CHAPTER 1

INTRODUCTION

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs (1). The number of higher plant species on earth is about 250,000. It is estimated that 35,000 to 70,000 species have been used in some cultures for medicinal purposes (2). Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in low socioeconomic areas where it offers the only affordable remedy. Since herbal medicines are from nature, most people consider that they are safe. However, the safety of herbal remedies is of particular importance in that the majority of these products are self-prescribed and are used to treat minor and often chronic conditions (2, 3). In recent years, herbal medicines are becoming popular as alternative medicine and have been used by many people. Herbal medicine is a readily available, affordable, effective and culturally-acceptable health care modality (2). Herbal products are available in the UK through various retail outlets such as pharmacies, health food shops, mail order companies, supermarkets and departmental stores. During 1990 sales of OTC phytomedicines in 7 EU countries were estimated to represent US$ 2.4 billion in total selling price, highlighting the enormous popularity of complementary therapies in Europe (3). In Thailand, traditional medicine and medicinal plants have been used by Thai people for the treatment of both common illnesses and some chronic disease before the Sukhothai period (4).

There have been many reports of compounds with anti-inflammatory activity being found in plant. In addition, many plants with a medicinal reputation for treating inflammatory diseases, and also diseases related to inflammation, have shown anti-inflammatory action against animal or biochemical models used by pharmacologists to screen compounds for anti-inflammatory activity (5).

The side effects of the anti-inflammatory drugs (e.g., gastric intolerance, bone marrow depression, and water and salt retention, etc.) are of the major problems in developing medicine today (6). Therefore, new anti-inflammatory drugs lacking these adverse effects are being searched for all over the world as alternatives to non-steroidal anti-inflammatory drugs (NSAIDs). During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, and have less side effects. In addition, according to WHO, about 80% of the world population still rely mainly on plant-based drugs (7).

Many plants show interesting biological activities with potential therapeutic applications, for example, in the year 2006, Kassuya CAL *et al*. reported that the extracts obtained from *Phyllanthus amarus*, and some of the lignans isolated from it, exhibit anti-inflammatory and antiallodynic action which are probably mediated through its direct antagonistic action on the PAF receptor binding sites (8). *Vitex negunclo*, family Verbenaceae, is a small tree of which the water extract of mature leaves are used in Ayurveda medicine as anti-inflammatory, analgesic and anti-itching agents (9). Another report showed anti-inflammatory effects of *Polygala japonica* extract which inhibits ear swelling induced by picryl chloride in mice, markedly inhibits footpad edema induced by histamine in rats, and decreases PGE_2 content in carrageenan induced air-pouch (10). Another plant with anti-inflammatory activity is the methanol extract of *Solanum trilobatum* that showed anti-inflammatory activity against carrageenan induced paw edema in rats. Moreover, a chloroform extract of *Salvia triloba* showed anti-inflammatory activity in both acute and chronic model, and the ulcerogenic effects of the extract has been found to be less than that of acetyl salicylic acid (11). Some studies in Thailand reported that aqueous extracts of *Oroxylum indicum* and *Derris scandens* significantly reduce myeloperoxide release, eicosanoid production is reduced by the aqueous extracts of *Acanthus ebracteatus* and

D. scandens. In addition, *D. scandens* extract also shows potent inhibitory activity against generation of leukotriene B4 and also displays antioxidant activity (12).

1.1 Inflammation

Inflammation is the reaction of body tissues to injury such as physical trauma, foreign bodies, chemical substances, surgery, radiation, and electricity. The area affected will undergo a series of changes as the body processes attempt to wall off, heal, and/or replace the injured tissue (13). The sequence of events in inflammation may be summarized as follows: (1) cellular injury; (2) local vasodilation, increased capillary permeability, cellular adhesion, and exudation to bring inflammatory cell and chemical mediators to the injured area; (3) destruction of injurious agents by phagocytes; (4) clearing away of cellular debris; and (5) deposition of a protein framework for tissue healing. During the course of inflammation, cellular mediators are released, and in the plasma, chain reaction (cascade) responses activate other protein mediator. These reaction result in formation of substances that augment the inflammatory response and produce systemic signs and symptoms (14). At the macroscopic level the characteristics of the inflammatory response are that the area is reddened, swollen, hot and painful and that there is intereference with, or alteration of function (15). Inflammation is characterized by a number of features: (1) vasodilatation leading to redness; (2) increased vascular permeability leading to swelling of tissues; (3) recruitment of leukocytes into tissues; and (4) if 1-3 occur chronically, this can lead to alterations in tissue functions (16).

