CHAPTER 1

INTRODUCTION

1.1 INFLAMMATION

Inflammation is the body's defensive mechanism response to infection, irritation, or injury to its tissues and cells by pathogens (i.e., bacteria, viruses, fungi and parasites) or noxious stimuli (i.e., chemical, radical, thermal, mechanical and physical injury) (1-3). The classic symptoms of inflammation consist of warmth, pain, redness, swelling and loss of function. The primary purposes of the inflammatory responses include destruction, localization and elimination of the injurious agents and removal of damaged tissue components (2, 4).

The important reactions involve in inflammation including vascular and cellular responses (5). The summary of inflammatory reactions and inflammatory mediators is shown in Table 1. Immediately after injury, vasodilatation and increase vascular permeability are the prominent vascular responses. These reactions cause fluid and protein complements leaking into the extracellular spaces. Increasing dilatation and permeability of microvascular are the results of the chemical mediators such as histamine, bradykinin, prostaglandins (PGs), and nitric oxide (NO). The cellular response produces rapidly recruitment of phagocytes, particularly leukocytes and monocytes in acute and chronic phases, respectively. Goals of phagocytic activity are to against injurious agents, remove debris, and restore normal tissue structure and function (5, 6). Inflammation is often classified as two distinct acute and chronic phases, each apparently mediated by different mechanisms (6).

Acute phase of inflammation is primary physical effect of the inflammatory response, which lasts from few minutes to several days (5). This phase is mainly regulated by the release of lipid autacoids (7). The hallmark effects of acute inflammation include 1) accumulation of fluid and plasma components in the affected area, 2) intravascular stimulation of platelets, and 3) leukocytes migration and accumulation in the inflamed area, particularly neutrophils which known as

Table 1 Summary of inflammatory reactions and inflammatory mediators (6)

Reactions	Mediators
Vasodilatation	- Prostaglandins (PGs) - Nitric oxide (NO) - Histamine
Increased vascular permeability	- Vasoactive amines - C3a and C5a - Bradykinin - Leukotriene (LT) C_4, D_4, E_4 - Platelet activating factor (PAF) - Substance P
Chemotaxis, leukocyte recruitment and activation	- C5a - LTB_4 - Chemokine - Tumor necrotic factor (TNF) - Interleukin-1 (IL-1)
Fever	- TNF - IL-1 - PGs
Pain	- PGs - Bradykinin - Substance P
Tissue damage	- Neutrophil and macrophage lysosomal enzymes - NO

polymorphonuclear leukocytes (PMNs). Increased of fluid and plasma components in extracellular spaces occur by increased capillary permeability that referred to as fluid exudate (6, 8). The fluid exudate contains a variety of mediators including the complement system, the coagulation system, the fibrinolytic system and the kinin system (9). The migration of leukocytes requires the release of chemical mediators known as chemotactic stimuli (i.e. complement system, arachidonic acid (AA) metabolites, and cytokines). PMNs are the first cells to be recruited to sites of inflammation, which they exert their effect by phagocytosis the injurious agents (5-6). A normal inflammatory response is an acute process that resolves after removal of the inciting stimulus. Diseases of inflammation and immunity can occur due to inappropriate inflammatory response or when the inflammation progresses to chronic state, either because of a long-term inappropriate response to a stimulus (e.g. allergy) or because the offending agent is not removed (e.g. chronic infection, transplantation, and autoimmunity) (10).

Chronic phase of inflammation is a persistent inflammatory reaction that may last for weeks, months, or even years (5, 7). The hallmark effects of this phase are characterized by 1) infiltration with macrophages, 2) fibroblast proliferation, and 3) scar formation. The effects of macrophages involve injurious killing, clearing up cellular and tissue debris, and also seem to be very important in remodeling the tissues. The modified macrophages resemble epithelial cells that called epithelioid cells. Epithelioid cells may clump in a mass, form a multinucleated giant cell that attempts to surround the injurious agents. The fibrous tissue eventually encapsulates the injurious agents and isolates it, these cells referred to as foreign-body giant cells and to form a ball of cells. This process involves the infiltration of fibroblasts, collagen, and new endothelial cells (angiogenesis) that cause scar formation for healing and repairing processes (2, 6). Chronic inflammation is regulated by inflammatory mediators including adhesion molecules, protein complements, platelet-derived growth factor (PDGF), transforming growth factor-α (TGF-α), etc (6).

