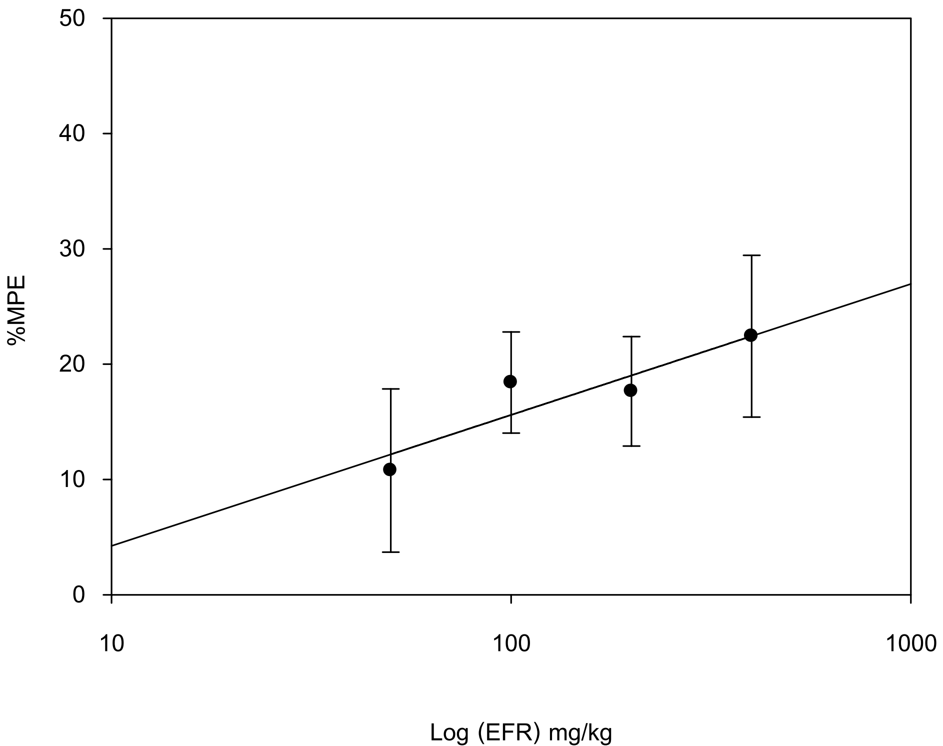

Figure 21 Linear regression of %MPE values at the time point at which peak antinociceptive response were observed after intraperitoneal administration of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50–400 mg/kg). N=10 for all groups. The regression equation was Y= 4.9316*Ln(x) -7.1146, r^2 = 0.8327.

Mouse Tail-flick Test

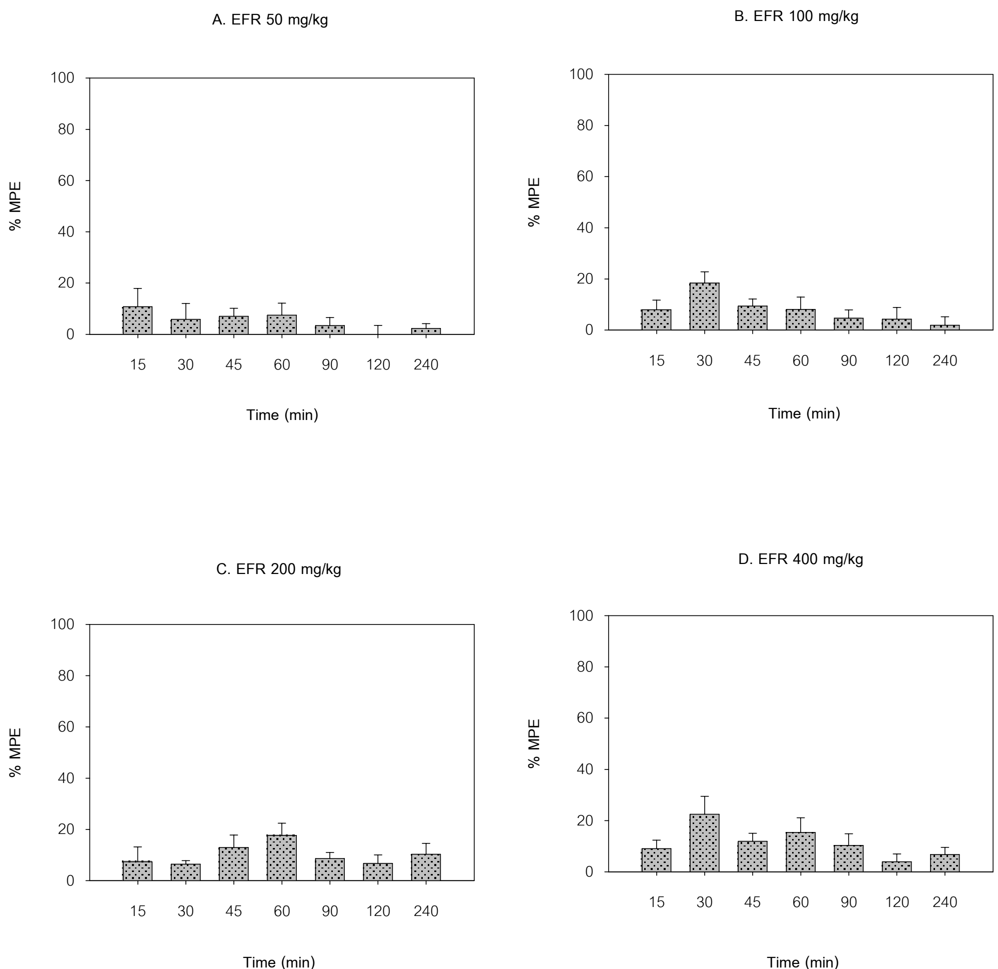

Figure 22 Individual time courses of the response (%MPE versus time (min)) after intraperitoneal administration of the ethanolic extract of *Ficus racemosa* L. root. A; EFR 50 mg/kg, B; EFR 100 mg/kg, C; EFR 200 mg/kg, D; EFR 400 mg/kg. N=10 for all groups.

ACETIC ACID-INDUCED WRITHING IN MICE

To demonstrate the validity of the acetic acid-induced writhing method following drug administration, mice received indomethacin (IND; 10 mg/kg) i.p. 30 min before i.p administration of 0.6% acetic acid and were observed for the writhing response during the subsequent 30 min period. As expected IND significantly ($p<0.001$) decreased writhing response by 94.59% producing a mean number of writhes of 1.80±1.28 compared with that of NSS (33.30±5.03; Figure 23)

Studies then utilized the acetic acid-induced writhing method in mice to examine the efficacy of the ethanolic extract of *Ficus racemosa* L. root (EFR) in producing analgesia. Mice were administered intraperitoneally 2%tween 80 or various doses of EFR (50, 100, 200 and 400 mg/kg). Control animals produced a mean number of writhes of 38.2±4.86, while EFR produced a mean number of writhes of 5.10±1.91, 8.10±2.51, 7.90±1.73 and 5.10±1.15, respectively. All doses of EFR significantly ($p<0.001$, $p<0.01$, $p<0.01$, $p<0.01$, respectively) decreased the number of writhes induced by acetic acid by 86.65%, 78.80%, 79.32% and 86.65%, respectively when compared to vehicle controls (Figure 24). IND showed the highest analgesia response compared to all test groups (Figure 25).

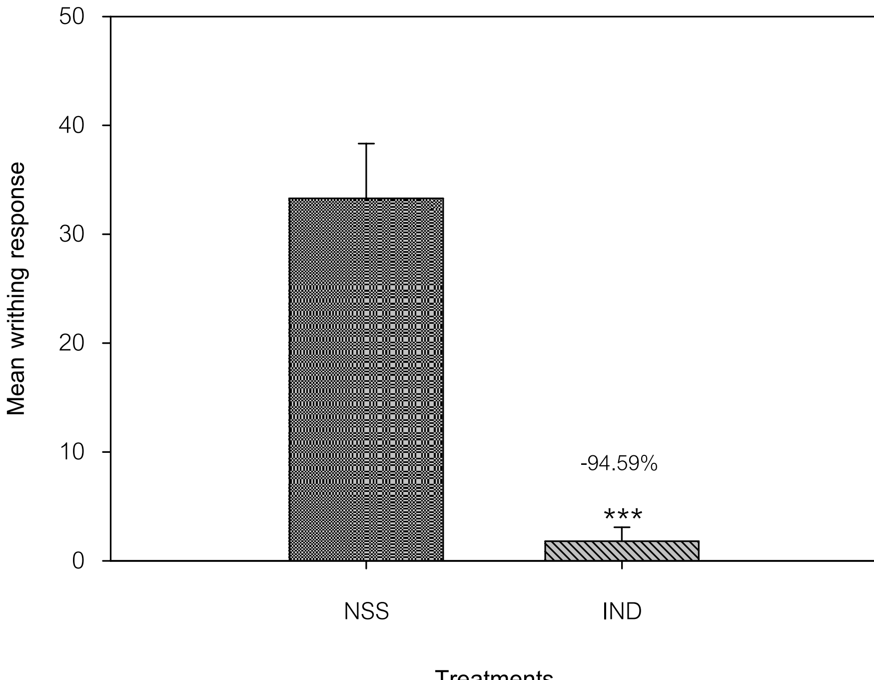

Figure 23 Mean writhing response after intraperitoneal administration of 0.9% normal saline solution (NSS) and indomethacin (IND; 10 mg/kg). N=10 for all groups. ***p<0.001 significantly different compared to NSS.

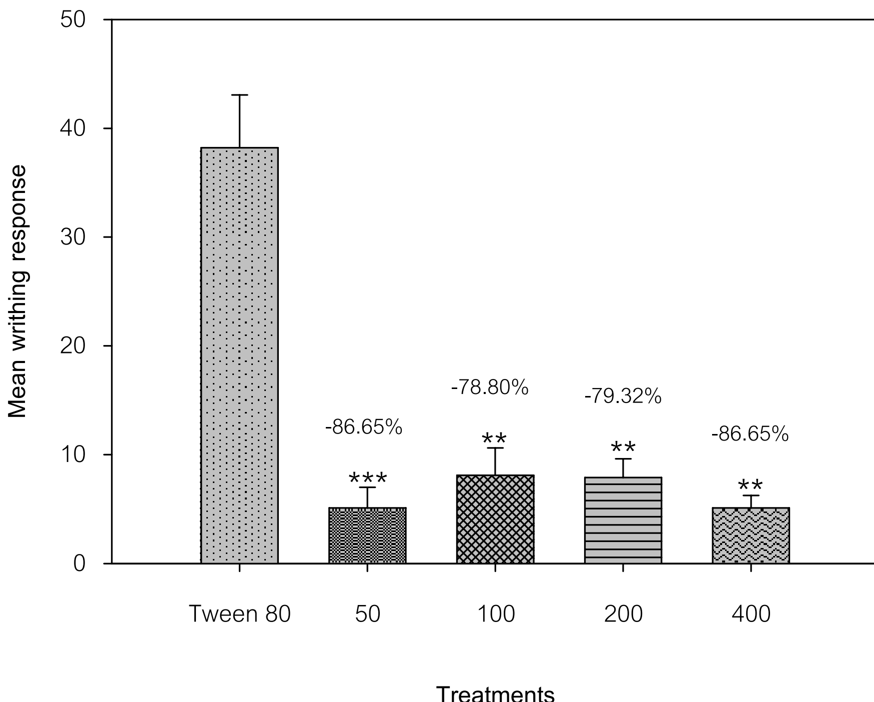

Figure 24 Mean writhing response after intraperitoneal administration of 2% tween 80 and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-400 mg/kg). N=10 for all groups. **$p<0.01$, ***$p<0.001$ significantly different compared to 2% tween 80.