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the injured tissues. The hallmarks of acute inflammation include (1) accumulation of fluid and plasma components in the affected tissue, (2) intravascular stimulaion of platelets, and (3) the presence of polymorphonuclear leukocytes (PMNs). Prolonged inflammation, known chronic inflammation, leads to a progressive shift in the types of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory processes. The characteristic cell components of chronic inflammation are lymphocytes, plasma cells, and macrophages (17, 18).

Acute inflammation is of short duration, lasting from minutes to several days (19). It is the initial response to tissue injury; it is mediated by the release of autacoids and usually precedes the development of the immune response. Some of the autacoids involved are listed in Table 1 (20). Acute inflammation is characterized by vascular and cellular changes. The vascular events are an initial dilatation of the small arterioles and channels in the capillary bed close, causing increased blood flow to the injured tissues, followed by slowing and then stasis of the blood and an increase in the permeability of the postcapillary venules, with exudation of fluid. The vasodilatation is brought about by various mediators (histamine, PGE_2 and I_2 or prostacyclin, etc) produced by the interaction of the microorganism with the tissue. Some of these mediators (e.g., histamine, platelet-activating factor (PAF) and cytokines released by toll like receptors-pathogen associated molecular patterns (TRL-PAMPs) interactions) are also responsible for the initial phase of increased vascular permeability. The fluid exudate contains a variety of mediators, which influence the cells in the vicinity and the blood vessels themselves. These include the components for four proteolytic enzyme cascades: the complement system, the coagulation system, the fibrinolytic system, the kinin system (19, 21). These events cause outflow of water and ions into the extravascular tissue leading to swelling. The microvascular loss of protein-rich fluid causes the red blood cells to become more concentrated, thereby increasing blood viscosity and slowing the circulation (22-25). The cellular phase involves the movement of neutrophil in a process called extravasation. Neutrophils are highly mobile cell and arrive first at the site of injury to begin phagocytosis and release inflammatory mediators capable of digesting proteins, including elastin and collagen. The cells accomplish extravasation through margination, pavementing, adhesion, emigration, and chemotaxis (19,21). Chronic inflammation is a persistence of the inflammation process and may follow a less predictable course than acute inflammation (19). It is considered to be inflammation of prolonged duration (weeks or months). It associates histologically with the presence of the cellular components such as macrophages, plasma cells, dendritric cells, lymphocytes, and in certain conditions, eosinophils together with the proliferation of blood vessels, fibrosis, and tissue necrosis (18, 23, 26). The chronically inflamed area usually is infiltrated by mononuclear leukocytes, mostly macrophages and lymphocytes. However, certain

types of chronic inflammation, such as osteomyelitis, may contain neutrophils for months, and some types of acute inflammation have increased numbers of lymphocytes in the early phase. When macrophages are the predominant cell, they divide, multiply and release chemotactic substances that attract more macrophages. The inflammatory process may begin as a low-grade, poorly cleared inflammation or on acute inflammation that is not totally resolved by the body. Chronic inflammation results in infiltration of the site with fibroblasts, increased amounts of collagen deposits, and varying amounts of scar tissue formation. The scar tissue and underlying inflammation often cause organ dysfunction. Chronic inflammation may begin insidiously with a low-grade fever and no other apparent symptoms (19). Chronic inflammation involves the release of number of mediators that are not prominent in the acute response. Some of these are listed in Table 2. One of the most important conditions involving these mediators is rheumatoid arthritis, in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to severe disability and in which systemic changes occur that can result in shortening of life (20).

Table 1 Pharmacological properties of the mediators of acute inflammation.

Mediator	**Vasodilation**	**Vascular permeability**	**Chemotaxis**	**Pain**
Histamine	++	↑↑↑	-	-
Serotonin	+/-	↑	-	-
Bradykinin	+++	↑	-	+++
Prostalandins	+++	↑	+++	+
Leukotrienes	-	↑↑↑	+++	-

Table 2 Mediators of chronic inflammation.

