CHAPTER 5

DISCUSSION AND CONCLUSION

Kaempferia galanga L. (*K. galanga*) (Zingiberaceae) is called "Proh Hom" in Thai. It posseses several biological activities and is widely used in Thai traditional medicine. *K. galangal*, an aromatic rhizomatous herbal spice, is an ingredient of many Ayurvedic drug preparations, and the aromatic essential oil from the rhizome is valuable to perfumery (Chithra *et al*., 2005). The constituents of this rhizome, hitherto reported, include cineol, borneol, 3-carane, camphene, kaempferol, kaempferide, cinnamaldehtyde, *p*-methoxycinnamic acid, ethyl cinnamate and ethyl *p*-methoxycinnamate (Nakao and Shibu, 1924; Kanjanapothi *et al*., 2004). The rhizomes of the plant, which contain essential oils, have been used in decoction or powder for indigestion, cold, pectoral and abdominal pains, headache and toothache. Its alcoholic maceration has also been applied as liniment for rheumatism (Key, 1976; Lieu, 1990; Kanjanapothi *et al*., 2004). In Chinese medicine *K. galanga* rhizomes have been used as an aromatic, and also as incense (Kanjanapothi *et al*., 2004). In Malaysia, this plant is commonly prescribed as traditional treatment for hypertension, rheumatism and asthma (Zakaria and Mustafa, 1994; Othman *et al*., 2006). Pharmacological activities of ethanolic and water extract and volatile oil of *K. galanga* have been reported to possess anti-allergic activity (Tewtrakul and Subhadhirasakul, 2006) In addition, the chloroform extract of *K. galanga* had a vasorelaxant effect (Mustafa *et al*., 1996). It is also applied externally for abdominal pain in women and used topically for treatment of rheumatism (Hirschhornn, 1983).

Acute toxicity test

In the acute toxicity test of this study, it was not possible to measure the plant LD_{50} due to the lack of death in the mice and the rats even at high concentration of the methanolic extract of *K. galanga* up to 5 g/kg orally, as a single dose, and the extract failed to produce any clinical signs of toxicity such as, including convulsion, hyperactivity, sedation, respiratory depression, and loss of righting reflex. This result indicated that the plant has a low toxicity. This

is in accordance with the study of Mokkhasmit and colleagues (1971), who administered 50% ethanolic extract of *K. galanga* to groups of Yoken Denken Tokyo mice. They reported that 10 g/kg of the extract, which was administered by oral and subcutaneous routes, did not produce any toxic symptoms and Kanjanapothi and colleagues (2004) have reported that oral administration of 5 g/kg ethanolic extract of *K. galanga*, appeared to be preferably non-toxic. There must be a point, however, at which it can be concluded that the test substance is practically non-toxic or non-lethal after an acute exposure. This test limit for acute oral toxicity is generally considered to be 5.0 g/kg body weight. If no motality is observed at this dose level, a higher dosage is generally not necessary (Hayes, 1989).

Analgesic activity

In analgesic activity test, the result in chapter four confirms that *K. galanga* exerts a significant effect against pain in the four current antinociceptive models in mice and rats. The methods for investigating antinociception were selected for both peripherally and centrally mediated effects. The acetic acid and formalin test (chemical stimuli) elucidated peripherally and the central activity, while the hot plate and the tail flick test (thermal stimuli) investigated the central activity.

The methanolic extract showed the antinociceptive activity in the doses tested. This was evident in all the nociceptive models, which indicates that it possesses both central and peripherally mediated activities.

