ฤทธิ์ระงับปวดและฤทธิ์ต้านการอักเสบของเคอร์คิวมินในสัตว์ทดลอง

นางสาววรวรรณา บัวดอนไพร

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ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF CURCUMIN IN ANIMAL MODELS

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วรวรรณา บัวดอนไพร : ฤทธิ์ระงับปวดและฤทธิ์ต้านการอักเสบของเคอร์คิวมินในสัตว์ทดลอง. (ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF CURCUMIN IN ANIMAL MODELS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.ร.ท.หญิง ภัสราภา โตวิวัฒน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.ดร.พรชัย โรจน์สิทธิศักดิ์, 118 หน้า.

เคอร์คิวมินเป็นสารสำคัญที่ได้จากขมิ้นชันและมีฤทธิ์ในการรักษาโรคต่างๆมากมาย การทดลองครั้งนี้เริ่มต้นศึกษาฤทธิ์ระงับปวดของเคอร์คิวมินที่ได้จากการสังเคราะห์ในขนาดต่างๆ กัน ด้วยวิธี hot-plate จับเวลาที่หนูเม้าส์เพศผู้สามารถทนอยู่บนแผ่นร้อนได้ (hot-plate latencies) ก่อนให้น้ำเกลือ ทางช่องท้อง มอร์ฟีน (10 มก./กก.) ทางช่องท้อง คาร์บอกซีเมทิลเซลลูโลส (0.5%) โดยการป้อน หรือเคอร์คิวมินสังเคราะห์ขนาด 25, 50, 100, 200 และ 400 มก./กก. โดยการป้อน และจับเวลาที่หนูสามารถทนอยู่บนแผ่นร้อนได้ ที่เวลา 15, 30, 45, 60, 90, 120 และ 240 นาที หลังให้สารทดสอบ โดยให้หนูเม้าส์อยู่บนแผ่นความร้อนไม่เกิน 45 วินาทีในการทดสอบแต่ละครั้ง (cut-off time) คำนวณเปอร์เซ็นต์สูงสุดที่หนูเม้าส์สามารถทนต่อความร้อนได้ (%MPE) แล้วนำมาคำนวณหาพื้นที่ใต้กราฟระหว่าง %MPE และเวลา (area of analgesia) พบว่า เคอร์คิวมินทุกขนาดที่ใช้ในการทดสอบมีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ ฤทธิ์ระงับปวดของเคอร์คิวมินที่ขนาด 200 มก./กก. ถูกยับยั้งได้ด้วยนาลอกโซน แสดงว่ากลไกการออกฤทธิ์ระงับปวดของเคอร์คิวมินเกี่ยวข้องกับตัวรับ opioid ในการทดลองด้วยวิธี tail-flick ทำการจับเวลาที่หนูเม้าส์ทนต่อความร้อนได้โดยไม่กระดกหางหนี (tail-flick latencies) ก่อนให้ น้ำเกลือ มอร์ฟีน คาร์บอกซีเมทิลเซลลูโลส (0.5%) หรือเคอร์คิวมินสังเคราะห์ขนาด 25-400 มก./กก. และทำการทดสอบหลังจากได้รับสารอีก 7 ครั้ง ในช่วงเวลา 4 ชม. โดยหางหนูจะได้รับความร้อนจากการส่องไฟแต่ละครั้งไม่เกิน 4 วินาที (cut-off time) พบว่าเคอร์คิวมินทุกขนาดที่ใช้ในการทดสอบไม่แสดงฤทธิ์ระงับปวด ส่วนการทดสอบที่เหนี่ยวนำให้หนูเม้าส์เกิดการบิดงอของลำตัว (writhing) ด้วยกรดอะซิติก จะทำการฉีดกรดอะซิติก 0.6% ในขนาด 10 มล./กก. เข้าทางช่องท้องของหนูเม้าส์ที่เวลา 1 ชั่วโมง หลังจากป้อนน้ำเกลือ อินโดเมทาซิน (10 มก./กก.) คาร์บอกซีเมทิลเซลลูโลส (0.5%) หรือเคอร์คิวมินสังเคราะห์ขนาด 25-400 มก./กก. แล้วนับจำนวนครั้งที่หนูเม้าส์เกิดการบิดงอของลำตัวเป็นเวลา 30 นาที พบว่าเคอร์คิวมินขนาด 200 และ 400 มก./กก. สามารถลดการบิดงอของลำตัวของหนูเม้าส์ได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม สำหรับการทดสอบด้วยวิธี Randall-Selitto จะฉีดคาราจีแนนเข้าที่อุ้งเท้าหลังข้างซ้ายของหนูแรทที่เวลา 1 ชม. หลังจากป้อน น้ำเกลือ อินโดเมทาซิน (10 มก./กก.) คาร์บอกซีเมทิลเซลลูโลส (0.5%) หรือเคอร์คิวมินสังเคราะห์ขนาด 25-400 มก./กก. และทำการวัดแรงกดที่อุ้งเท้าที่หนูสามารถทนได้ (paw withdrawal thresholds) ในช่วงเวลา 4 ชม. หลังจากให้คาราจีแนน พบว่าเคอร์คิวมินขนาด 200 มก./กก. ทำให้หนูแรททนต่อแรงกดที่อุ้งเท้าได้เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเป็นเวลา 4 ชม.

ในทดสอบฤทธิ์ต้านการอักเสบของเคอร์คิวมินสังเคราะห์ในหนูแรท ด้วยการเหนี่ยวนำให้อุ้งเท้าหนูบวมด้วยคาราจีแนน พบว่าเคอร์คิวมินทุกขนาดที่ใช้ในการทดสอบสามารถลดการบวมของอุ้งเท้าได้อย่างมีนัยสำคัญทางสถิติในระยะที่ 2 ของการบวม จากผลการทดลองทั้งหมดแสดงว่า เคอร์คิวมินสังเคราะห์มีฤทธิ์ระงับปวดทั้งในระดับประสาทส่วนกลางและระดับประสาทส่วนปลาย และกลไกการออกฤทธิ์ระงับปวดมีความเกี่ยวข้องกับตัวรับของ opioid ส่วนกลไกการต้านการอักเสบของเคอร์คิวมินสังเคราะห์มีส่วนเกี่ยวข้องกับการยับยั้งการสร้างพรอสตาแกลนดิน

ภาควิชา.....เภสัชวิทยา.....ลายมือชื่อนิสิต.....วรวรรณา นัวดอนไพร.....
สาขาวิชา.....เภสัชวิทยา.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....ร.ท.หญิง ภัสราภา โตวิวัฒน์.....
ปีการศึกษา.....2551.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEY WORD: CURCUMIN/ ANTINOCICEPTION/ ANTI-INFLAMMATORY/ HOT-PLATE TEST/ TAIL-FLICK TEST/ RANDALL-SELITTO/ CARRAGEENAN-INDUCED PAW EDEMA

WARAWANNA BUADONPRI: ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF CURCUMIN IN ANIMAL MODELS. THESIS PRINCIPAL ADVISOR: ASST. PROF. LT. PASARAPA TOWIWAT, PH.D., THESIS CO-ADVISOR: ASST. PROF. PORNCHAI ROJSITTHISAK, PH.D. 118 pp.

Curcumin is the important active ingredient responsible for the biological activity of *Curcuma longa* L. We initially determined the antinociceptive property of a range of synthetic curcumin doses in the mouse hot-plate test. Hot-plate latencies (cut-off 45 sec) were determined in male ICR mice prior to the administration of 0.9% normal saline solution (NSS; 10 ml/kg, i.p.), morphine (MO; 10 mg/kg, i.p.) 0.5% carboxymethylcellulose (CMC; 10 ml/kg, p.o.) or various doses of synthetic curcumin (25-400 mg/kg, p.o.). Hot-plate latencies were subsequently determined at 15, 30, 45, 60, 90, 120 and 240 min. The percent maximum possible effect (%MPE) was calculated and used in the determination of the area of analgesia (%MPE-min). All doses of curcumin tested produced a significant analgesic response. Curcumin 200 mg/kg produced analgesic response that was naloxone-sensitive suggesting opioid-mediated mechanism. In the mouse tail-flick test, tail-flick latencies (cut-off 4 sec) were determined prior to the administration of NSS, MO, 0.5% CMC or various doses of synthetic curcumin (25- 400 mg/kg) and were subsequently determined at 7 intervals over a 4 hr period. All doses of curcumin tested failed to produce analgesic response. In acetic acid-induced writhing in mice, the animals were induced with intraperitoneal injection of 0.6% acetic acid (10 ml/kg) 1 hr after the administration of NSS, indomethacin (IND, 10 mg/kg, p.o.), 0.5% CMC or various doses of synthetic curcumin (25-400 mg/kg, p.o.) and the mean writhing response was determined for 30 min. Curcumin 200 and 400 mg/kg significantly ($p<0.05$) decreased the mean writhing response compared to vehicle controls. In the Randall-Selitto test, rats were injected with carrageenan into the plantar surface of the left hind paw 1 hr after the administration of NSS, indomethacin (IND; 10 mg/kg, p.o.), 0.5%CMC or various doses of synthetic curcumin (25-400 mg/kg, p.o.) and the mean paw withdrawal threshold were determined over a 4 hr period. Curcumin 200 mg/kg significantly ($p<0.05$) increased mean paw withdrawal thresholds up to 4 hr after carrageenan administration.

Studies then determined the anti-inflammatory property of orally administered synthetic curcumin (25-400 mg/kg) using carrageenan-induced paw edema test in rats. All doses of curcumin significantly reduced paw volume during the second phase of edema. Taken together these results demonstrated that synthetic curcumin produced both central and peripheral analgesic activity and mechanism of action seems to be related to opioid receptors. The mechanism of anti-inflammatory effect of curcumin may involve in the inhibition of prostaglandin synthesis.

Department: Pharmacology Student's signature: Warawanna Buadonpri

Field of study: Pharmacology Principal Advisor's signature: Lt. Pasarapa Towiwat

Academic year: 2008 Co-advisor's signature: Pornchai Rojsitthisak

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LIST OF ABBREVIATIONS

β	= beta
δ	= delta
°C	= degree of Celsius
μ	= mu
κ	= kappa
μl	= microlitre
α	= alpha
γ	= gamma
%MPE	= percentage of the maximum possible effect
/	= per
ADI	= acceptable daily intake
AUC	= area under the curve (area of analgesia)
BW	= body weight
cm	= centimeter
CMC	= carboxymethylcellulose
CNS	= central nervous system
ED_{50}	= median effective dose
et al.	= et alii (and other)
g	= gram
hr	= hour
IASP	= International Association for the Study of Pain
IND	= indomethacin
i.p.	= intraperitoneal
mg/kg	= milligram per kilogram
min	= minute
ml/kg	= milliliter per kilogram
MO	= morphine sulphate
N	= sample size
NAL	= naloxone

NCI	= National Cancer Institute
NO	= nitric oxide
NOS	= nitric oxide synthase
NSAIDs	= Nonsteroidal anti-inflammatory drugs
NSS	= normal saline solution
PAF	= platelet-activating factor
PWTs	= paw withdrawal thresholds
sec	= second
TNF-α	= tumor necrosis factor-alpha

CHAPTER I
INTRODUCTION

Background and Rationale

Pain is a common problem in the general population, with research showing that in an adult population between 7–59% (Crombie et al., 1994; Croft et al., 1993; Verhaak et al., 1998; Elliott et al., 1999) suffer from pain. The impact on health economy is huge as chronic pain is estimated to be the third largest health problem in the world (Latham & Davis, 1994).

Presently, a variety of drug classes are used to treat chronic noncancer pain, including antidepressants, anticonvulsants, anti-inflammatory agents, muscle relaxants, antiarrhythmics, opioids, local anesthetics, and topical capsicin. Thus far, these agents primarily have been directed toward the disease state associated with the pain. Although opioids and nonsteriodal anti-inflammatory drugs are still the mainstays of analgesic therapy, these drugs possess a number of adverse effects. For example, opioids frequently cause respiratory depression, sedation, constipation and nausea while NSAIDs produce gastrointestinal ulceration, renal dysfunction and platelet inhibition. Therefore, the investigation of novel analgesic and anti-inflammatory agents without those side effects has been received considerable attention.

The use of medicinal plants or their active components in prevention and treatment of chronic disease is based on experience from traditional systems of medicine from various ethnic societies. Curcuma longa L., commonly called turmeric is extensively used as traditional medicine in India, China and Southeast Asia. Current traditional Indian medicine claims the use of turmeric against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Chinese traditional medicine uses turmeric in diseases associated with abdominal pains, icterus, etc. Its medicinal properties have been attributed mainly to the yellow pigment called curcuminiods, with curcumin as a main component. It has also been known to be safe for human consumption because turmeric is widely used as spice, food preservative and coloring agent. To search for a novel analgesic and anti-inflammatory agent, curcumin could therefore be served as a great candidate.

Purpose of the study

To evaluate the antinociceptive and anti-inflammatory effects of synthetic curcumin in comparison with the reference drugs. In addition, the possible mechanisms involved were also investigated.

Hypothesis

Curcumin has antinociceptive and anti-inflammatory effects in various animal models.

Research design

Experimental Research

Expected benefit and application

The knowledge obtained from the studies of antinociceptive and anti-inflammatory effects of synthetic curcumin could lead to the development of a new analgesic and/or anti-inflammatory agent.

Key words

Curcumin
Antinociception
Anti-inflammatory
Hot-plate test
Tail-flick test
Acetic acid-induced writhing test
Randall-Selitto test
Carrageenan-induced paw edema

CHAPTER II
LITERATURE REVIEWS

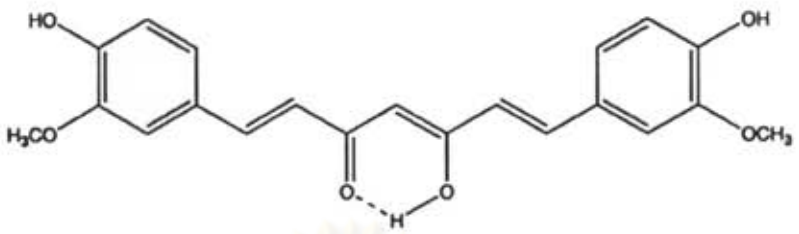
CURCUMIN

Curcuma longa L. is an herbaceous plant in the family of Zingiberaceae (Figure 1). The common name is turmeric. It is extensively used as spice, coloring agent and traditional medicine in India, China and Southeast Asia. Current traditional Indian medicine claims the use of turmeric against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Chinese traditional medicine uses turmeric in diseases associated with abdominal pains, icterus, etc (Ammon, 1991; Aggarwal et al., 2003). Its medicinal properties have been attributed mainly to the yellow pigment called curcuminiods comprising curcumin (commonly called diferuloylmethane), desmethoxycurcumin and bisdesmethoxycumin (Figure 2). Curcuminoids are presented in 3–5% of turmeric rhizome. The commercial grade curcuminoids typically contain curcumin 70-80%, demethoxycurcumin 15-25% and bisdesmethoxycurcumin 2.5-6.5%. (Strimpakos and Sharma, 2008; Maheshwari et al., 2006; Kohli et al., 2005; Sharma et al., 2005)

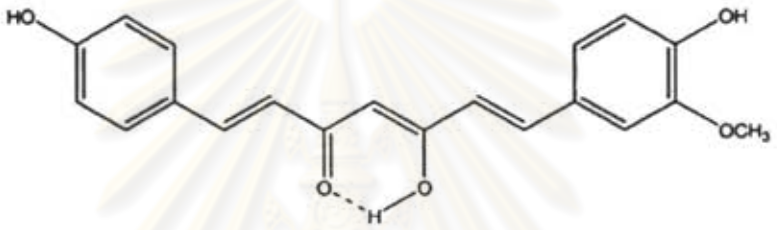
Curcumin is the major active ingredient responsible for the biological activity of turmeric. It was first isolated in 1815. The crystalline form of curcumin was obtained in 1910 and Lampe solved its structure in 1913. Its molecular formula is $C_2H_{20}O_6$ with the molecular weight of 368.4 g/mol. The IUPAC name of curcumin is 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5 dione which is also known as diferuloylmethane. Curcumin has an orange-yellow color and its crystalline solid has a melting point of 187°C. It is relatively insoluble in water, but soluble in ethanol, alkali, acetone, acetic acid and chloroform. Curcumin is unstable at neutral and basic pH values and is degraded to ferulic acid (4-hydroxy-3-methoxycinnamic acid) and feruloylmethane (4-hydroxy-3 methoxycinnamoyl-methane) (Rahman et al., 2006). Although the exact mechanism of degradation is still not fully obvious, the idea that an oxidative mechanism may be involved gains ground from observations that the presence of antioxidants such as ascorbic acid, N-acetyl-L cysteine, or glutathione completely blocks the degradation of curcumin at pH 7.4. However, the stability of curcumin increases in an acidic pH condition. Therefore, curcumin could be

stable in the stomach and small intestine because the pH is between 1 and 6, and degradation of curcumin is extremely slow under these conditions (Rahman et al., 2006).

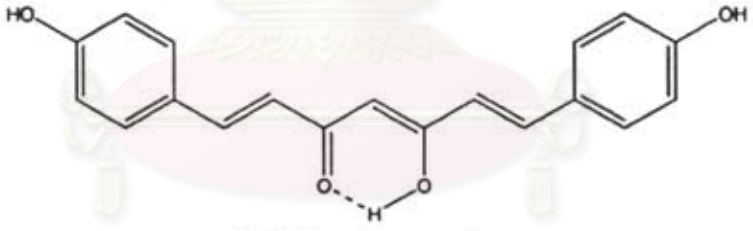
Figure 1 Curcuma longa L. (Turmeric)



(a) Curcumin



(b) Demethoxycurcumin



(c) Bis-demethoxycurcumin

Figure 2 Molecular structures of curcuminoids: (a) Curcumin; (b) Demethoxycurcumin; (c) Bis-demethoxycurcumin (Joe et al., 2004).

Absorption, metabolism, tissue distribution and pharmacokinetics of curcumin

Over the past three decades, the absorption, metabolism and tissue distribution of curcumin has been extensively studied in at least 10 studies which performed in rodents In an early study. At a dose of 1 g/kg administered to rats in the diet, about 75% of the dose was excreted in the feces and negligible amounts appeared in the urine. A few years later, a study of oral curcumin administered to rats demonstrated 60% absorption of curcumin and presented evidence for the presence of glucuronide and sulphate conjugates in urine (Sharma et al., 2005).

Pharmacokinetic measurements have revealed that about 40–85% of ingested curcumin is unaltered in the gastrointestinal tract, most of the absorbed curcumin being metabolized in the intestinal mucosa and liver. Curcumin undergoes O-conjugation to curcumin glucuronide and curcumin sulfate. It is also reduced to tetrahydrocurcumin, hexahydrocurcumin, and hexahydrocurcuminol in rats and mice in vivo and in human hepatic cell suspensions. Products of curcumin reduction are also subject to glucuronidation. Certain curcumin metabolites, such as tetrahydrocurcumin, possess anti-inflammatory and antioxidant activities similar to those of their metabolic progenitor. However, recent data indicated that the anti-inflammatory property was lost when curcumin is reduced to tetrahydrocurcumin, although its antioxidant property was still intact. It has been suggested that the intestinal tract plays an important role in the metabolic disposition of curcumin, a notion which is based predominantly on experiments with [^{3}H] labeled curcumin. Metabolites of curcumin such as curcumin glucuronide, curcumin sulfate, tetrahydrocurcumin, and hexahydrocurcumin were identified in intestinal and hepatic microsomes and hepatic cell cytosol from humans and rats (Figure 3).

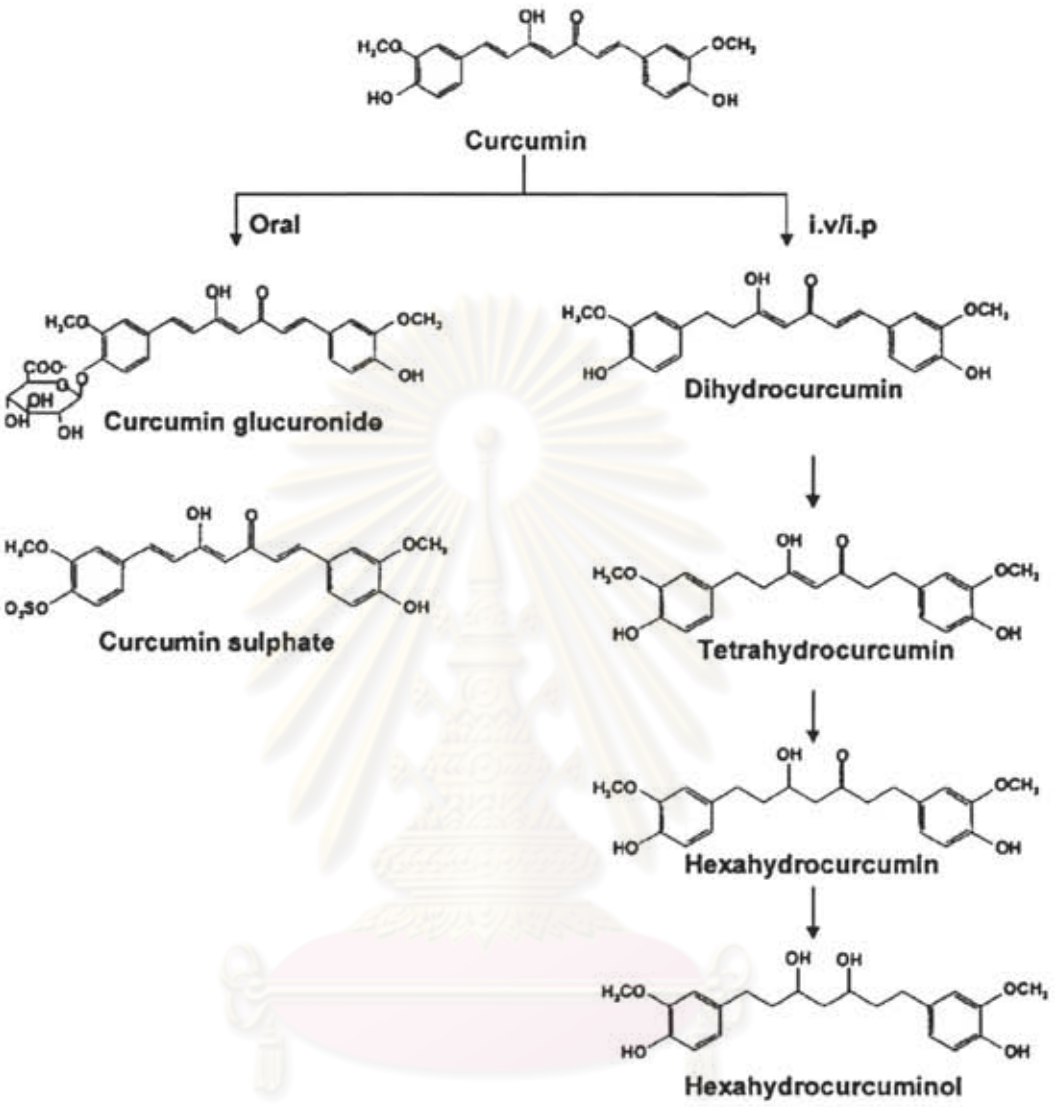


Figure 3 Structure of curcumin and its metabolites (Anand et al., 2007)

Biological activities of curcumin

Effect on gastrointestinal system

Stomach: Turmeric powder has beneficial effect on the stomach. It increases mucin secretion in rabbits and may thus act as gastroprotectant against irritants. However, controversy exists regarding antiulcer activity of curcumin. Both antiulcer and ulcerogenic effects of curcumin have been reported but detailed studies are still lacking. Curcumin has been shown to protect the stomach from ulcerogenic effects of phenylbutazone in guinea pigs at 50 mg/kg dose. It also protects from 5-hydroxytryptamine-induced ulceration at 20 mg/kg dose. However, when 0.5% curcumin was used, it failed to protect against histamine-induced ulcers. In fact, at higher doses of 50 mg/kg and 100 mg/kg, it produces ulcers in rats. Though the mechanism is not yet clear, an increase in the gastric acid and/or pepsin secretion and reduction in mucin content have been implicated in the induction of gastric ulcer. Recent studies indicated that curcumin can block indomethacin, ethanol and stress-induced gastric ulcer and can also prevent pylorus-ligation-induced acid secretion in rats. The antiulcer effect is mediated by scavenging of reactive oxygen species by curcumin (Chattopadhyay et al., 2004; Strimpakos and Sharma, 2008).

Intestine: Curcumin has some good effects on the intestine also. Antispasmodic activity of sodium curcuminate was observed in isolated guinea pig ileum. Antiflatulent activity was also observed in both in vivo and in vitro experiments in rats. Curcumin also enhances intestinal lipase, sucrase and maltase activity (Chattopadhyay et al., 2004; Strimpakos and Sharma, 2008).

Liver: Curcumin and its analogues have protective activity in cultured rat hepatocytes against carbon tetrachloride, D-galactosamine, peroxide and ionophore-induced toxicity. Curcumin also protects against diethylnitrosamine and 2-acetylaminofluorine-induced altered hepatic foci development. Increased bile production was reported in dogs by both curcumin and essential oil of C. longa (Chattopadhyay et al., 2004; Strimpakos and Sharma, 2008).

Pancreas: 1-phenyl-1-hydroxy-n-pentane, a synthetic derivative of p-tolylmethylcarbinol (an ingredient of C. longa) increases plasma secretion and bicarbonate

levels. Curcumin also increases the activity of pancreatic lipase, amylase, trypsin and chymotrypsin (Chattopadhyay et al., 2004; Strimpakos and Sharma, 2008).

Effect on cardiovascular system

Curcumin decreases the severity of pathological changes and thus protects from damage caused by myocardial infarction. Curcumin improves Ca^{2+}-transport and its slippage from the cardiac muscle sarcoplasmic reticulum, thereby raising the possibility of pharmacological interventions to correct the defective Ca^{2+}homeostasis in the cardiac muscle. Curcumin has significant hypocholesteremic effect in hypercholesteremic rats (Chattopadhyay et al., 2004).

Effect on nervous system

Curcumin and manganese complex of curcumin offer protective action against vascular dementia by exerting antioxidant activity (Chattopadhyay et al., 2004).

Effect on lipid metabolism

Curcumin reduces low density lipoprotein and very low density lipoprotein significantly in plasma and total cholesterol level in liver along with an increase of α-tocopherol level in rat plasma, suggesting in vivo interaction between curcumin and α-tocopherol that may increase the bioavailability of vitamin E and decrease cholesterol levels. Curcumin binds with egg and soy-phosphatidylcholine, which in turn binds divalent metal ions to offer antioxidant activity. The increase in fatty acid content after ethanol-induced liver damage is significantly decreased by curcumin treatment and arachidonic acid level is increased (Chattopadhyay et al., 2004).