Mediator	Sources	Primary Effects
Interleukins (IL) 1, 2 and 3	Macrophages, T lymphocytes	Lymphocyte activation, PG production
GM-CSF[1]	T lymphocytes, endothelial cells, fibroblasts	Macrophage and granulocyte activation
TNF-α[2]	Macrophages	PG production
Interferons (IFN)	Macrophages, endothelial cells, T lymphocytes	Many
PDGF[3]	Macrophages, endothelial cells, fibroblasts, platelets	Fibroblast chemotaxis, proliferation

[1]Granulocyte-macrophage colony-stimulating factor.

[2]Tumor necrosis factor alpha.

[3]Platelet-derived growth factor.

1.1.1 Mediators of inflammation

An initial inflammatory stimulus triggers the release of chemical mediators from plasma or connective tissue cells. Such soluble mediators, acting together or in sequence, amplify the initial inflammatory responses and influence its evolution by regulating the subsequent vascular and cellular responses. The inflammatory response is terminated when the injurious stimulus is removed and the inflammatory mediators have been dissipated, catabolized or inhibited (24).

The criteria used to determine whether an endogenous substance can be positively considered as an inflammatory mediator, were first considered by Dale (1911) and restated by Vane (1972)(27, 28). These criteria are as follows:

1. The mediator should be detectable, at the site of inflammation, at the right time, in amounts adequate to account for the effects under consideration.
2. The mediator, when administered in concentrations of the order of those found in the lesion, should produce the observed effects, and no others.

3. Specific blocking agents or antagonists of the effects of the proposed mediator should prevent or attenuate the effects.
4. Prevention of release of the mediator should abolish or prevent the effects.
5. Agents or procedures preventing the breakdown or removal of the mediator should prolong or potentiate the effects .

Mediators which suit the above criteria and are specified as inflammatory mediators.

I. Vasoactive amines

Histamine

Histamine is a basic amine formed from histidine by histidine decarboxylase. It is found in most tissues of the body but is present in high concentrations in the gastrointestinal (GI) tract. At the cellular level, it is found largely in mast cells and basophils, associated with heparin, but non-mast-cell histamine occurs in "histaminocytes" in the stomach and in histaminergic neurons in the brain (21). Histamine exerts its physiologic effects by interacting with target cell receptors, designated H_1, H_2, H_3 (29, 30); the most recently described H_4 receptor is more widely distributed, especially in organs associated with the immune system (31, 32). Its receptors are functionally coupled to G-proteins (33-36). Histamine is released by mast cell degranulation in response to a variety of stimuli such as physical injury, immune reaction and anaphylatoxins. In humans, histamine is an important mediator of immediate allergic and inflammatory reactions associated with mast cell degranulation but it has a lesser basis for implication in chronic inflammation (37). It is considered to be the principal mediator of the immediate phase of increased vascular permeability, edema formation, inducing venular gaps. It act on the vasculature by binding to specific H_1 receptor in vascular wall resulting to vasoconstriction of larger blood vessels. Stimulation of both H_1 and H_2 receptors induces pruritus, vasodilatation, and cardiac irritability (23, 37-39). It is also powerful stimulant of sensory nerve endings, especially those mediating pain and itching. Soon after its release, histamine is inactivated by histaminase (24, 38, 40).

Serotonin

Serotonin (5-hydroxtryptamine [5-HT]) is present in central and peripheral serotonergic neurons. 5-HT is present in high concentrations in the enterochromaffin cells throughout the GI tract, platelets and specific regions of the CNS (41-43). It is released after tissue injury, and it exerts algesic and analgesic effects depending on the sites of action and the receptor subtypes (44). 5-HT is also a preformed vasoactive mediator with effects similar to those of histamine. In the microcirculation, 5-HT can also cause vasodilatation through 5-HT_1 receptors (45), together with constriction of venules, with the result that capillary pressure rises and fluid escapes from the capillaries. In large vessels, both arteries and veins are usually constricted by 5-HT. This is a direct action on vascular smooth muscle cells, mediated through 5-HT_{2A} receptors. 5-HT acting in combination with other inflammatory mediators, may ectopically excite and sensitize afferent nerve fibers, thus contributing to peripheral sensitization and hyperalgesia in inflammation and nerve injury (44, 45). Another mediator, that plays an important role in nociceptive processing is substance P (SP). SP is secreted by nerves and inflammatory cells such as macrophages, eosinophils, lymphocytes, and dendritic cells and acts by binding to the neurokinin-1 (NK-1) receptor (46, 47). SP is thought to transmit nociceptive information and contribute to occurrence of pathological pain states such as inflammation and nerve injury (51); it regulates vessel tone and moderates vascular permeability (48).