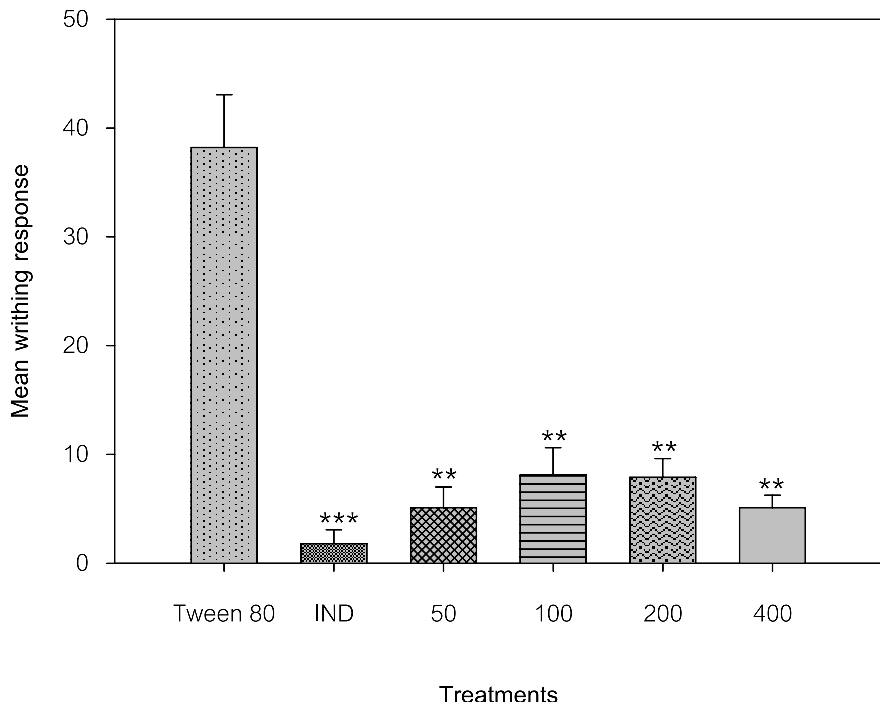

Figure 25 Mean writhing response after intraperitoneal administration of 2% tween 80, indomethacin (IND; 10 mg/kg) and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-400 mg/kg). N=10 for all groups. **p<0.01, ***p<0.001 significantly different compared to 2% tween 80.

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 4 hr (1.84°C) giving the maximum mean rectal temperature of 38.44±0.15°C after which there was a decrease (Figure 26). At the same period, the maximum mean rectal temperature of normothermic rats was 36.89±0.03°C, thus, LPS significantly ($p<0.001$) increased the rectal temperature (Table 3).

Acetylsalicylic acid (ASA; 300 mg/kg) significantly ($p<0.001$) reduced the increased rectal temperature produced by LPS over a period of 7 hr with a maximum reduction at 4 hr. The maximum reduction of mean rectal temperature produced by LPS in the presence of ASA was 36.01±0.18°C. Rectal temperature of ASA group was reduced to normal temperature within 3 hr after LPS injection. All doses of the ethanolic extract of *Ficus racemosa* L. root (EFR) significantly reduced the increased rectal temperature produced by LPS over the entire time tested (1-7 hr). EFR 50 mg/kg significantly ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, respectively) reduced LPS induced increase in rectal temperature over a period of 7 hr with a maximum reduction at 5 h. EFR 100 mg/kg significantly reduced the increased rectal temperature produced by LPS ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.05$, respectively) over a period of 7 hr with a maximum reduction at 3 hr. Both EFR 200 and 400 mg/kg significantly ($p<0.001$) reduced LPS induced increase in rectal temperature, over a period of 7 hr with a maximum reduction at 6 and 5 h, respectively. EFR 800 mg/kg significantly ($p<0.001$, $p<0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, respectively) reduced LPS induced increase in rectal temperature, over a period of 7 hr with a maximum reduction at 7 h. The antipyretic effect of all doses of EFR started as early as 1 hr and the effect was maintained for 7 hr after LPS injection (Table 3).

Rectal temperature of EFR groups at the doses of 50, 100, 200, 400 and 800 mg/kg was reduced to normal temperature within 3, 1, 3, 3 and 3 hr after LPS injection. The maximum reduction of mean rectal temperature produced by LPS in the presence of 50, 100, 200, 400 and 800 mg/kg of the EFR were 36.40±0.21°C, 36.54±0.15°C, 35.99±0.16°C, 36.36±0.20°C and 36.60±0.20°C, respectively. All doses of EFR used in this study were found to be as potent as ASA (Table 3).

Table 3 Effect of the ethanolic extract of *Ficus racemosa* (EFR) in lipopolysaccharide-induced fever in rats.

Treatment	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 rat[a]	36.90±0.14	36.70±0.12	36.89±0.03	36.56±0.07	36.77±0.12	36.36±0.09	36.37±0.13	36.49±0.12	36.47±0.10
Control[b]	36.84±0.12	36.60±0.11	38.21±0.07[###]	38.28±0.06[###]	38.41±0.11[###]	38.44±0.15[###]	38.36±0.13[###]	38.14±0.14[###]	37.78±0.12[###]
ASA 300 mg/kg	36.82±0.14	36.63±0.19	36.81±0.10***	36.74±0.15***	36.62±0.08***	36.01±0.18***	36.14±0.17***	36.27±0.13***	36.71±0.15**
EFR 50 mg/kg	36.62±0.19	36.88±0.10	36.99±0.16***	36.97±0.21***	36.63±0.20***	36.64±0.22***	36.40±0.21***	36.47±0.20***	36.69±0.24**
EFR 100 mg/kg	36.84±0.17	36.75±0.14	36.56±0.18***▲	36.63±0.15***	36.54±0.15***	36.54±0.24***	36.69±0.23***	36.94±0.14***	36.85±0.14*
EFR 200 mg/kg	36.98±0.13	36.65±0.26	36.89±0.12***	36.72±0.18***	36.39±0.15***	36.62±0.11***	36.43±0.15***	35.99±0.16***✦✦	36.22±0.21***
EFR 400 mg/kg	37.05±0.08	36.94±0.15	37.00±0.16***	37.04±0.18***	36.71±0.18***	36.38±0.23***	36.36±0.20***	36.49±0.18***	36.47±0.20***
EFR 800 mg/kg	36.84±0.09	36.96±0.11	37.22±0.13***	37.22±0.10*	36.94±0.12***	36.77±0.20***	36.78±0.23***	36.71±0.25***	36.60±0.20***

Each value represents mean ± SEM (n=8),. [a]Normal rats received 0.9% normal saline solution instead of lipopolysaccharide. [b]Control received 2%tween 80 solution.

[###]p<0.001 significantly different compared to normal rat values for the corresponding hour. *p<0.05, **p<0.01, ***p<0.001 significantly different compared to control values for the corresponding hour.

▲p<0.05 significantly different compared to EFR 800 mg/kg values for the corresponding hour. ✦✦p<0.01 significantly different compared to EFR 100 mg/kg values for the corresponding hour.

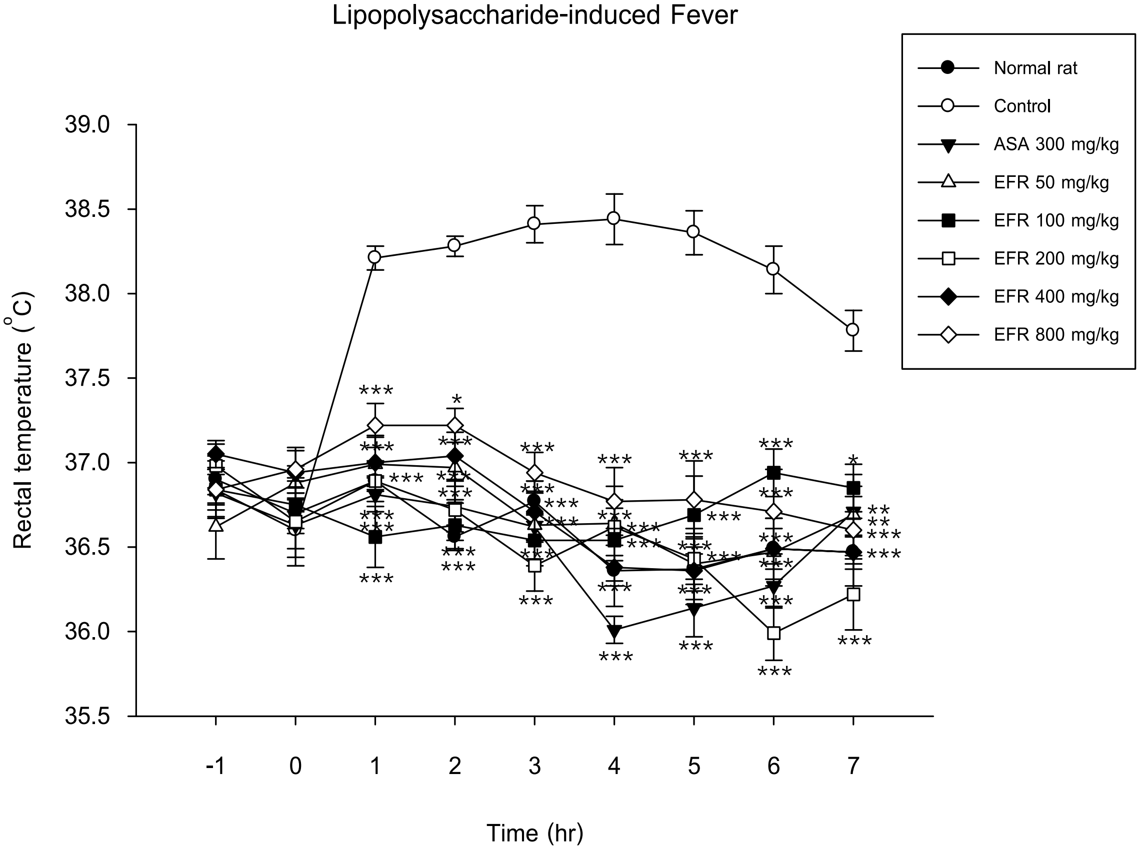

Figure 26 Changes in rectal temperature after oral administration of 2% tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-800 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 hr. All drugs were administered 1 hr before LPS. N = 8 for all groups. *p<0.05, **p<0.01, ***p<0.001 significantly different compared to control animals.

YEAST-INDUCED PYREXIA IN RATS

The results of the antipyretic effect of the ethanolic extract of *Ficus racemosa* L. root (EFR) were presented in (Table 4). Eighteen hours after subcutaneous injection of brewer's yeast, a significant increase in rectal temperature of approximately 2.24°C was observed in control animals ($p<0.001$) producing a mean rectal temperature of 38.92±0.18°C. At the same time, the mean rectal temperature of saline-injected normal rats was 36.59±0.15°C showing no febrile response occurred. The pyrexia induced by brewer's yeast was sustained throughout the observation period of 7 hr (Figure 27).

Acetylsalicylic acid (ASA; 300 mg/kg) significantly ($p<0.001$) reduced fever induced by brewer's yeast at 1 hr after oral administration by approximately 2.09°C and decreased the rectal temperature of rats at all time tested. Rectal temperature of ASA group was reduced to normal temperature within 1 hr after ASA administration. The ethanolic extract of *Ficus racemosa* L. root (EFR) at 50 mg/kg decreased rectal temperature significantly ($p<0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, respectively) at 1, 2, 3, 4, 5, 6 and 7 hr after oral administration. The EFR 100 mg/kg significantly ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, respectively) decreased rectal temperature at 1, 2, 3, 4, 5, 6 and 7 hr after administration. The EFR at the doses of 200 and 400 mg/kg significantly ($p<0.001$) decreased the rectal temperature at all time tested. The antipyretic effect of all doses of the EFR started as early as 1 hr and the effect was maintained for 7 hr after oral administration. Rectal temperature of EFR groups at the doses of 50, 100, 200 and 400 mg/kg was reduced to normal temperature at 6, 7, 6 and 6 hr, respectively after oral administration (Table 4).