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Papagallo, 2005). Pain may be felt because of inflammation, infection, ischemia, tissue necrosis, stretching of tissue, chemicals or burns. In the stomach and intestines, pain may result from inflammation of the mucosa or from distension or muscle spasm. Depending on the cause, pain may be sudden and short-term marked primary by reflex withdrawal (Gould, 2002) which are generated by mechanical thermal or chemical stimuli. When an injury occurs, pain is first evoked by the stimulation of the nociceptor (A δ fibers and C fibers) causing potassium and kinins to be released from the damaged cells. These stimulate the receptor directly resulting in the release of neuropeptides such as substance P

from nociceptor terminals and can cause the release of histamine from the mast cells with the production of platelet-activating factor (PAF) which in turn releases serotonin from the platelets. Histamine is also released from the mast cell, starting an inflammatory reaction leading to vasodilation and edema (Cambell, 2004). Bradykinin is released upon tissue injury and is present in inflammation exudates. Bradykinin is an important substance to stimulate nociception and prostaglandins sensitize nociceptor sending impulses to the spinal cord. This state is called peripheral sensitization. When the spinal cord becomes hyperexcitable, signal transduction comes to post central gyrus in thalamus, which is responsible for the conscious perception of pain. This state is called central sensitization (Mutschelor and Derendorf, 1995).

Animal exposed to painful stimuli show escape or avoidance behavior from the stimuli, retain the pain-related information in the working memory, and learn to avoid the aversive situation by anticipation of painful events. It is considered that these processes are produced by the negative affective component of pain (LeDoux *et al*., 1998; Gewirtz and Devis, 1997; Killcross *et al*., 1997; Tanimoto *et al*., 2003). Thus, pain clearly includes both sensory and negative effective components. Therefore, before testing the antinociceptive effect, it is necessary to make sure that neurology of motor deficits are not involved in the response. In the present study the results indicated that the methanolic extract of *K. galanga* significantly showed an analgesic effect in four pain models used in this study, chemical stimuli in the acetic acid-induced writhing, and the formalin test, the thermal painful stimuli in the hot plate and the tail flick test.

In the acetic acid-induced writhing assay, a chemical stimuli is used to screen both peripherally and centrally acting analgesic activity (Panthong *et al*., 2007). Intraperitoneal injection of 0.6% acetic acid that was irritating to serous membranes provokes a stereotypical behavior in rodents that is characterized by abdominal contractions, whole body movements, contortions of the abdominal muscle, and reduced motor activity and incoordination. The writhing test can predict effective analgesic doses for agents that can be used in humans (Dubinsky *et al*., 1987; Eaton, 2003).

Acetic acid cause pain by liberating endogenous substances including serotonin, histamine, prostaglandin, bradykinin and substance P and many others that excite pain nerve endings (Collier *et al*., 1968; Raj, 1996; Intahphuak *et al*., 2004). Several mediators take part in

visceral pain model nociception and transmission of nociception from the viscera (Cervero and Laird, 1999; Shafiee et al., 2003) and reported to be mediated at least in part, through supraspinal sites (Tanimoto, 2003). The writhing response of the mouse to an injection of noxious chemical is not a very specific nociception model, it is important to reveal a general antinociceptive effect of the extract under study (Marchioro *et al*., 2005). This test is used to screen for both peripherally and centrally acting analgesic activity (Raj, 1996).

The methanolic extract at the doses of 50, 100 and 200 mg/kg significantly reduced the number of writhing in mice induced by 0.6% acetic acid which suggested that *K. galanga* had the effect to decrease visceral pain through peripheral and central mechanism. Acetic acid-induced writhing is related to the increase in the peritoneal fluid levels of PGE_2 and $PGF_{2\alpha}$ (Derardt *et al.,* 1980). The abdominal contraction response is though to involve in part, local peritoneal receptors (Jais *et al*., 1997). The analgesic action of NSAIDs is exerted both peripherally and centrally. Their analgesic action is usually associated with their anti-inflammatory action and results from the inhibition of prostaglandin synthesis via cyclo-oxygenase. Prostaglandins produces little pain by themselve, but potentiate the pain caused by other mediators (e.g. histamine and bradykinin) (Neal, 1992). Analgesic action of NSAIDs as well as aspirin decrease the production of prostaglandin E series and the sensitivity to release of bradykinin (Simon, 2003). Bradykinin and related peptides are primary mediators of pain. After tissue injury, bradykinin directly activates and sensitizes nociceptors, in addition to promoting vasodilation, thereby potentiating hyperalgesia (Rupniak *et al*., 2000).

