CHAPTER 1
INTRODUCTION

1.1 PAIN

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. It is protective in nature because it provides warning signal for tissue damage and inflammation (1). Tissue damage causes the release of numerous chemicals, including prostaglandins (PGs), leukotrienes (LTs), thromboxanes (TXs), substance P, acetylcholine (ACh), bradykinins (BKs), serotonin (5-hydroxytryptamine or 5-HT), histamine, potassium ions (K^+), acids (H^+) and platelet activating factor (PAF). These chemicals play a causal role in pain associated with inflammation, trauma and a variety of other pathophysiologic conditions (2).

Pain can be classified according to location, site of referral, and duration. Acute and chronic pains are commonly classified according to its duration (3). Acute pain is evoked by tissue damage, the presence of an underlying pathologic process and activation of nociceptive receptors stimuli. It is typically of short duration and remits when the underlying pathologic process has resolved (3, 4). It generally resolves within minutes, hours, or days. On the contrary, chronic pain is persistent pain which can persist for years (5).

1.1.1 Nociceptors

The sensation of pain is a neural-biochemical phenomenon. When acute tissue damage occurs, neurochemical reactions at the site of injury activate the free nerve endings of special nerves called nociceptors (2). By definition, nociceptors respond selectively to noxious stimuli. Theses nociceptors are free nerve endings with cell bodies in the dorsal root ganglia and terminate in the superficial layers of the dorsal horn of the spinal cord. Three basic types of nociceptive receptors are described as follows (6) :

1. Chemical receptors are stimulated by chemicals such as 5-HT, histamine, K^+ or ACh released from damage tissues or cells. In addition, damage can also cause other inflammatory mediators to be synthesized such as BKs or cytokines.
2. Mechanical receptors respond to direct mechanical pressure such as squeezing or crushing, or to the pressure occurring as a consequence of tissue inflammation or edema.
3. Thermal receptors respond to extremes of heat or cold.

Nociceptors are also indentified as C (chemical) and A-delta (Aδ) polymodal nociceptors. The C-fibers nociceptors are nonmyelinated fibers that the most common and compose 80% of all peripheral nociceptors. Whiles the Aδ nociceptors, thinly myelinated fibers, respond to high-intensity mechanical stimulation and are therefore termed high-threshold mechanoreceptors. Moreover, some the Aδ nociceptors also respond to thermal stimuli and are termed mechanothermal receptors (7).

1.1.2 Nociceptor mediators

Tissue damage causes the release of proteolytic enzymes, which act locally on tissue proteins to liberate substances that excite peripheral nociceptors. In addition, direct stimulation of nociceptors releases polypeptide mediators that enhance pain perception.

Primary afferent nociceptors also have a neuroeffector function. Most nociceptors contain polypeptide mediators that are released from their peripheral terminals when they are activated (Figure 1). Substance P is released from primary afferent nociceptors and has multiple biologic activities. It causes vasodilation and degradation of mast cells. It is also a chemoattractant for leukocytes, and increases the production and release of inflammatory mediators (8).