The percent reduction in fever of ASA at 1, 2, 3, 4, 5, 6 and 7 hr after oral treatment were 5.37%, 5.71%, 5.93%, 5.32%, 5.68%, 5.69% and 5.92%, respectively. ASA showed the maximum % reduction of fever at 3 hr after oral administration. The maximum % reduction in fever after oral treatment of the EFR at the doses of 50, 100, 200 and 400 mg/kg were 4.68%, 5.01%, 6.14% and 5.69% respectively. The maximum

antipyretic effects of EFR were observed at 6, 7, 7 and 7 hr, respectively after oral administration (Figure 28). The EFR at the doses of 200 and 400 mg/kg appeared to be equally potent with ASA (Figure 28).

Table 4 Effect of the ethanolic extract of *Ficus racemosa* (EFR) in yeast-induced pyrexia in rats.

Treatment	Rectal temperature (°C) after treatment								
	Before yeast	18 hr after yeast	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Normal rat[a]	36.82±0.07	36.59±0.15	36.82±0.16	36.74±0.16	36.66±0.15	36.69±0.08	36.63±0.11	36.54±0.09	36.53±0.09
Control[b]	36.69±0.11	38.92±0.18###	39.08±0.14###	39.02±0.12###	38.92±0.16###	38.75±0.20###	38.48±0.26###	38.30±0.22###	37.96±0.19###
ASA 300 mg/kg	36.75±0.14	38.84±0.15	36.75±0.29***▲	36.62±0.25***▲▲✖	36.53±0.20***▲▲▲✖	36.77±0.13***	36.63±0.16***	36.63±0.15***	36.54±0.20***
EFR 50 mg/kg	36.62±0.17	38.73±0.20	37.93±0.19*	37.73±0.14***	37.74±0.15***	37.44±0.18***	37.14±0.20***	36.91±0.18***	37.01±0.17**
EFR 100 mg/kg	36.42±0.15	38.80±0.15	37.64±0.20**	37.57±0.17***	37.34±0.17***	37.21±0.17***	36.97±0.21***	36.92±0.19***	36.86±0.21**
EFR 200 mg/kg	36.66±0.16	38.97±0.15	37.50±0.27***	37.16±0.21***	37.14±0.15***	36.96±0.20***	36.93±0.17***	36.66±0.16***	36.58±0.21***
EFR 400 mg/kg	36.73±0.09	38.73±0.22	37.33±0.28***	37.19±0.24***	36.95±0.22***✦	36.91±0.19***	36.84±0.22***	36.71±0.17***	36.52±0.14***

Each value represents mean ± SEM (N=8), [a]Normal rats received 0.9% normal saline solution instead of 20% brewer's yeast. [b]Control received 2%tween 80 solution.

###p<0.001 significantly different compared to normal rat values for the corresponding hour. *p<0.05, **p<0.01, ***p<0.001 significant different compared to control values for the corresponding hour.

▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001 significantly different compared to EFR 50 mg/kg values for the corresponding hour. ✖p<0.05 significantly different compared to EFR 100 mg/kg values for the corresponding hour.

✦p<0.05 significantly different compared to EFR 50 mg/kg values for the corresponding hour.

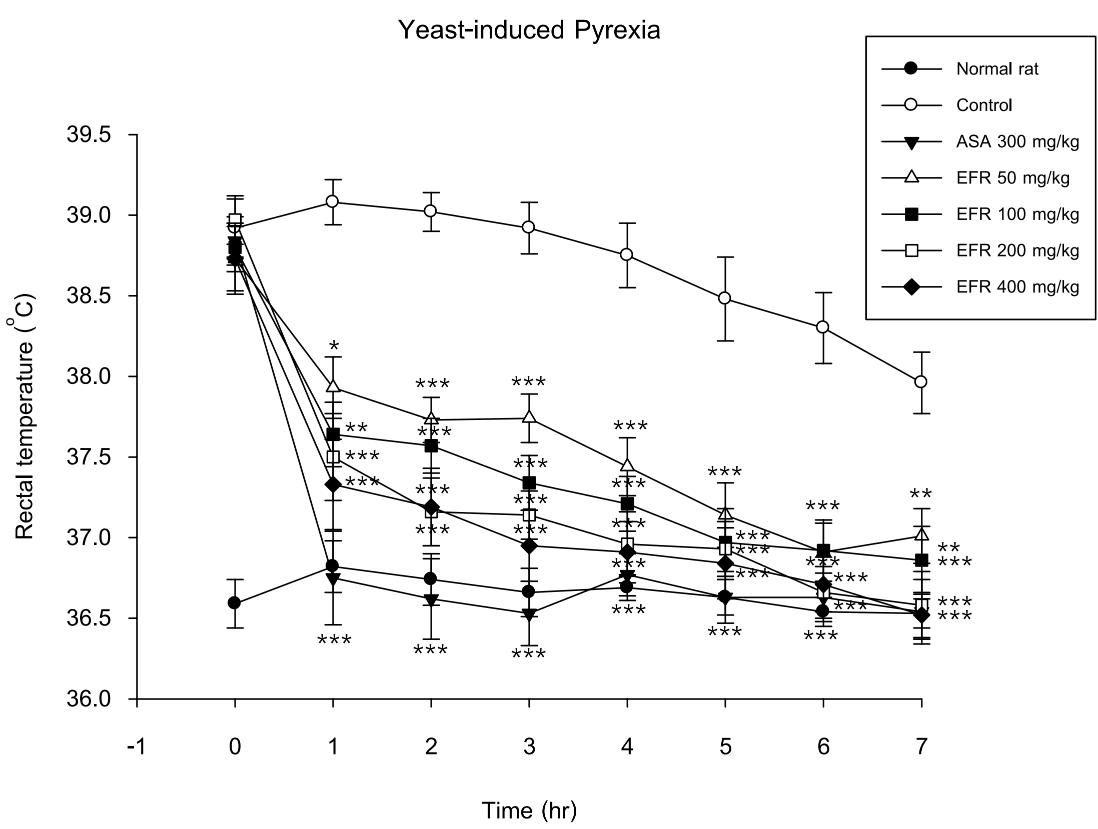

Figure 27 Changes in rectal temperature after oral administration of 2% tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-400 mg/kg) to rats with pyrexia. Pyrexia was induced by subcutaneous injection of 20% brewer's yeast (10 ml/kg) and 18 hr later the rats were used for the study. All drugs were administered at 0 hr. N = 8 for all groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to control animals.

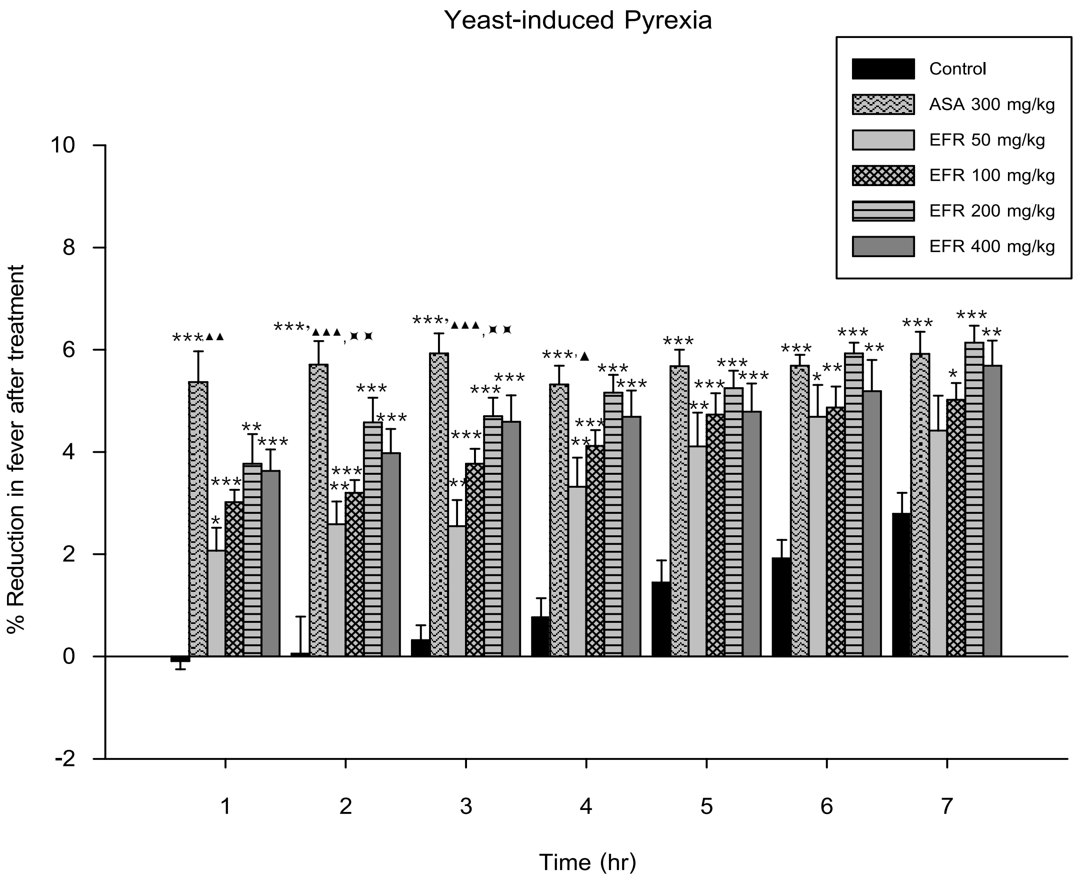

Figure 28 Percent reduction in fever after oral administration of 2% tween 80, acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-400 mg/kg). N=8 for all groups. *p<0.05, **p<0.01, ***p <0.001 significantly different compared to 2% tween 80. ▲p<0.05, ▲▲p< 0.01, ▲▲▲p< 0.001 significantly different compared to EFR 50 mg/kg.⌘⌘p< 0.01 significantly different compared to EFR 100 mg/kg.

CARRAGEENAN-INDUCED PAW EDEMA TEST

To demonstrate the validity of the carrageenan-induced paw edema test following drug administration, rats received intraperitoneal (i.p) administration of indomethacin (IND; 5 mg/kg) one hour before injection of carrageenan into the plantar surface of rat's right hind paw and were tested during the subsequent 6 hr period. As expected IND significantly ($p<0.001$) decreased paw edema at 1, 2, 3, 4, 5 and 6 hr after carrageenan injection compared with that of normal saline solution (NSS), producing a percentage of inhibition of edema of 46.15%, 52.44%, 55.10%, 61.40%, 51.79% and 53.41% respectively. The maximum inhibition of paw edema of IND (61.40%) was observed at 4 hr following carrageenan injection (Table 5).