The results in acetic acid-induced writhing test revealed that the *K. galanga* extract reduced the writhing responses in mice which suggested that the mechanism of action of antinociceptive activity of the extract may be linked partly to the cyclo-oxygenase enzymes in the peripheral tissue via the inhibition of prostaglandin in synthesis and probably due to interfere with the synthesis or release of endogeneous substances that excite pain nerve endings (Collier *et al*., 1968). Othman and colleagues (2002) reported that the ethyl cinnamate isolated from the rhizomes of the *K. galanga* showed a vasorelaxant effect on the rat aorta and inhibited the tonic contractions of smooth muscle. It is possibly due to ethyl cinnamate involved in inhibition of Ca^{2+} influx and release of nitric oxide (NO) and prostacyclin from the endothelial cells, thus it

might be possible that *K. galanga* partly inhibited writhing response in mice through the action of ethyl cinnamate which is one of the main components of this plant.

The advantage of using the formalin model of nociception is that it can discriminate pain in central and peripheral components (Tjolsen *et al*., 1992) termed early and late phase. The formalin test was conducted to confirm and study the possible analgesic mechanism of action of the extract. The test consists of two distinct phases that possibly reflect different types of pain mechanisms (Panthong *et al* ., 2007). Drug that act primarily on the central nervous system such as morphine inhibit both phase and NSAIDs such as aspirin acting supraspinally which inhibit prostaglandin synthesis can reduce the pain in both phases (Martindale *et al*., 2001). Thus the formalin test is best used to examine opioid mimetics (Eaton, 2003). Inflammation pain is selectively inhibited by the NSAIDs because these agents reduce inflammation. However, it has also been proposed that the NSAIDs may have analgesic effects independent of their anti-inflammatory activity (Hunskaar *et al*., 1985). The formalin test consists of two different phases which can be separated in time; the early phase (0-5 min) is neurogenic phase which is also known as non-inflammatory pain. This phase is mediated by the central effect through a direct activation of the nociceptors by C fibers activation. The direct stimulation of nerve ending caused substance P release with cooperation with bradykinin, while the late phase which is also termed as inflammatory pain, is a pain due to inflammation and mediated by the peripheral effect via the release of some chemical transmitter such as histamine, serotonin, bradykinin and prostaglandins (Shibata *et al*., 1989). Inhibition of the late phase is due to inflammation with release of serotonin, histamine, bradykinin and prostaglandins, which to some degree can cause the sensitization of central nociceptive neurons (Verma *et al*., 2007).

The methanolic extract of *K. galanga* significantly showed an inhibitory effect in both early and late phases, so it was indicated that *K. galang* had central and peripheral analgesic properties. It has been widely agreed that the nociceptive behaviors manifested during the acute early phase may be caused by the direct stimulation of the sensory nerve fibers by formalin, so the extract might reduce the licking time in the early phase of formalin test via blockade on nociceptor or inhibited releasing of substance P and bradykinin. Then, it is followed by the second phase which might be resulted from chronic inflammatory nociception responses. Since

the *K. galanga* could reduce the licking time in the late phase, it might be due to the inhibition of the inflammatory mediator or blockade on receptors. In addition all the doses of the extract (50, 100 and 200 mg/kg) had strong analgesic activity, the activity of the extract was more pronounced in the late phase, which was commonly associated with inflammatory pain. Thus, it might be concluded that analgesic effect on the early phase of the extract was due to the direct effect on the nociceptor via blockade on the nociceptor or the inhibit releasing the substance P or bradykinin.