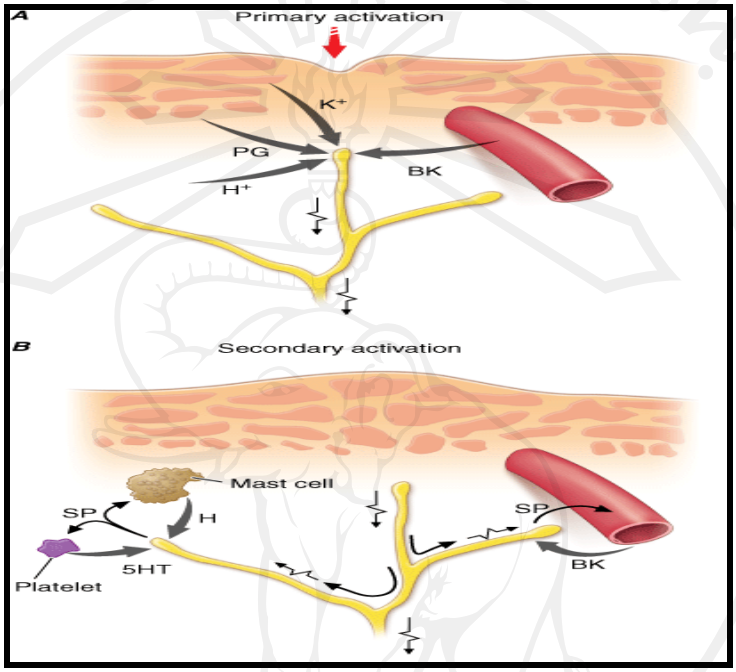

Figure 1 Events leading to activation, sensitization, and spread of sensitization of primary afferent nociceptor terminals (8)

A. Direct activation by intense pressure and consequent cell damage. Cell damage induces lower pH (H^+) and leads to release of K^+ and to synthesis of PGs and BKs.

B. Secondary activation, impulses generated in the stimulated terminal propagate not only to the spinal cord but also into other terminal branches where they induce the release of substance P. It causes vasodilation and neurogenic edema with further accumulation of BKs, also causes the release of histamine from mast cells and 5-HT from platelets.

1.1.3 Pain pathway

The processes of perception and response to pain may be summarized as four phases (6, 7):

1. Transduction is the process by which noxious stimuli are converted to electrical signals in the nociceptors.
2. Transmission is the second stage of noxious signals process. The noxious information from the peripheral nociceptors are relayed to the spinal cord, then to the thalamus, and finally to the cortex.
3. Modulation is a third stage and this stage represents changes that occur in the nervous system in response to noxious stimuli. The noxious signals are modified by the other activity in the body, which may be activity of other peripheral nerves or may occur in the central nervous system (CNS).
4. Perception in the brain perceives the sensation.

1.2 INFLAMMATION

1.2.1 Overview of inflammation

Inflammation is basically a protective response and a complex reaction in tissues, the ultimate goal of which is to eliminate the offending irritant and to repair the damage tissues. The cardinal signs of inflammation are redness, swelling, heat, pain and loss of function. Inflammation process involves changes in blood flow, increased vascular permeability, destruction of tissues via the activation and migration of leukocytes with synthesis of reactive oxygen derivatives (oxidative burst) and the synthesis of local inflammatory mediators. Disease results from failure of the regulatory process of inflammation, persistence of a pro-inflammatory stimulus, an impairment of the mechanisms that stop inflammation or a clonal disorder with pro-inflammatory consequences (9, 10).

1.2.2 Acute and chronic inflammation

The inflammatory reaction is generally divided into acute and chronic responses. Acute inflammation is rapid in onset (minutes) and of short duration; lasts from minutes to a few days. The major local manifestations of this process are (a) vascular dilation and increased blood flow (causing erythema and warmth);

(b) extravasation and extravascular deposition of plasma fluid and proteins (edema); and (c) emigration and accumulation of leukocyte (mostly neutrophils) in the site of injury (Figure 2). The failure to eliminate and repair the acute inflammatory response can progress to a chronic phase. Chronic inflammation is prolong in duration (lasts for weeks to months) and is associated with the presence of macrophages and lymphocytes, the proliferation of blood vessels, fibrosis, and tissue destruction. It is the cause of tissue damage in some of human diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis and pulmonary fibrosis (10).

1.2.3 Inflammatory cells and mediators

The cells involved in the inflammatory response are neutrophils (polymorphonuclear leucocytes; PMNs), macrophages, lymphocytes, mast cells, endothelial cells and fibroblasts. The nature of the leukocyte infiltrate varies with the age of the inflammatory response and the type of stimulus. In acute inflammation, neutrophils predominate in the inflammatory infiltrate during the first 6 to 24 h and are replaced by monocytes in 24 to 48 h (10). Neutrophils, an essential part of innate immune system, are released from bone marrow (half-life 4-10 h). Their cytoplasm contains granules and secretory vesicles which include antimicrobial or cytotoxic substances, proteinases, hydrolases and cytoplasmic membrane receptors (11, 12).