1.1.1 The chemical mediators of inflammation

The structural and functional changes in inflammatory reaction associated with vascular and cellular responses. Signs and symptoms of inflammatory response are produced by a large range of chemical mediators, which can be derived from plasma proteins or secreted by cells. The chemical mediators of inflammation are soluble and diffusible molecules, they act together or in sequence at the inflammatory site. They amplify the initial inflammatory response and influence its evolution by regulating the subsequent vascular and cellular responses (6, 11).

(1) Vasoactive amines

These mediators are preformed and stored in the granules of mast cells, basophils, and platelets.

(1.1) Histamine

Histamine is a basic vasoactive amine and widely distributed throughout the body and is highly concentrated in the lungs, skin, and gastrointestinal tract. It is produced by the decarboxylation of histidine by histidine decarboxylase. It is readily available and stored in intracellular granules of mast cells and basophils (12). Mast cells, which contain most of histamine granules, are the effector cells involved in the immediate inflammatory and allergic response. The mediators are secreted by degranulation of exocytosis granules in response to a variety of stimuli including tissue injury and immune reactions involving mast cells binding of IgE, protein complements such as C3a and C5a, and also cytokines especially interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) (13). Histamine acts on different receptors designated H_1, H_2, H_3, and H_4 receptors on target cells to produce its effects (12). Early inflammatory response provokes mast cells to release and degradation of histamine granules, thereafter its binding to H_1-receptor on endothelium causes vasodilatation, increased capillaries permeability, swelling and flare. In addition, the binding of histamine to H_1-receptors provokes smooth muscle contraction including ileum, bronchi, bronchioles, and uterus. Moreover, histamine induces the release of other inflammatory mediators such as IL-1, TNF-α, PGs, platelet activating factor (PAF), bradykinin, adhesion molecules, and of itself (14).

(1.2) Serotonin

Serotonin or 5-hydroxytryptamine (5-HT), is a monoamine primary known as a neurotransmitter modulates a variety of behaviors. Its actions is mediated through interactions at different receptors (5-HT_{1-7}) (15-16). It is present in a variety of central and peripheral tissues including in serotonergic neurons and constituents of the immune system. 5-HT is synthesized by enterochromaffin cells from gastric and intestinal mucosa and is stored in large amounts in peripheral tissues by mast cells and platelets. Inflammatory reactions cause the induction of PAF to activate the releasing of 5-HT leading to an increase in its concentration at the inflammatory site (17-18). 5-HT modulates immune function through several 5-HT receptors, particularly the 5-HT_{1A} and 5-HT_{2A} receptors that may have possible roles in immune reactions (15). The effect of serotonin is similar to histamine, which is also capable to cause vasodilatation, increase capillary permeability, and produce nonvascular smooth muscle contraction. 5-HT effects are mediated directly and/or in combination with other inflammatory mediators on membrane ion channel proteins to change vascular permeability and excitability of afferent nerve fibers. It contributes to peripheral sensitization and hyperalgesia in inflammation and to nerve damage (12).

(2) Plasma proteases

(2.1) Complement system

The complement system, a group of 20 plasma proteins, is an important factor involved in immunity and inflammation. The complement consists of nine proteins including C1-C9, which are inactive in normal condition and are split into fragments during activation. Each of the complement activation pathways culminates with the formation of a C3 cleaving enzyme (C3 convertase). These indigenous complement enzymes mediate the cleavage and activation of large numbers of C3 molecules (6, 19).

The important complements in inflammatory reaction include C3a and C5a, which are anaphylatoxins because they activate mast cells to release histamine. C5a activates phospholipid membrane of neutrophils and monocytes to produce AA metabolites such as PGs and LTs. Moreover, C5a also acts as chemotactic agent for inflammatory cells, particularly neutrophils and monocytes. Indeed, C5a contributes

to inflammatory reactions by simulating the release of other mediators. Therefore, inflammatory response begun by complement activation may be prolonged and potentiated by the actions of mediators released by C5a (20).