Anti-inflammatory activity

Curcumin is effective against carrageenin-induced oedema in rats and mice. The volatile oil and also the petroleum ether, alcohol and water extracts of C. longa show anti-inflammatory effects. The antirheumatic activity of curcumin has also been established in patients who showed significant improvement of symptoms after administration of curcumin. That curcumin stimulates stress-induced expression of stress proteins and may act in a way similar to indomethacin and salicylate, has recently been reported. Curcumin offers anti-inflammatory effect through inhibition of NFkB activation. Curcumin has also been shown to reduce the TNF-a-induced expression of the tissue factor gene in bovine aortic-endothelial cells by repressing activation of both AP-1 and NFkB. The anti-inflammatory role of curcumin

is also mediated through downregulation of cyclooxygenase-2 and inducible nitric oxide synthetase through suppression of NFkB activation. Curcumin also enhances wound-healing in diabetic rats and mice, and in H_2O_2-induced damage in human keratinocytes and fibroblasts (Araujo and Leon, 2001; Chattopadhyay et al., 2004; Jayaprakasha et al., 2005; Strimpakos and Sharma, 2008).

Antioxidant effect

The antioxidant activity of curcumin was reported as early as 1975. It acts as a scavenger of oxygen free radicals. It can protect haemoglobin from oxidation. In vitro, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical generation by activated macrophages, which play an important role in inflammation. Curcumin also lowers the production of ROS in vivo. Its derivatives, demethoxycurcumin and bis-demethoxycurcumin also have antioxidant effect. Curcumin exerts powerful inhibitory effect against H_2O_2-induced damage in human keratinocytes and fibroblastsand in NG 108-15 cells (Araujo and Leon, 2001; Chattopadhyay et al., 2004; Jayaprakasha et al., 2005; Strimpakos and Sharma, 2008).

Anticarcinogenic effect – induction of apoptosis

Curcumin acts as a potent anticarcinogenic compound. Among various mechanisms, induction of apoptosis plays an important role in its anticarcinogenic effect. Curcumin also suppresses tumour growth through various pathways. Nitric oxide (NO) and its derivatives play a major role in tumour promotion. Curcumin inhibits iNOS and COX-2 production by suppression of NFkB activation. Curcumin also increases NO production in NK cells after prolonged treatment, culminating in a stronger tumouricidal effect. Curcumin also induces apoptosis in AK-5 tumour cells through upregulation of caspase-3. Reports also exist indicating that curcumin blocks dexamethasone induced apoptosis of rat thymocytes. Recently, in Jurkat cells, curcumin has been shown to prevent glutathione depletion, thus protecting cells from caspase-3 activation and oligonucleosomal DNA fragmentation. Curcumin also inhibits proliferation of rat thymocytes. These strongly imply that cell growth and cell death share a common pathway at some point and that curcumin affects a common step, presumably involving modulation of AP-1 transcription factor (Araujo and Leon, 2001; Chattopadhyay et al., 2004; Strimpakos and Sharma, 2008).

Pro/antimutagenic activity

Curcumin exerts both pro- and antimutagenic effects. At 100 and 200 mg/kg body wt doses, curcumin has been shown to reduce the number of aberrant cells in cyclophosphamide-induced chromosomal aberration in Wistar rats. Turmeric also prevents mutation in urethane (a powerful mutagen) models. Contradictory reports also exist. Curcumin and turmeric enhance g-radiation-induced chromosome aberration in Chinese hamster ovary. Curcumin has also been shown to be non-protective against hexavalent chromium-induced DNA strand break. In fact, the total effect of chromium and curcumin is additive in causing DNA breaks in human lymphocytes and gastric mucosal cells (Chattopadhyay et al., 2004).

Anticoagulant activity

Curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation in vitro as well as in vivo in rat thoracic aorta (Chattopadhyay et al., 2004).

Antifertility activity

Petroleum ether and aqueous extracts of turmeric rhizomes show 100% antifertility effect in rats when fed orally. Implantation is completely inhibited by these extracts. Curcumin inhibits 5α-reductase, which converts testosterone to 5α-dihydrotestosterone, thereby inhibiting the growth of flank organs in hamster. Curcumin also inhibits human sperm motility and has the potential for the development of a novel intravaginal contraceptive (Chattopadhyay et al., 2004).

Antidiabetic effect

Curcumin prevents galactose-induced cataract formation at very low doses. Both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes in rat. Curcumin also decreases advanced glycation end products induced complications in diabetes mellitus (Chattopadhyay et al., 2004; Joe et al., 2004).

Antibacterial activity

Both curcumin and the oil fraction suppress growth of several bacteria like Streptococcus, Staphylococcus, Lactobacillus, etc. The aqueous extract of turmeric rhizomes has antibacterial effects. Curcumin also prevents growth of Helicobacter pylori

CagA+ strains in vitro (Araujo and Leon, 2001; Chattopadhyay et al., 2004; Jayaprakasha et al., 2005).

Antifungal effect

Ether and chloroform extracts and oil of C. longa have antifungal effects. Crude ethanol extract also possesses antifungal activity. Turmeric oil is also active against Aspergillus flavus, A. parasiticus, Fusarium moniliforme and Penicillium digitatum (Chattopadhyay et al., 2004; Jayaprakasha et al., 2005).

Antiprotozoan activity

The ethanol extract of the rhizomes has anti-Entamoeba histolytica activity. Curcumin has anti-Leishmania activity in vitro. Several synthetic derivatives of curcumin have anti-L. amazonensis effect. Anti-Plasmodium falciparum and anti-L. major effects of curcumin have also been reported (Araujo and Leon, 2001; Chattopadhyay et al., 2004; Jayaprakasha et al., 2005)

Antiviral effect

Curcumin has been shown to have antiviral activity. It acts as an efficient inhibitor of Epstein-Barr virus (EBV) key activator Bam H fragment z left frame 1 (BZLF1) protein transcription in Raji DR-LUC cells. EBV inducers such as 12-0 tetradecanoylphorbol-13-acetate, sodium butyrate and transforming growth factor-beta increase the level of BZLF1 m-RNA at 12–48 h after treatment in these cells, which is effectively blocked by curcumin. Most importantly, curcumin also shows anti-HIV (human immunodeficiency virus) activity by inhibiting the HIV-1 integrase needed for viral replication. It also inhibits UV light induced HIV gene expression. Thus curcumin and its analogues may have the potential for novel drug development against HIV (Araujo and Leon, 2001; Chattopadhyay et al., 2004; Joe et al., 2004; Jayaprakasha et al., 2005).

Antifibrotic effect

Curcumin suppresses bleomycin-induced pulmonary fibrosis in rats. Oral administration of curcumin at 300 mg/kg dose inhibits bleomycin-induced increase in total cell counts and biomarkers of inflammatory responses. It also suppresses bleomycin-induced alveolar macrophage-production of TNF-α, superoxide and nitric oxide. Thus curcumin acts as a potent antiinflammatory and antifibrotic agent (Chattopadhyay et al., 2004).

Wound-healing properties

Curcumin enhances cutaneous wound healing in rats and guinea pigs by increasing the formation of granulation tissue, biosynthesis of extracellular matrix proteins, and TGF-β1 in wounds. Curcumin also accelerated wound healing in streptozotocin-induced diabetic swiss albino rats and genetically diabetic (C57/KsJdb+/db+) mice by increasing the formation of granulation tissue, faster re-epitheliatization, and increased collagenization. Systemic treatment with curcumin after local muscle injury leads to faster restoration of normal tissue architecture, as well as an increased expression of biochemical markers associated with muscle regeneration (Joe et al., 2004)

Safety and toxicity

Studies of curcumin in animals have confirmed a lack of significant toxicity since an early report in which doses up to 5 g/kg were administered orally to Sprague–Dawley rats. Systematic preclinical studies funded by the Prevention Division of the US National Cancer Institute did not discover adverse effects in rats, dogs or monkeys of doses up to 3.5 g/kg body weight (BW) administered for up to 3 months (Sharma et al.,2005).

Although curcumin and turmeric are natural products used in the diet, the doses administered in clinical trials exceed those normally consumed in the diet. This fact underlines the need for systematic safety and toxicity studies. Turmeric is Generally Recognized As Safe ("GRAS") by the U.S. FDA, and curcumin has been granted an acceptable daily intake (ADI) level of 0.1–3 mg/kg-BW by the Joint FAO/WHO Expert Committee on Food Additives, 1996. In terms of dietary use in different countries, according to a study from Nepal, dietary consumption of turmeric up to 1.5 g per person per day, equivalent to 50 mg/day of curcumin, does not appear to be associated with adverse effects in humans. In India, where the average intake of turmeric can be as high as 2.0–2.5 g per day (corresponding to 60–100 mg of curcumin daily), no toxicities or adverse effects have been reported at the population level. More valuable than such population dietary studies, which are potentially confounded by multiple variables and interactions, are the systematic preclinical studies funded by the Prevention Division of the U.S. National Cancer Institute. These studies did not demonstrate any adverse effects in rats, dogs, or monkeys at doses of curcumin up to 3.5 g/kg-body weight (BW) administered for up to 90 days. A single report

of curcumin-induced gastric ulceration in albino rats was reported in 1980, but this finding has not been replicated in subsequent studies. More recently, no toxicity has been observed in a preclinical study of the administration of 2% dietary curcumin (1.2 g/kg BW) to rats for 14 days or in a study of 0.2% dietary curcumin (300 mg/kg BW) administered to mice for 14 weeks. Furthermore, a two-generation reproductive toxicity study in Wistar rats found no toxicity, reproductive or otherwise, related to oral curcumin administration (up to 1 g/kg-BW daily) in two successive generations of rats. Contrary to the lack of toxicity with studies of curcumin, a rarely cited carcinogenicity study of turmeric oleoresin reported: (a) hyperplasia of the mucosal epithelium in the cecum and colon of male and female rats; (b) an increased incidence of hepatocellular adenoma in male and female mice; (c) a significantly increased incidence of thyroid gland follicular cell hyperplasia in female mice; and (d) small but significant increases in sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. Further studies with this preparation of turmeric oleoresin have not been performed (Rahman et al., 2006; Strimpakos and Sharma, 2008).

Similar to the conclusions regarding the safety of curcumin in preclinical models, clinical trials have documented minimal toxicity from administration of curcumin or turmeric, although it has not been clearly stated by the reporters of most of these studies which methodologic criteria have been used to assess potential toxicity. In a study performed in India, administration of 1.2–2.1 g of oral curcumin to patients with rheumatoid arthritis daily for 2–6 weeks did not cause any toxicity. In another study of high-dose oral curcumin by Cheng and colleagues in Taiwan, administration of up to 8 g daily of curcumin for 3 months to patients with preinvasive malignant or highrisk premalignant conditions had no adverse effects. In a phase I clinical trial of oral curcumin in patients with advanced colorectal cancer in which the U.S. National Cancer Institute (NCI) criteria were used to assess potential toxicity, curcumin was well tolerated at all dose levels up to 3.6 g daily for up to 4 months. Adverse events probably related to curcumin consumption reported by patients in these studies were mainly gastrointestinal (nausea and diarrhea). Diarrhea (U.S. NCI toxicity grades 1 and 2, respectively) was experienced by one patient consuming 0.45 g curcumin daily and by another patient consuming 3.6 g daily, 1 and 4 months into treatment, respectively.

A third patient, consuming 0.9 g of curcumin daily, reported nausea (NCI toxicity grade 2), which resolved spontaneously despite continuation of treatment. Two abnormalities were detected in blood tests in this trial, both possibly related to treatment: An increase in serum alkaline phosphatase level was observed in four patients (two were NCI grade 1, and two were grade 2); and three other patients had serum lactate dehydrogenase increases to 1.5 times the upper limit of normal. It is unclear whether these abnormal blood test results were related to the activity of the malignant disease in these patients or to treatment toxicity (Strimpakos and Sharma, 2008)

PAIN

Physiologic pathways

Specialized receptors provide information to the central nervous system (CNS) about the state of the environment in the vicinity of the organism. Each receptor is specialized to detect a particular type of stimulus (e.g., touch, temperature, pain, etc.) Those receptors in the skin and other tissues that sense pain are free nerve endings, while those for temperature detection can be free nerve endings, bulbs of Krouse or Ruffini's corpuscles. Receptors are distributed with varying densities in different tissues. Pain receptors may be stimulated by mechanical damage, extremes of temperature, or by irritating chemical substances. While certain pain receptors are responsive to only one of the above stimuli, most can be stimulated by two or more. When the pain receptors in peripheral tissues (such as skin) are stimulated, the nociceptive (pain) impulses are transmitted to the CNS by two distinct types of neurons - the A-delta and C nerve fibres. The A-delta fibres are large-diameter, fast conducting myelinated fibres, which transmit "first" pain - sharp, prickling, and injurious. The C fibres are small diameter, slower conducting unmyelinated fibres that are responsible for "second" pain - dull, aching and visceral type (Figure 4).

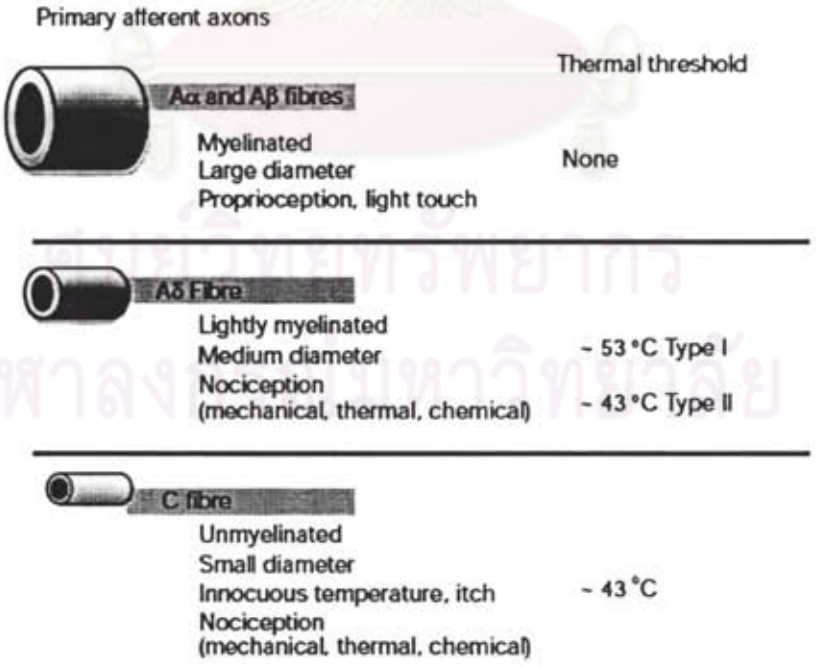


Figure 4 Different nociceptors detect different types of pain (Julius and Basbaum, 2001)

The primary afferent sensory neurons from the periphery then enter the spinal cord and synapse with neurons in the dorsal horn. The second-order neurons, arising from the dorsal horn, have long axons that decussate in the anterior commissure commissure and travel cephalad in the contralateral anterolateral pathway (also known as spinothalamic tract). Some of the long axons that synapsed with type C neurons do not decussate, but pass cranially in the ipsilateral anterolateral spinal pathway. The anterolateral spinal pathway fibres terminate in the thalamus, from which neuronal relays are sent to other CNS centers and the sensory cortex. These higher centers are responsible for the perception of pain and the emotional components that accompany it. There are four distinct processes in the sensory pathway: transduction, transmission, modulation and perception (Figure 5) (Kelly MRCPI FFARCSI et al., 2001).

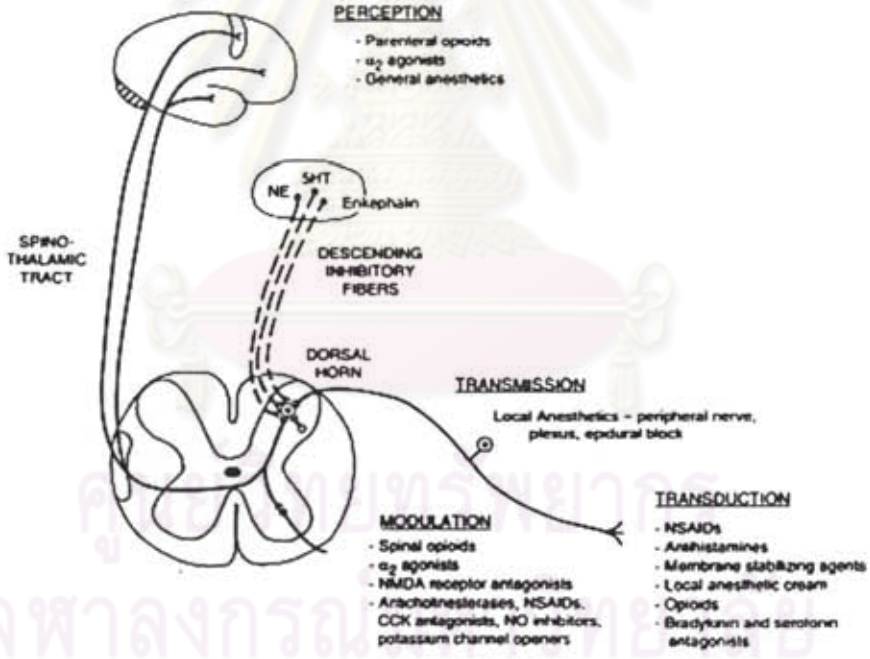


Figure 5 Diagrammatic representation of the four processes involved in the sensory pathway: transduction, transmission, perception, and modulation (Kelly MRCPI FFARCSI et al., 2001).

Each of these processes presents a potential target for analgesic therapy; therefore their physiology is described in some detail below.

1. **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 nociceptors 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) (Leon-Casasola,2007).

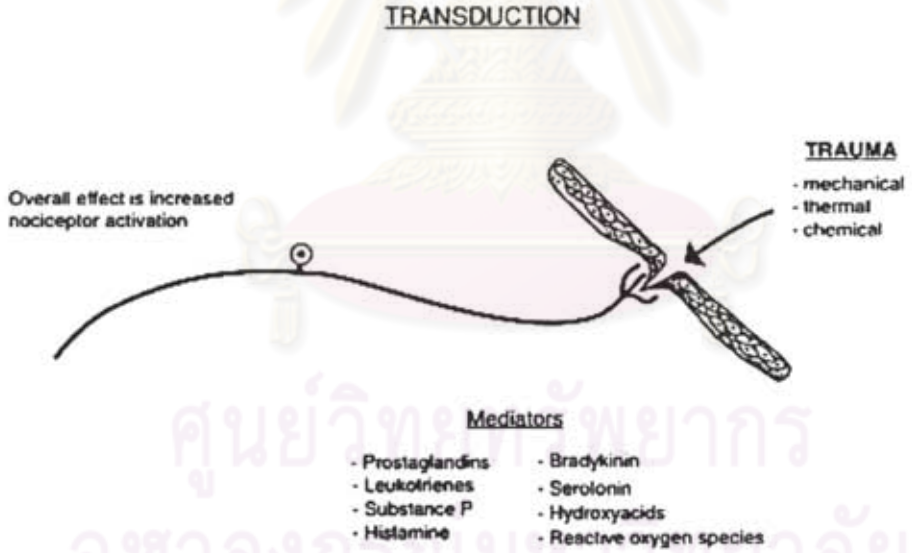


Figure 6 Representation of the transduction process and the mediators of inflammatory processes that lead to peripheral sensitization of nociceptors (Kelly MRCPI FFARCSI et al., 2001).

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 (Leon-Casasola,2007).

2. 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 (Leon-Casasola,2007).

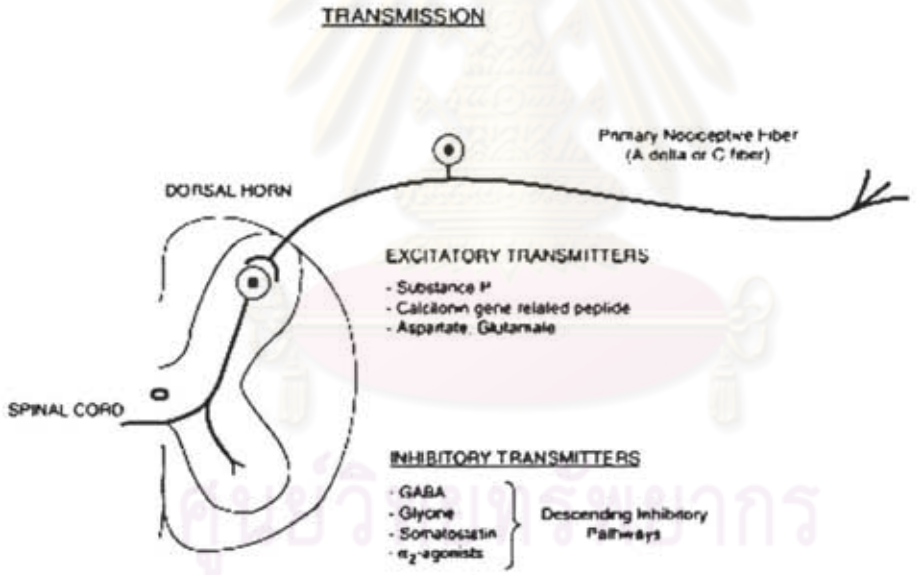


Figure 7 Representation of the transmission process by primary afferent (A-delta and C) fibres from periphery to the dorsal horn of the spinal cord. The balance between excitatory and inhibitory transmitter release determines the intensity of afferent information and the state of sensitization that occurs following peripheral injury (Kelly MRCPI FFARCSI et al., 2001).

3. **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, ß-endorphin) and enkephalins. These peptides represent 2 families of endogenous peptides that are believed to produce pain relief, mainly under situations of stress. This is critically important to you as a physician because when you relieve your patients' pain with narcotics; you give drugs that mimic the actions of these endogenous neurotransmitters. 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". Remember, of course, that it is morphine that mimics the action of the endorphins, since these are the physiological transmitters involved in the modulatory process (Leon-Casasola, 2007).

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

Nociceptive 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 order, supraspinal neurons integrate signals from the spinal neurons and project to the subcortical and cortical areas where pain is finally perceived (Lemke, 2004).

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. 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 (tetrodotoxin [TTX]-resistant) that differs from that found in nonnociceptive Aδ fibers (TTX-sensitive), and are potential targets for therapeutic intervention (Lemke, 2004).

Most axons from nociceptive-specific and wide dynamic range neurons cross midline and communicate with supraspinal centers through 1 of 3 major ascending nociceptive pathways. The spinothalamic pathway is the major ascending nociceptive pathway; it is divided into medial and lateral components. The medial component projects to medial thalamic nuclei and then (via 3rd-order neurons) to the limbic system; it is responsible for transmission of nociceptive input involved with the affective-motivational aspect of pain. The lateral component projects to lateral thalamic nuclei and then to the somatosensory cortex; it is responsible for transmission of nociceptive input involved with the sensory-discriminative aspect of pain.

The spinoreticular pathway projects to the reticular formation in the medulla and pons, to thalamic nuclei, and then to the somatosensory cortex. The reticular formation is critical to the integration of nociceptive input. Ascending reticular activity increases cortical activity, while descending reticular activity blocks other sensory activity.

The spinomesencephalic tract projects to the reticular formation and to the periaqueductal gray matter. The periaqueductal gray matter plays a central role in the integration and modulation of nociceptive input at the supraspinal level. Two smaller ascending pathways are also involved in nociception.

The cervicothalamic tract originates from neurons in the upper 2 cervical segments and projects to thalamic nuclei. The spinohypothalamic tract originates from neurons in dorsal horn and projects to autonomic control centers in the hypothalamus. The spinohypothalamic pathway is responsible for transmission of nociceptive input involved with cardiovascular and neuroendocrine responses to noxious stimuli, and it probably mediates some of the autonomic responses (changes in heart rate, arterial blood pressure, and respiratory rate) observed in animals anesthetized for surgical procedures (Lemke, 2004).

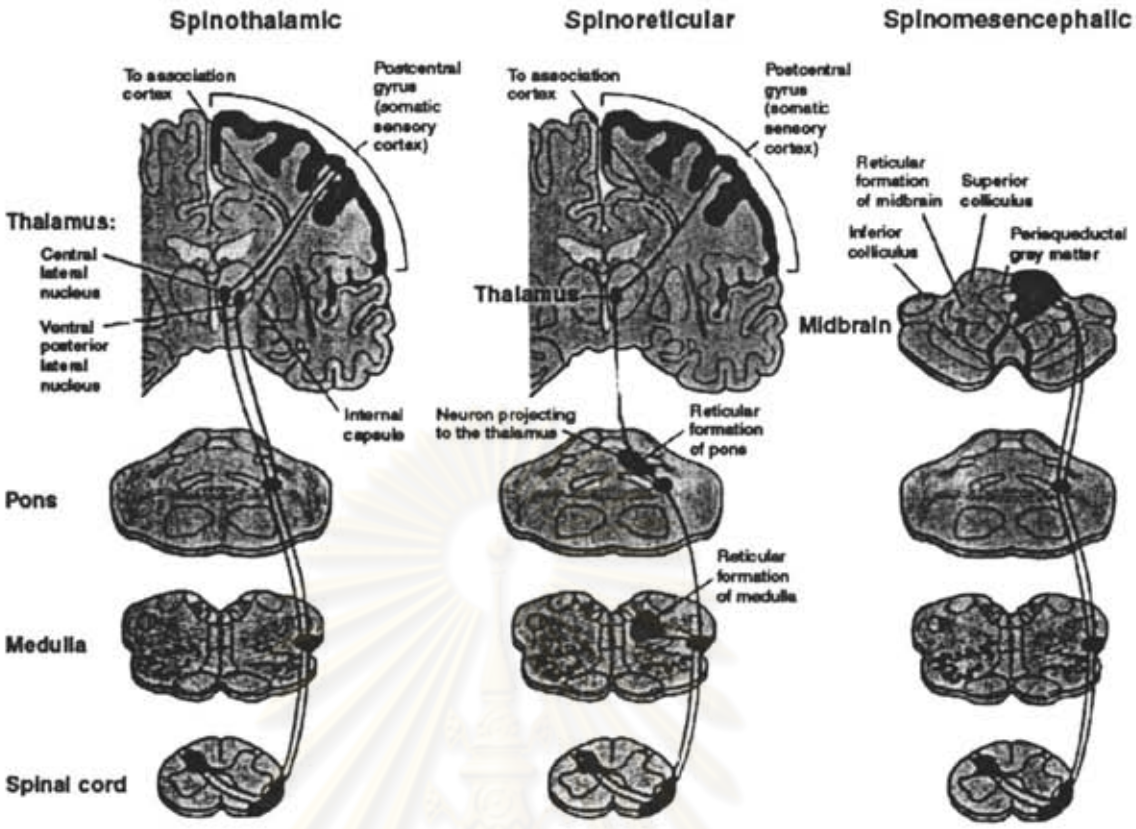


Figure 8 Ascending nociceptive pathways (Schwartz and Jessell, 2000).