Macrophages are the dominant cellular players in chronic inflammation. Mononuclear phagocytes derive from a common precursor in the bone marrow, which gives rise to blood monocytes. From the blood, monocytes migrate into various tissues such as liver (Kupffer cells), spleen and lymph nodes (sinus histiocytes), lungs (alveolar macrophages), and CNS (microglia). The half-life of blood monocytes is about 1 day, whereas the life span of tissue macrophages is several months or years. Activation of macrophages results in increased levels of lysosomal enzymes, reactive oxygen and nitrogen species, and production of cytokines. Macrophages are important producers of arachidonic acid metabolites, release up to 50% of their arachidonic acid from membrane phospholipid (11).

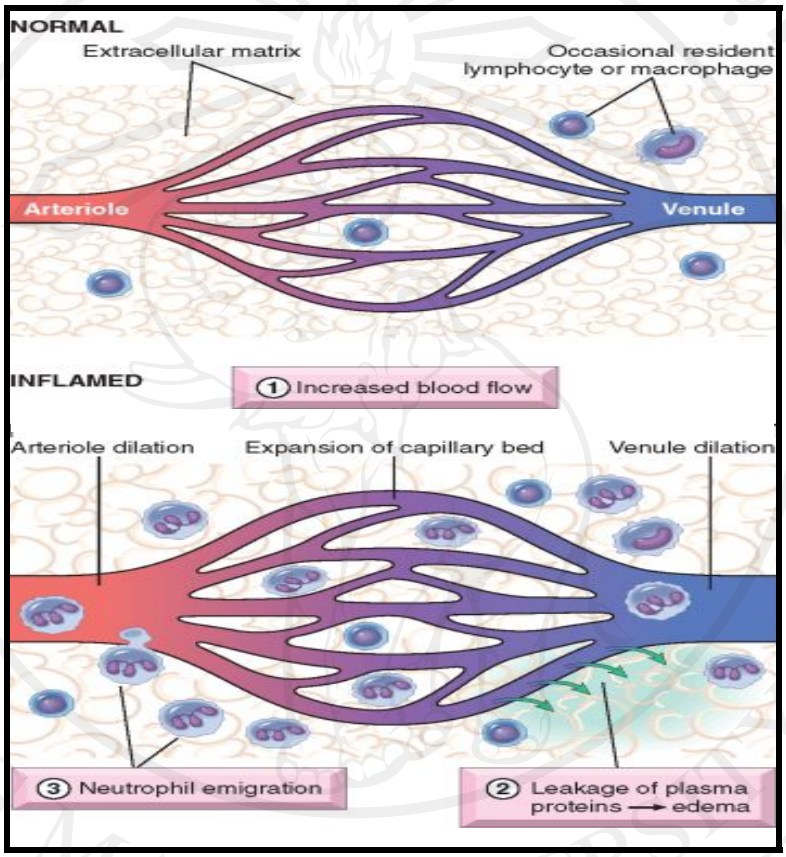

Figure 2 The major local manifestations of acute inflammation, compared to normal (10)

Lymphocytes are mobilized in both antibody-mediated and cell-mediated immune reactions. Antigen-stimulated (effector and memory) lymphocytes of different types (T and B cells) use various adhesion molecule pairs (selectins, integrins and their ligands) and chemokines to migrate into inflammatory sites. Cytokines from activated macrophages, mainly tumor necrosis factor (TNF), interleukin-1 (IL-1) and chemokines, promote leukocytes recruitment and result in the persistence of inflammatory response. Lymphocytes and macrophages interact in a bidirectional way, and these reactions play an important role in chronic inflammation (10).