(2.2) Kinin system

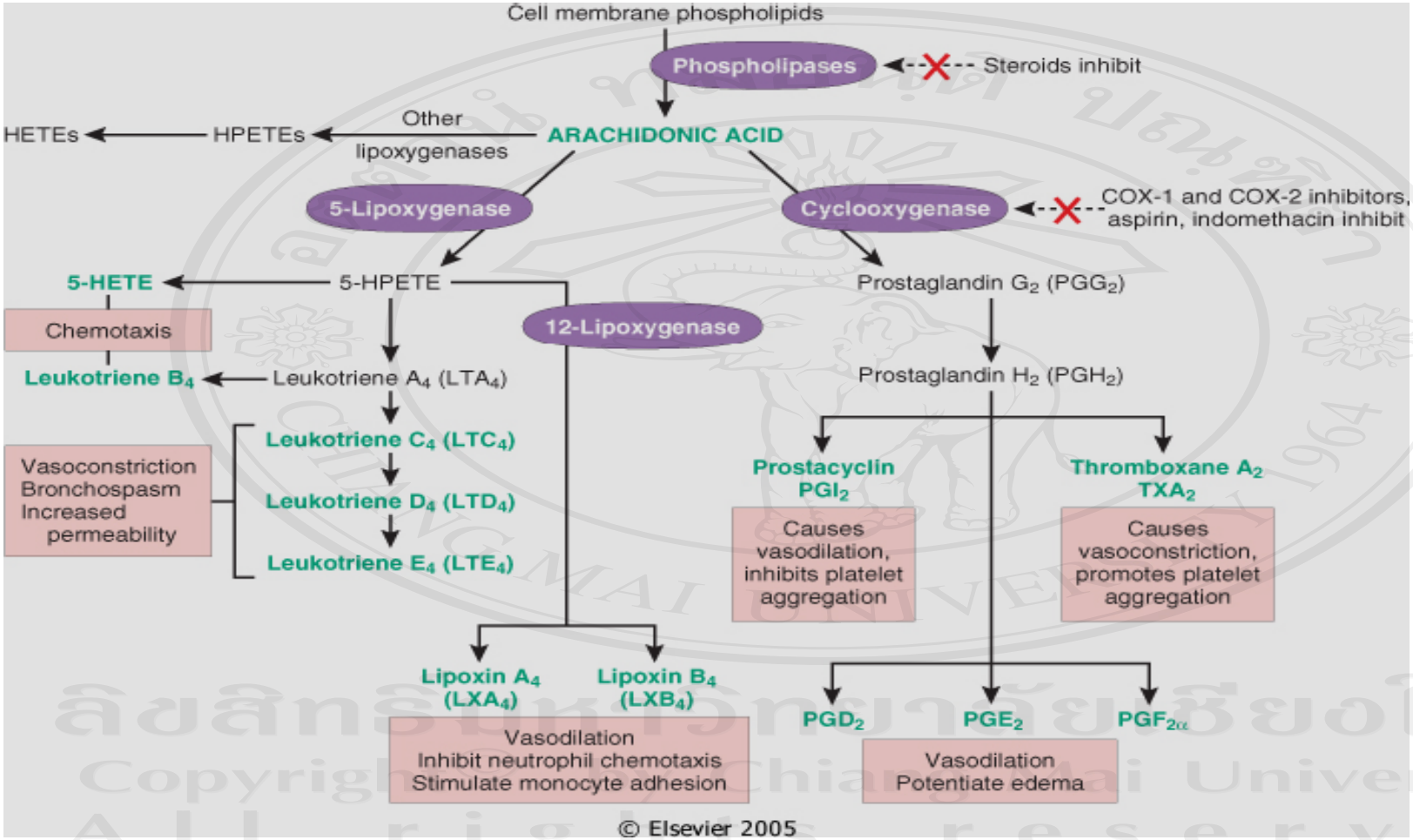
Kinins appear to participate in both acute and chronic inflammation (21). These peptides are potent and ubiquitous pro-inflammatory peptides inducing the cardinal signs of inflammation: hyperalgesia, vasodilatation and increased microvascular permeability. The kinins form in plasma, urine and tissue by the action of serine protease kallikreins (kinin-forming enzymes) on specific plasma glycoproteins termed kininogens. These substances are present normally in an inactive form. Three kinin peptides have been identified, i.e. bradykinin, lysyl-bradykinin and methionyl-lysyl-bradykinin (22). Bradykinin is one of the important mediators of inflammatory reaction which is an extremely potent vasodilator and enhancer of vascular permeability. Its functions on inflammatory reaction are probably due to their ability to amplify the inflammatory response by stimulating local tissues and inflammatory cells to generate additional mediators such as PGs (e.g. PGE_2 and PGI_2), cytokines (e.g. IL-1 and TNF-α), and NO (23). It is a potent pain-producing agent, an effect that is potentiated by PGs (24). These effects of bradykinins are mediated via one of two types of their receptors, B_1 and B_2 (25). B_1 receptors are absent under normal conditions but their expression is increased rapidly during inflammation and tissue damage; cytokines such as IL-1 and TNF-α are mainly responsible for this induction. Bradykinin does not directly activate B_1 receptors but its metabolites can activate this site (26). B_1 receptor activation stimulates leukocyte migration and synergizes the inflammatory reaction including swelling, heat, redness, and pain (27-28). By contrast, B_2 receptors are constitutively expressed in most normal tissues and cells, which are activated by bradykinin but not by its metabolites. B_1 receptor is considered as potential therapeutic target of anti-inflammatory drugs in inflammatory diseases (29).