Antinociceptive pathways

In addition to having ascending nociceptive pathways, animals have descending antinociceptive pathways that modulate nociceptive input at supraspinal and spinal levels. Practitioners must have a working knowledge of these normal pain-control pathways before they can appreciate the effects of anesthestic and analgesic drugs on these pathways and understand many of the principles of perioperative pain management. The descending antinociceptive pathways begin at the supraspinal level and project to neurons in the dorsal horn of the spinal cord. The periaqueductal gray matter (midbrain), locus ceruleus (pons), and nucleus raphe-magnus (medulla) are important structures in the modulation of nociceptive input. The periaqueductal gray matter receives direct input from the thalamus, hypothalamus, and reticular formation, and indirect input from the cerebral cortex. These midbrain neurons sendprojections to the nucleus raphe-magnus and then to neurons in the

dorsal horn. The locus ceruleus sends projections directly to dorsal horn neurons, and it may also receive input from the periaqueductal gray matter (Lemke, 2004).

Endogenous opioids (β-endorphins, enkephalins, dynorphins), serotonin (5-HT), and norepinephrine are the main neurotransmitters involved in the descending antinociceptive pathways. Axons that originate in the nucleus raphe-magnus release serotonin in the dorsal horn and comprise the "serotonergic" pathway. Similarly, axons that originate in the locus ceruleus release norepinephrine in the dorsal horn and comprise the "noradrenergic" pathway. Supraspinal release ergic" of opioid peptides
activates both antinociceptive pathways, and supraspinal release of γ-aminobutyric acid (GABA) (mediated by $GABA_A$ receptors) inhibits both antinociceptive receptors) inhibits both antinociceptive pathways (Lemke, 2004).

Opioid peptides modulate nociceptive input at spinal and supraspinal levels. All 3 types of opioid receptors (μ, κ, and δ) are present on nociceptive afferents (1^{st}order neurons) and on dorsal horn projection neurons, and μ and δ receptors are more common in the periaqueductal gray matter. At the supraspinal level, opioid peptides not only activate the descending antinociceptive pathways, they also inhibit GABA-mediated inhibition of these same pathways (disinhibition). At the spinal level, opioid peptides act presynaptically to inhibit release of glutamate and neuropeptides from primary afferent neurons, and postsynaptically to inhibit (hyperpolarize) projection neurons (Lemke, 2004).

Norepinephrine and α_2 adrenergic receptors also modulate nociceptive input at spinal and supraspinal levels, and they play a central role in modulation of
nociceptive input. α_2 receptors on noradrenergic neurons are sometimes called autoreceptors, and those on nonnoradrenergic neurons (nociceptive afferents) are
called heteroceptors. At the supraspinal level, noradrenergic neurons in the locus ceruleus tonically inhibit the neurons of the noradrenergic pathway. Release of norepinephrine within the locus ceruleus activates postsynaptic autoreceptors, inhibiting the tonically active inhibitory neurons and activating the noradrenergic pathway (another example of disinhibition). At the spinal level, norepinephrine activates heteroceptors presynaptically, to inhibit release of glutamate and neuropeptides from primary afferent neurons, and postsynaptically, to inhibit (hyperpolarize) projection neurons. Other receptors ($GABA_B$,

gabapentin, cannabinoid) also appear to play important roles in spinal modulation of nociceptive input, and they are potential targets for therapeutic intervention (Lemke, 2004).

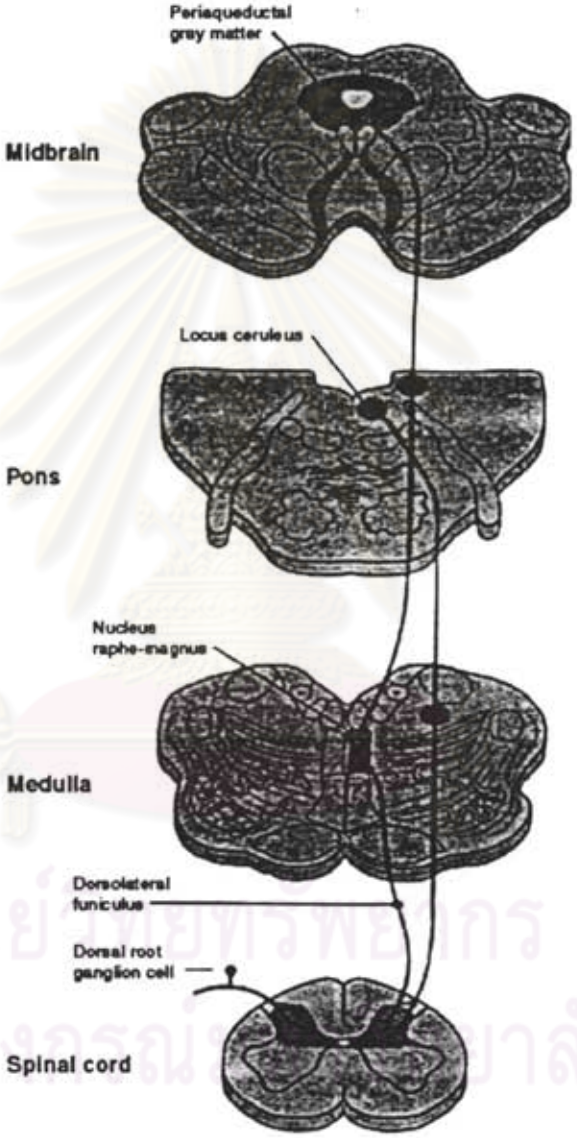


Figure 9 Descending antinociceptive pathways (Schwartz and Jessell, 2000).

INFLAMMATION

Inflammation is an immune response to cellular/tissue injury or infection by pathogens. It is clinically characterized by features such as redness, warmth, swelling and pain. The process itself is not considered a disease, but failure to contain it and successfully resolute it in a timely fashion result in exacerbation of tissue damage and modulation of cell signaling pathways (Serhan and Savill, 2005). Tissue injury induced by this trauma results in the release of inflammatory mediators including the cytokines and tumor necrosis factor (TNF-α), interleukin-1 (IL-1) from leukocytes, monocytes and macrophages (Paterson et al., 2003). Inflammation is complex, it is mainly divided into two parts i.e. acute and chronic which could either be beneficial or detrimental.

Acute inflammation is characterized by rapid onset and is of short duration. It is characterized by the exudation of fluids and plasma proteins; and the migration of leukocytes, most notably neutrophils into the injured area. This acute inflammatory response is believed to be a defense mechanism aimed at killing of bacteria, virus and parasites while still facilitating wound repairs.

Chronic inflammation is of a more prolonged duration and manifests histological by the presence of lymphocytes and macrophages, resulting in fibrosis and tissue necrosis. The persistent chronic inflammation increases the development of the degenerative diseases such as rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer, asthma, acquired immunodeficiency disorder (AIDS), cancer, congestive heart failure (CHF), multiple sclerosis (MS), diabetes, infections (bacteria, fungi, parasites), gout, IBD-inflammatory bowel disease, aging and other neurodegenerative CNS depression, all of which are associated with immunopathological that appears to play a key role in the onset of the condition (O'Byrne and Dalgleish, 2001)

Mechanistically, inflammatory pathways can be classified into arachidonic acid (AA)-dependent and AA-independent pathways (Yoon and Baek, 2005). Cyclooxygenase (COX), lipooxygenase (LOX) and phospholipase A2 (PLA2) pathways involve the metabolism of AA and therefore are considered AA-dependent. On the other

hand, nitric oxide synthase (NOS), NF-kB, peroxisome proliferator activated receptors (PPAR) and NSAID activated gene-1 (NAG-1) are classified as AA-independent (Figure 13).

Phospholipases, including PLA2, are a family of enzymes that release free fatty acids such as AA from the phospholipid layers of the plasma membrane (Six and Dennis, 2000). AA is then metabolized via either the COX pathway to produce prostaglandins (PGs) and thromboxane A2 or the LOX pathway to produce hydroperoxyeicosateraenoic acids (HETEs) and leukotrienes (LTs). Products from both pathways have been shown to be key players in the process of inflammation (Claria and Romano, 2005; Conrad, 1999; Moore and Weiss, 1985; Samuelsson, 1991; Spokas et al., 1999)

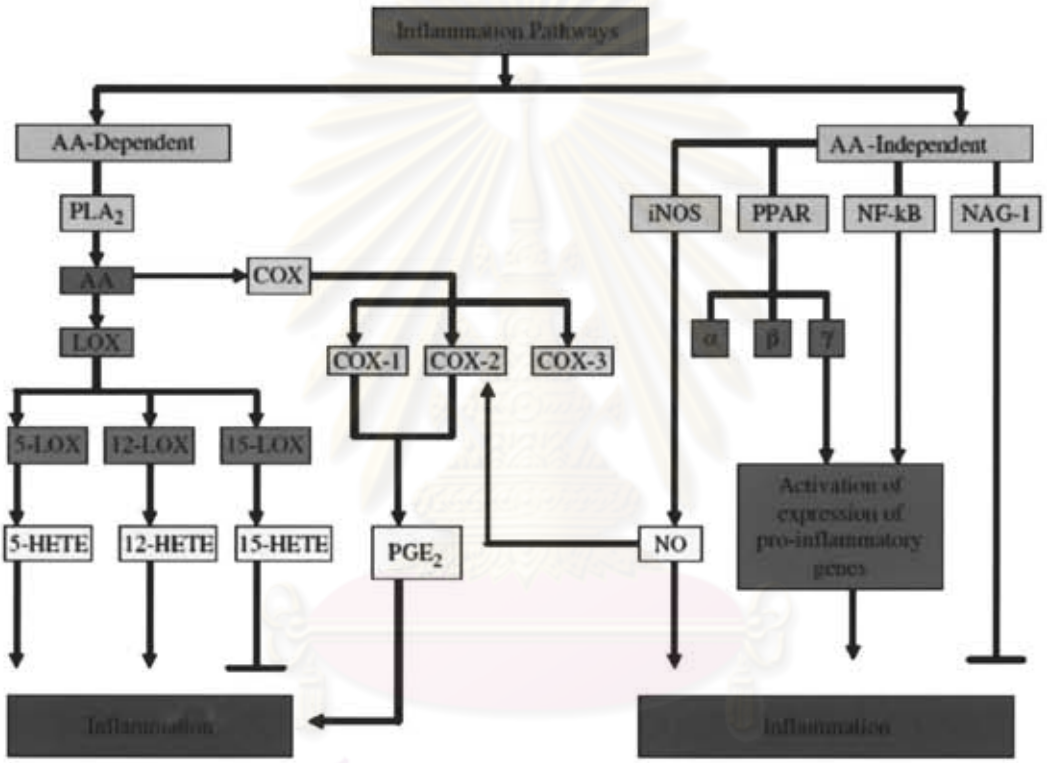


Figure 10 Pathways of inflammation. Inflammatory cell signaling pathways can be mechanistically classified into arachidonic acid (AA)-dependent and AA-independent. 15-hydroperoxyeicosateraenoic acid (15-HETE) and the gene product of NSAID activated gene-1 (NAG-1) are anti-inflammatory mediators. On the other hand, 5-HETE, 12-HETE, prostaglandin E2 (PGE2) and nitric oxide (NO) are all pro-inflammatory mediators. Peroxisome proliferators activated receptor g (PPARg) and nuclear factor kappa B (NK-kB) activates the expression of pro-inflammatory genes. COX: cyclooxygenase, iNOS: inducible nitric oxide synthase (Issa et al., 2006).

The anti-inflammatory properties of several phytomedicines origin, that contain substances like phytoestrogens, flavonoids and its derivatives, phytosterol, tocopherol, ascorbic acid, curcumin, genistein, and others can be the inhibitors of the molecular targets of pro-inflammatory mediators in inflammatory responses.

Pharmacological methods of pain management

1. Nonopioid Drugs

Non-opioid and multimodal analgesia is used extensively in day surgery, where the key to success is avoiding postoperative pain and symptoms such as sedation and nausea (both of which may be precipitated by opioids). Avoidance of opioids reduces or eliminates postoperative nausea and vomiting enabling the patient to be converted rapidly to oral analgesia and discharged. However, persisting pain remains a major problem in day surgery. This may be reduced by effective prescribing, using multimodal techniques (e.g. a combination of paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs)) (Hebbes and Lambert, 2007)

Paracetamol

Paracetamol is related to this group, although its mode of action is poorly understood. Paracetamol has been shown clinically to be an effective analgesic in both acute and chronic pain and cancer pain. It is effective alone, although there is an enhanced effect when used in combination with an opioid or other analgesics. The advantages of paracetamol are that it is devoid of the classical adverse effects of gastric irritation and renal impairment associated with the NSAIDs (Hebbes and Lambert, 2007). The mechanism by which paracetamol exerts its antinociceptive effects remains controversial, and may well be multimodal (MacPherson, 2000). There is evidence that paracetamol acts on the central COX-3 variant, sparing the peripheral COX- 1 and COX-2. This also explains why it does not exhibit anti- inflammatory effects, except at very high doses (when the effect may be due to enzyme homology between COX-1, COX-2 and COX-3) (Hebbes and Lambert, 2007).

Salicylates

The salicylates describe a group of compounds related to salicylic acid and its precursors. The classical example is aspirin. Whilst aspirin is relatively a nonselective COX-inhibitor its effects at COX-2 are irreversible. It has antiplatelet effects when given in low

doses, due to differential inhibition of the procoagulatory thromboxane A_2. This property is common to all NSAIDs and is a contributory factor to gastrointestinal haemorrhage. The salicylates inhibit COX to varying extents and share a mechanism of action (Hebbes and Lambert, 2007).

Nonsteroidal Antiinflammatory Drugs (NSAIDs)

Blockade of the proinflammatory mediators by NSAIDs will reduce the inflammatory response (and subsequent pain). Classically, their effect is anti inflammatory, analgesic, and antipyrexial because of the direct inhibition of prostaglandin production (Hebbes and Lambert, 2007). Inhibition of the production of gastroprotective prostaglandins and the prothrombotic thromboxane A_2 is thought to be related to an increased risk of gastrointestinal haemorrhage in NSAID use. In addition to the peripheral antiinflammatory effects, there is evidence that NSAIDs have a central antinociceptive mechanism of action that augments the peripheral effect. This may involve inhibition of central nervous system (CNS) prostaglandins or excitatory amino acids. However, a 20% to 50% reduction in opioid consumption with improved quality of analgesia has been reported using different NSAIDs following various types of surgery (Leung, 2004).

Cyclooxygenase isoenzyme

COX-1, also referred to as the constitutive form of the enzyme, is found in a range of tissues, an is involved in the production of PGI_2, PGE_2, thromboxane A_2 (MacPherson, 2000). Cyclooxygenase-1 appears to be expressed in many tissues and produces prostaglandins, which regulate normal cellular functions (Leung, 2004).

COX-2, the inducible isoenzyme, is found at a range of inflammatory sites, such as synovium, macrophage, and endothelial cells and the result of its stimulation is production of PGs responsible for the promotion of inflammation. It production is increased by inflammatory stimuli, surgical trauma, endotoxins, interleukin-1, and hypoxia (Leung, 2004).

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). 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).

Local Anesthetics

Although infiltrating the surgical site with local anesthetic before the incision can be impractical in emergency obstetric surgery, it has shown its potential benefit of decreasing postoperative analgesic requirements in elective lower abdominal surgery. One study of obstetric patients showed that during tubal ligation the infiltration of uterine tubes and mesosalpinx with 0.5% bupivacaine significantly enhanced analgesia both in the immediate postoperative setting and on the seventh postoperative day compared with infiltration with sodium chloride (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).

2. Opioids

Opioid receptors are synthesized or upregulated in the sensory neurons both centrally and peripherally. Endogenous opioid binding to these receptors reduces the excitability of afferent nerve fibers. Centrally the opioid receptors act as presynaptic receptors. In the dorsal horn, opioid inhibits pain transmission via interneurons (Leung, 2004; Rang et al, 2003).

Oral Opioids

Oral opioids remain the main pain management modality for postpartum patients. Oral opioids are also frequently used as supplemental analgesics for patients who have received neuraxial opioids. Short-acting oral opioids such as hydrocodone with acetaminophen are the common choices. However, the use of ibuprofen with hydrocodone or oxycodone has provided an alternative with equal efficacy. Oral opioid analgesic has its limitations. For example, factors such as slow gastric emptying after lower abdominal surgery and/or postoperative nausea and vomiting can significantly limit the potential usage of oral opioids (Leung, 2004).

Parenteral Opioids

Intravenous or subcutaneous patient-controlled analgesia (PCA) allows the patient to titrate her analgesic requirement against the side effects of opioids, such as sedation. The use of PCA also eliminates delay that may occur because of the screening and workload requirements of the nursing staff. Morphine is by far the most commonly used opioid for PCA because of its rapid onset of analgesic action and intermediate duration of action. In opioid-naive patients, the use of basal rate opioid infusion in the initial PCA setting should be cautioned because of the risk of respiratory depression. In

addition, the use of basal rate morphine infusion has not shown to be effective in decreasing resting pain, but is associated with a reduction in movement-associated pain scores. On the other hand, the use of basal rate in hydromorphone PCA showed significant reduction of pain visual analog scores with both rest and movement. Although one study showed that there is no significant difference in pain scores and side effects of PCA using morphine, fentanyl, or meperidine, the reports of seizures

that are associated with the use of meperidine has made the medication a less desirable drug of choice. Morphine PCA should also be avoided in patients with renal failure because of the increased risk of accumulation of the active metabolite, morphine-6-glucuronide (Leung, 2004).

Tramadol

Tramadol, a synthetic opioid of the aminocyclohexanol group, is a centrally acting analgesic with weak opioid agonist properties, and acts via noradrenergic and serotonergic neurotransmission. These opioid and nonopioid modes of action appear to act synergistically. Tramadol has proven effective in myocardial emergencies, and in both trauma and obstetric pain. In addition, tramadol has been shown in Europe to provide effective analgesia after both IM and IV administration for the treatment of

postoperative pain. Currently the drug is only available in the United States in oral preparation. The most common adverse events are nausea and vomiting. In contrast

to drugs such as morphine and meperidine, clinically relevant respiratory depression is rarely observed during tramadol administration at equipotent doses. It is also associated with significantly less dizziness and drowsiness than morphine. Although the onset of tramadol may be slightly slower than NSAIDs or opioids, comparative studies have generally shown that tramadol is more effective than NSAIDs in controlling postoperative pain, with a better side-effect profile. The use of a combination of tramadol and NSAIDs allows the tramadol dose to be reduced and results in a lower incidence of adverse effects. Because of the lack of long-term controlled studies in pregnant women that show a risk to the fetus, tramadol is currently under the Federal Drug Administration (FDA) Pregnancy Classification Category C, interpreted as "risk cannot be ruled out". Although the validity of the current FDA Pregnancy Drug Classification is controversial, a trial of Category A analgesics (safe for pregnancy) is preferred for postpartum pain before the use of tramadol (Leung, 2004).

ANIMAL MODELS

Central analgesic activity testing

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

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

A. Test based on the use of thermal stimuli

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

1) The Tail-flick test

a. The tail-flick test using radiant heat.

The tail-flick test using radiant heat is an extremely simplified version of the method used on human subjects by Hardy et al. (1940). The application of thermal radiation to the tail of an animal provokes the withdrawal of the tail by a brief vigorous movement. It is the reaction time of this movement that is recorded (often referred to as "tail-flick latency"). This is achieved by starting a timer at the same time as the application of the heat source. By using a rheostat, the intensity of current through the filament and therefore of radiant heat emission can be controlled in such a way that one can empirically predetermine the time until the withdrawal of the tail. This is usually between 2 and 10 s (most commonly between 2 and 4 s), although it can be much longer. A photoelectric cell stops the timer and switches off the lamp at the moment the tail is withdrawn. A lengthening of the reaction time is interpreted as an analgesic action. It is advisable not to prolong the exposure to radiant heat beyond 10 to 20 s, otherwise the skin may be burned. The advantages of this method are its

simplicity and the small interanimal variability in reaction time measurements under a given set of controlled conditions (Bars et al., 2001).

One can demonstrate that the tail-flick is spinal reflex in that, at least in its shorter latency form, it persists after section or cold block of upper parts of the spinal cord. As with all reflexes, it is subject to control by supraspinal structures. Details of the spinal pathways implicated in this reflex can be found elsewhere. It is triggered by C fibers when it is elicited by heat delivered by a CO_2 laser (Bars et al., 2001).

The tail-flick reflex may not always be purely spinal, notably when the heating slope is slower and there is an increase in the reaction time. Under these conditions, the tail-flick can disappear in the spinal animal. It is possible that the tail-flick is not a purely spinal reflex but is a more complicated one involving higher neural structures (Bars et al., 2001).

From a pharmacological point of view, there is a consensus that this test is truly efficient only for revealing the activity of opioid analgesics (but not of opioid partial agonists). In this context, it is adequate for predicting their analgesic effects in humans. For morphine itself, it is not difficult to construct dose-response curves for intravenous doses between 1 and 10 mg/kg (Bars et al., 2001).

As far as opioid partial agonists are concerned, some have been shown to increase the tail-flick reaction time when slow rate of heating are applied. It is probable that this pharmacological observation resulted from the aforementioned fact that supraspinal structures are involved when the test is carried out in this fashion (Bars et al., 2001).

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

The use of immersion of the tail is apparently a variant of the test described above. The most obvious difference is that the area of stimulation is far greater. Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. This test is actually quite different from the previous one insofar as immersion of the tail in a hot liquid increase its temperature very quickly and in a more or less linear fashion. The main interest in this response- which arguably has not been exploited sufficiently- lies in the possibility of applying different temperatures. Thus, lower temperatures can be used to seek evidence for the effects of minor analgesics. This also applies to using a bath in which the temperature increases slowly (Bars et al., 2001).

2) The Paw Withdrawal Test

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

3) The Hot Plate test

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

As far as analgesic substances are concerned, the paw-licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol, especially when the temperature of the plate is 50°C or less or if the temperature is increased in a progressive and linear fashion, e.g., from 43 to 52°C at 2.5°C/min. The specificity and sensitivity of the test can be increased by measuring the reaction time of the first evoked behavior regardless of whether it is paw-licking or jumping, or by lowering the temperature. The behavior is relatively stereotyped in the mouse but is more complex in the rat, which sniffs, licks its forepaws, licks its hind paws, straightens up, stamps its feet, starts and stops washing itself, among other things. These behaviors have been labeled "chaotic defensive movements" (Bars et al., 2001).

4) Test Using Cold Stimuli

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

B. Test based on the use of mechanical stimuli

The preferred sites for applying nociceptive mechanical stimuli are the hind paw and the tail. Tests using constant pressure have been abandoned progressively for those applying gradually increasing pressures. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. When the pressure increases, one can see successively the reflex withdrawal of the paw, a more complex movement whereby the animal tries to release its trapped limb, then a sort of struggle, and finally a vocal reaction. If the first of these reactions is undoubtedly a proper spinal reflex, the last two clearly involve supraspinal structures. This type of mechanical stimulation has a certain number of disadvantages: 1) it is sometimes difficult to measure the intensity of the stimulus with precision; 2) repetition of the mechanical stimulus can produce a diminution or conversely an increase in the sensitivity of the stimulated part of the body-in the latter case, this carries the risk that the tissues may be altered by inflammatory reaction that could call into question the validity of repeated tests; 3) the necessity of applying relatively high pressures-which explains the weak sensitivity of the method and the relatively small number of substances that have been shown to be active by this test; and 4) a non-negligible level of variability of the responses (Bars et al., 2001).

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

2. Long-Duration Stimuli Tests (Tonic Pain)

These tests use an irritant, foreign chemical agent as the nociceptive stimulus. They differ from most other pain tests in that (1) they do not measure a threshold response; (2) they quantitatively measure the resulting behavior after the stimulus, which varies in potency with time; and (3) they are not models of chronic pain, since the duration of the behaviors is short, usually minutes or tens of minutes. Hence, long-duration stimuli tests are considered models of tonic pain. They are usually based on intradermal or intraperitoneal injections of the agent (Eaton, 2003).

Intradermal Injection (The formalin test)

Formalin, a 37 percent solution of formaldehyde, is the most commonly used agent for intradermal paw injection (the formalin test). Other agents less commonly used are hypertonic saline, Freund's adjuvant, ethylene diamine tetra-acetic acid, capsaicin, or bee sting.

A 0.5 to 15 percent solution of formalin (usually about 3.5%) injected into the dorsal or plantar surface of the rat fore- or hind paw produces a biphasic painful response of increasing and decreasing intensity for about 60 min after the injection. Typical responses include the paw being lifted, licked, nibbled, or shaken; these responses are considered nociceptive, since formalin predominantly evokes activity in C fibers, and not in Aδ afferents. The initial phase of the response, which lasts 3 to 5 min, is probably due to direct chemical stimulation of nociceptors; this is followed by 10 to 15 min during which animals display little behavior suggestive of nociception. The second phase of this response starts about 15 to 20 min after the formalin injection and lasts 20 to 40 min, initially rising with both number and frequency of nociceptive behaviors, reaching a peak, then falling off. The intensities of these nociceptive behaviors are dependent on the concentration of formalin used, and the second phase involves a period of sensitization during which inflammatory phenomena occur. These inflammatory phenomena are possibly a result of central processes triggered by the neuronal activation during the first phase (Eaton, 2003).

Peripheral analgesic activity testing

1) Intraperitoneal Injections of Irritants (Writhing Test)

Intraperitoneal injection of agents (originally phenylbenzoquinone) that are irritating to serous membranes provokes a stereotypical behavior in rodents that is characterized by abdominal contrictions, whole body movements, contortions of the abdominal muscles, and reduced motor activity and incoordination. In this test, commonly called the "writhing test" the behaviors are considered reflexive, and are evidence of peritoneovisceral or visceral pain associated with visceral chemoreceptor (Eaton, 2003). The most utilized screening assay for nonnarcotic analgesic agents is the abdominal constriction assay in mice (Lombardino, 1985). Unfortunately, the frequency of cramps decreases spontaneously with time to such an extent, and with such variability, that is difficult to evaluate the effect of an analgesic on the behaviors of any single animal. Even with multiple modifications in the nature of the chemical irritant used, the concentration, temperature, and volume of the injectant, and other modifications to simplify the test and measurements of behaviors, the test lacks specificity, because these test work so well for all major and minor analgesics, as well as nonanalgesic substances such as muscle relaxants. Even with poor specificity of action, the writhing test can predict effective analgesic doses for agents that can be used in human (Eaton, 2003). In this test both central and peripheral analgesics are detected. The test, therefore, has been used by many investigators and can be recommending as a simple screening method. However, it has to be mentioned that other drugs such as clonidine and haloperidol also show a pronounced activity in this test. Because of the lack of specificity, caution is required in interpreting the results, until other tests have been performed. Nevertheless, a good relationship exists between the potencies of analgesics in writhing assays and their clinical potencies (Vogel, 2002).