Inflammatory mediators are soluble and diffusible molecules that act locally at the sites of inflammation as well as at remote sites. The cell-derived mediators are normally sequestered in intracellular granules and can be rapidly secreted by granule exocytosis (e.g., histamine in mast cell granules) or are synthesized *de novo* (e.g., PGs, cytokines) in response to stimuli (Table 1). The plasma protein-derived mediators (e.g., complement system and kinin) are produced mainly in the liver and present in the circulation as inactive precursors that must be activated, usually by a series of proteolytic cleavages, to acquire their biologic properties.

1.2.3.1 Arachidonic acid (AA) metabolites: Prostaglandins and leukotrienes

AA is a polyunsaturated fatty acid that contains 20-carbon atoms (5,8,11,14-eicosatetraenoic acid). It circulates in plasma in free and esterified forms and is a natural constituent of the phospholipid domain of cell membranes. It is mobilized for releasing from the membrane by diverse stimuli including physical perturbation and hormones, mediate a calcium-dependent translocation of cytosolic phospolipase A_2 ($cPLA_2$) to the nuclear membrane and the endoplasmic reticulum (ER) (10, 13). AA can be metabolized by three major enzymatic pathways: prostaglandin G/H synthetase (colloquially known as cyclooxygenase; COX), which catalyzes the formation of PGs and TXs: lipoxygenase (LOX), which results in the formation of LTs: and cytochrome P-450 isozymes, which generate epoxyeicosatrienoic acids (EETs) (10, 14).

Table 1 The sources of the cell-derived mediators and their roles in the inflammatory reaction (10)

Cell-derived mediators	Sources	Actions
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Serotonin	Platelets	Vasodilation, increased vascular permeability
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion and activation
Platelet-activating factor (PAF)	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Reactive oxygen species (ROS)	Leukocytes	Killing of microbes, tissue damage
Nitric oxide (NO)	Endothelium, macrophages	Vascular smooth muscle relaxation, killing of microbes
Cytokines (TNF, IL-1)	Macrophages, endothelial cells, mast cells	Local endothelial activation (expression of adhesion molecules), fever/pain/anorexia/hypotension, decreased vascular resistance (shock)
Chemokines	Leukocytes, activated macrophages	Chemotaxis, leukocyte activation

In COX pathway, prostaglandin G/H synthetase contains two reaction steps to metabolize AA to the unstable cyclic endoperoxides PGG_2 and PGH_2. The first is the COX reaction (the transformation of AA into PGG_2) and the second is the peroxidase reaction (the formation of PGH_2 from PGG_2). Afterwards, the unstable PGH_2 is converted by tissue-specific synthetases and isomerases into various prostanoids including PGD_2, PGE_2, PGI_2 (prostacyclin) and TXA_2 (Figure 3) (13).

PGs are formed in numerous types of cell within organism. They are released from cells in response to chemical stimuli or physical trauma. These PGs play a major role in the development of pain, inflammation and fever. They promote tissue inflammation by stimulating inflammatory cell chemotaxis, causing vasodilation and increasing capillary permeability and edema. Moreover, they also sensitize nociceptors and thereby generate pain. There are two COX enzymes, COX-1 and COX-2, which are products of separate genes and have different biologic functions based on their different temporal and tissue-specific expression. COX-1 is a housekeeping enzyme that is found in relatively constant levels in various tissues. It participates in the synthesis of PGs that are involved in homeostatic functions such as fluid and electrolyte balance in the kidneys, cytoprotective effect on the gastrointestinal (GI) tract and the platelet aggregation and hemostasis. On the contrary, COX-2 is an inducible enzyme that is established in normally very low level in most tissues. Nevertheless, it is rapidly up-regulated during the inflammatory process by pro-inflammatory substances such as cytokines, endotoxins, carcinogens and tumor promoters (9, 10, 15). The comparison of COX enzymes is shown in Table 2.