Studies then utilized the carrageenan-induced paw edema test to examine the efficacy of the ethanolic extract of *Ficus racemosa* L. root (EFR) in producing anti-inflammation. Rats were administered i.p. 2% tween 80 or various doses of EFR (50, 100, 200, 400 and 800 mg/kg) one hour before carrageenan injection. All doses of the EFR tested (50, 100, 200, 400 and 800 mg/kg) significantly decreased the paw edema induced by carrageenan injection. The EFR 50 mg/kg decreased the paw volume significantly ($p<0.05$, $p<0.05$, respectively) and EFR 100 mg/kg decreased the paw volume significantly ($p<0.05$, $p<0.01$, respectively) at 2 and 4 hr after carrageenan injection compared to vehicle control. The EFR 200 mg/kg decreased the paw edema significantly ($p<0.05$, $p<0.01$, $p<0.05$, $p<0.05$, respectively) and EFR 400 mg/kg decreased the paw volume significantly ($p<0.05$, $p<0.001$, $p<0.01$, $p<0.01$, respectively) at 1, 2, 3, 4 hr after carrageenan injection compared to the vehicle control. The EFR 800 mg/kg decreased the paw volume significantly ($p<0.01$, $p<0.001$, $p<0.01$, $p<0.001$, $p<0.05$, respectively) at 1, 2, 3, 4, 5 hr after carrageenan injection compared to vehicle control. The EFR dose of 50, 100, 200, 400 and 800 mg/kg showed the maximum inhibition of paw edema of 39.82%, 40.71%, 53.10%, 61.06% and 64.60%, respectively at 2 hr following carrageenan injection (Table 7). Percentage of inhibition from 1-6 hr after i.p. administration of 2% tween 80 and various doses of EFR (50- 800 mg/kg) were shown in the Table 6.

When the log of EFR dose was plotted versus percentage of inhibition at 2 hr after carrageenan injection, a significant linear correlation coefficient (r^2) equal to 0.9439 was observed (Figure 29). ED_{50} was calculated from the log of EFR dose and % inhibition line and was equal to 166.35 mg/kg (Figure 29).

Table 5 Change of edema volume (ml) after intraperitoneal administration of 0.9% normal saline solution (NSS) and indomethacin (IND; 5 mg/kg) from 1-6 hr after carrageenan injection.

Treatments	Paw edema ± S.E.M. (ml) (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
NSS	0.39±0.02	0.82±0.05	0.98±0.05	1.14±0.03	1.12±0.03	1.03±0.04
IND 5 mg/kg	0.21±0.02***	0.39±0.04***	0.44±0.05***	0.44±0.07***	0.54±0.06***	0.48±0.06***
	(46.15%)	(52.44%)	(55.10%)	(61.40%)	(51.79%)	(53.41%)

N=6 for all groups. Inhibition was reported as percent compared to the vehicle control.

***$p<0.001$ significantly different compared to NSS.

Table 6 Change of edema volume (ml) after intraperitoneal administration of 2% tween 80 and various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-800 mg/kg) from 1-6 hr after carrageenan injection.

Treatments	Paw edema ± S.E.M. (ml) (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% tween 80	0.45±0.05	1.13±0.10	1.29±0.15	1.41±0.08	1.26±0.09	1.15±0.11
EFR 50 mg/kg	0.32±0.04 (28.89%)	0.68±0.12* (39.82%)	0.92±0.11 (28.68%)	1.04±0.04* (26.24%)	1.08±0.02 (14.29%)	1.08±0.06 (6.09%)
EFR 100 mg/kg	0.29±0.06 (35.56%)	0.67±0.09* (40.71%)	0.92±0.11 (28.68%)	0.93±0.10** (34.04%)	0.88±0.10 (30.16%)	0.93±0.12 (19.13%)
EFR 200 mg/kg	0.22±0.05* (51.11%)	0.53±0.10** (53.10%)	0.77±0.11* (40.31%)	1.01±0.10* (28.37%)	0.98±0.12 (22.22%)	0.88±0.15 (23.48%)
EFR 400 mg/kg	0.21±0.03* (53.33%)	0.44±0.07*** (61.06%)	0.64±0.06** (50.39%)	0.95±0.08** (32.62%)	0.90±0.13 (28.57%)	0.89±0.12 (22.61%)
EFR 800 mg/kg	0.17±0.03** (62.22%)	0.40±0.05*** (64.60%)	0.67±0.06** (48.06%)	0.73±0.08*** (48.23%)	0.66±0.11* (47.62%)	0.66±0.12 (42.61%)

N=6 for all groups. Inhibition was reported as percent compared to the vehicle control.

*$p<0.05$, **$p<0.01$, ***$p<0.001$ significantly different compared to 2% tween 80.

Carageenan-induced Paw Edema

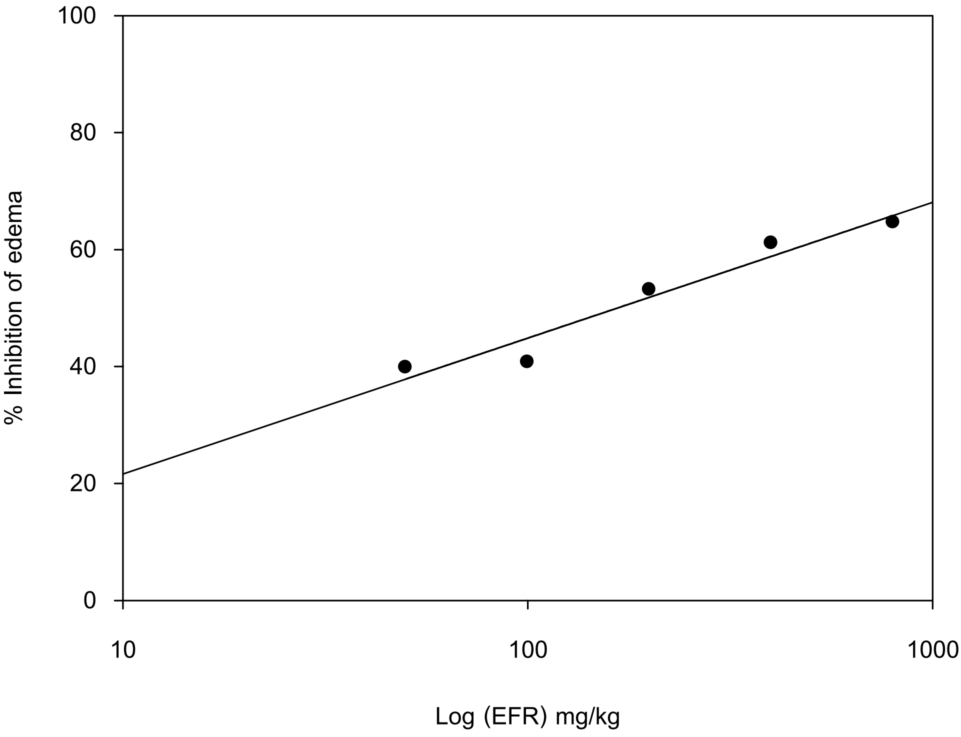

Figure 29 Linear regression of percentage of inhibition of edema after intraperitoneal administration of various doses of the ethanolic extract of *Ficus racemosa* L. root (EFR; 50-800 mg/kg) at 2 hr after carrageenan injection. N=6 for all groups. The regression equation was Y = 10.0859*Ln(x) – 1.5802, r^2 =0.9439. ED_{50}= 166.35 mg/kg.

CHAPTER V
DISCUSSION AND CONCLUSION

These studies have demonstrated the antinociceptive, antipyretic and anti-inflammatory effects of the ethanolic extract of *Ficus racemosa* L. root (EFR) in various animal models. Antinociceptive activity was assessed utilizing thermal (tail-flick test) and chemical (acetic acid-induced writhing test) models. Antipyretic effect was assessed utilizing lipopolysaccaride-induced and yeast-induced fever models. Anti-inflammatory effect was assessed utilizing an acute inflammation model (carrageenan-induced paw edema).

Initial attempts to investigate the analgesic effect of EFR utilized the mouse tail-flick technique, a central analgesic activity testing model that measures spinal reflex. 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") (Bars et al., 2001). This model usually employs morphine as a reference drug. Morphine (MO) administered intraperitoneally (i.p.) showed potent analgesic effect on the response in this model indicating the sensitivity of this test (Figure 17). The EFR was administered i.p. by suspending in 2% (w/v) aqueous tween 80 solution. Only EFR doses of 200 and 400 mg/kg produced significant analgesic response compared to vehicle treated controls during 240 min period (Figure 18). EFR produced milder analgesic effect in the spinal level compared to morphine (Figure 19). The linear regression equation between the log dose of EFR and the response (area of analgesia; %MPE-min) in the mouse tail-flick test was Response = 663.1075*Ln(x) -1725.41, r^2 = 0.8222 (Figure 20). The linear regression equation between the log dose of EFR and %MPE values at the time point at which peak antinociceptive response was observed was Y= 4.9316*Ln(x) -7.1146, r^2 = 0.8327 (Figure 21).

The acetic acid-induced writhing test was chosen to measure the analgesic effect of EFR against chemical stimuli. This test is commonly used for measuring peripheral analgesic activity and considered as a model of visceral inflammatory pain. Several mediators including kinin, acetylcholine, substance P and prostaglandins are involved in this visceral pain model of nociception (Vogel and Vogel, 1997) and

transmission of nociception from the viscera (Cervero and Laird, 1999). Intraperitoneal injection of agents (originally phenylbenzoquinone) that are irritating to serous membranes provokes a stereotypical behavior in rodents that is characterized by contraction of abdominal, twisting and turning of the trunk, and extension of the hind limbs (Svender and Hau, 1994). In this test, commonly called the "writhing test" indomethacin (IND), a non-steroidal anti-inflammatory drug, was used as a reference drug. Intraperitoneal administration of IND (10 mg/kg) produced significant analgesic response compared to NSS treated controls (Figure 23). All doses of EFR used in this study (50-400 mg/kg) produced significant analgesic response compared to the vehicle controls (Figure 24) and appeared to produce similar analgesic response compared to IND (Figure 25). It can be concluded that EFR exerts antinociceptive effect at the periphery and the maximum effect was obtained at the lowest dose (50 mg/kg) of EFR. The future research should be done to investigate the analgesic effect of EFR at the doses lower than 50 mg/kg.

Fever is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), macrophage protein-1 (MIP-1) and prostaglandins (Kluger, 1991, Roth and De Souza, 2001). Lipopolysaccharide (LPS) has been shown to produce fever in laboratory animals such as guinea pigs and rabbits by stimulating the production of endogenous TNF-α. (Kluger 1991, Roth and Zeisberger, 1995). Prostaglandin synthesis can be activated by TNF-α or phospholipase A_2 (Aronoff and Neilson, 2001). Brewer's yeast model induces both TNF-α and prostaglandin synthesis (Kluger, 1991). For better characterization of the antipyretic activity of the ethanolic extract of *Ficus racemosa* L. root (EFR), two models of pyresis including LPS-induced and brewer's yeast-induced fever were employed in this study. 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 thermoregulatory sites. They suppress peripheral production of pyrogenic cytokines such as TNF-α and interleukin-1β while lower the thermoregulatory

set point by blocking central cyclooxygenase production of prostaglandin E_2 (PGE_2) (Aronoff and Neilson, 2001).

In the LPS-induced fever model, 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 (Table 3). EFR (50-800 mg/kg) was administered orally by suspending in tween 80 solution. The oral administration was chosen in order to imitate the normal human consumption of “Bencha-Loga-Wichian” an antipyretic herbal formula. All doses of EFR showed antipyretic activity in LPS-induced fever model of rats at all time tested indicating that the antipyretic effect of EFR may be due to the suppression of TNF-α or the inhibition of its synthesis and block prostaglandin synthesis and therefore attenuates fever. The antipyretic effect of all doses of EFR occurred shortly within 1 hr after LPS injection and was sustained for up to 7 hr similar to ASA (Figure 26). The efficacy of all doses of the EFR (50-800 mg/kg) was comparable to that of ASA.