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

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

The mean applied force is determined for each time interval tested. The percentage increase in pain threshold is calculated by subtracting the applied force of the vehicle control from the applied force of the drug group which is divided by the applied force of the vehicle control in order to give the percentage of increase in pain threshold of the drug group (Vogel, 2002). The applied force is continuously monitored by an indicator moving along a linear scale calibrated in grams x 10 with a pointer riveted to slide, e.g., 11.5=115 grams. The scale can be multiplied by 2 or 3 by placing on the slide one or two discs, respectively. The application of force is stopped when the rat starts to struggle (vigorous attempt to withdraw the paw) to a noticeable degree (whether or not accompanied by shrill vocalization).

The method originally described by RANDALL and SELITTO has been used by many investigators and has been proven to detect central analgesics as well as peripheral analgesics. Peripherally acting analgesics such as the nonsteroidal anti-inflammatory drugs increase only the threshold of the inflamed paw, whereas opiate analgesics increase also the threshold of the intact paw (Vogel, 2002).

Anti-inflammatory activity testing

1) Oxazolone-induced ear edema in mice

The oxazolone-induced ear edema model as first described by Evans (1971) in mice is a model of delayed contact hypersensitivity that permits the quantitative evaluation of the topical and systemic anti-inflammatory activity of a compound following topical administration.

Average values of the increase of weight are calculated for each treated group and compared statistically with the control group. A 0.003% solution of hydrocortisone and a 1% solution of indomethacin were found to be active.

The method is suitable for both steroidal and non-steroidal compounds as well as for the evaluation of various topical formulations (Vogel, 2002).

2) Paw edema

One of the cardinal signs of inflammation is the presence of edema. It is not surprising, then, that edema tests are among the moat prominent models used to assess the efficacy of drugs for treating inflammatory disease such as arthritis (Lombardino,1985).

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, sulfate polysaccharides like carrageenin or naphthoylheparamine.

The effect can be measured in several ways. The hind limb can be dissected at the talocrural joint and weighed. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared to the controls. The increase of paw volume after 3 or 6 h is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema (Vogel, 2002). Maintenance of a constant temperature in the laboratory is important since the amount of paw swelling and the degree of inhibition achieved by NSAIDs decrease with decreasing temperature (Lombardino,1985). The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The differences at the various time intervals give some hints for the duration of the anti-inflammatory effect. A dose-response curve is run for active drugs and ED_{50} values can be determined (Vogel, 2002).

3) Cotton wool granuloma

The method has been described first by Meier et al. (1950) who showed that foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of compressed cotton. After several days, histological giant cells and undifferentiated connective tissue can be observed besides the fluid infiltration. The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal. More intensive granuloma formation has been observed if the cotton pellets have been impregnated with carrageenin. The average weight of the pellets of the control group as well as of the test group is calculated. The percent change of granuloma weight relative to vehicle control group is determined (Vogel, 2002).

CHAPTER III

MATERIALS AND METHODS

ANIMALS

Male ICR mice weighing 18-25 g, male Wistar rats weighing 130-175 g and male Sprague Dawley rats weighing 100-150 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 Faculty of Pharmaceutical Sciences, Chulalongkorn University (Appendix H).

PREPARATION OF CURCUMIN

Pure, synthetic curcumin was obtained from Assistant Professor Dr Pornchai Rojsitthisak, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

DRUGS

Morphine, indomethacin, acetic acid and carrageenan were dissolved in 0.9% sodium chloride solution. Curcumin was suspended in 0.5% carboxymethylcellulose. The control animals were given with an equivalent volume of vehicle in the same route. Morphine sulphate (10 mg/kg, Thai FDA) and indomethacin (10 mg/kg, Sigma Chemical Co., USA) were used as standard analgesic drugs. Naloxone (5 mg/kg, Sigma Chemical Co., USA), Carrageenan (1% in 0.9% sodium chloride solution (50 µl/ rat), Sigma Chemical Co., USA) and acetic acid (0.6% in 0.9% sodium chloride solution (10 ml/kg), Merck, Germany).

EXPERIMENTAL METHODS

Hot-plate Analgesic Testing

The male ICR mice weighing 18-25 g were used (N=10 per group). Analgesic testing was determined using the hot-plate method. The surface of the hot plate (measuring 28x28 cm) was set at 55±0.5 °C and was surrounded by a clear Plexiglas wall cylinder, 20 cm in diameter and 30 cm in height to confine the animal to the heated surface during testing. On the day of testing, animals were randomly assigned to one of eight treatment groups and underwent 3 pre-drug baseline trials on the hot-plate spaced 5-10 min apart. Only those animals which had a pretreatment hot-plate latency time of less than 45 sec were utilized in these studies. Mice were then administered various doses of treatments and retested. Each mouse was placed on the hot-plate from an elevation of 5 cm and the latency to the licking of a rear paw or a vigorous jumping up from the surface of the metal plate was used as the end point and recorded with a stopwatch. If this behavior was not observed within 45 sec the animal was removed from the hot-plate, given a score of 45 for its paw-lick latency and returned to its cage (the maximum time allowed for an animal to remain on the surface of the plate during testing was 45 sec). The average of the last two trials served as the baseline pre-drug paw-lick latency.

Immediately, after the third baseline trial on the hot-plate, the drug administration took place with vehicle (10 ml/kg) and morphine sulphate (10 mg/kg) intraperitoneally (i.p.) or 0.5% CMC and various doses of curcumin (25-400 mg/kg) orally (p.o.). All animals were placed on the hot-plate for 7 subsequent trials at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of hot-plate latency are expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

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

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

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

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

Analysis of the mechanism of antinociceptive action of curcumin

The possible participation of the opioid system in the antinociceptive effect of curcumin was investigated using the model of mouse hot-plate test. Animals were pretreated with naloxone (5 mg/kg, i.p.) 10 min before curcumin (200 mg/kg, p.o.) administration (Miranda et al., 2001).

Figure 11 Hot-plate Analgesiometer

Tail-flick Analgesic Testing

These studies employed the tail-flick assay described by D'Amour and Smith in 1941, with minor modifications. Male ICR mice, weighing 18-25 g were used (N=10 per group). Mice were placed in individual Plexiglas restrainers with an opening to allow the tail to protrude. Each tail rested in a shallow groove housing a light sensitive sensor. A beam of radiant heat (24-V, high amperage 150-watt light bulb situated 8 cm above the tail) was aimed at the middle of 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 animal within any given experiment. Animals failing to respond within 1.5 sec were excluded from testing. On the day of testing, all animals were tested for 3 pre-drug tail-flick baseline 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 vehicle (10 ml/kg) and morphine sulphate (10 mg/kg) intraperitoneally (i.p.) or 0.5% CMC and various doses of curcumin (25-400 mg/kg) orally (p.o.). Tail-flick latencies were recorded at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of tail-flick latency are expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

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

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

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

Figure 12 Tail-flick Analgesia meter

Acetic acid-induced writhing test in mice

Male ICR mice weighing 18-25 g were used (N=6 per group). Analgesic testing was determined using the acetic acid-induced writhing method described by Koster et al. in 1959. On the day of testing, animals were randomly assigned to one of eight treatment groups. Mice were then administered various doses of treatments one hour before intraperitoneal administration of 0.6% acetic acid (10 ml/kg, i.p.) which used to induce the constriction response.

The drug administration took place with indomethacin (10 mg/kg) or various doses of curcumin (25-400 mg/kg) or vehicle (10 ml/kg) p.o one hour 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 is reported as percentage of inhibition of writhing response compared with the vehicle control group. The percentage of inhibition of writhing response was calculated using the following formula:

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

Note: Wr = mean writhing response

Figure 13 Writhing response

Randall-Selitto Test

Male Wistar rats weighing 130-175 g were used (N=8 per group). For this assay, hind paw withdrawal thresholds (PWTs) to a noxious mechanical stimulus were determined using an analgesymeter as described by Hargreaves et al. in 1988. The analgesymeter was fitted with a single weight such that the maximum pressure exerted on the paw was 500 g.

The pressure on the paw was increased at a constant rate of 32 g/sec. The application of force was stopped when the rat started to struggle (vigorous attempt to withdraw the paw). Cut-off was set at 500 g and endpoint was taken as complete paw withdrawal. PWTs were determined at the withdrawal response. Animals failing to react to a 500 g force were given scores corresponding to the full scale. The animals was held during this test but not restrained. On the day of testing, all animals underwent 3 pre-drug paw pressure baseline trial conducted at 10-15 min intervals. The score from the last two trials served as the baseline measure for each animal. Animals were pretreated orally with indomethacin (10 mg/kg, p.o.), vehicle or various doses of curcumin (25-400 mg/kg, p.o.) one hour before an intraplantar injection of 1% carrageenan (50 μl) to the left hind paw. Rats were then tested for mechanical hyperalgesia during the subsequent 240 min period. The paw withdrawal thresholds (PWTs) were expressed as percentage inhibition of hyperalgesia, according to the following formula:

$$\% \text{ inhibition of hyperalgesia} = \frac{\text{PWTs (control)} - \text{PWTs (sample)}}{\text{PWTs (control)}} \times 100$$

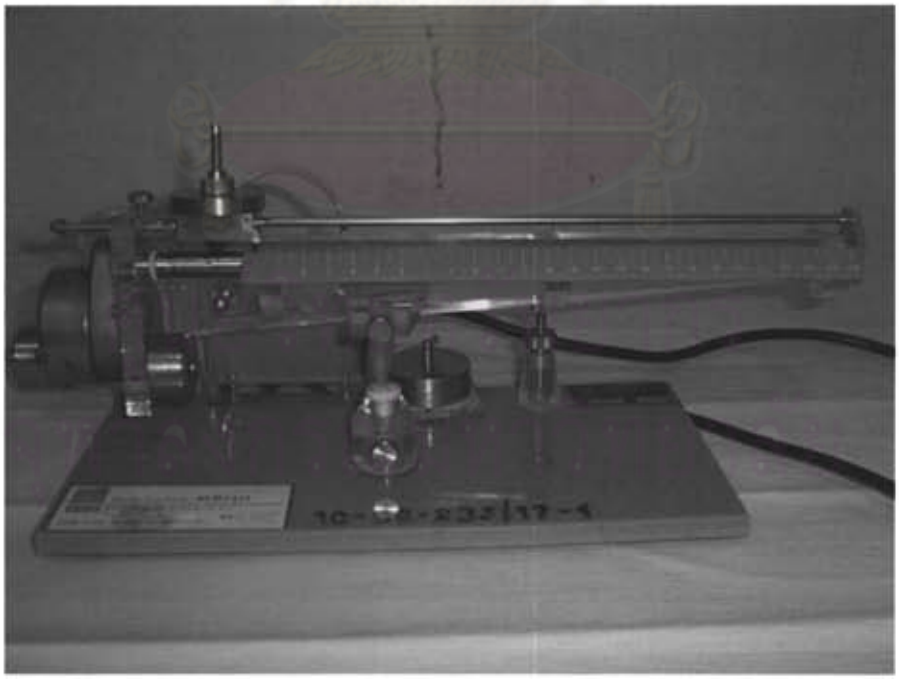


Figure 14 Analgesymeter

Carrageenan-induced paw edema Testing

The anti-inflammatory activity of curcumin was determined using carrageenan-induced paw edema test in hind paws of rats as described by Winter et al. in 1962. Male Sprague-Dawley rats (N=6 per group), 100-150 g, were fasted overnight before the experiment with free access to water. The animals were treated orally with indomethacin (10 mg/kg), vehicle control (0.9% NSS or 0.5% CMC 10 ml/kg) or various doses of curcumin (25-400 mg/kg). One hour later, the rats were challenged by subcutaneous injection of 50 µl of 1% solution of carrageenan into the plantar surface of the left hind paw (Amanlou et al., 2005). 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:

$$\% \text{ inhibition of edema} = 100\ (1-Vt/Vc)$$

Note: Vc = edema volume in control group; Vt = edema volume in tested group

Figure 15 Plethysmometer

Rota-rod test

To rule out the possibility of motor impairment or sedation from oral administration of curcumin, mice were tested for their ability to perform a rota-rod test after administration of curcumin at dose of 200 mg/kg compared to vehicle controls (N=10). Mice were placed on a horizontal rod (3.5 cm diameter) rotating at a speed of 16.5 revolutions per minute. The mice that were capable of remaining on the top for 60 sec or more, in three successive trials were selected for the study (Chattopadhyay et al., 2003). Each group of the animals was then placed on the rod at an interval of 15, 30, 45, 60, 90, 120 and 240 min after oral administration of curcumin. The results were expressed as the time (seconds) in which animals remained on the rota-rod. The cut-off time used was 60 sec (Otuki et al., 2001).

Figure 16 Rota-rod Apparatus

DATA TREATMENT AND STATISTICAL ANALYSE

Statistical analyses were performed on the dose-response curves by analysis of variance (ANOVA). Post hoc analyses were performed using Fisher's LSD test (SPSS version 13.0 for windows). The minimum level of statistical significance was set at $p<0.05$.

CHAPTER IV
RESULTS

MOUSE HOT-PLATE TEST

To demonstrate the validity of the hot-plate analgesic testing following drug administration, mice received morphine sulphate (MO; 10 mg/kg) intraperitoneal (i.p.) and were tested during the subsequent 240 min period. As expected MO significantly ($p<0.01$) increased hot-plate latency producing an area of analgesia of 14414±1725 %MPE-min compared with that of normal saline solution (NSS) (852±641 %MPE-min; Figure 17)

Initial studies utilizing the hot-plate test in mice to examine the efficacy of curcumin in producing analgesia. Mice were administered orally 0.5% carboxymethylcellulose (CMC) or various doses of curcumin (25, 50, 100, 200, 400 mg/kg). Curcumin doses of 25 mg/kg or higher significantly ($p<0.05$) increased hot-plate latency when compared to the vehicle group (Figure 18).

When the log of curcumin dose was plotted versus area of analgesia (%MPE-min), a linear correlation was observed. When all five doses of curcumin (25, 50, 100, 200 and 400 mg/kg) were plotted a linear correlation coefficient (r^2) equal to 0.3512 was observed, while the plotting of only four doses (25, 50, 100 and 200 mg/kg) revealed a significant linear correlation coefficient of 0.8003 (Figure 19&20). ED_{50} was calculated from the log dose and %MPE line and was equal to 133.10 mg/kg (Figure 21). The analgesic peak effects of curcumin (25, 50, 100, 200 and 400 mg/kg) were reached within 45, 45, 60, 90, 45 min after oral administration, respectively. Individual time courses of the responses are shown in Figure 22.

In order to investigate any role of the opioid receptor in curcumin actions, mice were then administered NSS (10 ml/kg, i.p.), naloxone (NAL; 5 mg/kg, i.p.), a short-acting opioid receptor antagonist, 0.5% CMC (10 ml/kg, p.o.), curcumin (200 mg/kg, p.o.) or the combination of naloxone and curcumin (5/200 mg/kg). Naloxone alone failed to produce significant response when compared to vehicle control. Curcumin 200 mg/kg produced significant ($p<0.05$) response when compared to vehicle control. The inclusion of naloxone with curcumin significantly ($p<0.05$) attenuated the analgesic response due to curcumin

indicating that opioid receptors are involved in the analgesic response produced by curcumin (Figure 23). Individual time courses of the responses are shown in Figure 24.

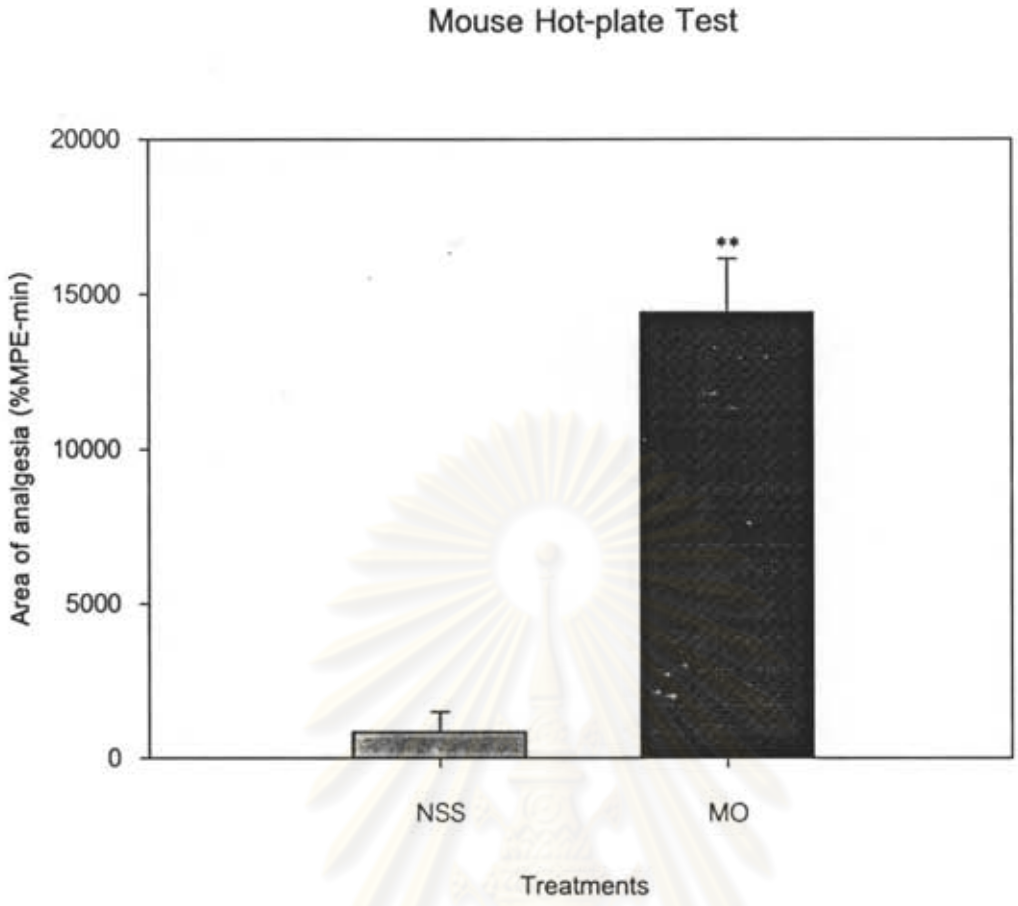


Figure 17 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. **$p<0.01$ significantly different compared to NSS.

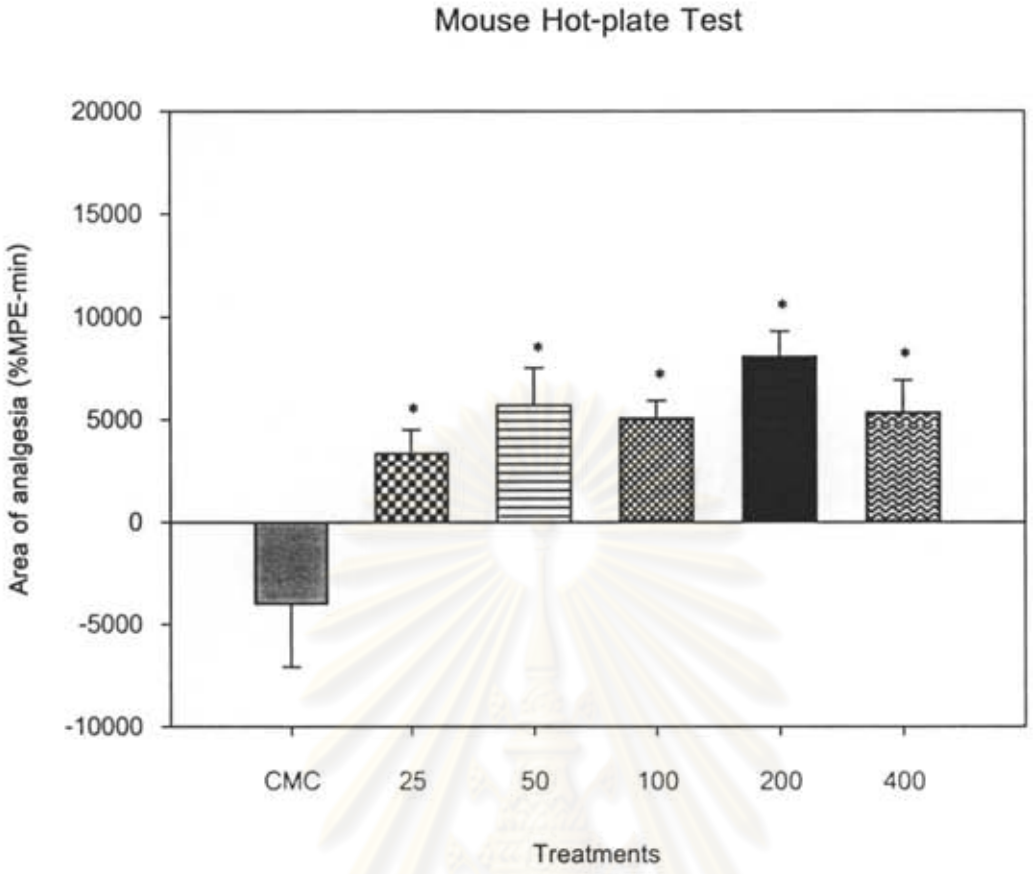


Figure 18 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 0.5% carboxymethylcellulose (CMC) and various doses of curcumin (25-400 mg/kg). N=10 for all groups. *$p<0.05$ significantly different compared to CMC.

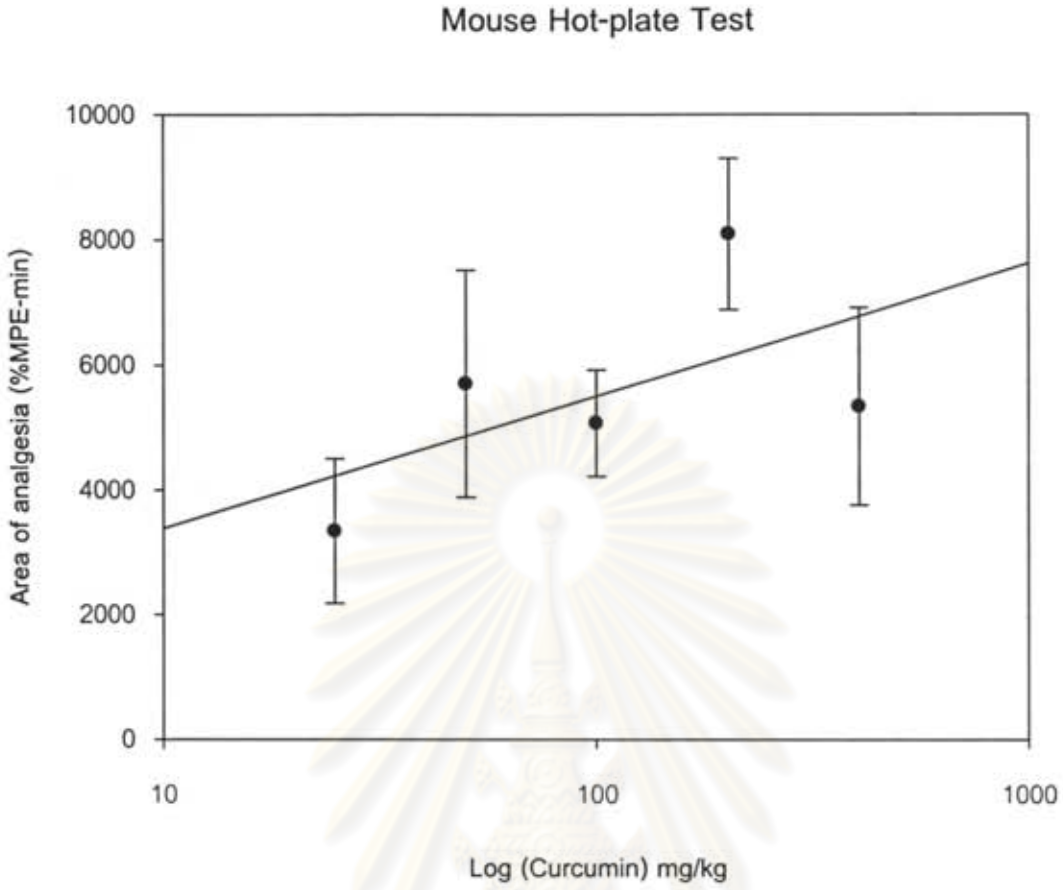


Figure 19 Linear regression of area of analgesia (%MPE-min) from 0-240 minutes after oral administration of curcumin (25-400 mg/kg). N=10 for all groups. The regression equation was Y = 921.1299*Ln(x) + 1270.8080, r^2 = 0.3512.