The analgesic effect of the late phase of the methanolic extract of *K. galanga* is due to the inhibition of the synthesis or releasing of inflammatory mediators such as histamine, serotonin and bradykinin. The formalin test is best to examine opioid mimetics because opioid analgesics provide analgesia for both phases of behavioral response but the late, delayed phase is more sensitive (Eaton, 2003). The results reveal that the methanolic extract of *K. galanga* provides analgesia for both phases and the late phase is more sensitive. So the mechanism of action of *K. galanga* was opioid mimetics and similar to aspirin.

To check for possible central antinociceptive effect of the *K. galanga* extract, the hot plate and the tail flick models are considered specific tests for evaluation of the central pain (Marchioro *et al*., 2005) at the supraspinal and spinal levels (Wong *et al*., 1994), respectively, possibly acting on either ascending and descending inhibitory pain pathway (Richardson *et al*., 1998).

In the hot plate test, the extract considerably increased the mice reaction time to the heat stimulus. Only mice showed nociceptive responses in hot plate test such as jumping or licking the pain less than 15 seconds were used. The methanolic extract of *K. galanga* at doses of 50 and 100 mg/kg significantly increased latency of nociceptive response at 90 minutes ($p<0.05$ and $p<0.01$, respectively) when compared to the control while *K. galanga* at the dose of 200 mg/kg significantly increased the pain latency in mice at 45 ($p<0.05$), 60 ($p<0.01$), 75 ($p<0.01$) and 90 ($p<0.01$) minutes after administration, compared to the control. The results indicated that the methanolic extract of *K. galanga* at the dose of 50 mg/kg slightly but significantly delayed the pain responses in the mice. The methanolic extract of *K. galanga* at the dose of 200 mg/kg exhibited more potent effects to delay the latency of nociceptive response than the extract at doses of 50 and 100 mg/kg. To confirm the central analgesic effect of the extract, the antagonistic action

of naloxone on effects of morphine or *K. galanga* (200 mg/kg) on the latency of nociceptive response was also investigated. The results showed that naloxone given before morphine also antagonized the effect of morphine at 45, 60, 75 and 90 minutes ($p<0.01$) compared to the morphine administered alone. Furthermore, naloxone given before the methanolic extract of *K. galanga* (200 mg/kg) significantly decreased the latency of nociceptive response of the extract at 75 and 90 minutes ($p<0.01$) when compared to the methanolic extract of *K. galanga* at the dose of 200 mg/kg given alone.

The hot plate method is one of the most common tests evaluating the analgesic efficacy of drugs in rodents (Somchit *et al*., 2004). However, care must be taken for drugs that produce false positive results by modifying the behavior of the rodents (Tjolsen *et al*., 1991). The drug that reduces the nociceptive response indicated by cutaneous thermic stimuli in the hot plate test might exhibit central analgesic properties or supraspinal analgesia (Matheus *et al*., 2005). Thermic painful stimuli are known to be selective to centrally, but not peripherally acting analgesic drugs (Chau, 1989).

Study using the hot plate test, which is usually used to determine the involement of central nociceptive mechanism has, at least, comfirmed the ability of the extract to influence the central mechanism as seen with the formalin test. The antinociceptive action of the extract was blocked by naloxone, an opioid receptor antagonist, indicating that the antinociception was partly mediated via opioid mechanism. This could be due to the direct agonist activity of opioidiomimetric constituents in the extract and/or due to increase release of endogeneous opioid peptides (Deranigagala *et al*., 2003).

In addition, in the tail flick test, only rats showed nociceptive responses such as withdrawal of the tail from light beam less than 3 seconds in tail flick test were used. The antinociceptive effect of the extract at doses of 50, 100 and 200 mg/kg began at the time 45 ($p<0.05$), 75 ($p<0.05$) and 45 ($p<0.01$) minutes, respectively after the oral administration when compared to the control and persisted until the time of 75 minutes. Morphine (5 mg/kg), a centrally acting analgesic drug , significantly increased ($p<0.01$) the tail flick latency at all time intervals measured in the tail flick test in rats, but its antinociceptive effect still remained at the time of 90 minutes.