II. Plasma protease

Kinins

Kinins appear to participate in the acute and chronic phases of inflammatory reaction (49). Kinins are a group of potent vasodilator peptides. They are formed enzymatically by the action of enzymes known as kallikreins or kininogenase. Three kinins have been identified in mammals: bradykinin, lysylbradykinin (kallidin) and methionyllysylbradykinin. Each kinin is formed from a kininogen by action of a different enzyme. Bradykinin is released by plasma kallikrein, lysylbradykinin by glandular kallikrein and methionyllysylbradykinin by pepsin and pepsin-like enzymes. The three kinins have been found in plasma and urine (50). Bradykinin

causes vasodilation and increased vascular permeability. Its vasodilator action is partly a result of generation of PGI_2 and release of NO. It is a potent pain-producing agent, an effect that is potentiated by the PGs. Bradykinin is spasmogenic for several types of smooth muscle including that of the intestine and the contraction is slow and sustained in comparison with that produced by histamine (21). Bradykinin effects are in most cases mediated by interaction with one of two types of bradykinin receptors (B_1 and B_2) present on many cell types, including vessel endothelial cell, smooth muscle, nerve cells and synovial lining cells. Both are typical G-protein-coupled receptors (21, 51). B_1-receptors are absent in most normal tissues, but are strongly inducible within a few hours under conditions of inflammation and tissue damage; cytokines such as IL-1 and TNF-α are mainly responsible for this induction. B_1-receptors respond to the bradykinin metabolite, but not to bradykinin itself, and are selectively blocked by various peptide antagonists. It is likely that B1-receptors play a significant role in inflammation and hyperalgesia. B_2-receptors are constitutively present in many normal cells and tissues and activated by bradykinin and lysylbradykinin, but not by the bradykinin metabolite (21, 51-52). Kinins are rapidly degraded to inactive products by kininases and therefore have rapid and short-lived functions. Perhaps the most significant function of kinins is their ability to amplify the inflammatory response by stimulating local tissue cells and inflammatory cells to generate additional mediators, including prostanoids, cytokines (especially TNF-α and ILs), NO, and tachykinins (26).

Complement system

The complement system is a biochemical cascade which helps clear pathogens from an organism. It is part of the innate immune system and underlies one of the main effector mechanisms of antibody-mediated immunity. Three biochemical pathways activate the complement system: the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway. The three pathways all generate homologous variants of the protease C3-convertase. The classical complement pathway typically requires antibodies for activation, while the alternative and mannose-binding lectin pathways can be activated by C3 hydrolysis or antigens without the presence of antibodies. In all three pathways, a C3-convertase

cleaves and activates component C3, creating C3a and C3b and causing a cascade of further cleavage and activation events. C3b binds to the surface of pathogens leading to greater internalization by phagocytic cells by opsonization. C5a is an important chemotactic protein, helping recruit inflammatory cells. Both C3a and C5a have anaphylatoxin activity, directly triggering degranulation of mast cells as well as increasing vascular permeability and smooth muscle contraction. C5b initiates the membrane attack pathway, which results in the membrane attack complex (MAC), consisting of C5b, C6, C7, C8, and polymeric C9. MAC is the cytolytic endproduct of the complement cascade; it forms a transmembrane channel, which causes osmotic lysis of the target cell. Kupffer cells and other macrophage cell types help clear complement-coated pathogens (53,54).

Clotting system

In the clotting system, factor Xa, an intermediate in the clotting cascade causes increased vascular permeability and leukocyte emigration. Thrombin participates in inflammation by enhancing leukocyte adhesion to endothelium and by generating fibrinopeptides that increase vascular permeability and are chemotactic for leukocytes. While activated Hageman factor is inducing clotting, it is simultaneously activating the fibrinolytic system. This mechanism exists to counter-regulate clotting by cleaving fibrin, thereby solubilizing the fibrin clot. Fibrin degredation products increase vascular permeability, while plasmin also cleaves the complement C3 component to C3a, resulting in vasodilation and increased vascular permeability (55, 56).