(3) Arachidonic Acid metabolites

AA is a 20-carbon polyunsaturated fatty acid, primary derived from the two essential fatty acids, linoleic and alpha-linolenic acid, and forms as a component of phospholipid membranes (30). The AA pathway is one of the main mechanisms for the production of mediators of pain and inflammation, and of mediators which also control homeostatic functions. Cellular phospholipases, especially phospholipase A_2 (PLA_2), are activated during inflammation and hydrolyzed phospholipids to AA. Metabolites of AA, i.e. eicosanoids, mediate both acute and chronic phases of inflammation. Eicosanoids are synthesized via two major pathways catalyzed by cyclooxygenase (COX) and lipoxygenase (LOX) (31-32). The scheme of AA metabolism is shown in Figure 1.

COX, known as prostaglandin H synthetase (PGHS), is the catalyze enzyme committed step in the metabolism of AA to prostanoids including PGs and thromboxanes (TXs) (33-34). PGs, mainly PGE_2 and PGI_2, have been detectable during inflammation. These mediators are the important modulators of the classical signs of inflammation, i.e. induction of swelling and pain. PGE_2 and PGI_2 do not appear to have direct effects on vascular changes but they have been shown to synergize the effects of other inflammatory mediators, for example, histamine, 5-HT and bradykinin to cause increased vascular permeability and vasodilation. The other effect of PGE_2 and PGI_2 is the sensitization of the peripheral nerve endings to painful stimuli by lowering the threshold of nociceptors. Centrally, PGE_2 can increase excitability in pain transmission of neuronal pathways in the spinal cord. Thus PGs contribute to pain both peripherally and centrally (4, 31, 35). Moreover, PGE_2 is a pyrogenic agent that causes fever, produced by endogenous pyrogens, especially IL-1 and TNF-α. In addition, major product of AA metabolism in platelets is TXA_2, which produces platelet aggregation and vasoconstriction (31).

In fact, three isoforms of COX exist; COX-1, COX-2 and COX-3. The first, COX-1, constitutively expressed in most tissues, e.g. platelets, endothelium, kidney and stomach mucosa, and has been presumed to function primarily in the maintenance of physiological functions such as cytoprotection of gastric epithelium (34). The second, COX-2, is usually undetectable under basal conditions but can be highly inducible in response to pro-inflammatory mediators, such as IL-1 and C5a (36-37).



5-HETE: 5-hydroxy eicosatetraenoic acid, 5-HPETE: 5-hydroperoxyeicosatetraenoic acid

Figure 1 Diagram inlustrating the arachidonic acid (AA) metabolism (6)

The selective inhibition of COX-2 has been more directly implicated for suppressing inflammatory response (38). Finally, COX-3, is a splice variant of COX-1 and mostly expressed in the brain and the heart (39). The action of COX-3 is inhibited by acetaminophen and is thus thought to mediate the antipyretic and analgesic effects but its mechanism of action has been questioned (40). Both COX-1 and COX-2 share similar properties to convert AA, which is released from the plasma membrane via the action of phospholipase A_2 into protanoids (32, 41).