In antagonistic studies, naloxone (2 mg/kg) completely antagonized the effect of morphine on the latency of nociceptive response at all time interval measured (p<0.01). Furthermore, naloxone at the dose of 2 mg/kg given before *K. galanga* at the dose of 200 mg/kg significantly decreased the latency of nociceptive response produced by the extract in the tail flick test at 30 (p<0.05), 45 (p<0.01), 60 (p<0.01), 75 (p<0.01) and 90 (p<0.05) minutes when compared to the extract at the dose of 200 mg/kg.

The tail flick model is considered specific test for evaluation of the central pain (Marchioro *et al*., 2005) at spinal level (Wong *et al*., 1994). The analgesic effect reflected in the tail flick test is dependent on centrally acting opioid like analgesic (Conner *et al*., 2000; Yonathan *et al*., 2006) Opioid receptors are widely distributed in several peripheral tissues and cutaneous nerves as well as the CNS, especially in the pain transmission pathways including in the dorsal horn at the spinal cord (Jin *et al*., 2006). Opioids have excitatory effect in multiple regions of the nervous system and this effect is generally attributed to the reduction on inhibitory pathway (Charles and Hales, 2004), possibly acting on a descending inhibitory pain pathway (Richardson *et al*., 1998). Descending control of spinal nociception is a major determinant of acute pain in different behavioral and emotional states (McMullan and Lumb, 2006). There are strong suggestions that analgesic system is heavily related to the endogenous opioid systems (Yaksh, 1999; Brooks and Tracey, 2005). Indeed, this is the commonest explanation for pain relief produced via acupuncture, which is believed to recruit descending pain control systems (Liu *et al*., 2004).

In the present study, in the hot plate and the tail flick tests all the doses of the MEKG prolong latency of nociceptive response especially the higher dose of 200 mg/kg. The extract had the more potent analgesic action on the tail flick test than in the hot plate test. So it is suggested that the methanolic extract of *K. galanga* had an analgesic activity-like morphine via central mechanism through the opioid receptor at the spinal level more than the supraspinal level.

Furthermore, to confirm the central analgesic action of the extract by using the pure opioid antagonist drug, naloxone, a morphine derivative with bulkier substituents at the N_{17} position. This agent has a relatively high affinity for μ opioid binding sites. It has lower affinity for the δ and κ receptors (Barber, 1997) but can also reverse antagonists at the δ and κ sites (Way

et al., 1992). In animal models, the opioid receptor μ, κ and δ receptor are responsible for supraspinal and spinal analgesia (Gutstein and Akil, 2001).

According to Lipman and Jackson (2004), opioid receptors are composed of ghycoproteins found in cellular membranes. These receptors are coupled to G proteins that modulate potassium and calcium ion conduction. When opioid agonists occupy either μ or δ opioid receptors, they open a potassium ion channel that permits an increase in potassium conductance. The hyperpolarization inhibits neuronal activity. In contrast κ receptor activation inhibits calcium entry via a calcium ion channel. Activation of the opioid receptors decreases transmission of signals from the primary peripheral afferent nerves to higher CNS centers, as well as the processing of the pain stimulus. Activation of the opioid receptors leads to analgesia as well as adverse effects.

All three opioid receptor types have known subtypes. Two μ subtypes have been best elucidated. Activation of μ_1 leads to supraspinal analgesia, whereas μ_2 activation leads to spinal analgesia (Stoelting and Hillier, 2006) and commonly thought to be responsible for the adverse effect such as sedation, respiratory depression, euphoria and constipation. Activation of δ (δ_1 and δ_2) leads to the spinal analgesia and potentiates μ receptor analgesia, activation of κ (κ_1 and κ_2) receptor leads to the spinal analgesia and κ_3 leads to the supraspinal analgesia (Lipman and Jackson, 2004).