III. Arachidonic acid metabolites

Arachidonic acid (AA) metabolites also called eicosanoids. These products derived from the metabolism of AA affect a variety of biologic processes, including inflammation and hemostasis (57). AA is a 20 carbon polyunsaturated fatty acid, derived primarily from dietary linoleic acid and is present in the body mainly in its esterified form as a component of cell membrane phospholipids. It is released from phospholipids via cellular phospholipases that have been activated by mechanical, chemical, or physical stimuli or by an inflammatory mediator such as C5a. AA metabolism proceeds along one of the two major pathways, cyclooxygenase (COX)

and lipoxygenase (LOX) (58). The scheme of the major metabolic transformations of AA is shown in Figure 1.

In the COX pathway, products are PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin), and thromboxane A_2 (TXA_2). TXA_2, a potent platelet aggregating agent and vasoconstrictor, is the major PG product from platelets. Endothelium possesses prostacyclin synthetase, which leads to formation of PGI_2, a vasodilator and a potent inhibitor of platelet aggregation. PGD_2 is the major metabolite of the COX pathway in mast cells, along with PGE_2 and $PGF_{2\alpha}$. It causes vasodilation and edema formation. The PGs are also involved in the pathogenesis of pain and fever in inflammation (57, 59). COX exists in two main isoforms, COX-1 and COX-2. COX-1 is expressed in gastric mucosa and mediates a "housekeeping" function (37, 47). It regulates several homeostatic processes such as renal blood flow, gastric cytoprotection and platelet aggregation, while COX-2 is induced in settings of inflammation by cytokines and inflammatory mediators. COX-2 is the enzyme responsible for generation of most of the inflammatory PGs (60, 61). There are several lines of evidence to support the notion that COX-derived products are important mediators of inflammation, i.e., PG synthesis is increased at sites of inflammation (24). COX is an important enzyme that is inhibited by aspirin and NSAIDs. Recently, an alternative splice variant of COX-1 that is selectively inhibited by paracetamol (acetaminophen) has been identified and called COX-3 (62, 63). There is considerable evidence that the analgesic effect of paracetamol is central and is due to activation of descending serotonergic pathways, but its primary site of action may be inhibition of PG synthesis (62).

The LOX pathway, 5-LOX is the predominant AA-metabolizing enzyme in neutrophils. The derivative of AA, 5-HPETE (5-hydroperoxy-eicosatetraenoic acid), is unstable and is either reduced to 5-HETE (hydroxyeicosatetraenoic acid) or converted into a family of compounds collectively called leukotrienes. Leukotrienes, the products of 5-LOX metabolism have been associated with immediate hypersensitivity reactions, anaphylaxis and asthma (64). Leukotriene A_4 (LTA_4), which in turn gives rise to LTB_4 or LTC_4. LTB_4 is a potent chemotactic agent and causes aggregation of neutrophils. LTC_4 and its subsequent metabolites, LTD_4 and LTE_4, are known as slow-reacting substances of anaphylaxis (SRS-A) (18), which cause vasoconstriction, bronchospasm, and increased vascular permeability (18, 24).