The LOX enzyme has five bioactive forms, i.e. 5-LOX, 12(S)-LOX, 12(R)-LOX, 15-LOX-1, and 15-LOX-2. Indeed, 5-LOX is the predominant of LOX that catalyzes synthesis of LTs, which play major roles in the development and persistence of inflammatory responses by increasing the vascular permeability and chemotactic attraction of leukocytes (31). Primary product of 5-LOX is LTA_4 which in turn gives rise to LTB_4 and LTC_4. LTB_4 is a potent chemotactic agent that causes migration of PMNs into inflammatory sites. LTC_4 generates subsequent metabolites including LTD_4 and LTE_4, which are collectively known as slow reacting substance of anaphylaxis (SRS-A). Their effects consist of bronchoconstriction, and increased vascular permeability (5-6).

(4) Platelet- Activating Factor (PAF)

PAF is a potent pro-inflammatory phospholipid mediator produced by macrophages, neutrophils, platelets, and endothelial cells in response to inflammation (42-43). Its biosynthesis involves the acetylation of a precursor released from membrane phospholipids by activated phospholipase A_2, which is closely coupled with AA (44). The synthesis of PAF may be stimulated during inflammation, which has been implicated in the release of cytokines such as IL-1 and TNF-α (45). PAF induces cellular activation by binding to a receptor, which is a member of the super-family of G-protein-coupled receptors (3). PAF plays an important role in the pathophysiology of inflammatory reactions including increased vascular permeability, platelet aggregation, and migration of PMNs and monocytes (46). The effect of PAF is dependent on amount of production. High concentration of PAF causes vasoconstriction and bronchoconstriction, in contrary, low concentration of PAF

induces vasodilation and increases vascular permeability, which appears to be 100 to 10000 times more potent to increase vascular permeability than histamine (47).

(5) Cytokines

Polypeptide products, cytokines, are produced during immune and inflammatory reactions. Most cytokines are not constitutively produced, but require cell activation for their synthesis. The pro-inflammatory cytokines including IL-1, IL-6, TNF-α, interferon gamma (IFN-γ), transforming growth factor beta (TGF-β), etc., are crucially synergistic in the inflammatory responses (48). Cytokines are primarily produced by leukocytes and monocytes but can also be generated by endotoxin, immune reactions, or a variety of inflammatory mediators. IL-1 and TNF-α are the most important inflammatory cytokines which induce endothelial activation with increased production of eicosanoids and NO, and expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1) and E-selectin (49-50). Both IL-1 and TNF-α activate tissue fibroblast, resulting in increased proliferation and production of extracellular matrix. These effects play essential roles to amplify the inflammatory responses on both acute and chronic phases of inflammation (3, 6).

1.2 PAIN

Pain is considered both a sensation and an emotion, which is a perception to the peripheral or central nociception, occurs when tissue damage by pathogens or noxious stimuli (51). Inflammatory mediators, i.e. histamine, 5-HT, bradykinin and PGs, can produce hyperalgesia or increase the sensitivity of nociceptors to noxious stimuli (52-53). Bradykinin, a substance causing pain receptor sensitization, is rapidly metabolized and therefore may be involved mainly acute pain (54). Release of PGs, particularly PGE_2, at the site of peripheral inflammation contributes to pain hypersensitivity by reducing the threshold and increasing the excitability of peripheral terminals of afferent nerve fibers (55). One of the strong hyperalgesia substances is substance P, which is considered to be neurotransmitter in pain fibers. It is released from the nerve terminals and sensitizes nociceptors. It causes the release of histamine and serotonin from platelets and mast cells, which contributes significantly to increase

vasodilation and permeability associated with inflammation. During inflammation, substance P contribute directly and indirectly to neurogenic inflammation and hyperalgesia in the periphery and to the excitability changes in the spinal dorsal horn associated with the transmission of pain signals (56). These chemical mediators produce pain by activating peripheral nociceptors, sensitizing nociceptors in peripheral endings, or stimulating the release of pain-producing substances (52-53). Nociceptors are abundant in the skin and underlying soft tissues, joint surfaces, arterial walls, and periosteum whereas most internal organ such as lung and uterine tissues contain few nociceptors (57). The nociceptive fibers consist of 2 types including A-delta (Aδ) fibers and C-fibers. The C fibers are more numerous and sensitive to inflammatory phenomena than Aδ fibers. However, Aδ fibers are more strong in intensity and speed of the pain sensation, but the thresholds of Aδ fibers are higher than those of C fibers. Overall, the quality of the pain evoked by Aδ fibers is less than the result of stimulating C fibers. These activated fibers generate impulses which pass to the axon in dorsal horn of spinal cord and then transmit along complex pathways through the spinal cord to the brain, in the hypothalamus and brain stem (57-58).