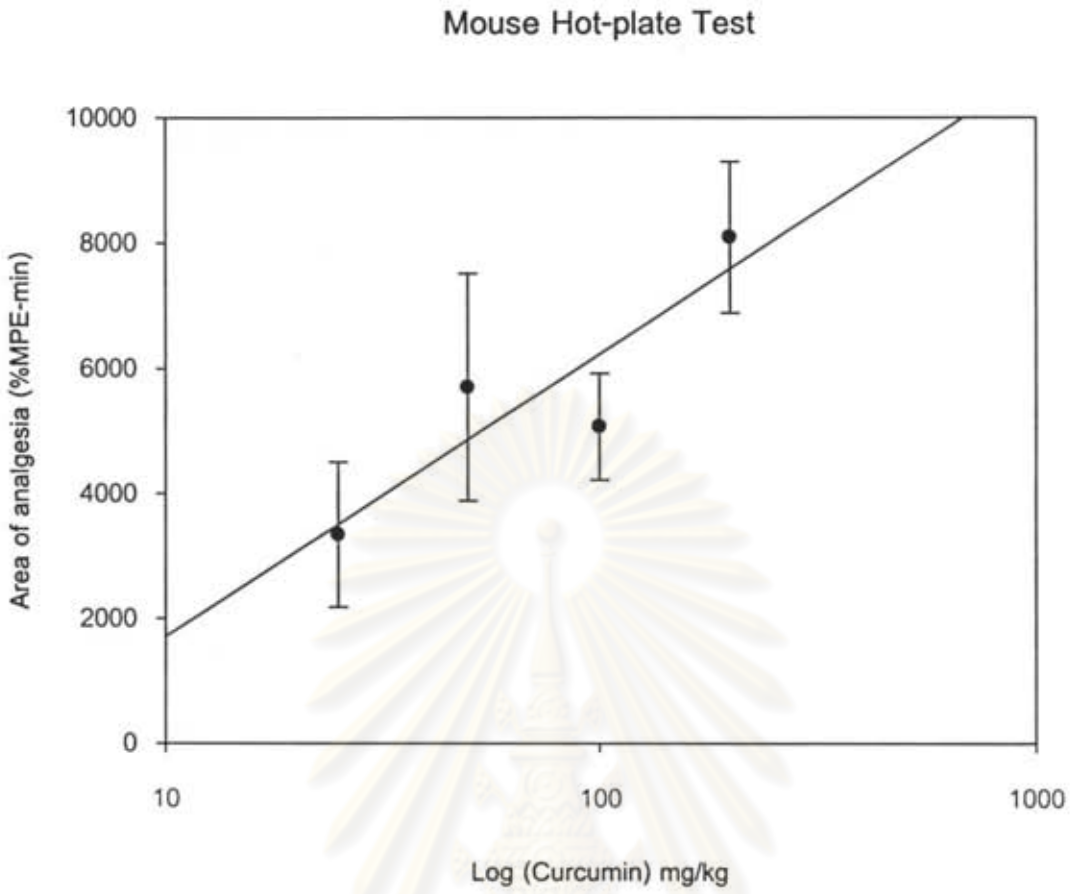


Figure 20 Linear regression of area of analgesia (%MPE-min) from 0-240 minutes after oral administration of curcumin (25-200 mg/kg). N=10 for all groups. The regression equation was $Y = 1963.3221 * Ln(x) - 2806.2717$, $r^2 = 0.8003$.

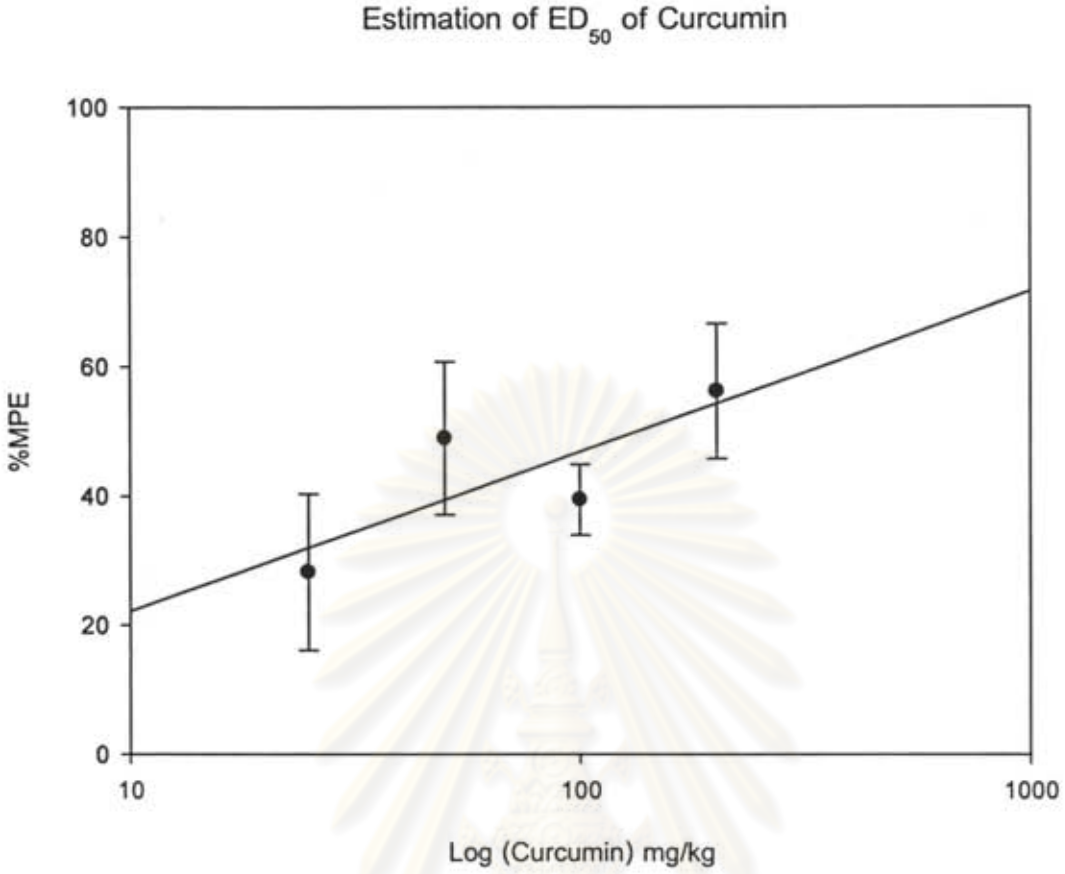


Figure 21 Linear regression of %MPE values at the time point at which peak antinociceptive response were observed after oral administration of various doses of curcumin (25-200 mg/kg) using hot-plate test. N=10 for all groups. The ED_{50} was calculated from the log dose and %MPE line as Y= 10.7524*Ln(x) - 2.5910, r^2 = 0.6288 and equal to 133.10 mg/kg.

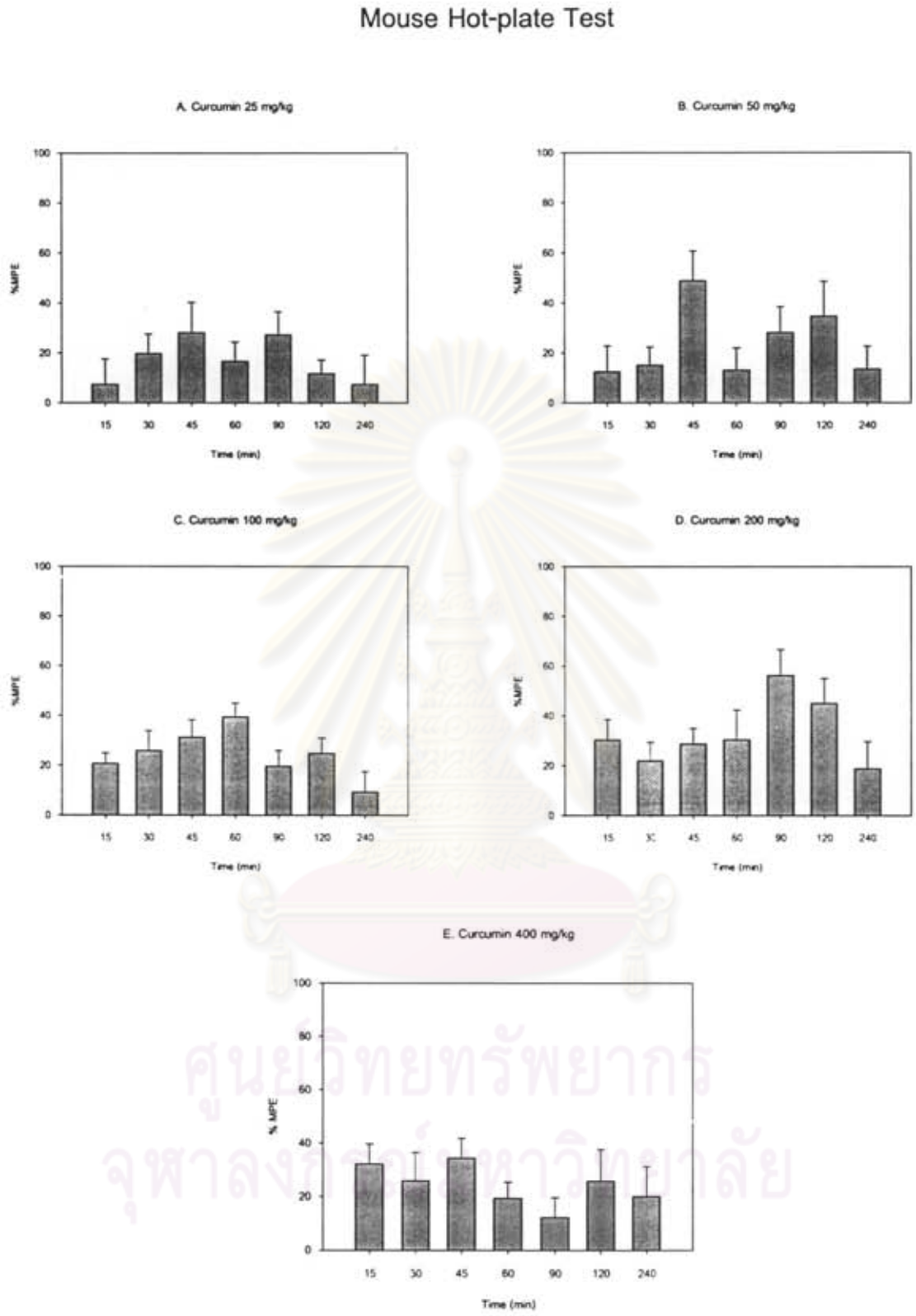


Figure 22 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of curcumin. A; Curcumin 25 mg/kg, B; Curcumin 50 mg/kg, C; Curcumin 100 mg/kg, D; Curcumin 200 mg/kg, E; Curcumin 400 mg/kg. N=10 for all groups.

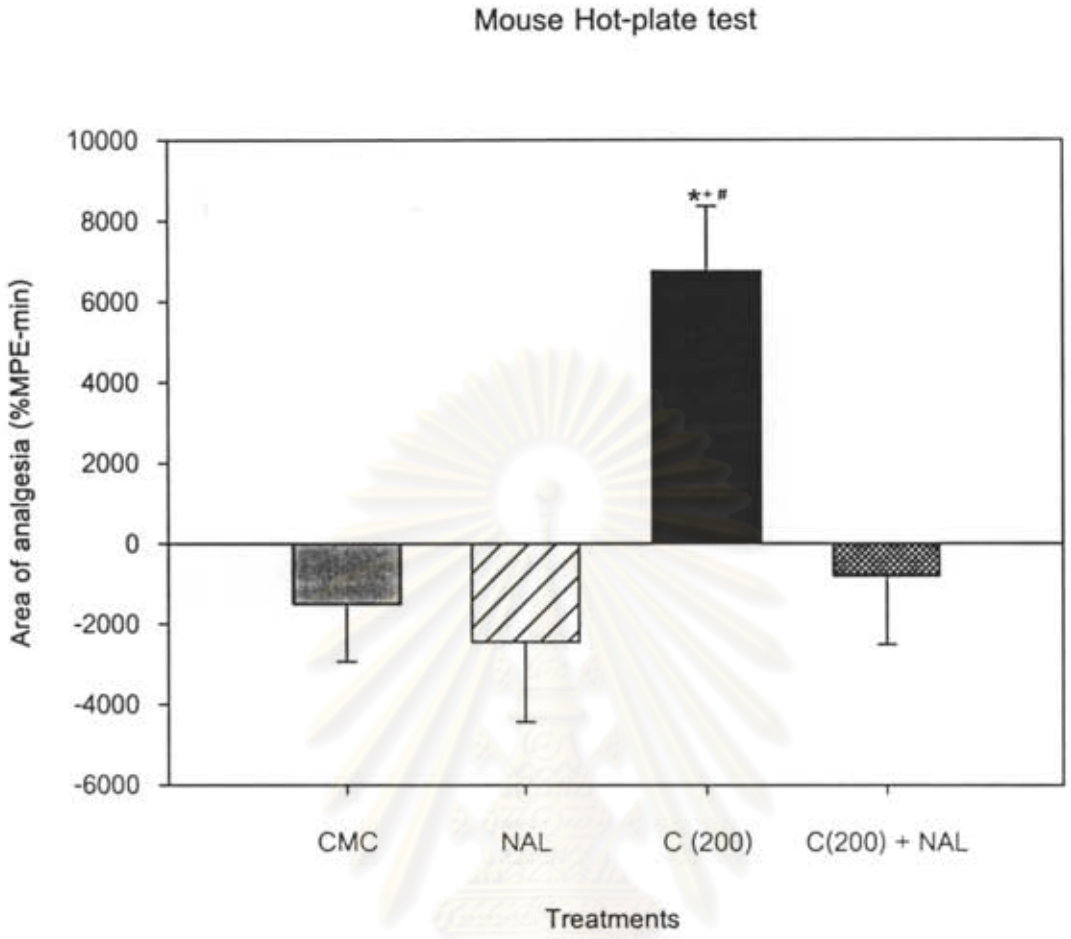


Figure 23 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 0.5% carboxymethylcellulose (CMC; 10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), curcumin (C; 200 mg/kg, p.o.) and the combination of naloxone and curcumin (5/200 mg/kg). N=10 for all groups. *$p<0.05$ significantly different compared to CMC; +$p< 0.05$ significantly different compared to naloxone; #$p<0.05$ significantly different compared to naloxone+curcumin.

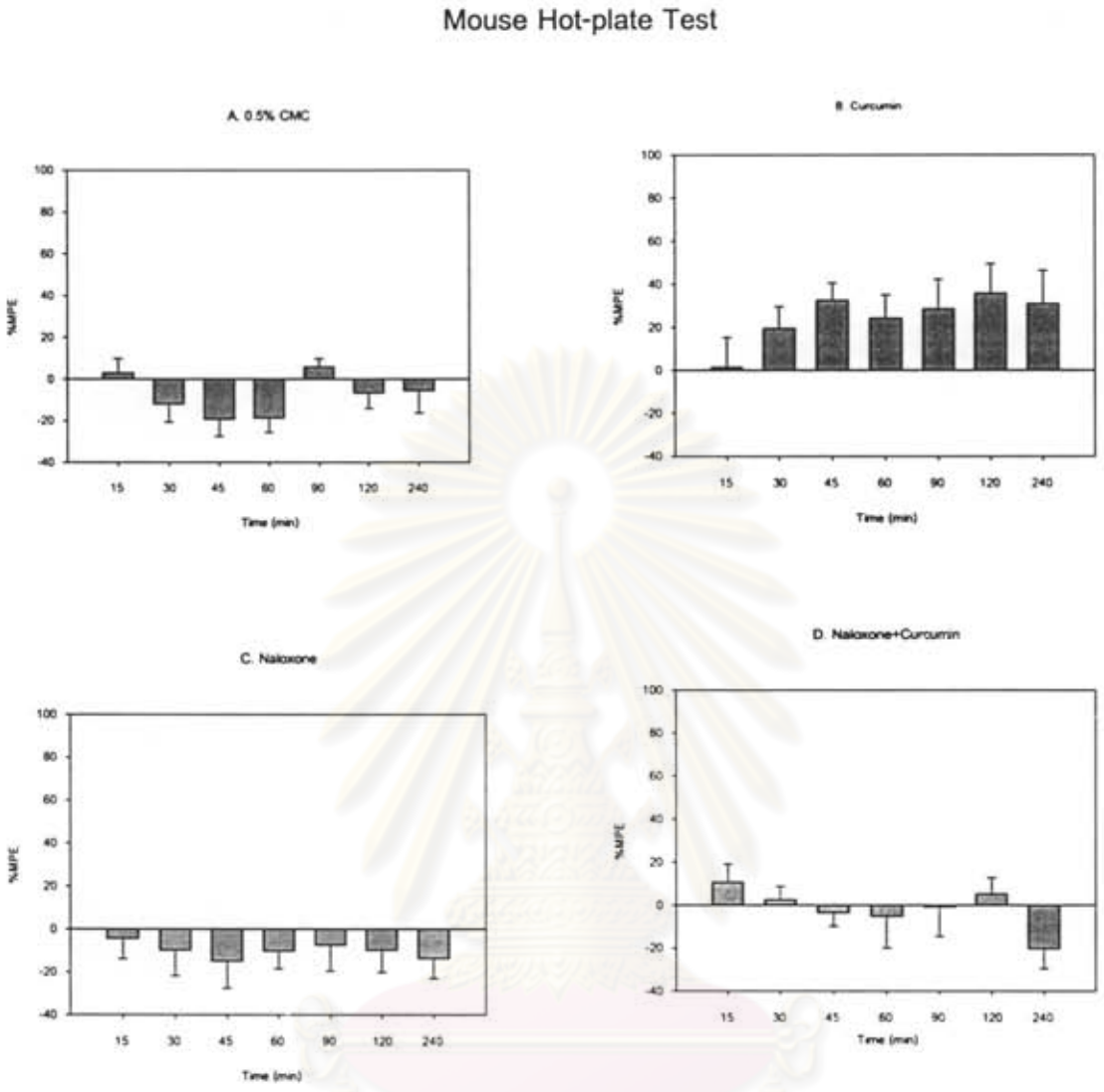


Figure 24 Individual time courses of the response (%MPE versus time (min) after administration of 0.5% carboxymethylcellulose (CMC, p.o.), curcumin 200 mg/kg (p.o.), naloxone 5 mg/kg (i.p.) and the combination of naloxone and curcumin (5/200 mg/kg). N=10 for all groups. A; Curcumin 25 mg/kg, B; Curcumin 50 mg/kg, C; Curcumin 100 mg/kg, D; Curcumin 200 mg/kg, E; Curcumin 400 mg/kg. N=10 for all groups.

MOUSE TAIL-FLICK TEST

To demonstrate the validity of the tail-flick analgesic testing following drug administration, mice received morphine sulfate (MO; 10 mg/kg) i.p. and were tested during the subsequent 240 min period. As expected MO significantly ($p<0.01$) increased tail-flick latency producing an area of analgesia of 15372± 2149 %MPE-min compared with that of normal saline solution (NSS) (-161± -222 %MPE-min; Figure 25).

Studies then utilized the mouse tail-flick method to examine the efficacy of curcumin in producing analgesia. Mice were orally administered CMC or various doses of curcumin (25, 50, 100, 200, 400 mg/kg). All doses of curcumin failed to produce analgesic response when compared to the vehicle group (Figure 26). Individual time courses of the responses are shown in Figure 27.

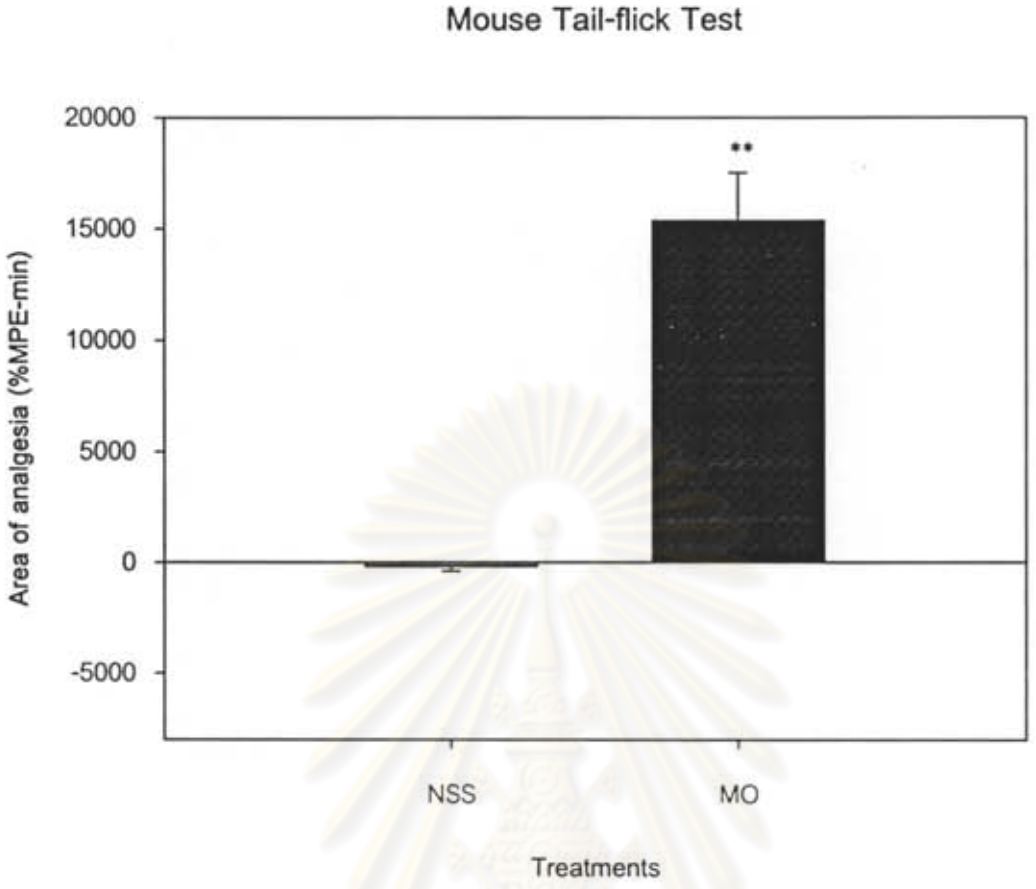


Figure 25 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. **$p < 0.01$ significantly different compared to NSS.

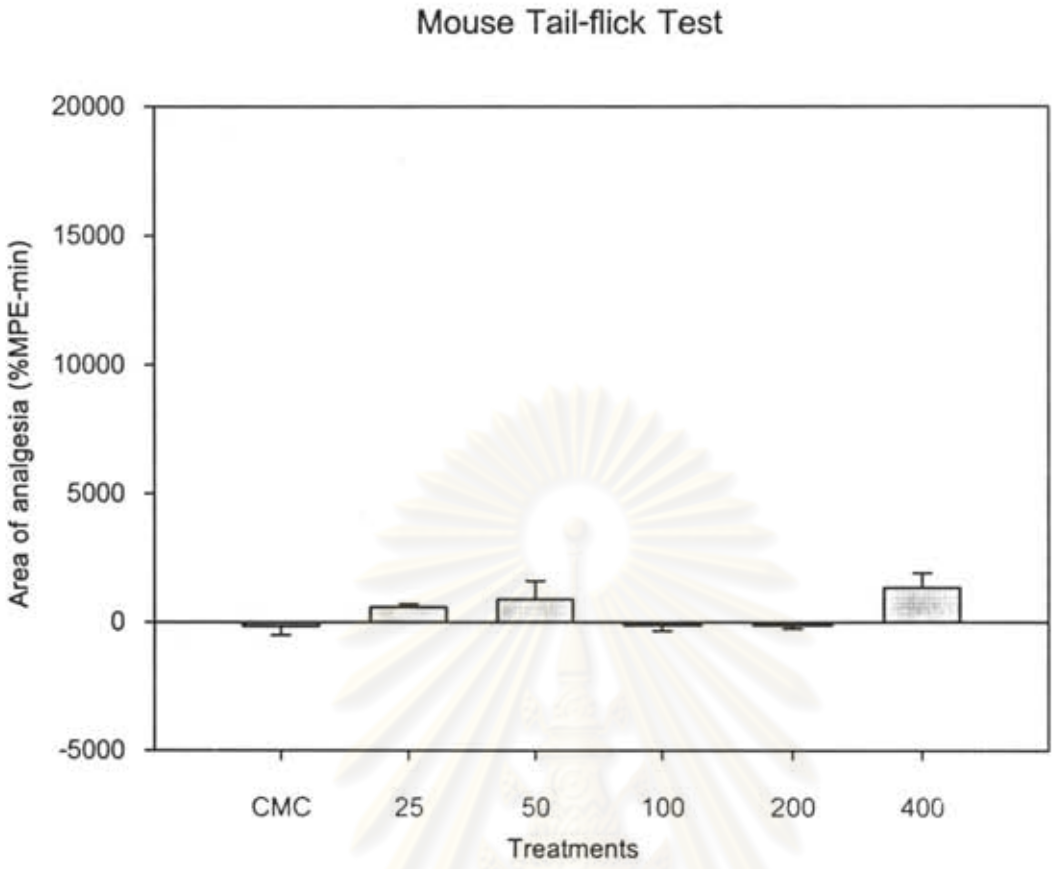


Figure 26 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 0.5% carboxymethylcellulose (CMC) and various doses of curcumin (25-400 mg/kg). N=10 for all groups.

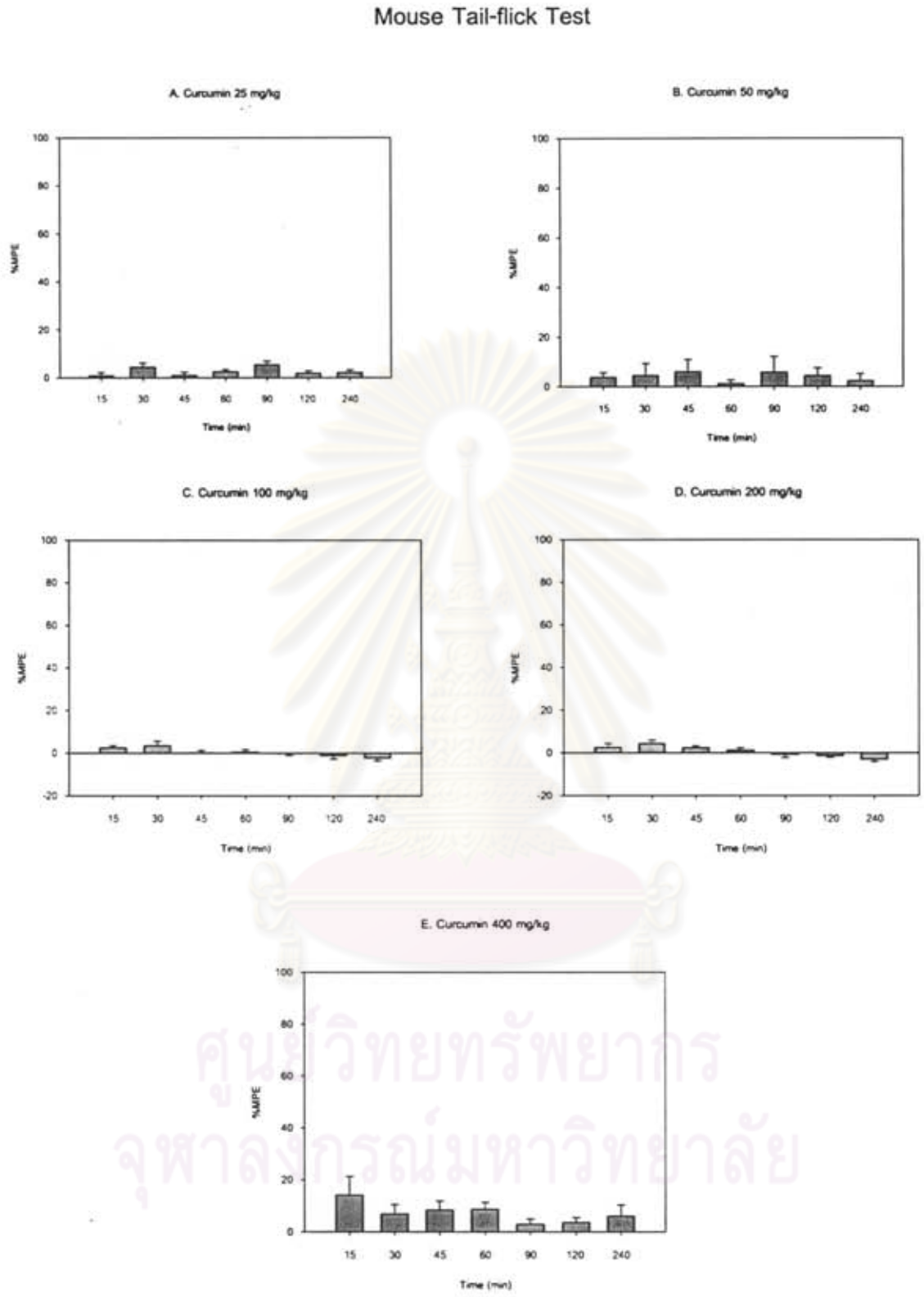


Figure 27 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of curcumin. A; Curcumin 25 mg/kg, B; Curcumin 50 mg/kg, C; Curcumin 100 mg/kg, D; Curcumin 200 mg/kg, E; Curcumin 400 mg/kg. N=10 for all groups.