The EFR (50-400 mg/kg) also significantly attenuated the fever induced by brewer’s yeast suggesting that the antipyretic mechanism of the EFR occurred through inhibition of TNF-α and prostaglandin synthesis. The onset of action of all doses of the EFR started within 1 hr after oral administration with duration of action of 7 hr similar to ASA (Figure 27). The high doses of EFR (200 and 400 mg/kg) had comparable efficacy to that of ASA. The efficacy of all doses of the EFR increased with time (Figure 28). In this study brewer’s yeast induced increasing in rectal temperature of rats by approximately 2.24°C, while LPS increased the rectal temperature by approximately 1.84°C. Therefore, the lowest doses of EFR (50 and 100 mg/kg) showed less antipyretic effect in the brewer’s yeast-induced pyrexia model compared to the LPS-induced fever model.

Carrageenan-induced paw edema is a standard experimental model of acute inflammation (Winter et al., 1962). Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Carrageenan-induced paw edema is a biphasic response; the first phase (1 hr) involves the release of serotonin and histamine and second phase

(over 1 hr) is mediated by prostaglandins, cyclooxygenase products, and the continuity between the two phases is provided by kinins (Perianayagam et al., 2006). In this study, indomethacin (IND; 5 mg/kg) i.p. significantly reduced paw edema at all times tested (1-6 hrs) after carrageenan injection compared with that of normal saline solution (NSS) (Table 5). The anti-edematous effect of IND is due to inhibition of those inflammatory mediators from reaching the injured site or from bringing out their pharmacological effects which normally ameliorate the inflammation and other symptoms by the fact that IND is a cyclooxygenase inhibitor, resulting in the reduction of prostaglandins. IND showed the maximum inhibition of paw edema of 61.40% at 4 hr after carrageenan injection. This result is consistent with the previous study that nonsteroidal anti-inflammatory drugs (NSAIDs), such as IND, reduce paw swelling in a dose-dependent manner to a maximum of ~ 60% (Whiteley and Dalrymple, 1998).

All doses of EFR showed significant reduction of paw edema with a maximum inhibition at 2 hr after carrageenan injection (Table 6). The anti-edematous effect of EFR during the second phase could be concluded that EFR involves in the reduction of prostaglandin through the inhibition of cyclooxygenase enzyme which is consistent with the *in vitro* studies that the ethanolic extract of several parts of *Ficus racemosa* inhibited activity of prostaglandin and cyclooxygenase enzyme (Li et al., 2003). Percent inhibition of edema of EFR increased with dose. Linear correlation between the log dose of EFR ranging from 50 to 800 mg/kg and the maximum percentage of inhibition of edema at 2 hr after carrageenan injection was found with r^2 = 0.9439 and gave the ED_{50} value of 166.35 mg/kg (Figure 29).

In conclusion, this present study has demonstrated that EFR exerts a pronounced antinociception when assessed in thermal and chemical models of nociception in rodents. EFR has both central and peripheral analgesic activities. Nevertheless, EFR has poor efficacy in central analgesic activities compared to morphine. The analgesic mechanisms of the EFR are not known at this time. EFR was demonstrated to reduce rectal temperature in both LPS and yeast-induced fever models. The results suggested that the antipyretic mechanism of EFR occurred through suppression of TNF-α and inhibition of prostaglandin synthesis via cyclooxygenase

pathway. Although this study had provided the information about the onset and duration of action of the extract of the *Ficus racemosa* L. root, further investigation is required to determine the pharmacokinetic profiles. In addition, EFR also has anti-inflammatory effect assessed with carrageenan-induced paw edema model. The anti-inflammatory mechanism is possibly due to the inhibition of prostaglandin synthesis by cyclooxygenase pathway.

FUTURE RESEARCH

In these studies there are some evidences that the ethanolic extract of *Ficus racemosa* L. root is capable of significantly produced antinociceptive, antipyretic and anti-inflammatory responses, and support the potential use of extract.

The future research can comprise of several objectives as listed below:

(1) To identify the active components of the extract.

(2) To investigate the antinociceptive and antipyretic effects of lower doses of EFR.

(3) To investigate the possible mechanisms of antinociceptive, antipyretic and anti-inflammatory effects of EFR.

(4) To investigate the antinociceptive effect of various doses of EFR in other various animal models including models which use mechanical stimuli and chronic pain model such as neuropathic pain model.

(5) To investigate the potential use of EFR in combination with other analgesics or nonsteroidal anti-inflammatory drugs.

(6) To investigate other routes of administration that might be more appropriate for the use of EFR and possibly enhance the analgesic, antipyretic or anti-inflammatory effects of EFR.

(7) To investigate the anti-inflammatory effect of various doses of EFR in other chronic inflammatory models.

(8) To test side effects and toxic effects of EFR at high doses.

These and other studies may provide important clues to help understand the mechanism underlying the analgesic, antipyretic and anti-inflammatory effects of EFR and further support the use of such compound in a clinical setting.

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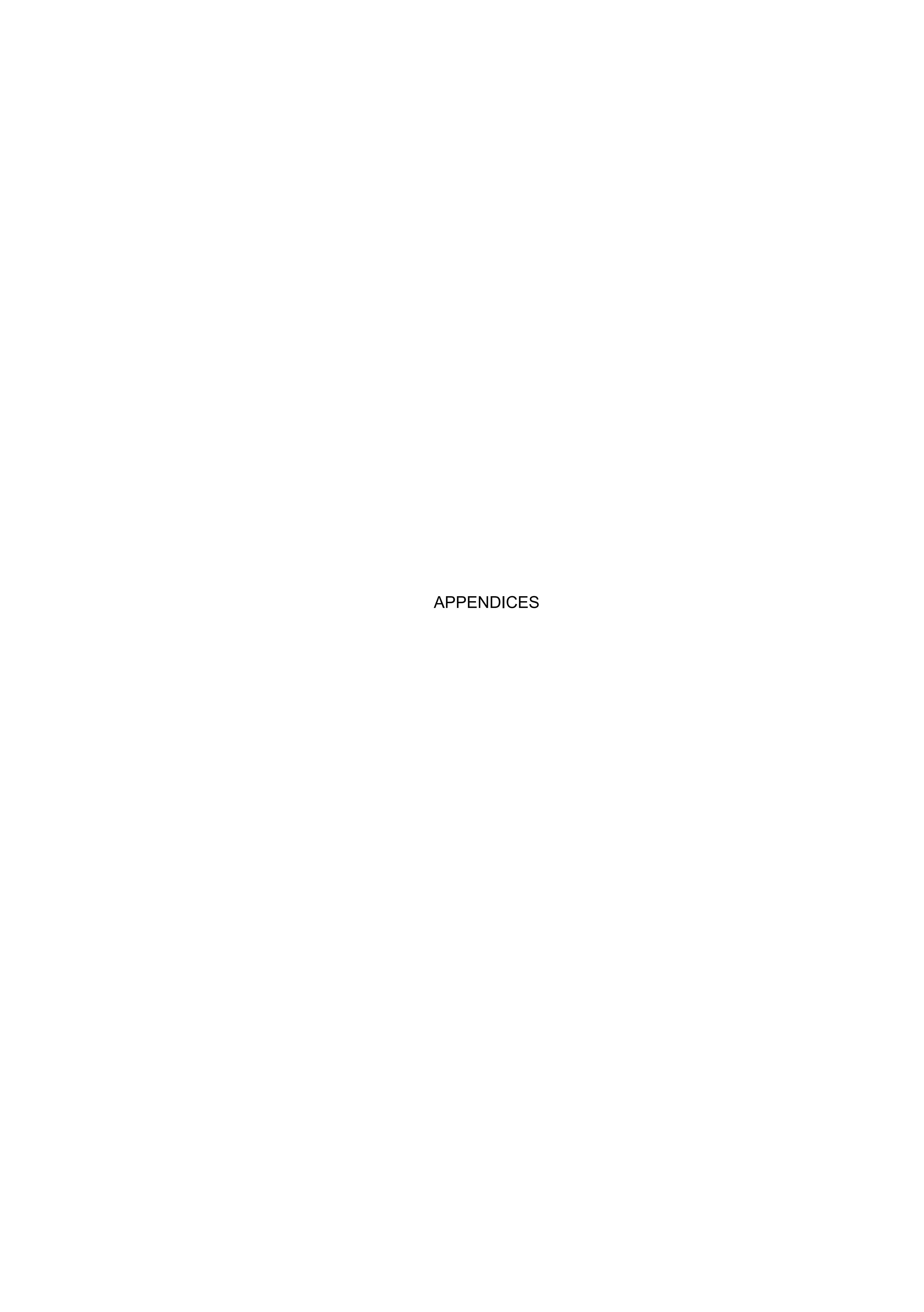
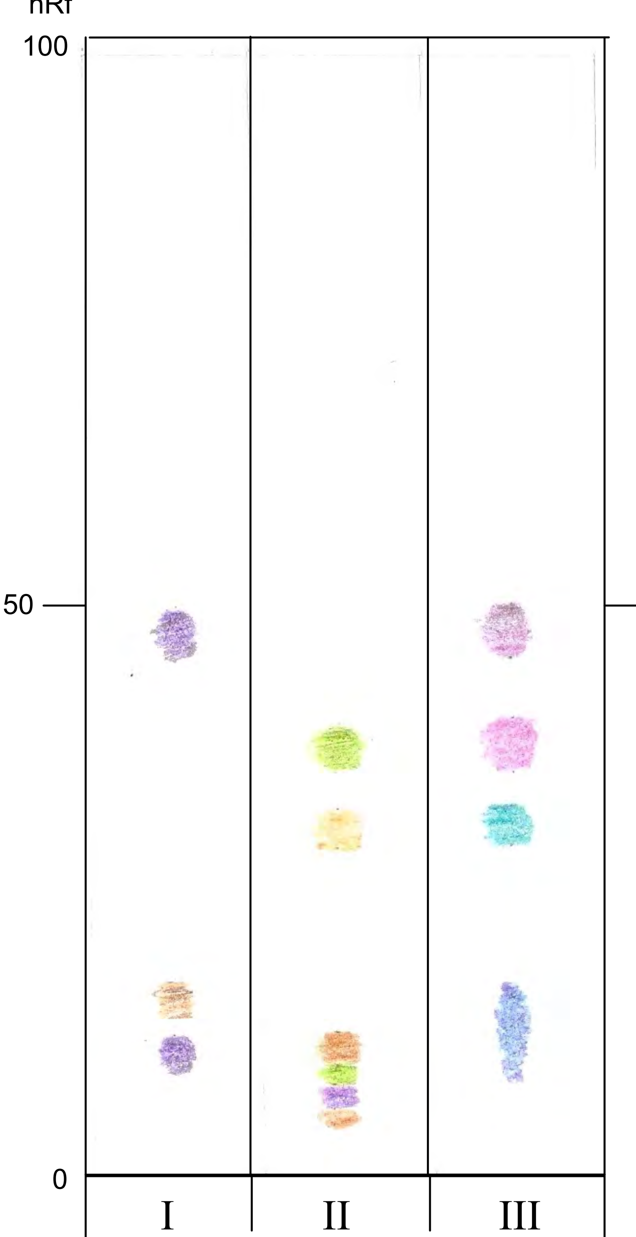


APPENDICES

Appendix A

Thin-layer chromatogram of the ethanolic extract of *Ficus racemosa* Linn. Root



Solvent system Chloroform

Stationary phase SiO_2 GF_{254}

Detection

I = detection under UV light 254 nm

II = detection under UV light 365 nm

III = detection with vanillin-sulfuric acid regent

Figure 30 Thin-layer chromatogram of the ethanolic extract of *Ficus racemosa* Linn. Root

Appendix B

Data of mouse tail-flick test

Table 7 Latency (sec) in mouse tail-flick test from 0-240 min after intraperitoneal administration of the various doses of EFR (50-400 mg/kg) N=10 for all groups. Data presented as mean±S.E.M.