1.3 FEVER

Fever is defined as the elevation of normal body temperature. It is known as febrile response, particularly of inflammation and infection. Hypothalamus regulates body temperature; it controls thermoregulatory mechanisms by balancing heat production and heat loss (59). Fever may be provoked by many stimuli including bacteria and their endotoxins, viruses, yeast, and immune reaction products. These substances are commonly called exogenous pyrogens; they are digested by macrophages which become activated and then release a substance called endogenous pyrogen. The *in vivo* animal experiments reveal that the main substances played as endogenous pyrogens are IL-1, IL-6 and TNF-α. Endogenous pyrogens stimulate the production of COX-2 metabolites, particularly PGE_2, in the organum vasculosa lamina terminalis (OVLT). Elevation of PGE_2 in the hypothalamus produces fever by raising the set-point of the thermoregulatory center in the preoptic area (POA) of the anterior hypothalamus, which in turn leads to vasoconstriction in the skin and

increased body temperature. This occurs through the prostaglandin E receptor 3 (EP_3) expressed in neurons surrounding the OVLT (60-61). Antipyretic drugs appear to act by inhibiting the synthesis and/or release of PGE_2 in the hypothalamus (61).

1.4 ANTI-INFLAMMATORY DRUGS

There are two primary goals for treatment of patients with inflammation: first, the relief of pain, which is often the presenting symptom and the major continuing complaint of the patient; and second, the slowing or arrest of the tissue-damaging process. Nonsteroidal anti-inflammatory drugs (NSAIDs) produce anti-inflammatory, analgesic and also antipyretic effects. Therefore, they are appropriate for the treatment of both acute and chronic inflammatory conditions (62).

NSAIDs are commonly used for the treatment of inflammation, pain, and fever. The mechanism of action of NSAIDs is suppression the PGs synthesis via inhibition of the COX enzyme. Therefore NSAIDs act to decrease the sensitivity of vessels to bradykinin and histamine as well as affect lymphokine production from T lymphocytes (30, 63). Furthermore, NSAIDs are classified as mild analgesic and antipyretic agents. The analgesic activities of NSAIDs are due to the attenuation of sensitized nociceptive nerve ending to inflammatory mediators, which play as hyperalgesia substances (3). Most traditional NSAIDs or nonselective COX inhibitors inhibit both COX-1 and COX-2. Inhibition of COX-1 in gastric epithelial cells depresses mucosal cytoprotective PGs, especially PGI_2 and PGE_2, thereby cause gastric ulceration (62). The new NSAIDs with highly selective COX-2 inhibition are developed such as celecoxib and etoricoxib; their properties are analgesic, anti-inflammatory, antipyretic and anti-platelet aggregation but cause fewer gastrointestinal damage than do traditional NSAIDs. In the light of recent information of selective COX-2 inhibitors on a potential cardiovascular hazard (rofecoxib: increase risk of myocardial infarction and stroke) and a life-threatening skin reactions (valdecoxib: toxic epidermal necrolysis, Stevens-Johnson syndrome and erythema multiform), it is therefore not advised to use coxibs as a first choice among the NSAIDs (38, 41).

Alternative agents for anti-inflammation with less severe adverse effects as do traditional, selective or specific NSAIDs or steroids are in deed required and extensively sought. One of the interesting candidates is medicinal plants (64).

1.5 PLANTS WITH ANTI-INFLAMMATORY EFFECT

Medicinal plants, known as herbal medicines, have been used worldwide since ancient time for the treatment of many diseases (65). Herbal medicines are considered primary health care for over 2,500 years ago. Scientists found that people in different parts of the globe use the same or similar plants for the same purposes. The World Health Organization estimates that about 80% of people worldwide use herbal medicines for some aspects of their primary health care (66). Herbal medicines are popular among majority of people in many countries due to their origin from natural source, being cheap and on the belief of being safe. The pharmacopoeia of scientific medicine in the 20th century has been developed primarily from native herbal lore, and so far herbal medicine continues to be used for the treatment of various diseases. Up to 25% of all prescription drugs have at least one active ingredient from plant extracts or synthesized plant compounds. Many conventional medicines are synthetic compounds designed to mimic the action of the bioactive compounds which are extracted from different parts of plants such as stems, seeds, roots, leaves, etc. (65-66).