ACETIC ACID-INDUCED WRITHING IN MICE

To demonstrate the validity of the acetic aid-induced writhing method following drug administration, mice received indomethacin (IND; 10 mg/kg) orally and were tested during the subsequent 30 min period. As expected IND significantly ($p<0.01$) decreased writhing response by 90.74% producing a mean number of writhes of 3.33 ± 0.80 compared with that of NSS (36±3.99; Figure 28).

Studies then utilized the acetic acid-induced writhing method in mice to examine the efficacy of curcumin in producing analgesia. Mice were administered orally CMC or various doses of curcumin (25, 50, 100, 200, 400 mg/kg). Curcumin doses of 200 and 400 mg/kg significantly ($p<0.05$) decreased the number of writhes induced by acetic acid by 74.07% and 56.67%, respectively when compared to 0.5% CMC (Figure 29). IND showed the highest analgesia response compared to all test groups (Figure 30).

When the log of curcumin dose was plotted versus mean writhing response, a linear correlation coefficient (r^2) equal to 0.5812 was observed (Figure 31). Linear correlation coefficient (r^2) of percentage of inhibition of writhing response after oral administration of curcumin (25-400 mg/kg) equal to 0.5813 (Figure 32).

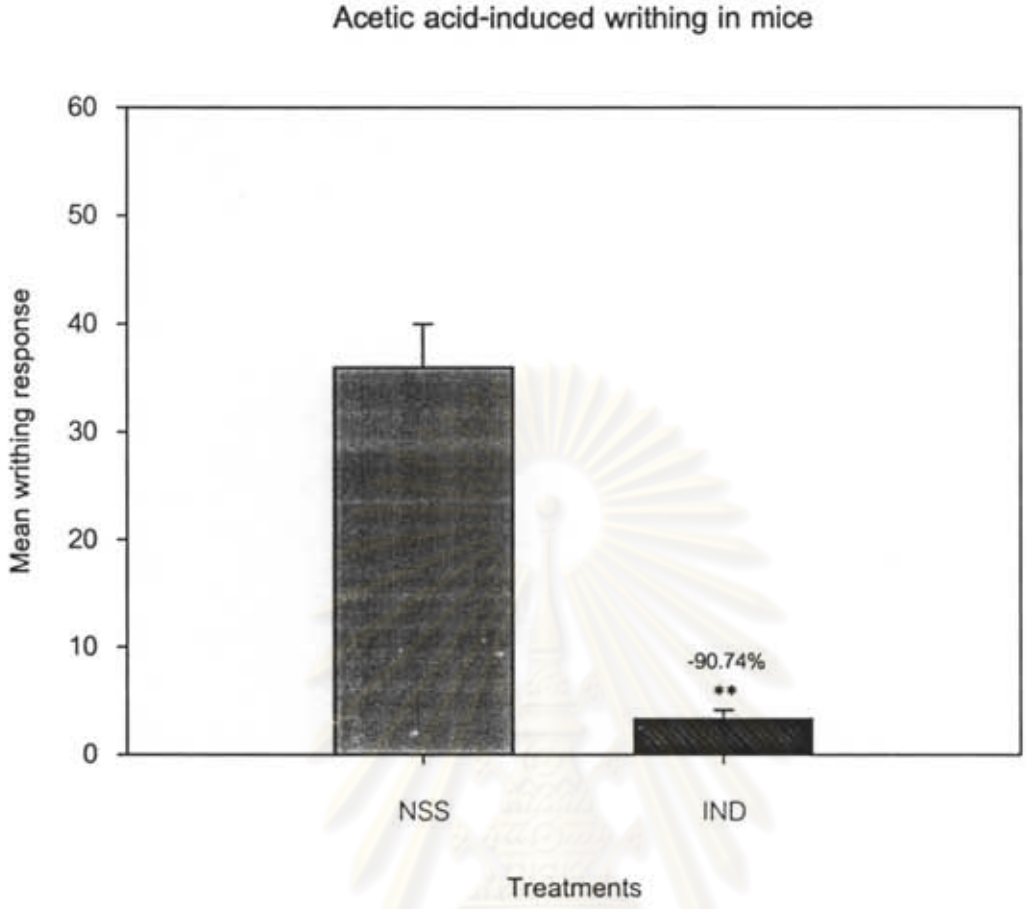


Figure 28 Mean writhing response after oral administration of 0.9% normal saline solution (NSS) and indomethacin (IND; 10 mg/kg). N=6 for all groups. **$p<0.01$ significantly different compared to NSS.

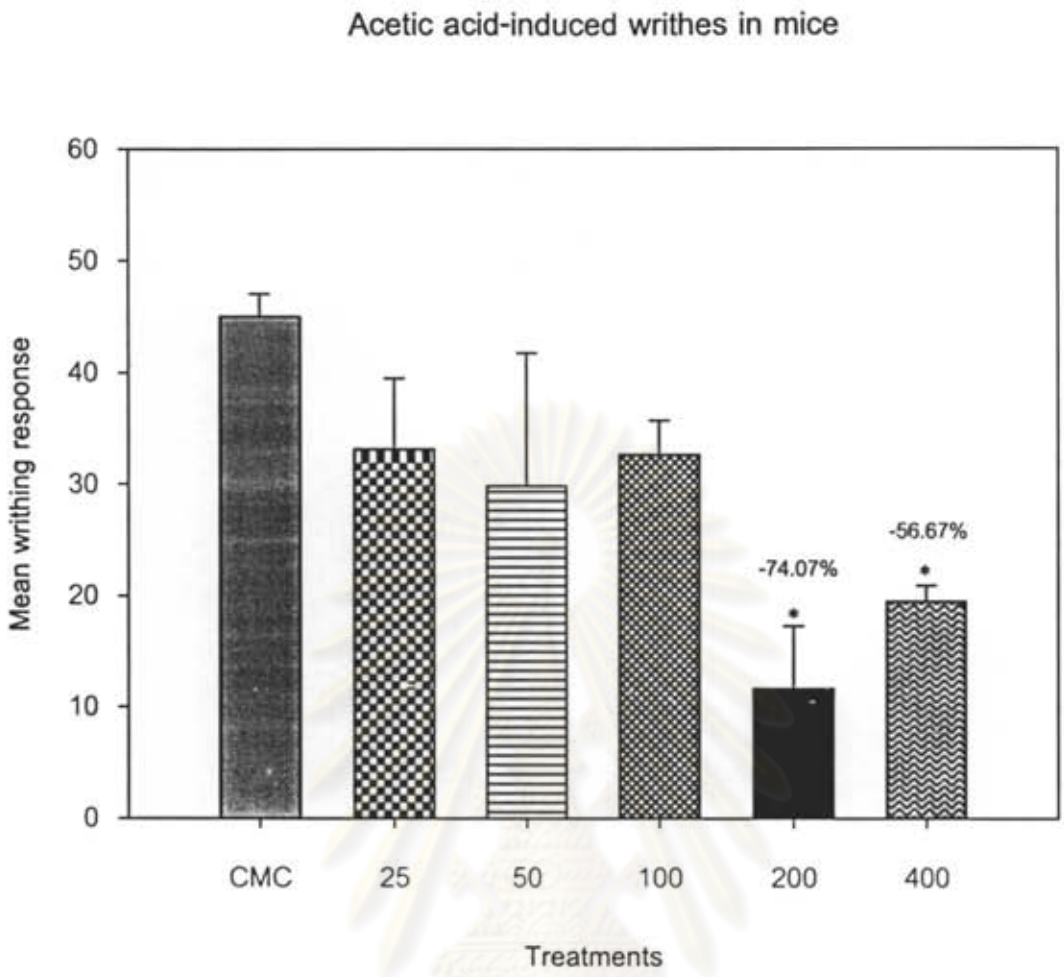


Figure 29 Mean writhing response after oral administration of 0.5% carboxy-methylcellulose (CMC) and various doses of curcumin (25-400 mg/kg). N=6 for all groups. *p< 0.05 significantly different compared to CMC.

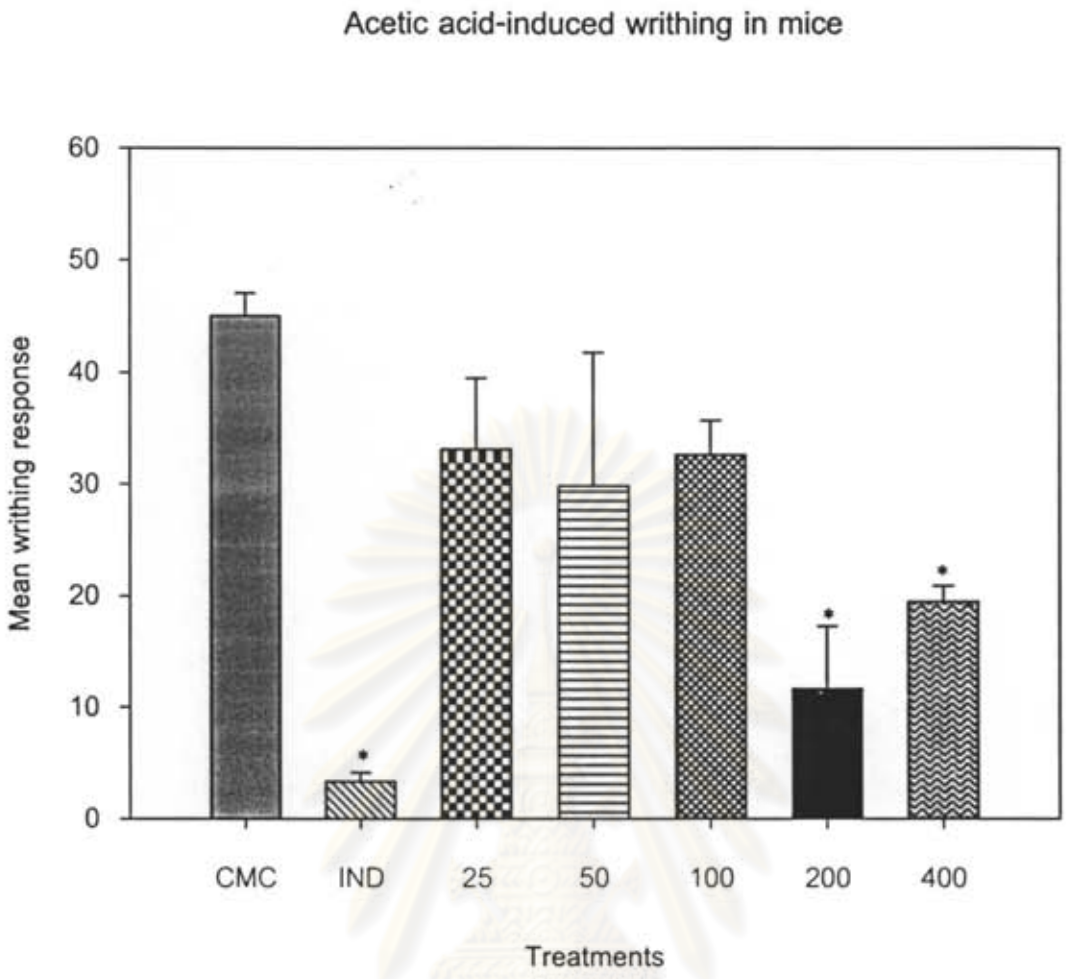


Figure 30 Mean writhing response after oral administration of 0.5% carboxymethylcellulose (CMC), indomethacin (IND; 10 mg/kg) and various doses of curcumin (25-400 mg/kg). N=6 for all groups. *$p<0.05$ significantly different compared to CMC.

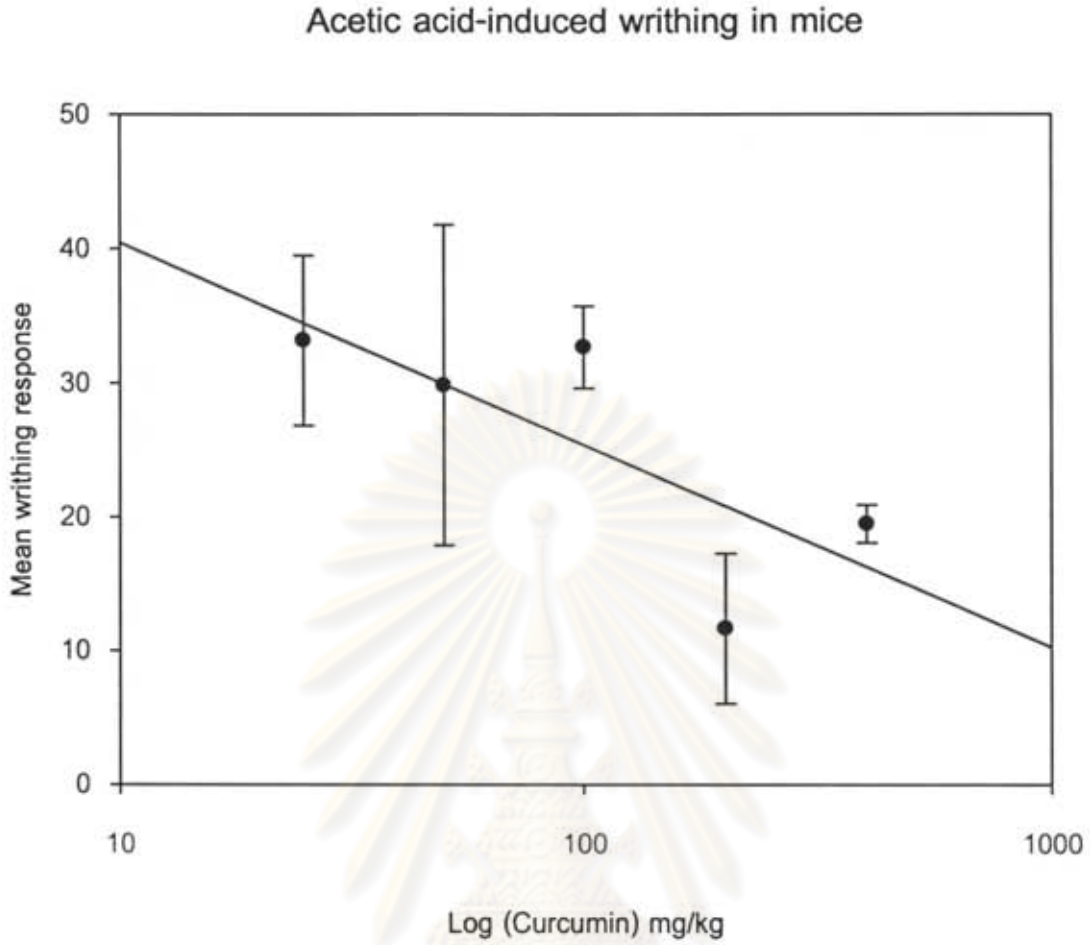


Figure 31 Linear regression of mean writhing response after oral administration of curcumin (25-400 mg/kg). N=6 for all groups. The regression equation was $Y = -6.5643 * Ln(x) + 55.5962$, $r^2 = 0.5812$.

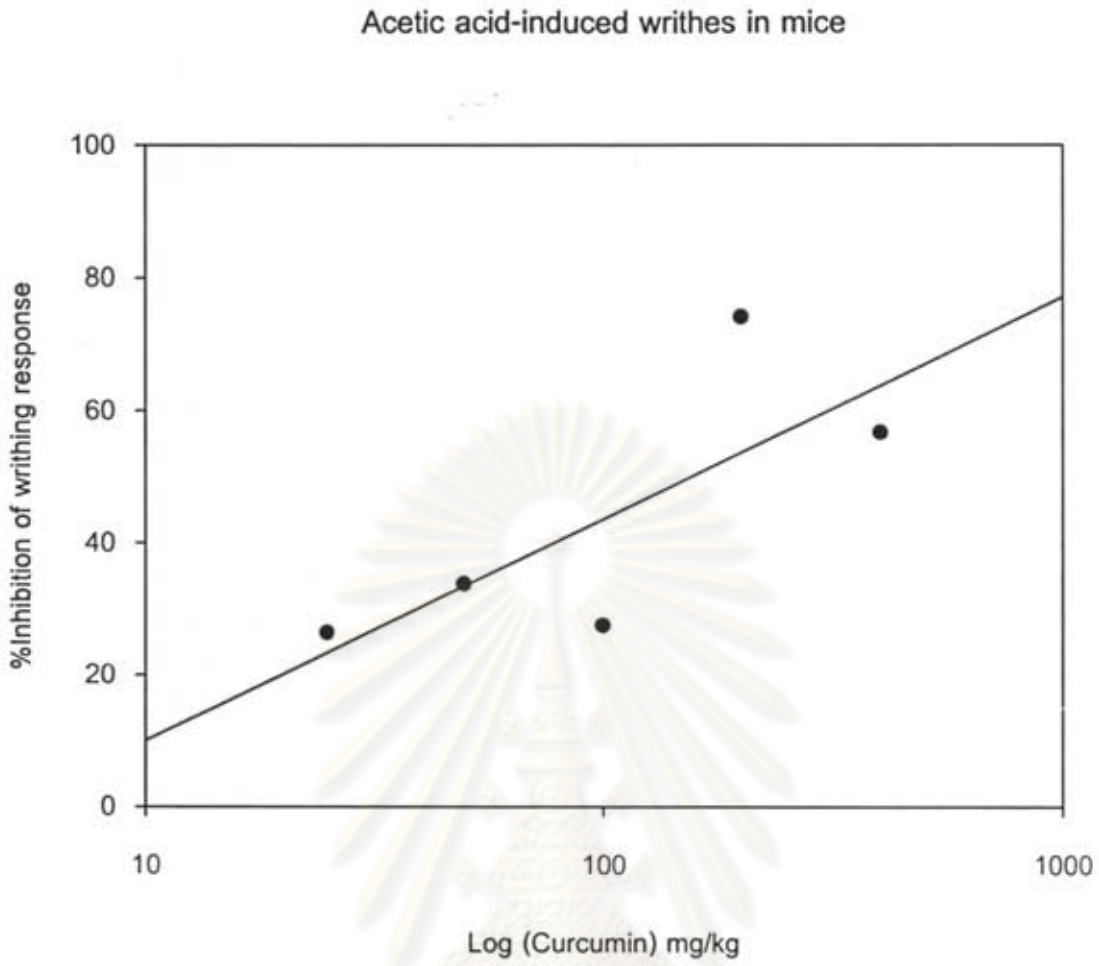


Figure 32 Linear regression of percentage of inhibition of writhing response after oral administration of curcumin (25-400 mg/kg). N=6 for all groups. The regression equation was $Y = 14.5885*Ln(x) - 23.5547$, $r^2 = 0.5813$. $ED_{50} = 155.77$ mg/kg.

Randall-Selitto Test

To determine the antinociceptive effect of curcumin in carrageenan induced paw inflammation, the Randall-Selitto paw pressure technique was used. Two hours after the carrageenan injection into the plantar surface of rat's left hind paw, the left hind paw was red and swollen. In order to demonstrate the validity of the Randall-Selitto test following drug administration, rats received oral administration of indomethacin (IND; 10 mg/kg, p.o.) 60 min prior to injection of carrageenan. Rats were then tested for mechanical hyperalgesia during the subsequent 240 min period. IND significantly ($p<0.01$) increased mean paw withdrawal threshold (PWT) compared to NSS at 90 and 120 min after carrageenan administration (Figure 33).

Studies then utilized the Randall-Selitto test to examine the antinociceptive effect of curcumin, rats were administered orally CMC or various doses of curcumin (25-400 mg/kg) 60 min before carrageenan administration and tested for mechanical hyperalgesia at 15, 30, 45, 60, 90, 120, and 240 min after carrageenan administration. Only curcumin 200 mg/kg significantly ($p<0.05$) increased PWT when compared to vehicle control at all time tested except for 45 min. Percentage of inhibition of hyperalgesia after oral administration of curcumin 200 mg/kg were 50.68%, 33.87%,
-10.81%, 47.06%, 41.67%, 34.00%, and 59.09%, respectively (Figure 34).

When the log of curcumin dose was plotted versus mean paw withdrawal threshold at 2 hr after carrageenan administration, a linear correlation was observed.
When all five doses of curcumin (25-400 mg/kg) was plotted a linear correlation coefficient (r^2) equal to 0.4261 was observed while the plotting of percentage of inhibition of hyperalgesia after oral administration of various doses of curcumin (25-400 mg/kg) at 2 hr after carrageenan administration revealed a linear correlation coefficient of 0.4261 (Figure 35&36).

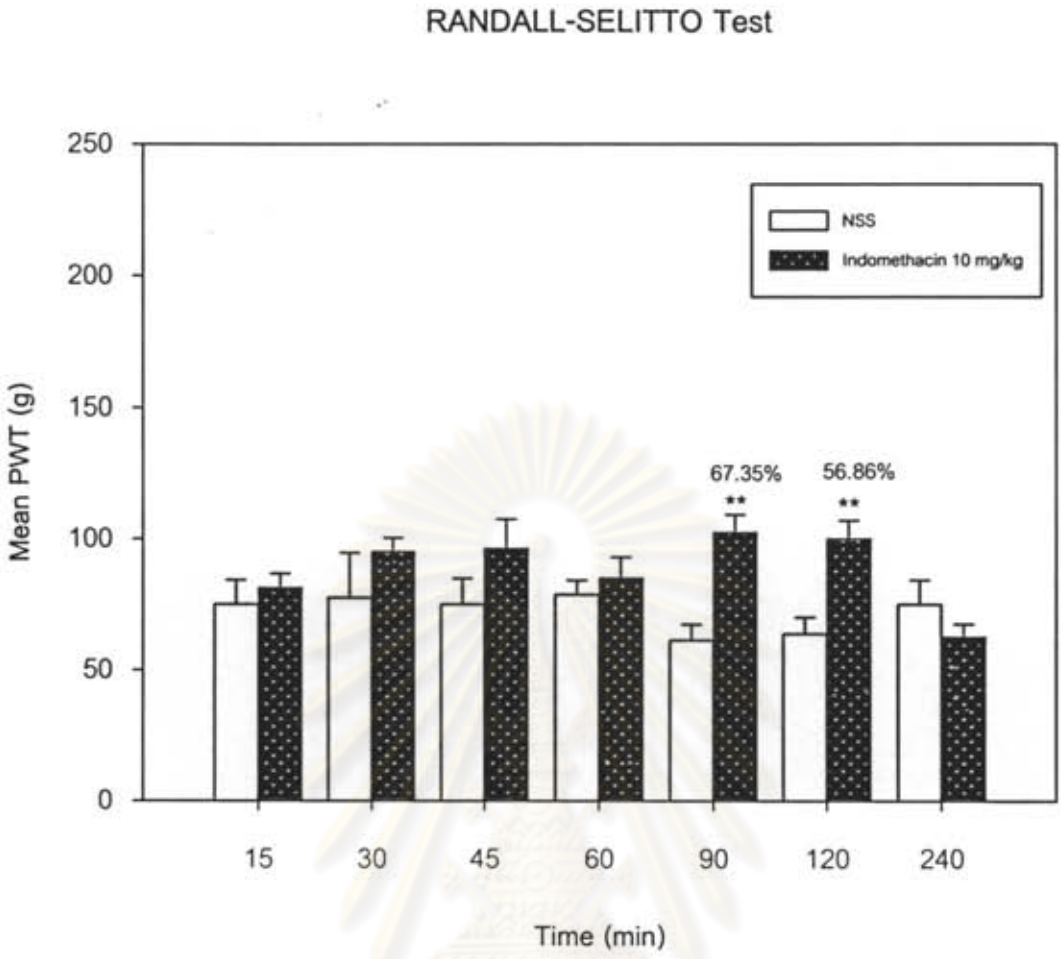


Figure 33 Mean paw withdrawal threshold (g) at 15, 30, 45, 60, 90, 120, and 240 min after oral administration of 0.9% normal saline solution (NSS) and indomethacin (IND; 10 mg/kg). N=8 for all groups. Inhibition is reported as percent compared to the vehicle control. **p< 0.01 significantly different compared to NSS.