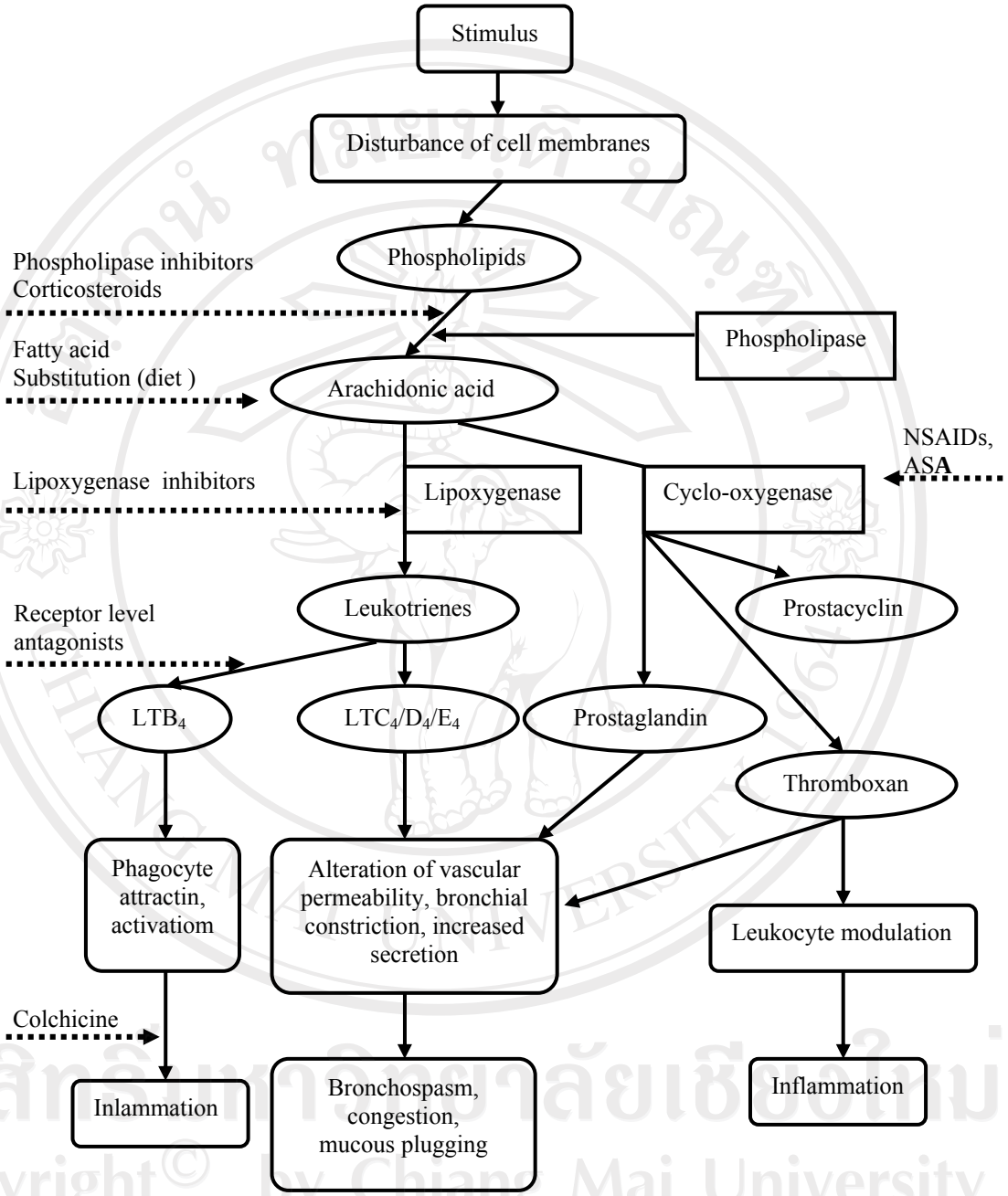

Figure 1. Scheme for mediators derived from AA and sites of drug action (65).

IV. Platelet-activating factor (PAF)

PAF is a biologically active lipid that can produce effects at exceedingly low concentrations (21). It is another phospholipid-derived mediator with a broad spectrum of inflammatory effects. PAF causes vasoconstriction and bronchoconstriction and is 100-10,000 times more potent than histamine in inducing vasodilation and increased vascular permeability (57). PAF is released indirectly from many activated inflammatory cells, endothelial cells, and injured tissue cells by phopholipase A_2 activity and acts on specific receptors in many cell types. Acting on specific receptors, PAF has a wide range of pathophysiological actions and is capable of producing many of the phenomena of inflammation. Injected locally, it produces not only vasodilatation, and thus erythema, but also increases vascular permeability and wheal formation. Higher doses produce hyperalgesia. It is a potent chemotaxin for neutrophils and monocytes and is important in recruiting eosinophils into the bronchial mucosa in the late phase of asthma. It can activate phospholipase A_2 to generate eicosanoids. On platelets, it causes shape change and the release of the granule contents. This effect is associated with metabolism of AA and thromboxane A_2 generation and is important in haemostasis and thrombosis (21, 26).