The people in Southeast Asian countries use many plants as folklore medicine that show interesting anti-inflammatory effects with potential therapeutic applications. For example, *Ardisia crispa* (family Myrsinaceae) is widely distributed in Malaysia, Philipines and Thailand. Various parts of this plant are used for treating throat pain, chest pain, rheumatoid arthritis (67), fever, diarrhea, and dysmenorrhea (68). The hexane fraction of the ethanol extract from the root of *A. crispa* possesses anti-inflammatory and analgesic effects in experimental models (69). The stem of *Cryptolepis buchanani* (family Asclepiadaceae) has been traditionally used for the treatment of inflammation, including arthritis and muscle and joint pain. The 50% ethanol extract of this plant shows anti-inflammatory effects on both acute and chronic phases in experimental animal models (70). The fresh stem and leave of *Cissus quadrangularis* (family Vitaceae) is used for the treatment of hemorrhoid,

menstrual disorder, scurvy and as anti-flatulence (71). The pharmacological study of the crude extract from *C. quadrangularis* reveals analgesic, anti-inflammatory and venotonic effects in animal models (72). Moreover, many species of Bauhinia genus such as *B. purpurea*, *B. racemosa*, and *B. tarapotensis* have been proved to possess analgesic, antipyretic and anti-inflammatory activities in animal models (73-75).

1.6 HISTORICAL BACKGROUND OF *BAUHINIA SIAMENSIS*

Bauhinia siamensis, a species of Leguminosae family, is a plant newly discovered in Phu Miang, Phitsanulok, Thailand. This plant is known in Thai as "Soi Siam" or "Siao Dang". The picture of *B. siamensis* is shown in Figure 2. *B. siamensis* is a climber plant with ovate leaves, 4-7.5 x 4-7 cm, its flower is light or dark pink colour, inflorescences elongate raceme, pendulous to 75 cm long, the seed is ovate, flat and dark brown of 1.5-2 cm long. Because this plant has been newly found in 2002, therefore its pharmacological effects have not yet been performed (76).

Although, no evidence of therapeutic effect of this plant has been mentioned, however, there are many studies of phytochemical and pharmacological effects of plants in Bauhinia genus. Bauhinia belongs to the Leguminosae family that comprises approximately 300 species. Native people from tropical regions use various parts of Bauhinia as medicine in infusions and other phytotherapeutic preparations (77-78). The aqueous extract of *B. purpurea* leaves was found to contain the active compounds which are flavonoids, triterpenes and saponins. This aqueous extract has been shown anti-inflammatory effect in carrageenin-induced rat paw edema model, and analgesic effect in both early and late phases of formalin test as well as reduces abdominal constriction in nociceptive test in mice (73). The methanol extract from *B. racemosa* stem bark possesses anti-inflammatory effects in both acute phase in histamine- and serotonin-induced rats paw edema and chronic phase in cotton pellet-induced granuloma. This methanol extract also potentiates morphine and aspirin-induced analgesic, as well as reduces pyrexia in animal model (74). The chloroform extract from *B. tarapotensis* leaves possesses anti-inflammatory properties in croton-oil-induced ear edema in mice (75).

Figure 2 "Soi Siam**"** (*Bauhinia siamensis* K. & S.S Larsen)

1.7 HYPOTHESIS

As mentioned above many plant species in the Bauhinia genus show anti-inflammatory, analgesic and antipyretic activities in animal models. Therefore it is possible that *B. siamensis* also possess these effects. The hypothesis of this study was therefore that methanol extract from *B. siamensis* possesses anti-inflammatory, analgesic and antipyretic effects.

1.8 PURPOSES OF THE STUDY

The purposes of the present study were to verify anti-inflammatory, analgesic and antipyretic effects of the methanol extract from *B. siamensis* in various animal models in comparison with reference drugs such as diclofenac, prednisolone and codeine. The mechanisms of action of the methanol extract from *B. siamensis* on the inflammatory process, pain and pyrexia pathways were also examined in comparison with reference drugs.