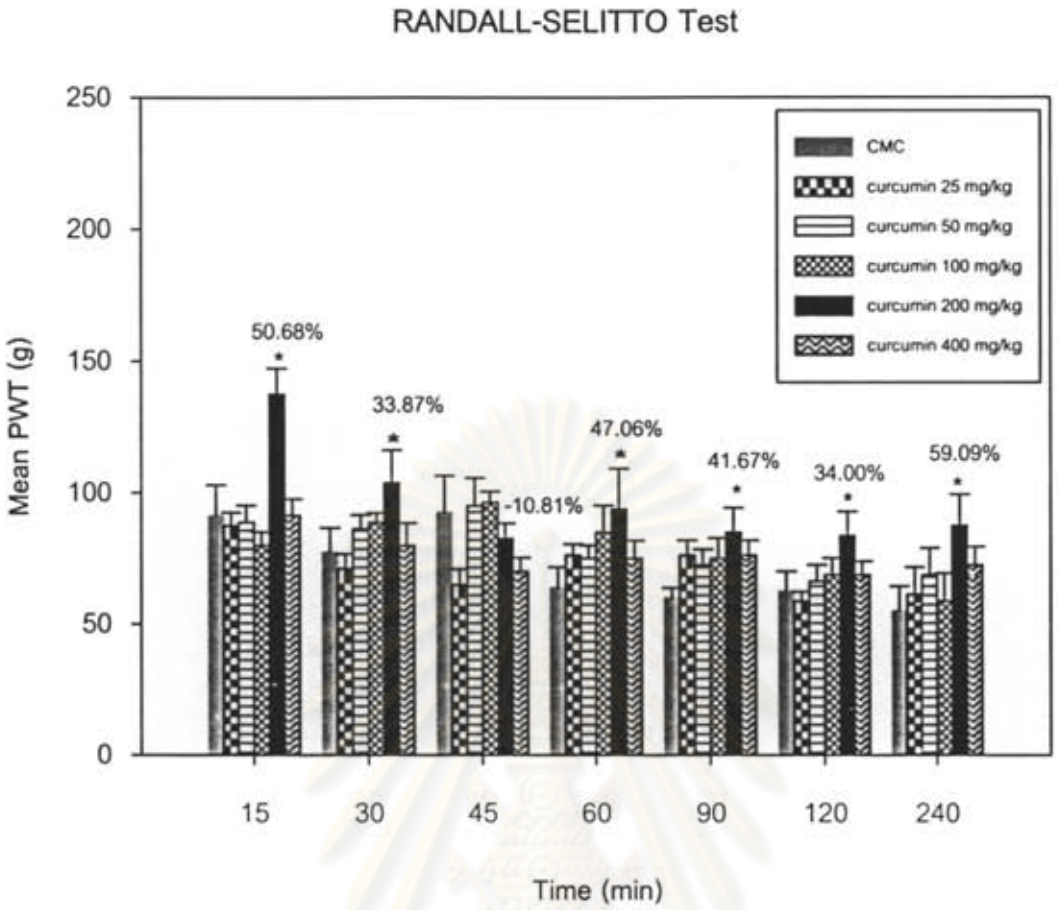


Figure 34 Mean paw withdrawal threshold (PWT) at 15, 30, 45, 60, 90, 120, and 240 min after oral administration of 0.5% carboxymethylcellulose (CMC) and various doses of curcumin (25-400 mg/kg). N=8 for all groups. Inhibition is reported as percent compared to the vehicle control. *$p<0.05$ significantly different compared to CMC.

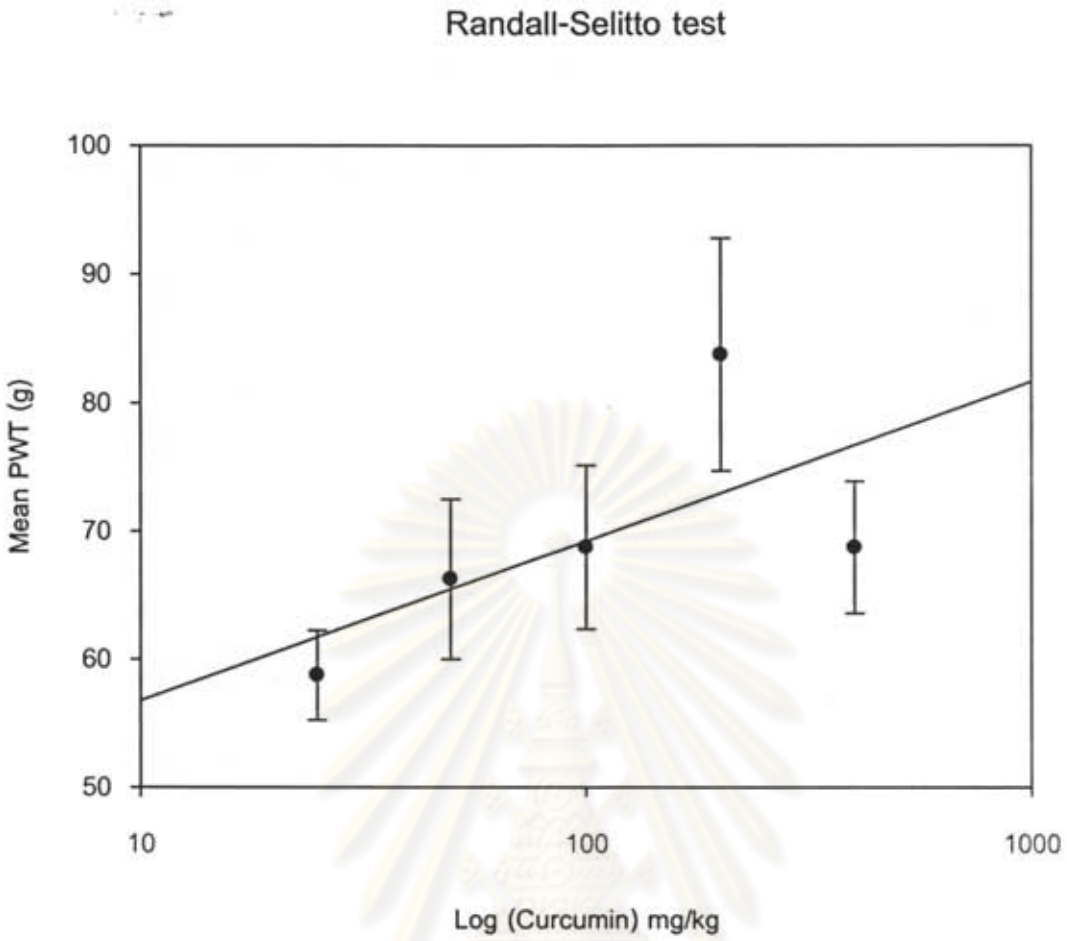


Figure 35 Linear regression of mean paw withdrawal threshold after oral administration of curcumin (25-400 mg/kg) at 2 hr after carrageenan administration. N= 8 for all groups. The regression equation was $Y = 5.4101*Ln(x) + 44.3355$, $r^2 = 0.4261$.

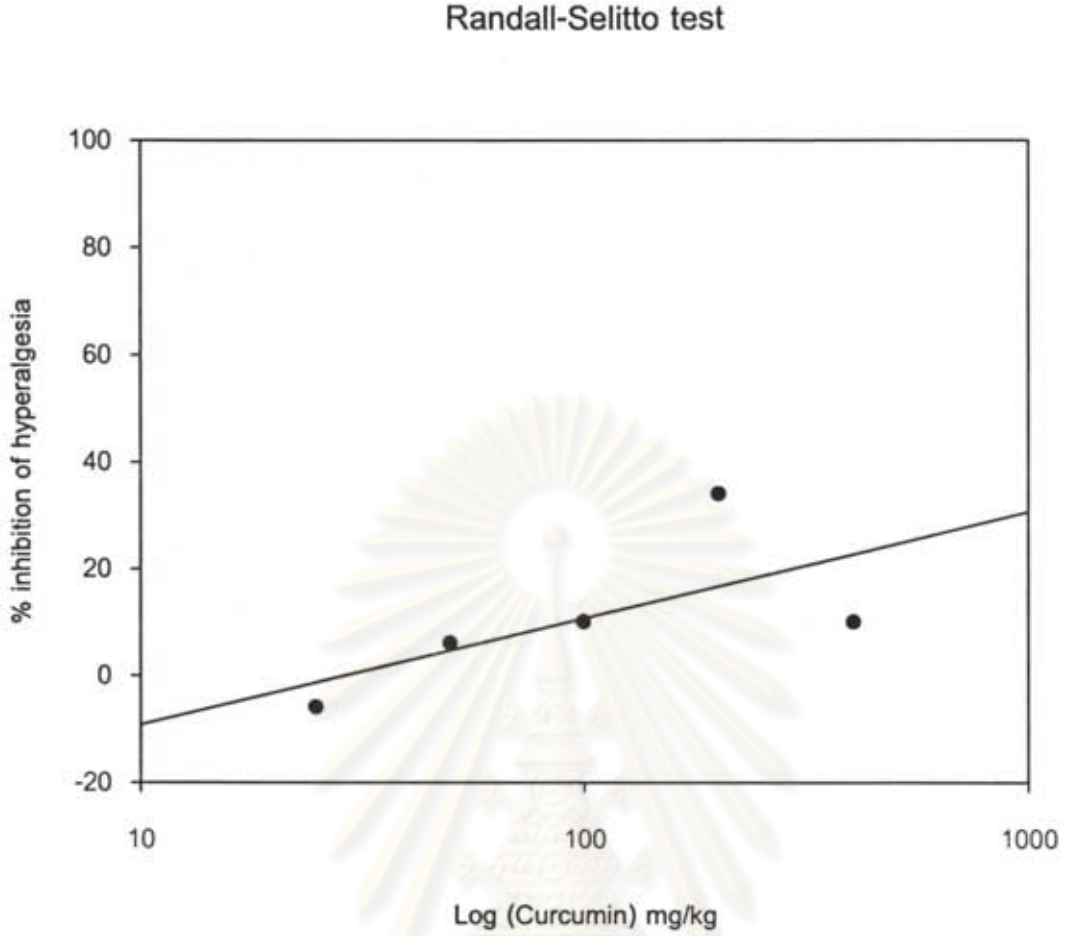


Figure 36 Linear regression of % inhibition of hyperalgesia after oral administration of various doses of curcumin (25-400 mg/kg) at 2 hr after carrageenan administration. N=8 for all groups. The ED_{50} was calculated from Y= 8.6562*Ln(x) -29.0631, r^2 = 0.4261.

CARRAGEENAN-INDUCED PAW EDEMA TEST

To demonstrate the validity of the carrageenan-induced paw edema test following drug administration, rats received oral administration of indomethacin (IND; 10 mg/kg) one hour before injection of carrageenan into the plantar surface of rat's left hind paw and were tested during the subsequent 6 hr period. As expected IND significantly ($p<0.01$) decreased paw edema at 2 hr and 3 hr after carrageenan administration compared with that of normal saline solution (NSS), producing a percentage of inhibition of edema of 46.87% and 65.71%, respectively (Table 1).

Studies then utilized the carrageenan-induced paw edema test to examine the efficacy of curcumin in producing anti-inflammation. Rats were administered orally CMC or various doses of curcumin (25-400 mg/kg) one hour before carrageenan administration. Curcumin 25 mg/kg decreased the paw volume significantly ($p<0.05$) at 4 hr, while curcumin doses of 50 and 100 mg/kg decreased the paw volume significantly ($p<0.05$) compared to vehicle control at 5 hr after carrageenan administration. Curcumin 200 mg/kg decreased the paw volume significantly ($p<0.05$) when compared to the vehicle group at 2 and 3 hr while curcumin 400 mg/kg decreased the paw edema significantly ($p<0.05$) at 2, 3, and 5 hr after carrageenan administration. Curcumin doses of 200 and 400 mg/kg showed a maximum inhibition of paw edema of 53.85%, 58.97%, respectively at 2 hr after carrageenan administration (Table 2). Percentage of inhibition from 1-6 hr after oral administration of 0.5% carboxymethylcellulose (CMC) and various doses of curcumin (25-400 mg/kg) were shown in the Table 2.

When the log of curcumin dose was plotted versus percentage of inhibition at 2 hr after carrageenan injection, a linear correlation was observed. When all five doses of curcumin (25, 50, 100, 200 and 400 mg/kg) were plotted a linear correlation coefficient (r^2) equal to 0.5800 (Figure 37).

Table 1 Change of edema volume (ml) of oral administration of 0.9% normal saline solution (NSS) and indomethacin (IND; 10 mg/kg) from 1-6 hr after carrageenan administration. N=6 for all groups. Inhibition is reported as percent compared to the vehicle control. *$p<0.05$ significantly different compared to NSS.

Treatment	Paw edema ± S.E.M.(% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
NSS	0.09±0.04	0.32±0.03	0.35±0.06	0.33±0.05	0.33±0.03	0.27±0.04
Indomethacin 10 mg/kg	0.11±0.05 (-22.22%)	0.17±0.04* (46.87%)	0.12±0.04* (65.71%)	0.21±0.07 (36.36%)	0.22±0.04 (33.33%)	0.27±0.08 (0%)

Table 2 Change of edema volume (ml) of oral administration of 0.5% carboxymethylcellulose (CMC) and various doses of curcumin (25-400 mg/kg) from 1-6 hr after carrageenan administration. N=6 for all groups. Inhibition is reported as percent compared to the vehicle control. *$p<0.05$ significantly different compared to CMC.

Treatment	Paw edema ± S.E.M.(%Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
0.5% CMC	0.21±0.01	0.39±0.04	0.43±0.04	0.43±0.03	0.46±0.05	0.26±0.05
Curcumin 25 mg/kg	0.17±0.04 (19.05%)	0.26±0.04 (33.33%)	0.33±0.04 (23.25%)	0.28±0.05* (34.88%)	0.33±0.02 (28.26%)	0.20±0.05 (23.08%)
Curcumin 50 mg/kg	0.11±0.04 (47.62%)	0.28±0.07 (28.21%)	0.33±0.06 (23.25%)	0.39±0.05 (9.30%)	0.31±0.04* (32.61%)	0.22±0.04 (15.38%)
Curcumin 100 mg/kg	0.13±0.06 (38.09%)	0.30±0.05 (23.08%)	0.41±0.07 (4.65%)	0.41±0.04 (4.65%)	0.32±0.06* (30.43%)	0.26±0.06 (0%)
Curcumin 200 mg/kg	0.15±0.05 (28.57%)	0.18±0.06* (53.85%)	0.26±0.04* (39.53%)	0.32±0.08 (25.58%)	0.34±0.04 (26.09%)	0.25±0.05 (3.85%)
Curcumin 400 mg/kg	0.1±0.03 (52.38%)	0.16±0.05* (58.97%)	0.27±0.04* (37.21%)	0.31±0.05 (27.91%)	0.31±0.05* (32.61%)	0.26±0.04 (0%)

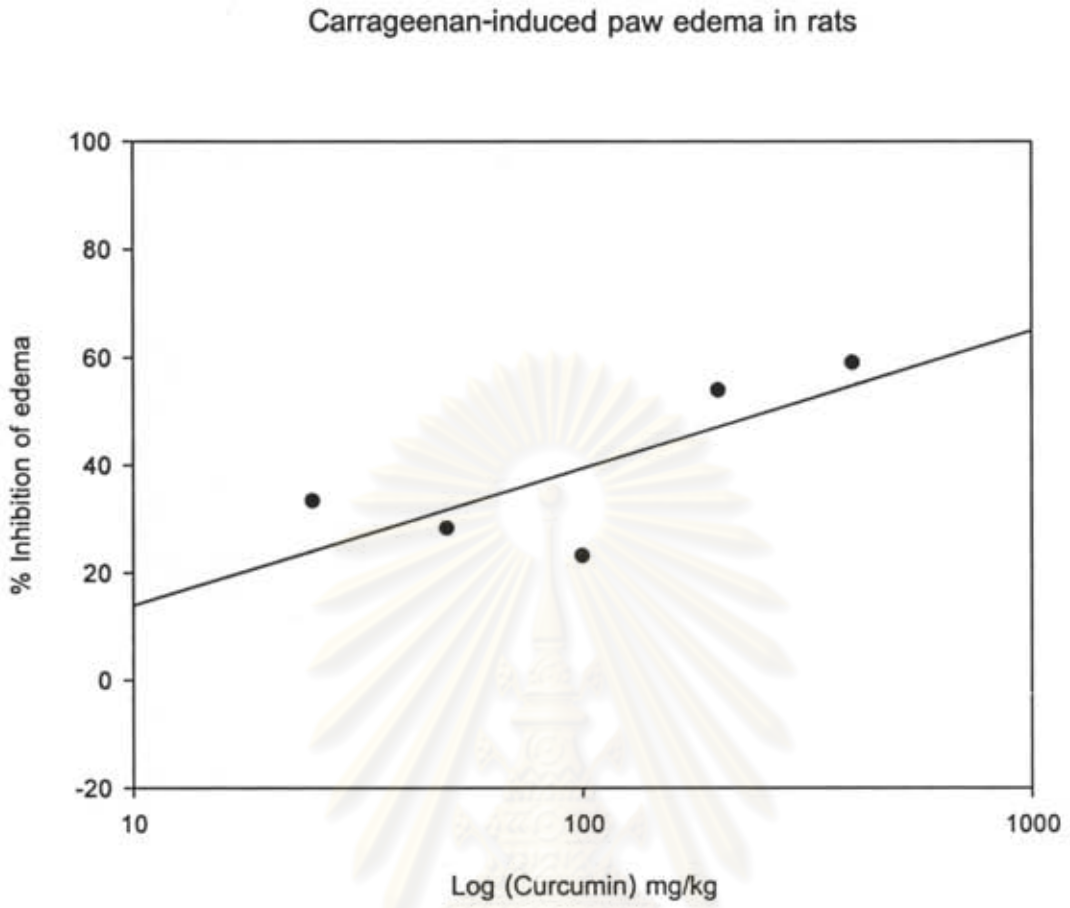


Figure 37 Linear regression of percentage of inhibition of edema of various doses of curcumin (25-400 mg/kg) at 2 hr after carrageenan injection. N=6 for all groups. The regression equation was Y = 11.0972*Ln(x) - 11.6165, r^2 = 0.5800.

ROTA-ROD TEST

In order to measure the effect of curcumin on motor performance, mice were then treated p.o. with curcumin dose of 200 mg/kg and tested on rota-rod for 60 sec. The rota-rod performance of mice was observed at 15, 30, 45, 60, 90, 120 and 240 min after curcumin administration. Data showed that curcumin at the dose tested did not significantly produce motor impairment (Figure 38).

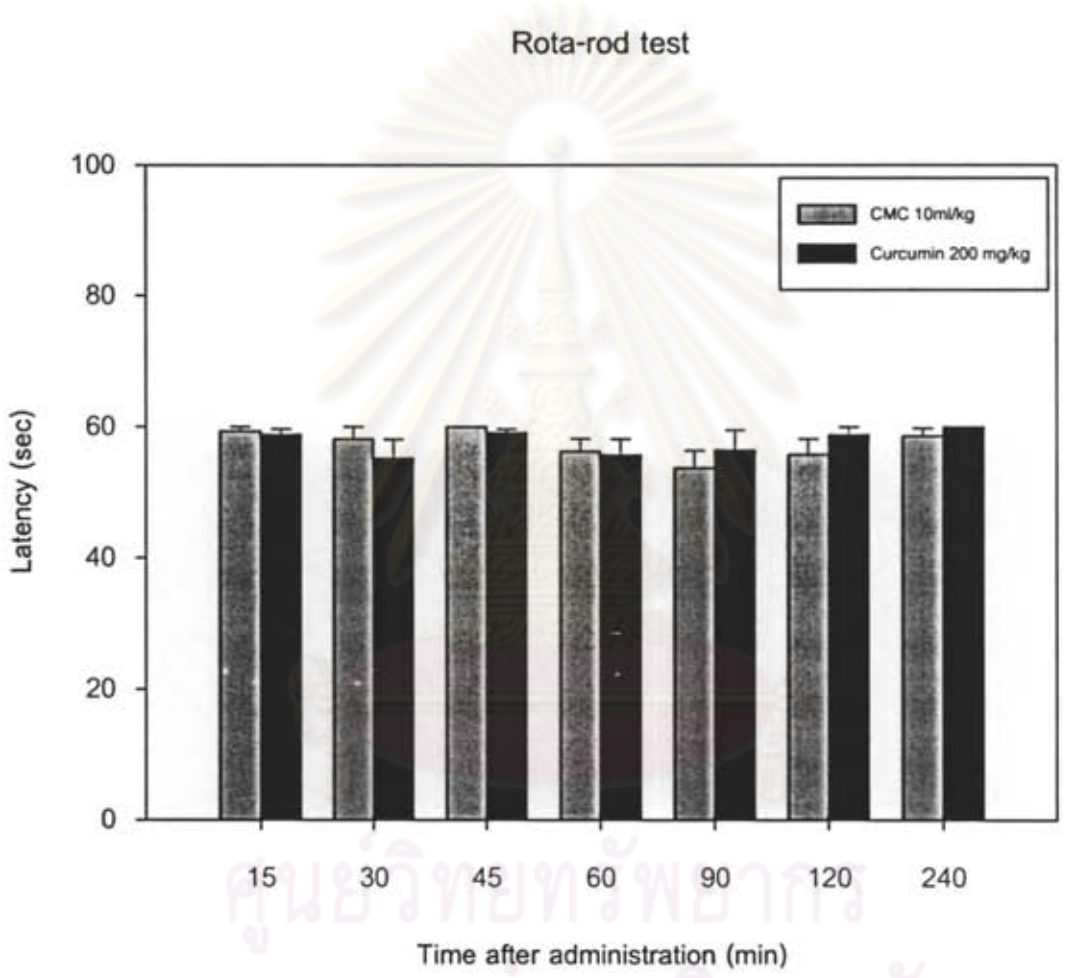


Figure 38 Rota-rod performance at 15, 30, 45, 60, 90, 120 and 240 minutes after oral administration of 0.5% carboxymethylcellulose and curcumin 200 mg/kg. N=10 for all groups.

CHAPTER V
DISCUSSION AND CONCLUSION

These studies have demonstrated the antinociceptive and anti-inflammatory effects of curcumin in various animal models. Antinociceptive activity was assessed utilizing thermal (hot-plate and tail-flick test), mechanical (Randall-Selitto test) and chemical (acetic acid-induced writhing test) models. Anti-inflammatory effect was assessed in acute inflammation model (carrageenan-induced paw edema).

Initial attempts to investigate the analgesic effect of curcumin utilized the standard mouse hot-plate test (Woolfe and MacDonald, 1944), a central analgesic activity testing model which measures two behavioral components including paw licking and jumping. Both are considered to be supraspinally integrated responses. This model usually employs morphine as a reference drug. Morphine (MO) showed potent analgesic effect on the response in this model indicating the sensitivity of this test (Figure 17). Curcumin was administered orally by suspending in CMC. The oral administration was chosen in order to imitate the normal human consumption of curcumin. The analgesic action of all doses of curcumin was observed during 240 min period (Figure 18). Significant linear correlation between the log dose of curcumin ranging from 25 to 400 mg/kg and the response (%MPE-min) in the mouse hot-plate test was not observed ($r^2 = 0.3512$). However, it is noted that the response tend to decline at the highest dose of 400 mg/kg. Therefore, after excluding the dose at 400 mg/kg, the linear correlation was obtained with $r^2 = 0.8003$ (Figure 19&20). Subsequently, the plot between %MPE and log dose was made and ED_{50} was found to be 133.10 mg/kg (Figure 21). Considering the dose from 25-200 mg/kg, the antinociceptive peak response of lower doses of curcumin was observed earlier than the antinociceptive peak response of the higher doses of curcumin (Figure 22). This phenomenon may partly due to the erratic absorption from the gastrointestinal tract and poor systemic bioavailability (~1%) after oral dosing of curcumin in rodents (Yang et al., 2007).

Naloxone, a short acting opioid antagonists, was utilized to investigate the involvement of opioid receptors in the analgesic effects of curcumin. The results showed the involvement of opioid receptors in analgesia produced by curcumin (Figure 23).

Studies were then undertaken to investigate the effectiveness of curcumin utilizing the mouse tail-flick technique, another central analgesic activity testing model that measures spinal reflex. MO administered i.p. produced significant analgesic response as expected (Figure 25). Curcumin all doses tested had no analgesic effect in this model (Figure 26). The results obtained from hot-plate and tail-flick tests suggested that curcumin had an effect on supraspinally integrated responses but not on spinally nociceptive reflex.

In order to measure an analgesic effect of curcumin against chemical stimuli, an acetic acid-induced writhing test was chosen. This test is commonly used for measuring peripheral analgesic activity and considered as a model of visceral inflammatory pain. Writhing responses consist of contractions of the abdomen, twisting and turning of the trunk, and extension of the hind limbs (Svendsen and Hau, 1994). Indomethacin (IND), a nonsteroidal anti-inflammatory drug, was used as a reference drug. Oral administration of IND (10 mg/kg) produced significant analgesic response compared to NSS treated controls (Figure 28). Curcumin doses of 200 and 400 mg/kg produced significant analgesic response compared to the vehicle control (Figure 29). However, curcumin showed less peripheral analgesic efficacy than IND (Figure 30). Linear correlation between the log dose of curcumin ranging from 25 to 400 mg/kg and the mean writhing response was found with $r^2 = 0.5812$. The plot between %inhibition of writhing response and the log dose of curcumin gave the ED_{50} value of 155.77 mg/kg (Figure 31&32).

Additionally, an analgesic effect of curcumin against mechanical stimuli was also investigated using the Randall-Selitto test. Randall-Selitto test is utilized to measure pain in inflamed tissue and considered as a hyperalgesia pain model. It has been used to distinguish between analgesic drugs acting in the CNS and analgesic drugs acting locally at the site of inflammation (Hargreaves, 1988). This test is sensitive to the non-narcotic analgesics (Svendsen and Hau, 1994). Oral administration of IND (10 mg/kg) produced significant analgesic responses only at 90 and 120 min after carrageenan administration compared to NSS treated controls (Figure 33). The maximum percentage of inhibition of hyperalgesia found at 90 min after carrageenan administration was 67.35%. From all tested doses (25-400 mg/kg) only curcumin dose of 200 mg/kg increased mean paw withdrawal threshold (PWTs) during the time intervals tested up to 4 hr after carrageenan administration, except at the 45 min (Figure 34). The maximum percentage of inhibition of

hyperalgesia of 59.09% was found at 240 min after carrageenan administration. The maximum inhibition of hyperalgesia results indicated that curcumin was less efficient in reducing hyperalgesia compared to IND. However, curcumin had longer duration of action in comparison with IND. The results obtained from both the acetic acid-induced writhing test in mice and the Randall-Selitto test clearly demonstrated that curcumin could reduce inflammatory pain induced by carrageenan by peripheral inhibition.

In order to evaluate the possible non-specific muscle relaxant or sedative effects of curcumin, treated mice were tested on the rota-rod. Animals were orally administered with vehicle or curcumin (200 mg/kg) 60 min before tested. Curcumin has no significant effect on the rota-rod performance compared to vehicle controls. This finding confirmed that the antinociceptive effect caused by curcumin is unrelated to the impairment of motor response.

Carrageenan-induced paw edema is a suitable experimental animal model to evaluate the anti-edematous effect. It is believed to be biphasic; 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 (Vinegar et al., 1969; Perianayagam et al., 2006). In this study, IND (10mg/kg) significantly reduced paw edema only at 2 and 3 hr (second phase) after carrageenan administration (Table1). The anti-edematous effect of IND at the second phase could be explained by the fact that IND is a cyclooxygenase inhibitor, resulting in the reduction of prostaglandins. This result is also consistent with the previous study that IND strongly inhibited the second phase without affecting the development of the first phase (Vinegar et al., 1968). All doses of curcumin showed significant reduction of edema at 2 hr or more, suggesting that curcumin produce an anti-edematous effect at the second phase. The effect of curcumin is similar to that of IND. Various studies have shown that curcumin exhibits anti-inflammatory effect by inhibition of cyclooxygenase enzyme (Goel et al., 2001; Kim et al.,2003; Strimpakos and Sharma, 2008), and hence, reduces prostaglandins. It could be concluded that the anti-inflammatory effect of curcumin involves in the reduction of prostaglandin through the inhibition of cyclooxgenase enzyme.