The results evaluated from the hot plate and the tail flick tests indicated that *K. galanga* had an analgesic activity, like morphine via central mechanism, in the level of spinal more than supraspinal mechanism to produce analgesia property. The above mentioned findings have revealed the extract effectiveness in inhibiting the centrally and thermally-induced nociception. Furthermore, the ability of the methanolic extract of *K. galanga* to affect both the chemically and thermally-induced nociceptive response is characteristic of strong analgesics like opioid agonists (Zakaria *et al*., 2007). Granger and colleagues (2005) reported that monoterpenes are the primary components of plant essential oils. (+)-Borneol is a bicyclic monoterpene present in the essential oils of numerous medicinal plants include *K. galanga*. (+)-Borneol and its structural analogue camphor are used for analgesia and anaesthesia in traditional Chinese and Japanese medicine.

Antipyretic activity

Fever may be a result of infection or one of the sequel of tissue damage, inflammation, graft injection or other disease states. Antipyretic agents are drugs, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature (Goodman and Gilman, 1996).

Yeast-induced fever is called pathogenic fever. Its etiology include production of prostaglandin, which set the thermoregulatory center at a higher set point. It could stimulate phagocytes to release endogenous pyrogen which circulates in blood to act on the thermoregulatory center in the hypothalamus. Endogenous pyrogen produced and activated IL-1 and prostaglandins, mainly PGE_2 which alter metabolism of thermoregulatory cells via cAMP secondary messenger-mediated mechanism. The result is an increase set point for thermoregulation to a higher temperature. So, inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as acetylsalicyclic acid (Howard, 1993; Rawlins and Postgrad, 1973).

In brewer's yeast induced pyrexia test, the result showed that *K. galanga* extract lacked effect on yeast-induced fever in rats while the reference drug aspirin suppressed fever induced by yeast in rats by inhibiting the synthesis of prostaglandin E_2 within the hypothalamus (Clark and Cumby, 1975; Hajare *et al*., 2000; Reanmongkol *et al*., 2007). It is concluded that *K. galanga* extract did not significantly reduce fever. However, our results showed that *K. galanga* extract had a slight effect to reduce fever in rats. It is possible that antipyretic effect of *K. galanga* for the treatment of malarial chill in traditional medicine might be resulted from antiplasmodial activity. Monbrison and colleagues (2006) reported that *K. galanga* have a direct antimalarial activity on several strains of *plasmodium falcipasum*.

Anti-inflammatory activity

Inflammation is a defense reaction of the organism and its tissue to injurious stimuli. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be included, maintained or aggravated by many diseases (Gupta *et al*., 2003). The aim is to repair the damage or at least to limit it and also to remove the cause, for example, bacteria or foreign bodies (Silbernagl and Lang, 2000). Cause of inflammation include direct damage (cuts, sprains), chemical such as acids, ischemia and cell necrosis or infarction, allergic reactions, physical agents (thermal injuries or burns, radiation), foreign bodies (splinters or dirt) and infection (Gould, 2002).

In acute inflammation, when tissues injure (damages cells), mast cell and platelets release chemical mediators such as histamine, serotonin, protaglandins and leukotrienes into the interstitial fluid and blood. These chemicals affect the blood vessels and the nerves in the area. First neutrophils and later monocytes and macrophages collect along the capillary wall and then move through the wider separations in the wall into the interstitial area. There they destroy and remove foreign materials, microorganisms and cell debris by phargocytosis (Gould, 2002).