In LOX pathway, LOX enzyme is responsible for the production of LTs, which are chemoattractants for leukocytes and secreted mainly by leukocytes. Three major isozymes have been classified according to their positional specificity of AA oxygenation: the 5-, 12-, and 15-LOX produce the 5-, 12-, and 15-hydroperoxy-eicosatetraenoic acid (HPETE), respectively (16). 5-LOX converts AA to 5-HPETE, which is chemotactic for inflammatory cells and the precursor of LTs. LTB_4 is a potent chemotactic agent and activator of neutrophils, causing aggregation and adhesion of the cells to vascular endothelium, generation of ROS and release of lysosomal enzymes. Moreover, it plays an important role in immune reactions by

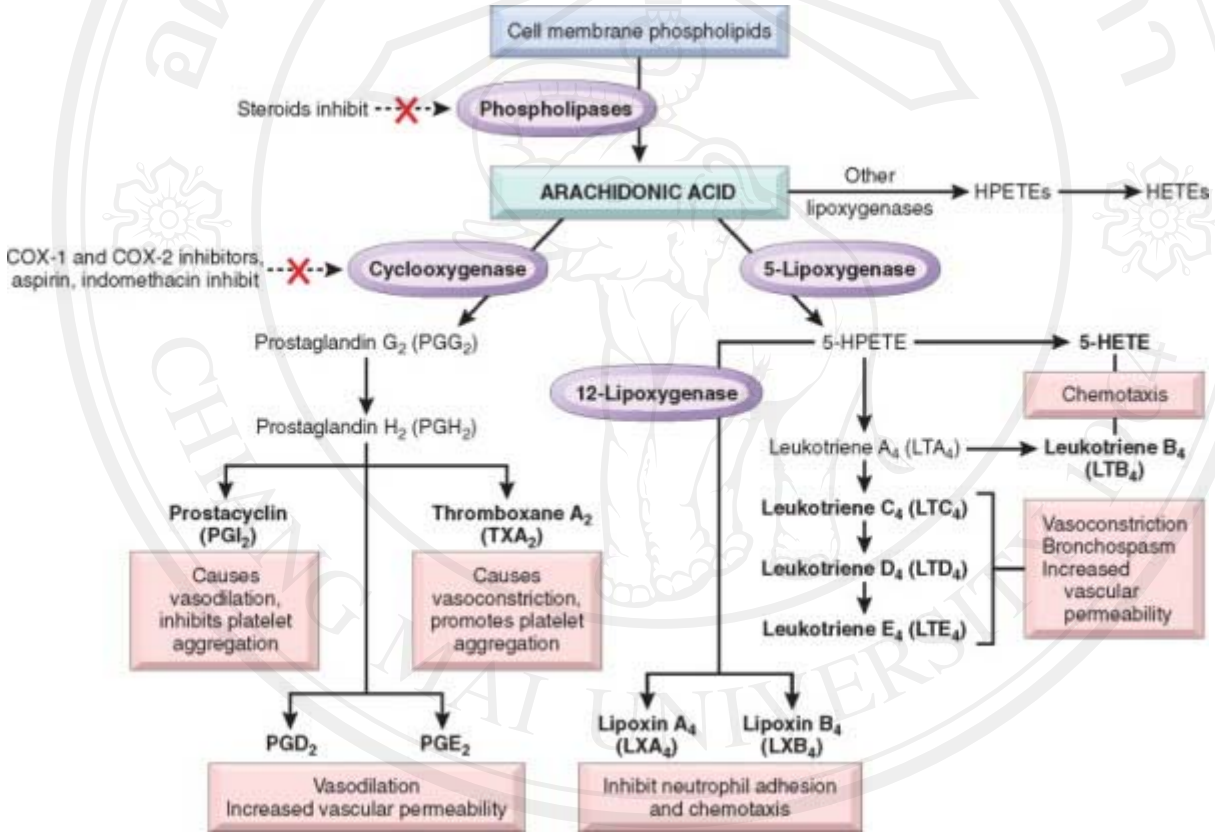

Figure 3 Arachidonic acid metabolites and their roles in inflammation (10)

Table 2 Comparison of the properties of COX-1 and COX-2 (14)