In conclusion, this present study has demonstrated that curcumin exerts a pronounced antinociception when assessed in thermal, chemical and mechanical models of nociception in rodents. Curcumin has both central and peripheral analgesic activities. The

analgesic mechanism is most likely involved with the opioid pathway. In addition, curcumin 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

The future research could comprise of several objectives as listed below

(1) To investigate the antipyretic effect of various doses of curcumin.

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

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

(4) To better understand the mechanism of curcumin that is involved in producing its analgesic and anti-inflammatory effects.

(5) To observe other opioid antagonist to better characterize the mechanism of curcumin effects.

(6) To test side effects and toxic effects of curcumin at high doses.

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

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APPENDICES

Appendix A

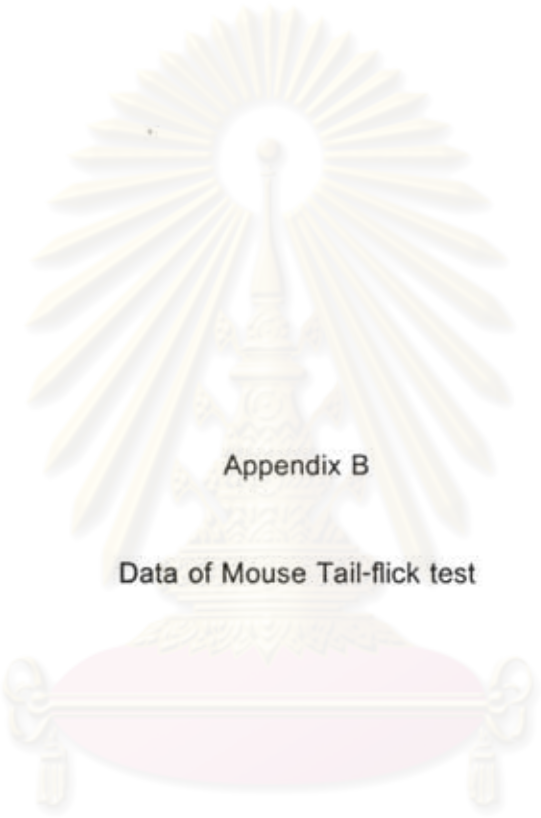
Data of Mouse Hot-plate test

Table 3 Latency (sec) in Mouse Hot-plate test from 0-240 min after oral administration of the various doses of curcumin (25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Curcumin (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
25	23.66±2.37	25.99±2.35	28.02±3.04	25.81±1.98	28.65±1.98	24.63±1.54	23.86±2.63
50	23.81±2.32	24.72±1.32	32.11±3.02	23.76±2.35	27.59±2.61	29.00±3.27	24.03±2.18
100	27.14±0.91	28.52±1.56	29.61±1.46	31.42±1.14	26.84±1.47	27.94±1.38	24.64±1.65
200	29.30±1.76	27.76±1.68	29.28±1.21	30.36±2.17	35.14±2.36	31.98±2.67	26.85±2.34
400	26.06±2.50	24.35±3.08	26.80±2.31	22.61±2.36	21.28±1.83	25.13±3.12	23.41±2.71

Table 4 %MPE-Time in Mouse Hot-plate test from 0-240 min after oral administration of the various doses of curcumin (25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Curcumin (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia (%MPE-min)
25	7.46±10.14	19.77±7.88	28.20±12.13	16.63±7.82	27.29±9.29	11.73±5.52	7.39±11.77	3347.25±1160.9
50	12.47±10.37	15.08±7.29	48.95±11.82	13.11±8.90	28.16±10.39	34.67±13.96	13.60±9.04	5703.98±1814.85
100	20.70±4.22	25.94±7.90	31.26±7.01	39.43±5.48	19.63±6.29	24.87±6.10	9.41±8.11	5074.29±851.21
200	30.36±8.12	21.98±7.39	28.66±6.25	30.41±11.97	56.22±10.42	45.00±10.05	18.87±10.78	8093.39±1205.61
400	32.29±7.45	25.97±10.54	34.40±7.47	19.43±6.05	12.27±7.43	25.88±11.73	20.15±11.09	5344.94±1580.56



Appendix B

Data of Mouse Tail-flick test

Table 5 Latency (sec) in Mouse Tail-flick test from 0-240 min after oral administration of the various doses of curcumin (25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Curcumin (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
25	0.87±0.03	0.98±0.06	0.87±0.06	0.92±0.03	1.01±0.06	0.90±0.03	0.91±0.04
50	1.15±0.08	1.16±0.16	1.20±0.17	1.08±0.04	1.19±0.21	1.16±0.12	1.10±0.12
100	1.07±0.04	1.10±0.06	1.00±0.04	1.01±0.03	0.99±0.03	0.96±0.05	0.93±0.04
200	1.06±0.04	1.11±0.06	1.05±0.05	1.02±0.02	0.96±0.04	0.94±0.04	0.89±0.02
400	1.41±0.22	1.19±0.10	1.24±0.09	1.24±0.09	1.07±0.05	1.09±0.05	1.16±0.13

Table 6 %MPE-Time in Mouse Tail-flick test from 0-240 min after oral administration of the various doses of curcumin (25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Curcumin (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia (%MPE-min)
25	0.87±1.33	4.43±1.69	0.96±1.37	2.49±0.91	5.41±1.54	1.85±0.95	2.19±1.06	582.12±123.84
50	3.83±1.92	4.56±4.81	5.98±4.99	1.20±1.49	5.80±6.37	4.39±3.20	2.32±2.97	884.97±715.13
100	2.50±0.99	3.45±2.14	0.16±1.04	0.45±1.12	-0.19±0.84	-1.18±1.58	-2.21±1.44	-124.95±218.90
200	2.54±1.77	4.31±1.72	2.35±0.91	1.25±1.08	-0.73±1.48	-1.31±0.84	-3.04±1.01	-135.85±124.18
400	14.12±7.15	6.83±3.66	8.43±3.43	8.64±2.72	2.88±2.05	3.56±1.95	6.00±4.30	1348.82±564.74

Appendix C

Data of Acetic acid-induced writhes in mice

Table 7 Dose-response and Time-course effects of curcumin on acetic acid-induced writhes in mice. Data presented as mean±S.E.M.

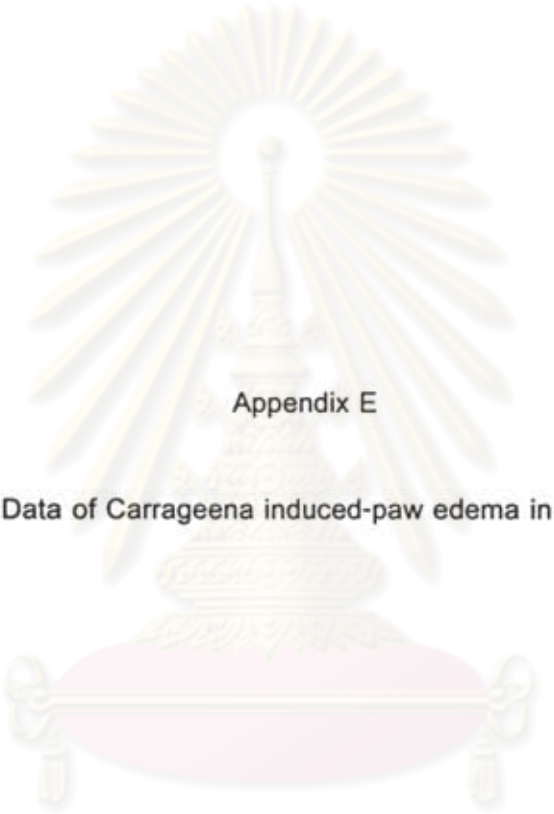
Treatment (mg/kg)	Time Course (5 min blocks)						Total
	1	2	3	4	5	6	
CMC 10 ml/kg	0.17±0.17	9.83±1.76	10.67±0.92	12.00±1.26	7.33±0.80	5.00±1.37	45.00±2.03
Curcumin 25	1.00±1.00	7.33±2.80	6.83±1.19	8.00±2.54	5.33±0.84	4.67±1.36	33.17±6.33
Curcumin 50	1.33±0.49	7.00±2.61	6.17±2.98	4.83±1.72	4.83±1.17	5.67±3.48	29.83±11.93
Curcumin 100	1.00±0.26	8.17±1.11	8.83±1.70	5.67±1.28	5.67±1.05	3.33±0.71	32.67±3.05
Curcumin 200	0±0	2.17±0.83	3.00±1.50	3.00±1.69	2.00±1.09	1.50±0.95	11.67±5.62
Curcumin 400	0.17±0.07	3.33±1.36	5.50±2.25	4.33±1.77	4.00±1.63	2.17±0.88	19.50±7.96

Appendix D

Data of RANDALL-SELITTO test

Table 8 Paw withdrawal threshold (g) from 0-240 min after oral administration of the various doses of curcumin (25-400 mg/kg). N=8 for all groups. Data presented as mean±S.E.M., %=percentage of inhibition of hyperalgesia.

Treatment (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
CMC 10 ml/kg	91.25±11.72	77.50±9.21	92.5±13.98	63.75±8.00	60.00±3.78	62.50±7.50	55.00± 9.45
Curcumin 25	87.50±4.91 (-4.11%)	71.25±5.49 (-8.06%)	65.00±5.98 (-29.73%)	76.25±4.20 (19.61%)	76.25±5.65 (27.08%)	58.75±3.50 (-6.00%)	61.25±10.43 (11.36%)
Curcumin 50	88.75±6.39 (-2.74%)	86.25±5.32 (11.29%)	95.00±10.69 (2.70%)	75.00±5.00 (17.65%)	72.50±5.90 (20.83%)	66.25±6.25 (6.00%)	68.75±10.25 (25.00%)
Curcumin 100	80.00±5.00 (-12.33%)	88.75±3.50 (14.52%)	96.25±4.20 (4.05%)	85.00±10.18 (33.33%)	75.00±7.79 (25.00)	68.75±6.39 (10.00%)	58.75±10.60 (6.82%)
Curcumin 200	137.5±9.77* (50.68%)	103.75±12.53* (33.87%)	82.50±5.90 (-10.81%)	93.75±15.46* (47.06%)	85.00±9.26* (41.67%)	83.75±9.05* (34.00%)	87.5±11.91* (59.09%)
Curcumin 400	91.25±6.39 (0%)	80.00±8.45 (3.23%)	70.00±5.35 (-24.32%)	75.00±6.81 (17.65%)	76.25±5.64 (27.08%)	68.75±5.15 (10.00%)	72.50±7.00 (31.82%)



Appendix E

Data of Carrageena induced-paw edema in rats

Table 9 Effect of 0.5%CMC 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		1.08	0.96	1.08	1.07	1.20	1.29		-
1 hr	Paw volume (ml)	1.15	1.04	1.05	1.1	1.17	1.14		-
	Volume of edema (ml)	0.25	0.23	0.19	0.2	0.22	0.19	0.25	
2 hr	Paw volume (ml)	1.4	1.27	1.3	1.14	1.37	1.21		-
	Volume of edema (ml)	0.5	0.46	0.44	0.24	0.42	0.26	0.5	
3 hr	Paw volume (ml)	1.39	1.18	1.37	1.3	1.46	1.23		-
	Volume of edema (ml)	0.49	0.37	0.51	0.4	0.51	0.28	0.49	
4 hr	Paw volume (ml)	1.35	1.33	1.2	1.39	1.31	1.37		-
	Volume of edema (ml)	0.45	0.52	0.34	0.49	0.36	0.42	0.45	
5 hr	Paw volume (ml)	1.29	1.43	1.2	1.48	1.3	1.4		-
	Volume of edema (ml)	0.39	0.62	0.34	0.58	0.35	0.45	0.39	
6 hr	Paw volume (ml)	1.24	1.16	0.91	1.26	1.15	1.18		-
	Volume of edema (ml)	0.34	0.35	0.05	0.36	0.2	0.23	0.34	

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Table 10 Effect of indomethacin 10 mg/kg (p.o.) 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		0.82	0.88	0.92	0.98	0.90	0.94	-	-
1 hr	Paw volume (ml)	1.13	0.96	1.13	1.03	0.82	1.01		
	Volume of edema (ml)	0.31	0.08	0.21	0.05	-0.08	0.07	0.11	-12.28
2 hr	Paw volume (ml)	1.02	1.14	0.96	1.06	1.16	1.12		
	Volume of edema (ml)	0.20	0.26	0.04	0.08	0.26	0.18	0.17	46.60
3 hr	Paw volume (ml)	1.06	1.05	0.95	0.97	1.04	1.09		
	Volume of edema (ml)	0.24	0.17	0.03	-0.01	0.14	0.15	0.12	66.04
4 hr	Paw volume (ml)	1.28	1.24	1.04	1.02	1.04	1.07		
	Volume of edema (ml)	0.46	0.36	0.12	0.04	0.14	0.13	0.21	37.50
5 hr	Paw volume (ml)	1.22	1.11	1.03	1.12	1.09	1.22		
	Volume of edema (ml)	0.40	0.23	0.11	0.14	0.19	0.28	0.22	32.83
6 hr	Paw volume (ml)	1.32	1.16	1.03	1.05	1.06	1.42		
	Volume of edema (ml)	0.50	0.28	0.11	0.07	0.16	0.48	0.27	2.44

Table 11 · Effect of curcumin 25 mg/kg (p.o.) 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		1.08	0.96	1.08	1.07	1.20	1.29	-	-
1 hr	Paw volume (ml)	0.97	0.83	0.92	0.91	0.93	0.85		20.31
	Volume of edema (ml)	1.00	1.10	1.20	1.03	1.08	1.02	1.07	
2 hr	Paw volume (ml)	0.03	0.27	0.28	0.12	0.15	0.17		
	Volume of edema (ml)	1.28	1.03	1.29	1.28	1.17	0.94	1.16	31.90
3 hr	Paw volume (ml)	0.31	0.20	0.37	0.37	0.24	0.09		
	Volume of edema (ml)	1.29	1.06	1.40	1.35	1.22	1.09	1.23	21.87
4 hr	Paw volume (ml)	0.32	0.23	0.48	0.44	0.29	0.24		
	Volume of edema (ml)	1.15	0.93	1.28	1.23	1.26	1.24	1.18	34.88
5 hr	Paw volume (ml)	0.18	0.10	0.36	0.32	0.33	0.39		
	Volume of edema (ml)	1.31	1.19	1.15	1.24	1.35	1.15	1.23	27.47
6 hr	Paw volume (ml)	0.34	0.36	0.23	0.33	0.42	0.30		
	Volume of edema (ml)	1.04	1.04	1.24	1.00	1.27	1.00	1.10	22.87

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Table 12 Effect of curcumin 50 mg/kg (p.o.) 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		0.98	0.87	0.94	0.79	0.89	0.89		-
1 hr	Paw volume (ml)	0.95	1.03	1.00	1.03	0.95	1.08		
	Volume of edema (ml)	-0.03	0.16	0.06	0.24	0.06	0.19	1.07	46.87
2 hr	Paw volume (ml)	1.05	1.17	1.30	0.87	1.26	1.36		
	Volume of edema (ml)	0.07	0.30	0.36	0.08	0.37	0.47	1.16	28.88
3 hr	Paw volume (ml)	1.05	1.21	1.32	1.29	1.25	1.22		
	Volume of edema (ml)	0.07	0.34	0.38	0.50	0.36	0.33	1.23	22.66
4 hr	Paw volume (ml)	1.21	1.32	1.40	1.30	1.14	1.31		
	Volume of edema (ml)	0.23	0.45	0.46	0.51	0.25	0.42	1.18	10.08
5 hr	Paw volume (ml)	1.12	1.12	1.26	1.23	1.19	1.30		
	Volume of edema (ml)	0.14	0.25	0.32	0.44	0.30	0.41	1.23	31.87
6 hr	Paw volume (ml)	1.07	1.03	1.08	1.18	1.19	1.14		
	Volume of edema (ml)	0.09	0.16	0.14	0.39	0.30	0.25	1.10	13.07

Table 13 Effect of curcumin 100 mg/kg (p.o.) 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		0.77	1.01	0.98	0.93	0.92	0.96		-
1 hr	Paw volume (ml)	1.15	0.98	1.14	1.00	1.04	1.04		
	Volume of edema (ml)	0.38	-0.03	0.16	0.07	0.12	0.08	0.13	39.06
2 hr	Paw volume (ml)	1.27	1.13	1.33	1.25	1.20	1.17		
	Volume of edema (ml)	0.50	0.12	0.35	0.32	0.28	0.21	0.30	23.28
3 hr	Paw volume (ml)	1.21	1.08	1.45	1.47	1.41	1.43		
	Volume of edema (ml)	0.44	0.07	0.47	0.54	0.49	0.47	0.41	3.12
4 hr	Paw volume (ml)	1.29	1.25	1.34	1.34	1.33	1.46		
	Volume of edema (ml)	0.52	0.24	0.36	0.41	0.41	0.50	0.41	5.43
5 hr	Paw volume (ml)	1.19	1.15	1.16	1.40	1.34	1.24		
	Volume of edema (ml)	0.42	0.14	0.18	0.47	0.42	0.28	0.32	30.04
6 hr	Paw volume (ml)	1.20	1.05	1.18	1.20	1.15	1.36		
	Volume of edema (ml)	0.43	0.04	0.20	0.27	0.23	0.40	0.26	-2.61

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Table 14 Effect of curcumin 200 mg/kg (p.o.) 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		0.98	0.94	1.02	0.89	1.05	1.09		-
1 hr	Paw volume (ml)	1.21	1.16	0.95	1.16	1.17	1.20		
	Volume of edema (ml)	0.23	0.22	-0.07	0.27	0.12	0.11	0.15	31.25
2 hr	Paw volume (ml)	1.25	0.97	1.06	1.28	1.16	1.33		
	Volume of edema (ml)	0.27	0.03	0.04	0.39	0.11	0.24	0.18	53.45
3 hr	Paw volume (ml)	1.34	1.15	1.21	1.08	1.25	1.48		
	Volume of edema (ml)	0.36	0.21	0.19	0.19	0.2	0.39	0.26	39.84
4 hr	Paw volume (ml)	1.43	1.27	1.07	1.47	1.25	1.43		
	Volume of edema (ml)	0.45	0.33	0.05	0.58	0.20	0.34	0.32	24.42
5 hr	Paw volume (ml)	1.35	1.35	1.24	1.33	1.38	1.34		
	Volume of edema (ml)	0.37	0.41	0.22	0.44	0.33	0.25	0.34	26.01
6 hr	Paw volume (ml)	1.27	1.31	1.04	1.18	1.29	1.38		
	Volume of edema (ml)	0.29	0.37	0.02	0.29	0.24	0.29	0.25	1.96

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Table 15 Effect of curcumin 400 mg/kg (p.o.) 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		0.99	0.92	0.97	0.97	1.13	0.96		-
1 hr	Paw volume (ml)	1.04	1.11	0.96	1.13	1.19	1.11		
	Volume of edema (ml)	0.05	0.19	-0.01	0.16	0.06	0.15	0.10	53.12
2 hr	Paw volume (ml)	0.98	1.12	1.02	1.15	1.39	1.25		
	Volume of edema (ml)	-0.01	0.20	0.05	0.18	0.26	0.29	0.16	58.19
3 hr	Paw volume (ml)	1.33	1.27	1.11	1.19	1.52	1.16		
	Volume of edema (ml)	0.34	0.35	0.14	0.22	0.39	0.20	0.27	35.94
4 hr	Paw volume (ml)	1.25	1.37	1.20	1.15	1.59	1.23		
	Volume of edema (ml)	0.26	0.45	0.23	0.18	0.46	0.27	0.31	28.29
5 hr	Paw volume (ml)	1.39	1.33	1.15	1.14	1.53	1.25		
	Volume of edema (ml)	0.40	0.41	0.18	0.17	0.40	0.29	0.31	32.23
6 hr	Paw volume (ml)	1.06	1.22	1.23	1.21	1.39	1.36		
	Volume of edema (ml)	0.07	0.30	0.26	0.24	0.26	0.40	0.25	-2.18

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Appendix F

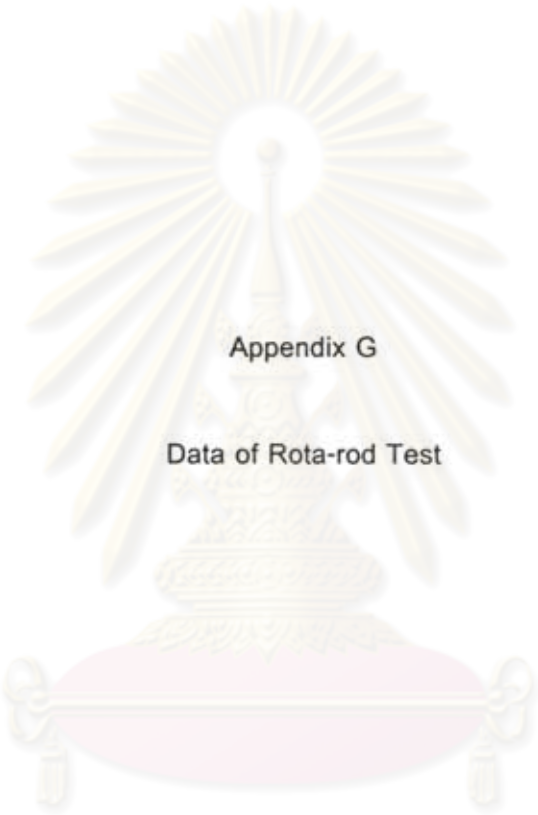
Study of mechanism of analgesic action involving Opioid pathays using Naloxone in mouse hot-plate test

Table 16 Latency (sec) in Mouse Hot-plate test from 0-240 min after administration of NSS (10 ml/kg, i.p.), naloxone (5 mg/kg, i.p.), 0.5% carboxymethylcellulose (10 ml/kg, p.o.), curcumin (200 mg/kg, p.o.) and the combination of naloxone and curcumin (5/200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatment (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
NSS	23.03±1.48	19.31±1.64	22.79±2.56	20.54±1.39	20.71±0.88	22.89±1.03	22.29±1.30
Naloxone 5 mg/kg	23.47±1.65	22.65±1.80	21.99±1.32	22.19±1.47	23.00±1.76	22.80±1.21	21.04±2.28
CMC 10 ml/kg	23.33±1.73	20.12±1.87	18.69±1.53	18.89±1.18	24.09±0.99	21.50±1.39	21.96±1.86
Curcumin 200 mg/kg	24.48±1.42	27.10±2.12	30.62±1.29	28.34±2.25	30.39±2.20	31.45±2.62	29.19±3.26
Naloxone + Curcumin	25.73±2.04	23.89±1.53	22.85±1.37	22.74±2.31	23.02±2.79	24.61±1.79	19.09±1.93

Table 17 %MPE-Time in Mouse Hot-plate test from 0-240 min after administration of NSS (10 ml/kg, i.p.), naloxone (5 mg/kg, i.p.), 0.5% carboxymethylcellulose (10 ml/kg, p.o.), curcumin (200 mg/kg, p.o.) and the combination of naloxone and curcumin (5/200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatment (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia (%MPE-min)
NSS 10 ml/kg	4.94±7.78	-12.42±11.18	4.32±12.57	-5.72±7.28	-4.39±4.25	4.80±5.16	1.21±8.26	124.25±1193.60
CMC 10 ml/kg	3.05±6.76	-11.89±8.71	-19.32±8.18	-18.55±7.02	5.81±3.99	-6.66±7.52	-5.55±10.74	-1498.42±1432.05
Naloxone 5 mg/kg	-4.34±9.53	-9.79±12.00	-14.90±12.68	-10.18±8.29	-7.35±12.19	-9.78±10.44	-13.75±9.33	-2443.36±1988.18
Curcumi 200 mg/kg	1.44±13.82	19.53±10.05	32.70±7.87	24.25±10.86	28.55±13.74	35.90±13.62	31.07±15.32	6763.75±1597.94
Naloxone + Curcumin	10.62±8.41	2.59±6.03	-3.49±6.32	-5.19±14.72	-0.81±13.64	5.28±7.48	-20.14±9.39	-807.39±1705.93



Appendix G

Data of Rota-rod Test

Table 18 Rota-rod latency of 0.5% carboxymethylcellulose 10 ml/kg, p.o.

No.	15 min	30min	45min	60min	90min	120min	240min
1	60	60	60	60	60	60	60
2	60	60	60	57	60	60	60
3	60	60	60	60	49	60	60
4	53	60	60	44	45	60	60
5	60	60	60	60	60	47	60
6	60	60	60	60	60	60	60
7	60	60	60	60	39	52	58
8	60	41	60	46	60	60	60
9	60	60	60	55	60	60	60
10	60	60	60	60	44	39	48
AVG	59.3	58.1	60.0	56.2	53.7	55.8	58.6

Table 19 Rota-rod latency of curcumin 200 mg/kg, p.o.

No.	15 min	30min	45min	60min	90min	120min	240min
1	60	60	60	60	60	60	60
2	53	60	60	60	60	48	60
3	60	60	59	54	60	60	60
4	60	60	60	60	60	60	60
5	60	60	60	41	30	60	60
6	60	60	60	60	60	60	60
7	55	55	60	60	60	60	60
8	60	60	55	60	60	60	60
9	60	35	57	60	55	60	60
10	60	42	60	42	60	60	60
average	58.8	55.2	59.1	55.7	56.5	58.8	60.0

Appendix H

Study Protocol Approval by Chulalongkorn University Animal Care and Use Committee, Bangkok, Thailand

Chulalongkorn University Animal Care and Use Committee

Certificate of Project Approval	☑ Original ☐ Renew
Animal Use Protocol No. 08-33-007	**Approval No. 08-33-007**
Protocol Title Antinociceptive and anti-inflammatory effects of curcumin in animal models	
Principal Investigator Pasarapa Towiwat, Ph.D.	
Certification of Institutional Animal Care and Use Committee (IACUC) This project has been reviewed and approved by the IACUC in accordance with university regulations and policies governing the care and use of laboratory animals. The review has followed guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand.	
Date of Approval March 24, 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 Warawanna Buadonpri was born in August 5, 1977 in Suphanburi, Thailand. She graduated with a Bachelor of Science in Pharmacy in 2001 from Naresuan University. After graduation, she had worked in the Pharmaceutical unit of Nong Ya Sai Hospital for one year. She is currently working in the Pharmaceutical unit of Bang Pla Ma Hospital, Suphanburi, Thailand.