EFR (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
50	1.43±0.22	1.30±0.18	1.34±0.08	1.34±0.15	1.23±0.10	1.14±0.10	1.20±0.07
100	1.40±0.13	1.70±0.13	1.45±0.08	1.41±0.14	1.32±0.89	1.31±0.13	1.24±0.10
200	1.38±0.16	1.35±0.07	1.53±0.15	1.68±0.12	1.42±0.06	1.36±0.11	1.46±0.13
400	1.48±0.07	1.84±0.20	1.55±0.10	1.63±0.18	1.51±0.12	1.33±0.09	1.41±0.08

Table 8 %MPE-time in mouse tail-flick test from 0-240 min after intraperitoneal administration of the various doses of EFR (50-400 mg/kg) N=10 for all groups. Data presented as mean±S.E.M.

EFR (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia (%MPE-min)
50	10.77±7.08	5.83±6.20	7.00±3.10	7.45±4.68	3.37±3.14	0.09±3.40	2.27±1.88	765.93±527.68
100	7.90±3.83	18.40±4.38	9.34±2.74	8.03±4.85	4.61±3.24	4.25±4.57	1.90±3.27	1285.87±683.00
200	7.36±5.81	6.42±1.36	12.93±4.88	17.64±4.74	8.61±2.35	6.75±3.33	10.31±4.17	2181.09±543.00
400	9.06±3.26	22.42±7.01	11.93±3.12	15.35±5.75	10.23±4.59	3.91±3.07	6.71±2.88	1999.62±291.42

Appendix C

Data of acetic acid-induced writhing in mice

Table 9 Dose-response and time-course effects of various doses of EFR (50-400 mg/kg) on acetic acid-induced writhing in mice. Data presented as mean±S.E.M.

Treatment (mg/kg)	Time Course (5 min blocks)						Total
	1	2	3	4	5	6	
Tween 10 ml/kg	1.70±0.68	10.80±1.23	8.30±1.22	8.10±1.70	5.80±1.02	3.50±0.81	38.20±4.86
EFR 50	0.20±1.33	1.20±0.59	1.70±0.70	0.90±0.46	0.80±0.55	0.30±0.21	5.1±1.91
EFR100	0.20±.20	1.80±1.24	1.80±0.59	2.40±0.75	1.30±0.42	0.60±0.34	8.10±2.51
EFR 200	0.10±0.10	0.90±0.41	2.20±0.44	2.30±0.52	1.70±0.62	0.70±0.34	7.90±1.73
EFR 400	0±0	0.30±0.15	1.20±0.57	1.00±.033	1.30±0.47	1.30±0.42	5.10±1.15

Appendix D

Data of lipopolysacchacide induced fever in rats

Table 10 Effect of NSS (10 ml/kg, i.m.) in rats in lipopolysacchacide-induced fever model (normal rats)

No.	Rectal temperature (°C) before and after treatment (NSS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.62	36.84	36.78	36.68	36.58	36.14	36.26	36.22	36.50
2	37.36	36.50	36.92	36.58	36.42	36.40	36.08	36.50	36.62
3	37.30	36.94	36.98	36.46	37.26	36.80	36.88	36.82	36.70
4	36.98	36.42	36.80	36.34	37.14	36.52	36.24	36.70	36.14
5	37.18	37.18	37.02	36.32	36.64	36.58	36.36	36.64	36.46
6	36.62	36.30	36.76	36.74	36.34	36.06	35.88	36.04	36.40
7	36.16	36.36	36.94	36.46	36.70	36.18	36.32	36.04	36.02
8	36.94	37.02	36.88	36.90	37.10	36.18	36.94	36.92	36.90
average	36.90	36.70	36.89	36.56	36.77	36.36	36.37	36.49	36.47

Table 11 Effect of 2% tween 80 (10 ml/kg, p.o.) in lipopolysacchacide-induced fever in rats

No.	Rectal temperature (°C) before and after treatment (LPS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.10	36.74	38.10	38.10	38.16	38.12	37.94	37.78	37.48
2	36.86	36.92	38.66	38.28	38.04	38.08	38.18	37.84	37.70
3	36.78	36.30	38.10	38.36	38.30	38.30	38.64	38.60	38.44
4	36.12	36.16	38.28	38.30	38.82	38.66	38.36	38.00	37.60
5	36.94	36.96	38.32	38.40	38.36	38.28	38.20	38.08	37.86
6	37.18	36.88	38.18	38.54	38.92	39.30	38.88	38.54	37.76
7	36.62	36.26	38.04	38.24	38.54	38.68	38.78	38.60	37.96
8	37.12	36.58	37.98	38.02	38.14	38.12	37.90	37.68	37.40
average	36.84	36.60	38.21	38.28	38.41	38.44	38.36	38.14	37.78

Table 12 Effect of ASA (300 mg/kg, p.o.) in lipopolysacchacide-induced fever in rats

No.	Rectal temperature (°C) before and after treatment (LPS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.16	35.50	36.70	37.00	36.66	35.00	35.64	36.10	36.32
2	37.22	36.92	36.72	36.60	36.52	36.54	36.40	36.16	36.78
3	36.00	36.60	37.08	36.86	36.52	35.92	35.68	35.96	37.32
4	36.82	36.80	37.04	36.92	36.68	36.30	36.36	36.64	36.64
5	36.62	37.04	36.88	36.64	37.02	36.34	36.14	36.24	36.24
6	37.04	37.08	37.04	37.20	36.56	36.24	36.44	36.18	36.46
7	36.64	36.20	36.18	35.82	36.20	36.16	36.90	37.00	37.40
8	37.04	36.88	36.82	36.86	36.80	35.58	35.56	35.88	36.50
average	36.82	36.63	36.81	36.74	36.62	36.01	36.14	36.27	36.71

Table 13 Effect of EFR (50 mg/kg, p.o.) in lipopolysacchacide-induced fever in rats

No.	Rectal temperature ($^{\circ}C$) before and after treatment (LPS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	35.78	36.68	36.68	36.70	36.58	36.10	36.02	36.06	35.62
2	37.14	36.44	36.10	35.80	35.60	35.46	35.36	35.40	35.78
3	37.02	37.28	36.78	37.32	37.22	37.26	36.38	36.20	37.38
4	36.36	37.16	37.28	37.64	36.68	36.44	36.22	37.20	37.26
5	36.40	36.68	37.48	37.54	37.48	37.18	37.26	36.66	36.62
6	37.38	37.14	37.06	36.92	36.76	37.06	36.94	37.02	37.28
7	36.78	36.78	37.18	36.76	36.46	36.82	36.66	36.66	36.54
8	36.12	36.84	37.36	37.04	36.24	36.78	36.36	36.56	37.00
average	36.62	36.88	36.99	36.97	36.63	36.64	36.40	36.47	36.69

Table 14 Effect of EFR (100 mg/kg, p.o.) in lipopolysacchacide-induced fever in rats

No.	Rectal temperature (°C) before and after treatment (LPS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.94	36.20	36.00	36.34	36.82	36.78	37.20	36.84	36.82
2	35.96	36.40	36.18	36.00	36.80	37.00	36.56	36.34	36.36
3	36.88	37.10	36.38	36.50	36.02	35.36	35.34	37.06	36.62
4	37.18	36.32	36.82	36.84	36.56	35.66	36.82	37.30	37.40
5	37.06	37.00	36.32	36.46	36.90	37.34	36.68	37.18	37.00
6	36.30	36.94	36.54	36.88	36.48	36.44	36.30	36.38	36.36
7	37.34	37.26	37.64	37.46	36.96	36.84	37.32	37.26	37.34
8	37.04	36.80	36.56	36.58	35.78	36.90	37.28	37.18	36.92
average	36.84	36.75	36.56	36.63	36.54	36.54	36.69	36.94	36.85

Table 15 Effect of EFR (200 mg/kg, p.o.) in lipopolysacchacide-induced fever in rats

No.	Rectal temperature (°C) before and after treatment (LPS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.92	35.32	36.82	36.74	36.68	36.56	36.62	36.04	36.44
2	37.42	36.18	37.02	36.90	36.46	36.08	36.00	35.40	35.82
3	37.36	37.44	36.84	37.14	35.96	36.64	36.32	36.16	36.50
4	36.68	36.50	36.50	36.16	36.26	37.04	36.70	35.58	35.66
5	37.36	37.42	37.18	36.32	36.50	36.94	35.80	35.70	35.72
6	36.90	37.08	37.06	36.96	37.12	36.72	36.26	35.84	35.62
7	36.38	36.16	36.38	36.00	35.72	36.68	37.20	36.50	37.08
8	36.78	37.10	37.34	37.50	36.38	36.26	36.56	36.68	36.88
average	36.98	36.65	36.89	36.72	36.39	36.62	36.43	35.99	36.22

Table 16 Effect of EFR (400 mg/kg, p.o.) in lipopolysacchacide-induced fever in rats

No.	Rectal temperature (°C) before and after treatment (LPS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.34	37.22	37.14	37.42	37.04	36.96	36.36	36.22	36.30
2	37.04	36.04	36.26	36.30	37.40	36.84	36.40	35.70	35.48
3	37.16	37.30	37.16	37.36	36.52	36.00	35.60	35.94	36.18
4	36.76	36.80	37.34	37.30	36.26	35.38	35.62	37.02	36.06
5	37.24	37.16	37.36	37.40	37.36	37.34	37.28	37.14	36.98
6	36.66	36.88	36.86	37.14	36.66	36.50	36.82	36.58	36.86
7	37.12	37.36	37.44	37.20	36.44	35.76	36.40	36.56	37.24
8	37.10	36.76	36.42	36.18	36.02	36.28	36.38	36.72	36.62
average	37.05	36.94	37.00	37.04	36.71	36.38	36.36	36.49	36.47

Table 17 Effect of EFR (800 mg/kg, p.o.) in lipopolysacchacide-induced fever in rats

No.	Rectal temperature (°C) before and after treatment (LPS injection)								
	-1 hr	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.08	36.60	37.66	37.66	37.12	36.10	36.02	37.52	37.18
2	36.66	37.32	36.60	37.18	36.74	37.00	36.40	35.74	35.84
3	36.66	37.34	37.50	37.14	36.90	36.84	37.14	37.02	37.16
4	36.88	37.02	37.44	37.38	36.88	37.32	37.44	37.08	37.02
5	36.76	37.10	37.46	37.44	37.58	37.42	37.30	36.72	36.76
6	36.46	37.02	37.30	37.28	37.08	36.72	37.00	36.74	36.32
7	36.94	36.72	36.92	36.76	36.46	35.78	35.66	35.54	35.76
8	37.26	36.56	36.86	36.94	36.72	36.98	37.26	37.30	36.76
average	36.84	36.96	37.22	37.22	36.94	36.77	36.78	36.71	36.60

Appendix E

Data of brewer's yeast induced fever in rats

Table 18 Effect of NSS (10 ml/kg, s.c.) in rats in brewer's yeast-induced fever model (normal rats)

No.	Before NSS	18 hr after NSS	Rectal temperature (°C) after treatment						
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.90	36.12	36.14	36.04	36.26	36.50	36.52	35.82	36.14
2	36.94	36.82	36.66	36.80	36.94	36.78	36.76	36.10	36.94
3	36.42	36.02	36.84	36.74	36.24	36.56	36.38	36.44	36.66
4	36.90	36.42	37.14	36.86	36.68	36.68	36.80	36.52	36.64
5	37.08	36.84	36.48	36.74	37.06	36.60	36.30	36.28	36.34
6	36.62	37.36	37.38	37.38	37.20	36.98	36.98	36.80	36.76
7	36.82	36.70	37.34	37.14	36.84	37.04	37.02	36.68	36.34
8	36.90	36.40	36.58	36.18	36.02	36.36	36.26	36.64	36.40
average	36.82	36.59	36.82	36.74	36.66	36.69	36.63	36.41	36.53