Property	COX-1	COX-2
Expression	Constitutive	Inducible; not normally present in most tissues
Tissue location	Ubiquitous expression	Constitutive in part of nervous system
Cellular localization	Endoplasmic reticulum (ER)	Inflamed and activated tissues, ER and nuclear membrane
Substrate selectivity	AA, eicosapentaenoic acids	AA, γ-linolenate, α-linolenate, linoleate, eicosapentaenoic acids
Role	Protection and maintenance function	Pro-inflammatory and mitogenic functions
Induction	Generally no induction; human chorionic gonadotropin (hCG) can up-regulate COX-1 in amnion	Induced by bacterial lipopolysaccharide (LPS), TNF-α, IL-1, IL-2, epidermal growth factor (EGF), IFN-γ mRNA rises 20- to 80-fold upon induction
Inhibition	Pharmacologic: NSAIDs (low-dose aspirin)	*In vivo*: Anti-inflammatory glucocorticoids, IL-1β, IL-4, IL-10, IL-13 Pharmacologic: NSAIDs, COX-2 selective inhibitor and steroids

enhancing the release of pro-inflammatory cytokines of the macrophages and lymphocytes. LTC_4, LTD_4 and LTE_4 constitute the biologic mixture previously known as "slow-reacting substance of anaphylaxis". These compounds play a pathophysiological role in immediate hypersensitivity reactions. They are potent constrictors of smooth muscles (100-1,000 times as potent as histamine) leading to vasoconstriction, bronchospasm and also increase vascular permeability and lead to edema (10, 16).

1.2.3.2 Vasoactive amines: Histamine and serotonin

Histamine and serotonin are the vasoactive amines that play a role in vasodilation and be released in early phase of inflammation. Histamine, a decarboxylated derivative of histidine, is synthesized and stored in the granules of tissue mast cells and basophils. It diffuses rapidly through tissues and into the blood stream and promotes many of the sequelae of acute inflammation, including vasodilatation, increased vascular permeability, and interactions with the peripheral nervous system (14, 17). Serotonin, a derivative of tryptophan, is stored in platelets, mast cells and enterochromaffin cells of the GI tract and is released through degranulation (17). Release of serotonin from platelets is stimulated when platelets aggregate after contact with collagen, thrombin, adenosine diphosphate and antigen-antibody complexes. Thus, the platelet release reaction, which is a key component of coagulation, also results in increased vascular permeability. This is one of several links between clotting and inflammation (10). Chemical mediators of inflammatory response are demonstrated in Table 3.

Table 3 Chemical mediators of the inflammatory response (11, 14)

Function	Mediators
Increased vascular permeability	Histamine Serotonin Substance P BK Complement components: C3a, C5a PGE_2, PGI_2, LTC_4, LTD_4,LTE_4 PAF Calcitonin gene-related peptide (CGRP)
Vasodilatation	PGI_2, PGE_1, PGE_2, PGD_2 NO
Vasoconstriction	TXA_2, LTB_4, LTC_4, LTD_4 C5a
Tissue damage	Neutrophil and macrophage lysosomal products Oxygen radicals NO
Fever	IL-1, IL-6, TNF-α LTB_4, LXA_4, LXB_4 PGE_2
Pain	PGE_2, PGI_2 BK

1.3 ANTI-INFLAMMATORY DRUGS

1.3.1 Nonsteroidal anti-inflammatory drugs (NSAIDs)

The main anti-inflammatory agents are the NSAIDs and the glucocorticoids. NSAIDs are the most frequently used drugs in the treatment of inflammatory diseases. They are frequently prescribed for rheumatic musculoskeletal complaints and are often taken without prescription for minor aches and pains. The mechanism of action of aspirin and other NSAIDs was based on the inhibition of PGs biosynthesis (18). The properties of PGs are important in the regulation of inflammatory processes, platelet aggregation, pain and fever induction, and many other processes. NSAIDs have three major pharmacologically desirable actions as describe below;

An anti-inflammatory action: the decrease in vasodilator prostaglandins (PGE_2, PGI_2) means less vasodilatation and edema. Accumulation of inflammatory cells is not reduced.