Carrageenan-induced inflammation test was selected, which is usually used to determine its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation (DiRosa *et al*., 1971; Ismail *et al*., 1997; Olajide *et al*., 1999). The relative potency estimates obtained from most drugs tend to reflect clinical experience (Winter *et al*., 1962). The local injection of carrageenan-induced inflammatory process in the rat involves three phases by several mediators released in ordinate sequence (DiRosa, 1972). An initial phase during the first 1.5 h, is caused by the release of histamine and serotonin, a second phase is mediated by bradykinin from 1.5 to 2.5 h and finally, a third phase, the mediator of which is suspected to be prostaglandin occur from 2.5 to 6 h after carrageenan injection. This third phase appears to be the most interesting compared to the two earlier phases. Thus, the maximal vascular response as determined with leukocyte migration to the inflamed area, also reaches its maximum level in this third phase (Vinegar *et al*., 1969).

The carrageenan-induced hind paw edema in rats is known to be sensitive to cyclo-oxygenase inhibitors, but not to lipoxygenase inhibitors, and has been used to evaluate the

effect of NSAIDs which primarily inhibit the cyclo-oxygenase involved in prostaglandins synthesis. It has been demonstrated that the suppression of carrageenan induced hind paw edema after the third hour correlates reasonably with therapeutic dose of most clinically effective anti-inflammatory agents (DiRosa, 1972; Panthong *et al*., 2007). Oral pretreatment of the animal with *K. galanga* resulted in a significant inhibition of carrageenan-evoked hind paw edema. The inhibitory effect of *K. galanga* at dose of 200 mg/kg on carrageenan-induced at the second phase might be due to the influence on the bradykinin released at the 1.5 to 2.5 h after carrageenan injection and inhibitory effect of the extract at the 3 to 5 hours, suggests that the main mechanism of action of *K. galanga* may involve the prostaglandin biosynthesis. The ability of the *K. galanga* to reduce the thickness of edematous paw suggested the presence of an anti-inflammatory activity. This finding could be supported by the earlier claim made by Attaway and Zobarsky (1993) that compounds with anti-inflammatory activity might also possess antinociceptive activity.

The anti-chronic inflammatory activity of the extract was also demonstrated in subcutaneous implantation of cotton pellets that provoked foreign body granuloma (Wagh *et al*., 2006). The response to subcutaneously implanted cotton pellet in the rat has been divided into three phases: a transudative phase, an exudative phase and a proliferative phase and is a typical feature of established chronic inflammatory reaction. The fluid absorbed by the pellet greatly influenced the wet weight of the granuloma and the dry weight correlates well with amount of granulomatous tissue formed (Swingle and Shideman, 1972). The anti-chronic inflammatory activity of the extract was also demonstrated in the cotton treat-induced granuloma in rats. Tissue granulation, one of the distinctive features of chronic inflammation, which is composed of marked infiltration macrophages and neovascularization, was induced by subcutaneous implantation of biomaterials. The implanted material induces a host inflammatory response and modulates the release of inflammatory mediators which finally leads to tissue proliferation and granuloma formation (Rames and Williams, 1992; Tang and Eaton, 1995; Hu *et al*., 2001). Monocyte infiltration and fibroblast proliferation rather than neutrophil infiltration and exudates take place in chronic inflammation. This proliferation becomes widespread by the proliferation of small vessels or granuloma (Hosseinzadeh *et al*., 2000). Nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the size of granuloma which results from cellular reaction by inhibiting

granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides (Suleyman *et al*., 1999; Ramprasath *et al*., 2004). The results of the extract on cotton pellet-induced granuloma formation in rats indicated that the extract at an oral dose of 50, 100 and 200 mg/kg significantly inhibited the granuloma dry and wet weight and granuloma formation. These results suggest that the extract inhibits the transudative, exudative and proliferative phase of inflammation. It is possible that *K. galanga* extract also inhibits monocytes infiltration and fibroblast proliferation. Activated monocytes can release a series of pro-inflammatory cytokines, inducing tumor necrosis factor-α (TNF-α) (Laupattarakasem *et al*., 2006). TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cell (Dore and Sirois, 1996). Additionally, TNF-α stimulates neutrophils to transcribe and release cytokines, and chemokines biosynthesis (Marucha *et al*., 1990; Fernandez *et al*., 1996). Interaction between these mediators thus enhances further inflammatory reactions (Gouwy *et al*., 2005) and inhibition of TNF-α release can reduce the severity of inflammation. Cellular accumulations and pro-inflammatory cytokines, TNF-α and IL-1β, were demonstrated within the first 2 weeks (Dalu *et al*., 2000). Kato et al (2002) also reported that TNF-α can accelerate both angiogenesis and matrix degrading by induction of vascular endothelial growth factor and various matrix metalloproteinases. Therefore, the inhibition of tissue granuloma by the extract, at least in part, might be through interference with TNF-α.

K. galanga composes of many bioactive compounds such as cinnamaldehyde which has been demonstrated to exhibit anti-tumor (Jeong *et al*., 2003), anti-bacterial (Gill and Holley, 2004), anti-proliferation (Koh *et al*., 1998), and inhibited TLR4 and TLR2 induced cytokines production (Chao *et al*., 2007). The anti-inflammatory activity of the extract might be, at least in part, due to its suppressive effects on the cytokine production. Furthermore, (-)-borneol exhibited the inhibition of NO production by 68.8%, which the IC_{50} value was 11.2 μg/ml. Kaempferol has the ability to modulate inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and reactive C-protein (CRP) expression (Garcia-Mediavilla *et al*., 2007). Shin and colleagues (1970) reported that *p*-methoxycinnamic acid (*p*-MCA) showed a significant decrease in carrageenan-induced edema of the rat hind paw, and increased capillary permeability by

anaphylaxis and chemical mediators of protamine sky blue in the mouse peritoneal cavity. It, however, showed only weak inhibitory effect on cotton pellet-induced granuloma formation of the rat (Shin *et al*., 1970). Thus, the anti-inflammatory activities of the extract may be resulted from the action of the variety chemical components of *K. galanga*.

As previously mentioned in the experiment, aspirin and morphine were used as the reference drugs to investigate the possible antinociceptive, antipyretic and anti-inflammatory activities of *K. galanga* extract incorporated with various essential tests for analgesic (writhing, formalin, hot plate and the tail flick tests), antipyretic (brewer's yeast-induced fever), anti-inflammatory (carrageenan-induced paw edema) and cotton pellet-induced granuloma formation activities. In addition, *K. galanga* extract showed analgesic effect similar to both morphine and aspirin and exhibited anti-inflammatory activity similar to aspirin. The *K. galanga* extract did not have antipyretic activity while aspirin significantly reduced fever in rats. It would be probably that there are variability of the active compound(s) contained in the crude drug, so analgesic and anti-inflammatory activities of the *K. galanga* extract may be resulted from the different active compound(s) of the extract.

In summary, in the present investigation it could be concluded that the methanolic extract of *K. galanga* at tested doses exhibited the pharmacological activities as follows:

1. In analgesic activity, the methanolic extract of *K. galanga* showed the antinociceptive response in acetic acid induced writhing , formalin, hot plate and the tail flick tests. The extract exhibited the analgesic activity via peripheral and central mechanism both at spinal and supraspinal levels.

2. In antipyretic activity, *K. galanga* extract did not have any antipyretic activity in rat-induced fever by the brewer's yeast injection.

3. In anti-inflammatory activity, the methanolic extract of *K. galanga* exhibited the anti-inflammatory activity in acute phase by inhibition of the rat paw edema. The possible mechanism of the observed anti-inflammatory activity might be related to its ability to inhibit the release of bradykinin or prostaglandins synthesis and the exhibit of the anti-inflammatory activity

in chronic phase by the interfering of the TNF-α release, neutrophil infiltration and exudates release.

Therefore the extract of *K. galanga* used in traditional medicine is proved to have an analgesic and anti-inflammatory activities.