Table 19 Effect of 2% tween 80 (10 ml/kg, p.o.) in brewer's yeast-induced fever in rats

No.	Before yeast	18 hr after yeast	Rectal temperature (°C) after treatment						
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.36	38.38	38.44	38.50	38.40	38.04	37.10	36.92	36.82
2	36.92	38.90	39.02	39.08	39.34	39.38	39.28	38.78	38.64
3	36.64	39.18	39.10	38.60	38.56	38.40	38.46	38.42	38.14
4	36.48	38.96	39.02	38.94	39.00	38.62	38.38	38.40	38.20
5	36.48	38.70	38.66	38.94	38.36	38.02	37.84	37.98	37.62
6	36.66	39.10	39.44	39.24	39.08	39.20	38.68	38.60	38.04
7	37.30	39.36	39.46	39.40	39.00	38.86	38.80	38.50	38.12
8	36.66	39.80	39.50	39.48	39.62	39.44	39.32	38.78	38.08
average	36.69	39.05	39.08	39.02	38.92	38.75	38.48	38.30	37.96

Table 20 Effect of ASA (300 mg/kg, p.o.) in brewer's yeast-induced fever in rats

No.	Before yeast	18 hr after yeast	Rectal temperature (°C) after treatment						
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	37.20	38.54	37.60	36.72	36.50	36.84	36.92	36.58	36.50
2	36.24	39.20	37.68	37.64	37.22	37.10	37.00	37.06	37.26
3	36.90	39.42	37.70	37.34	37.40	37.18	37.14	37.06	36.90
4	36.98	38.00	35.60	35.62	35.96	36.38	35.92	35.80	35.76
5	36.12	38.80	36.64	36.66	36.56	37.26	36.98	37.02	37.12
6	36.72	38.98	36.10	35.72	35.76	36.28	36.54	36.60	35.70
7	36.72	38.78	36.04	36.62	36.16	36.60	36.06	36.40	36.56
8	37.08	38.96	36.66	36.62	36.68	36.50	36.46	36.48	36.48
average	36.75	38.84	36.75	36.62	36.53	36.77	36.63	36.63	36.54

Table 21 Effect of EFR (50 mg/kg, p.o.) in brewer's yeast-induced fever in rats

No.	Before yeast	18 hr after yeast	Rectal temperature (°C) after treatment						
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	35.80	38.40	37.10	37.08	37.14	36.50	36.14	36.22	36.80
2	36.02	38.20	37.18	37.22	37.34	37.02	36.70	36.54	36.64
3	37.18	39.48	38.24	37.74	37.58	37.54	37.16	36.84	36.48
4	36.74	38.76	38.00	37.84	38.10	37.68	37.64	37.68	37.78
5	36.74	38.26	37.82	37.62	37.44	37.60	37.62	37.04	36.94
6	36.66	38.20	38.22	38.02	38.08	37.52	37.02	36.74	36.94
7	37.10	38.92	38.52	38.14	38.24	38.26	37.86	37.60	37.76
8	36.68	39.64	38.34	38.14	38.00	37.42	36.94	36.64	36.78
average	36.62	38.73	37.93	37.73	37.74	37.44	37.14	36.91	37.02

Table 22 Effect of EFR (100 mg/kg, p.o.) in brewer's yeast-induced fever in rats

No.	Before yeast	18 hr after yeast	Rectal temperature (°C) after treatment						
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.00	38.36	37.36	37.30	37.20	36.56	36.42	36.36	36.12
2	36.30	38.56	37.22	37.48	37.30	36.96	36.62	36.52	36.34
3	36.00	38.52	37.16	36.68	36.46	36.54	36.02	36.02	36.14
4	36.72	38.68	37.80	37.60	37.06	37.32	36.82	37.10	36.76
5	36.10	39.54	38.62	38.06	37.78	37.60	37.32	37.22	37.64
6	37.22	39.26	38.08	38.08	37.94	37.46	37.42	37.26	37.34
7	36.38	39.00	37.94	37.94	37.70	37.88	37.68	37.42	37.26
8	36.64	38.54	36.92	37.38	37.30	37.36	37.48	37.44	37.30
average	36.42	38.81	37.64	37.57	37.34	37.21	36.97	36.92	36.86

Table 23 Effect of EFR (200 mg/kg, p.o.) in brewer's yeast-induced fever in rats

No.	Before yeast	18 hr after yeast	Rectal temperature (°C) after treatment						
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.00	38.58	36.30	36.86	37.06	36.24	36.38	36.06	35.64
2	37.10	38.94	37.36	37.10	36.96	36.54	36.36	36.36	36.08
3	36.50	38.30	37.40	36.50	36.58	36.42	36.68	36.16	36.12
4	36.38	39.32	37.72	37.46	36.90	36.92	37.02	36.88	37.00
5	37.06	39.50	38.62	37.64	37.50	37.96	37.88	37.40	37.40
6	37.24	39.22	38.36	38.16	37.96	37.34	36.90	36.76	36.90
7	36.76	38.72	37.58	37.46	37.24	37.30	37.08	36.78	36.82
8	36.26	39.20	36.70	36.32	36.90	36.98	37.10	36.90	36.68
average	36.66	38.97	37.51	37.19	37.14	36.96	36.93	36.66	36.58

Table 24 Effect of EFR (400 mg/kg, p.o.) in brewer's yeast-induced fever in rats

No.	Before yeast	18 hr after yeast	Rectal temperature (°C) after treatment						
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	36.74	37.78	37.00	37.14	36.94	36.92	36.74	36.78	36.50
2	36.70	38.16	36.34	36.14	36.10	36.18	35.92	35.94	36.00
3	36.32	39.42	38.26	37.40	37.30	37.12	36.42	36.12	36.24
4	36.62	39.42	37.94	37.74	36.72	36.82	37.36	36.90	36.80
5	36.76	39.04	37.62	37.32	37.56	37.04	37.30	37.10	36.68
6	36.62	39.00	38.16	38.08	37.74	37.62	37.80	37.44	37.24
7	37.20	38.80	37.10	37.38	37.10	37.44	36.90	36.82	36.56
8	36.88	38.22	36.18	36.28	36.12	36.14	36.54	36.60	36.16
average	36.73	38.73	37.33	37.19	36.95	36.91	36.87	36.71	36.52

Table 25 Percent reduction in fever after oral administration of 2% tween 80

No.	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	-0.16	-0.31	-0.05	0.89	3.34	3.80	4.06
2	-0.31	-0.46	-1.13	-1.23	-0.98	0.31	0.67
3	0.20	1.48	1.58	1.99	1.84	1.94	2.65
4	-0.15	0.05	-0.10	0.87	1.49	1.44	1.95
5	0.10	-0.62	0.88	1.76	2.22	1.86	2.79
6	-0.87	-0.36	0.05	-0.26	1.07	1.28	2.71
7	-0.25	-0.10	0.91	1.27	1.42	2.18	3.15
8	0.75	0.80	0.45	0.90	1.21	2.56	4.32
average	-0.09	0.06	0.32	0.77	1.45	1.92	2.79

Table 26 Percent reduction in fever after oral administration of ASA 300 mg/kg

No.	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	2.44	4.72	5.29	4.41	4.20	5.09	5.29
2	3.88	3.98	5.05	5.36	5.61	5.46	4.95
3	4.36	5.28	5.12	5.68	5.78	5.99	6.39
4	6.32	6.26	5.37	4.26	5.47	5.79	5.89
5	5.57	5.52	5.77	3.97	4.69	4.59	4.33
6	7.39	8.36	8.26	6.93	6.26	6.11	8.41
7	7.07	5.57	6.76	5.62	7.01	6.14	5.72
8	5.90	6.01	5.85	6.31	6.42	6.37	6.37
average	5.37	5.71	5.93	5.32	5.68	5.69	5.92

Table 27 Percent reduction in fever after oral administration of EFR 50 mg/kg

No.	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	3.39	3.44	3.28	4.95	5.89	5.68	4.17
2	2.67	2.57	2.25	3.09	3.93	4.35	4.08
3	3.14	4.41	4.81	4.91	5.88	6.69	7.60
4	1.96	2.37	1.70	2.79	2.89	2.79	2.53
5	1.15	1.67	2.14	1.73	1.67	3.19	3.45
6	-0.05	0.47	0.31	1.78	3.09	3.82	3.30
7	1.03	2.00	1.75	1.70	2.72	3.39	2.98
8	3.28	3.78	4.14	5.60	6.81	7.57	7.21
average	2.07	2.59	2.55	3.32	4.11	4.68	4.42

Table 28 Percent reduction in fever after oral administration of EFR 100 mg/kg

No.	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	2.61	2.76	3.02	4.69	5.06	5.21	5.84
2	3.48	2.80	3.27	4.15	5.03	5.29	5.76
3	3.53	4.78	5.35	5.14	6.49	6.49	6.18
4	2.28	2.79	4.19	3.52	4.81	4.08	4.96
5	2.33	3.74	4.45	4.91	5.61	5.87	4.81
6	3.01	3.01	3.36	4.58	4.69	5.09	4.89
7	2.72	2.72	3.33	2.87	3.38	4.05	4.46
8	4.20	3.01	3.22	3.06	2.75	2.85	3.22
average	3.02	3.20	3.77	4.12	4.73	4.87	5.01

Table 29 Percent reduction in fever after oral administration of EFR 200 mg/kg

No.	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	5.91	4.46	3.94	6.07	5.70	6.53	7.62
2	4.06	4.73	5.08	6.16	6.63	6.63	7.34
3	2.35	4.70	4.49	4.91	4.23	5.59	5.69
4	4.07	4.73	6.15	6.10	5.85	6.21	5.90
5	2.23	4.71	5.06	3.90	4.10	5.32	5.32
6	2.19	2.70	3.21	4.79	5.92	6.27	5.92
7	2.94	3.25	3.82	3.67	4.24	5.01	4.91
8	6.38	7.35	5.87	5.66	5.36	5.87	6.43
average	3.77	4.58	4.70	5.16	5.25	5.93	6.14

Table 30 Percent reduction in fever after oral administration of EFR 400 mg/kg

No.	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
1	2.06	1.69	2.22	2.28	2.75	2.65	3.39
2	4.77	5.29	5.40	5.19	5.87	5.82	5.66
3	2.94	5.12	5.38	5.83	7.61	8.37	8.07
4	3.75	4.26	6.85	6.60	5.23	6.39	6.65
5	3.64	4.41	3.79	5.12	4.46	4.97	6.05
6	2.15	2.36	3.23	3.54	3.08	4.00	4.51
7	4.38	3.66	4.38	3.51	4.90	5.10	5.77
8	5.34	5.08	5.49	5.44	4.40	4.24	5.39
average	3.63	3.98	4.59	4.69	4.79	5.19	5.69

Appendix F

Data of carrageenan induced-paw edema in rats

Table 31 Effect of NSS (i.p.) on paw volume in carrageenan-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.34	1.25	1.19	1.34	1.25	1.19	_	_
1 hr	Paw volume (ml)	1.77	1.62	1.68	1.68	1.62	1.54		_
	Volume of edema (ml)	0.43	0.37	0.49	0.34	0.37	0.35	0.39	
2 hr	Paw volume (ml)	2.01	2.08	2.15	2.28	2.04	1.93		_
	Volume of edema (ml)	0.67	0.83	0.96	0.94	0.79	0.74	0.82	
3 hr	Paw volume (ml)	2.17	2.26	2.10	2.43	2.18	2.31		_
	Volume of edema (ml)	0.83	1.01	0.91	1.09	0.93	1.12	0.98	
4 hr	Paw volume (ml)	2.36	2.43	2.29	2.43	2.48	2.39		_
	Volume of edema (ml)	1.02	1.18	1.10	1.09	1.23	1.20	1.14	
5 hr	Paw volume (ml)	2.38	2.41	2.35	2.42	2.28	2.44		_
	Volume of edema (ml)	1.04	1.16	1.16	1.08	1.03	1.25	1.12	
6 hr	Paw volume (ml)	2.25	2.25	2.38	2.44	2.22	2.18		_
	Volume of edema (ml)	0.91	1.00	1.19	1.10	0.97	0.99	1.15	