An analgesic effect: decreased PG generation means less sensitization of nociceptive nerve endings to inflammatory mediators such as BK and serotonin. Relief of headache is probably a result of decreased prostaglandin-mediated vasodilatation.

An antipyretic effect: this is partly owing to a decrease in the mediator PGs (which is generated in response to the inflammatory pyrogen IL-1) that is responsible for elevating the hypothalamic set-point for temperature control, thus causing fever.

The traditional NSAIDs (tNSAIDs) are known to inhibit both COX-1 and COX-2. The classes of tNSAIDs include arylpropionic acids (ibuprofen, naproxen, flubiprofen, ketoprofen), indole acetic acids (indomethacin. etodolac), heteroaryl acetic acid (diclofenac, ketorolac), enolic acids (piroxicam, phenylbutazone), and alkanones (nabumeton) (13, 19). The major side effects of tNSAIDs such as gastroduodenal injury and impaired renal function are caused by the inhibition of COX-1, whereas their analgesic and anti-inflammatory activities result from the inhibition of COX-2 (20). The discovery of COX isozymes led to the development of selective COX-2 inhibitors, the first one is celecoxib. These selective inhibitors are effective anti-inflammatory drugs that developed for improved GI safety, base on selective sparing of COX-1 in the GI mucosa and platelets. However, the main findings of some clinical trials led the Food and Drug Administration (FDA) to black-

box warning about potential cardiotoxicity of all NSAIDs (except aspirin) in early 2005 (21). Recently, several studies have raised concerns that selective COX-2 inhibitors may increase the risk of cardiovascular and cerebrovascular events. The probable explanation for this event is that COX-2 inhibitors diminish endothelial cell production of PGI_2 (a vasodilator and inhibitor of platelet aggregation), while leaving intact the platelets production of TXA_2 (a vasoconstrictor and activator of platelet aggregation), then it promotes vascular thrombosis (10, 15).

1.3.2 Anti-inflammatory glucocorticoids

Glucocorticoid drugs including dexamethasone, prednisone and prednisolone have powerful anti-inflammatory and immunosuppressive effects. They inhibit both the early and the late phases of inflammation. Anti-inflammatory and immunosuppressive effects of glucocorticoids are described as suppression of the activation of T lymphocytes by ILs and nuclear factor *kappa* B (NF-κB), decrease in the synthesis of the pro-inflammatory cytokines and other substances (e.g., IL-1, TNF), decrease in the release of inflammatory mediators such as histamine, PGs and LTs by increasing the transcription of lipocortin which inhibits PLA_2 activity. They also increase the synthesis of annexin-1, an important substance that has anti-inflammatory action. In addition, glucocorticoids stabilize lysosomal membranes of neutrophils and prevent the release of catabolic enzymes (e.g., acid phosphatase). Finally, glucocorticoids cause vasoconstriction and decrease vascular permeability that are signs of inflammation (22, 23). Glucocorticoids are given orally, intramuscularly or intravenously. They may also be given topically-injected intra-articularly, given by aerosol into the respiratory tract, administered as drops into the eye or the nose, or applied in creams or ointments to the skin. When drugs are used in anti-inflammatory and immunosuppressive therapy, the metabolic action and the effects on water and electrolyte balance and organ systems are unwanted side-effects. Moreover, chronic glucocorticoid use is associated with many serious adverse effects, including osteoporosis, muscle wasting, and abnormal carbohydrate metabolism (14).

Consequent to these adverse effects associated with NSAIDs and glucocorticoids described above, development of new drugs from medicinal plants is regarded as important areas of health research in Thailand. Several lines of evidence have shown immense of medicinal plants in various diseases. Although Thai people have used Thai traditional medicine (TTM) for centuries to maintain good health and to treat common diseases in accordance with traditional folk beliefs (24), but this practice has judged its way to modern medicine and is fading away. In the past decade, awareness of adverse effects and high cost of modern medicine has coined the interest to herbal medicine and several clinical settings have accepted herbal medicine as part of therapeutic option. However, scientific validation of their therapeutic efficacy and safety are urgently needed in order to gain acceptance by health professionals and the public (25).