Table 32 Effect of indomethacin (5 mg/kg, i.p.) on paw volume in carrageenan-induced paw edema in rats

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.38	1.26	1.49	1.37	1.42	1.40	_	_
1 hr	Paw volume (ml)	1.65	1.46	1.64	1.54	1.68	1.62		46.15
	Volume of edema (ml)	0.27	0.20	0.15	0.17	0.26	0.22	0.21	
2 hr	Paw volume (ml)	1.84	1.69	1.70	1.71	1.82	1.89		52.44
	Volume of edema (ml)	0.46	0.43	0.21	0.34	0.40	0.49	0.39	
3 hr	Paw volume (ml)	1.90	1.63	1.76	1.72	2.00	1.94		55.10
	Volume of edema (ml)	0.52	0.37	0.27	0.35	0.58	0.54	0.44	
4 hr	Paw volume (ml)	1.92	1.59	1.70	1.79	2.13	1.85		61.40
	Volume of edema (ml)	0.54	0.33	0.21	0.42	0.71	0.45	0.44	
5 hr	Paw volume (ml)	1.91	1.70	1.82	1.95	2.17	1.98		51.79
	Volume of edema (ml)	0.53	0.44	0.33	0.58	0.75	0.58	0.54	
6 hr	Paw volume (ml)	1.93	1.62	1.84	1.83	2.18	1.82		53.40
	Volume of edema (ml)	0.55	0.36	0.35	0.46	0.76	0.42	0.48	

Table 33 Effect of 2% tween 80 (10 ml/kg, i.p.) on paw volume in carrageenan-induced paw edema in rats

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.34	1.59	1.69	1.39	1.62	1.37	_	_
1 hr	Paw volume (ml)	1.81	2.26	2.19	1.68	2.01	1.73		_
	Volume of edema (ml)	0.47	0.67	0.50	0.29	0.39	0.36	0.45	
2 hr	Paw volume (ml)	2.56	2.73	2.86	2.06	2.83	2.71		_
	Volume of edema (ml)	1.22	1.14	1.17	0.67	1.21	1.34	1.13	
3 hr	Paw volume (ml)	2.95	3.00	3.00	2.05	2.72	3.03		_
	Volume of edema (ml)	1.61	1.41	1.31	0.66	1.10	1.66	1.29	
4 hr	Paw volume (ml)	3.00	3.05	2.94	2.51	3.10	2.84		_
	Volume of edema (ml)	1.66	1.46	1.25	1.12	1.48	1.47	1.41	
5 hr	Paw volume (ml)	2.60	2.77	2.60	2.71	3.15	2.75		_
	Volume of edema (ml)	1.26	1.18	0.91	1.32	1.53	1.38	1.26	
6 hr	Paw volume (ml)	2.28	2.55	2.54	2.69	3.12	2.73		_
	Volume of edema (ml)	0.94	0.96	0.85	1.30	1.50	1.36	1.15	

Table 34 Effect of EFR (50 mg/kg, i.p.) on paw volume in carrageenan-induced paw edema in rats

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.25	1.35	1.34	1.33	1.48	1.40	_	_
1 hr	Paw volume (ml)	1.62	1.62	1.64	1.60	1.70	1.91		28.89
	Volume of edema (ml)	0.37	0.27	0.30	0.27	0.22	0.51	0.32	
2 hr	Paw volume (ml)	2.01	2.32	1.88	1.80	1.80	2.44		39.82
	Volume of edema (ml)	0.76	0.97	0.54	0.47	0.32	1.04	0.68	
3 hr	Paw volume (ml)	2.28	2.48	2.02	1.95	2.25	2.66		28.68
	Volume of edema (ml)	1.03	1.13	0.68	0.62	0.77	1.26	0.92	
4 hr	Paw volume (ml)	2.24	2.42	2.29	2.25	2.62	2.58		26.24
	Volume of edema (ml)	0.99	1.07	0.95	0.92	1.14	1.18	1.04	
5 hr	Paw volume (ml)	2.35	2.49	2.35	2.44	2.59	2.42		14.29
	Volume of edema (ml)	1.10	1.14	1.01	1.11	1.11	1.02	1.08	
6 hr	Paw volume (ml)	2.49	2.58	2.22	2.39	2.56	2.41		6.09
	Volume of edema (ml)	1.24	1.23	0.88	1.06	1.08	1.01	1.08	

Table 35 Effect of EFR (100 mg/kg, i.p.) on paw volume in carrageenan-induced paw edema in rats

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.45	1.22	1.42	1.44	1.42	1.37	_	_
1 hr	Paw volume (ml)	1.60	1.55	1.60	1.63	1.91	1.78		35.56
	Volume of edema (ml)	0.15	0.33	0.18	0.19	0.49	0.41	0.29	
2 hr	Paw volume (ml)	1.78	2.06	2.18	1.94	2.18	2.21		40.71
	Volume of edema (ml)	0.33	0.84	0.76	0.50	0.76	0.84	0.67	
3 hr	Paw volume (ml)	1.87	2.26	2.45	2.42	2.27	2.54		28.68
	Volume of edema (ml)	0.42	1.04	1.03	0.98	0.85	1.17	0.92	
4 hr	Paw volume (ml)	1.95	2.15	2.54	2.60	2.22	2.43		34.04
	Volume of edema (ml)	0.50	0.93	1.12	1.16	0.80	1.06	0.93	
5 hr	Paw volume (ml)	1.93	2.29	2.52	2.52	2.11	2.25		30.16
	Volume of edema (ml)	0.48	1.07	1.10	1.08	0.69	0.88	0.88	
6 hr	Paw volume (ml)	2.06	2.19	2.68	2.73	2.02	2.19		19.13
	Volume of edema (ml)	0.61	0.97	1.26	1.29	0.60	0.82	0.93	

Table 36 Effect of EFR (200 mg/kg, i.p.) on paw volume in carrageenan-induced paw edema in rats

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.53	1.34	1.81	1.48	1.50	1.50	_	_
1 hr	Paw volume (ml)	1.95	1.58	1.98	1.63	1.58	1.73		51.11
	Volume of edema (ml)	0.42	0.24	0.17	0.15	0.08	0.23	0.22	
2 hr	Paw volume (ml)	2.40	2.01	2.24	1.72	1.82	2.14		53.10
	Volume of edema (ml)	0.87	0.67	0.43	0.24	0.32	0.64	0.53	
3 hr	Paw volume (ml)	2.56	2.17	2.57	1.72	2.26	2.48		40.31
	Volume of edema (ml)	1.03	0.83	0.76	0.24	0.76	0.98	0.77	
4 hr	Paw volume (ml)	2.58	2.19	2.60	2.25	2.81	2.76		28.37
	Volume of edema (ml)	1.05	0.85	0.79	0.77	1.31	1.26	1.01	
5 hr	Paw volume (ml)	2.32	2.17	2.40	2.51	2.90	2.75		22.22
	Volume of edema (ml)	0.79	0.83	0.59	1.03	1.40	1.25	0.98	
6 hr	Paw volume (ml)	2.18	2.09	2.22	2.31	2.87	2.77		23.48
	Volume of edema (ml)	0.65	0.75	0.41	0.83	1.37	1.27	0.88	

Table 37 Effect of EFR (400 mg/kg, i.p.) on paw volume in carrageenan-induced paw edema in rats

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.49	1.50	1.57	1.48	1.58	1.47	_	_
1 hr	Paw volume (ml)	1.79	1.73	1.87	1.65	1.66	1.66		53.33
	Volume of edema (ml)	0.30	0.23	0.30	0.17	0.08	0.19	0.21	
2 hr	Paw volume (ml)	2.06	2.07	1.96	1.70	1.85	2.09		61.06
	Volume of edema (ml)	0.57	0.57	0.39	0.22	0.27	0.62	0.44	
3 hr	Paw volume (ml)	2.18	2.29	2.00	2.04	2.13	2.26		50.39
	Volume of edema (ml)	0.69	0.79	0.43	0.56	0.55	0.79	0.64	
4 hr	Paw volume (ml)	2.22	2.51	2.24	2.53	2.63	2.65		32.62
	Volume of edema (ml)	0.73	1.01	0.67	1.05	1.05	1.18	0.95	
5 hr	Paw volume (ml)	2.04	2.49	2.03	2.66	2.64	2.61		28.57
	Volume of edema (ml)	0.55	0.99	0.46	1.18	1.06	1.14	0.90	
6 hr	Paw volume (ml)	2.09	2.26	2.12	2.63	2.64	2.67		22.61
	Volume of edema (ml)	0.60	0.76	0.55	1.15	1.06	1.20	0.89	

Table 38 Effect of EFR (800 mg/kg, i.p.) on paw volume in carrageenan-induced paw edema in rats

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% inhibition
Paw volume before carrageenan injection		1.30	1.39	1.20	1.38	1.29	1.31	_	_
1 hr	Paw volume (ml)	1.57	1.47	1.42	1.53	1.45	1.46		62.22
	Volume of edema (ml)	0.27	0.08	0.22	0.15	0.16	0.15	0.17	
2 hr	Paw volume (ml)	1.89	1.79	1.69	1.77	1.56	1.56		64.60
	Volume of edema (ml)	0.59	0.40	0.49	0.39	0.27	0.25	0.40	
3 hr	Paw volume (ml)	2.06	1.98	2.05	2.11	1.74	1.93		48.06
	Volume of edema (ml)	0.76	0.59	0.85	0.73	0.45	0.62	0.67	
4 hr	Paw volume (ml)	2.09	2.17	2.22	2.13	1.83	1.83		48.23
	Volume of edema (ml)	0.79	0.78	1.02	0.75	0.54	0.52	0.73	
5 hr	Paw volume (ml)	2.07	2.20	2.24	1.88	1.75	1.66		47.62
	Volume of edema (ml)	0.77	0.81	1.04	0.50	0.46	0.35	0.66	
6 hr	Paw volume (ml)	2.02	2.14	2.36	1.89	1.79	1.62		42.61
	Volume of edema (ml)	0.72	0.75	1.16	0.51	0.50	0.31	0.66	

Appendix G

Study protocol approval by the Institutional Animal Care and Use Committee,

Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

Chulalongkorn University Animal Care and Use Committee

Certificate of Project Approval	☑ Original ☐ Renew
Animal Use Protocol No. 08-33-011	**Approval No. 08-33-011**
Protocol Title Antinociceptive antipyretic and anti-inflammatory effects of the ethanolic extract of ficus racemosa *Linn. root*	
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 April 25, 2008	**Date of Expiration** March 24, 2009
Applicant Faculty/Institution **Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Rd., Pathumwan BKK-THAILAND. 10330**	
Signature of Chairperson Withaya Janthasoot	**Signature of Authorized Official**
Name and Title **WITHAYA JANTHASOOT** **Chairman**	**Name and Title** **RUNGPETCH SAKULBUMRUNGSIL, Ph.D.** **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.

VITAE

Miss. Sirakarn Chomchuen was born in September 15, 1979 in Chiangmai, Thailand. She graduated with a Bachelor degree of Nursing in 2002 from Chiangmai University. After graduation, she worked in Surg unit of Maharaj Nakorn Chiangmai Hospital for two years. Now she is currently working in the intensive care unit of Bumrungrad International Hospital, Bangkok, Thailand.