1.4 THE HISTORICAL BACKGROUND OF *ALPINIA PURPURATA*

1.4.1 Description of *A. purpurata*

In Thailand, Zingiberaceae plants are comprised of 26 genera and more than 300 species (26). *A. purpurata* (Vieill.) K. Schum (synomym, *Guillainia purpurata* Vieill), or red ginger known in Thailand as "King Daeng", is a plant in this family and native to tropical and subtropical Asia. It is one of the species cultivated for ornamental plants (27). The picture of *A. purpurata* is shown in Figure 4. The flowers of *A. purpurata* are used for ornamental purposes and the plant grows well in rich soil, wet habitats and easily maintained. The plant is more productive when started from rhizomes. Herb of *A. purpurata* is perennial, coarse, unbranched, erect to 4 m high (13 ft) and arising from an underground rhizome. Its leaves are simple, alternate, two-ranked, subsessile atop a sheath, blade oblong and usually 15-70 x 5-20 cm. It flowers continuously through out the year. It is propagated by rhizome division and by the bulbils that are produced in the axils of the lowest inflorescence bracts. It is often grown as a border plant in the tropics and in the green house in temperate climates. It is advisable to cut back the stalks that have finished flowering. Its long-lasting colorful bracts make this ginger a favorite in cut flower arrangements (28).

Figure 4 *Alpinia purpurata* (Vieill.) K. Schum or **"**King Daeng**"** (29)

1.4.2 Phytochemical and pharmacological studies of *A. purpurata* and Zingiberaceous plants

The Zingiberaceous plants especially ginger are an importance natural resource that provides many useful products for food, traditional medicines and perfume (30,31). However, *A. purpurata* is scarcely cited in both phytochemistry and biochemical activity. The phytochemical constituents from *A. purpurata* are 3-methoxyflavone, steroidal glycosides (32), unstable labdane diterpene and alkaloid piperine (33). The hexane and ethyl acetate extracts of the rhizomes of *A. purpurata* were reported to contain the four compounds including 4-(1-acetoxyallyl) phenyl acetate, (*E*)-3-(4-acetoxyphenyl) allyl acetate, 4-hydroxybenzaldehyde and (*E*)-3-(4-hydroxyphenyl) acrylaldehyde (34).

The pharmacological effects of Zingiberaceous plants have been reviewed and described. The essential oil and the hydroalcoholic extract of *A. purpurata* have been reported to elicit notable antibacterial and vasodilator effects, respectively (35, 36). In the Zingiberaceous plans such as *Zingiber zerumbet* (37), *Z. officinale* (38-40), *A. galangal* (40), and *A. katsumadai* (41) have been reported to possess analgesic and anti-inflammatory activities. In addition, the curcumin of *Curcuma longa* L. exerts inhibitory effect on COX and LOX as well as nitric oxide synthetase (NOS) activities (9, 42). *A. zerumbet* possesses potent vasorelaxant (43) and antinociceptive effects (44). *A. katsumadai* used topically shows antipruritic and anti-inflammatory effects in animal model (41). *A. officinarum* possesses an anti-inflammatory effect in both *in vivo* (45) and *in vitro* models (46). To date, however, no analgesic and anti-inflammatory effects of *A. purpurata* have been reported.

1.5 HYPOTHESIS

The analgesic and anti-inflammatory effects of Zingiberaceous plants were described as above. *A. purpurata* is a plant in the Zingiberaceae family, and theoretically it may share the analgesic and anti-inflammatory activities as with plants in this family. Therefore, the hypothesis of this study is that *A. purpurata* possesses analgesic and anti-inflammatory effects.

1.6 PURPOSES OF THE STUDY

The purpose of the present study was to study the analgesic and anti-inflammatory activities of *A. perpurata* rhizome extract in animal models. The possible mechanisms of action of these activities were also examined. Moreover, acute toxicity test was conducted to verify its safety.