V. Cytokines

Cytokines are polypeptide products produced during immune and inflammatory responses. IL-1 and TNF are historically associated with cellular immune responses, additional effects that are important in the inflammatory response. Both IL-1 and TNF are produced by activated macrophages, and their secretion is stimulated by endotoxin, immune complexes, toxins, physical injury, or a variety of inflammatory mediators (24). IL-1 and TNF induce endothelial activation with increased expression of adhesion molecules, secretion of additional cytokines and growth factors, production of eicosanoids and NO, and increased endothelial thrombogenicity. TNF also causes aggregation and activation of neutrophils and the release of proteolytic enzymes from mesenchymal cells, thus contributing to tissue damage. Both cytokines activate tissue fibroblasts, resulting in increased proliferation and production of extracellular matrix (22). IL-1 and TNF also induce the systemic acute-phase responses typically associated with infection or injury,

include fever, lethargy, hepatic synthesis of various proteins, metabolic wasting, neutrophils release into circulation, inducing corticosteroid synthesis and release. TNF also plays an important role in mediating the hypotensive effects of septic shock, including diminished myocardial contractility and vascular smooth muscle relaxation (58).

1.1.2 Nonsteroidal anti-inflammatory drugs (NSAIDs)

All NSAIDs exhibit anti-inflammatory properties and are used as first-line agents for the symptomatic relief of inflammatory condition. The anti-inflammatory activity of NSAIDs is mediated chiefly through inhibition of biosynthesis of PGs by inhibition of COX-2 and COX-1 activities (66). The inhibition of COX-2 is thought to mediate, at least in part, the antipyretic, analgesic and anti-inflammatory action of NSAIDs, but the simultaneous inhibition of COX-1 results in unwanted side effects, particularly those leading to gastric ulcers (67). The side effects of these drugs, that result from blockage of the synthesis of endogenous PGs and TXA_2, include disturbances in GI (i.e., dyspepsia, gastric irritation and gastric ulceration), renal (i.e., salt water retention leading to edema) and platelet function (i.e., prolongation of bleeding time) (58, 61, 68). To preserve the anti-inflammatory effect of COX inhibition but prevent the harmful effect on gastric mucosa, highly selective COX-2 inhibitiors are now available (69). Therefore, agents that selectively block COX-2 such as meloxicam, celecoxib, and etoricoxib may offer a more favorable side effect profile yet still effectively decrease inflammation (66, 70). Analgesic effect of NSAIDs is due to a decrease of PGs generation resulting in less sensitization of nociceptive nerve endings to inflammatory mediatores. Antipyretic effect is also due to a decrease in those mediators, generated in response to inflammatory pyrogen IL-1, that is responsible for elevating the hypothalamic set point for temperature control in fever, but normal body temperature is not affected by antipyretics. LOX is not affected by any of the COX inhibitors, and in fact COX blockade may increase substrate access to the LOX pathway (71). Anti-inflammatory steroids such as cortisol inhibit the release by phlsoholipase A2 of AA from phospholipids and thus inhibit formation of all AA derivatives (72).

1.2 Pain

Pain is the sensation of discomfort, hurt, or distress. It is a common human ailment and may be mild or severe, acute or chronic. Pain occurs when tissue damage activates the free nerve endings (pain receptors or nociceptors) of peripheral nerves. Causes of tissue damage may be physical (e.g., heat, cold, pressure) or chemical (e.g., pain related to substances released from damaged cell and products of inflammation, including bradykinin, histamine, and PGs). Bradykinin, one of the strongest pain producing substances, is quickly metabolized and therefore may be involved mainly acute pain. PGs increase bradykinin's pain-provoking effects by increasing the sensitivity of pain receptors. Several other substances are also thought to produce pain, including acetylcholine, adenosine triphosphate, histamine, leukotrienes, potassium, serotonin, and SP (73). SP, which is considered to be neurotransmitter in pain fibers, is released from the nerve terminals and sensitizes nociceptors. SP causes the release of histamine and serotonin from platelets and mast cells, which contributes significantly to capillary vasodilation and permeability associated with inflammation (74). Although SP is not an algesic itself, it increases the permeability of blood vessels. This produces a leakage of fluid into the surrounding tissues which provides for wider diffusion of the algesia. In this manner a large area becomes painful (75). Overall, these chemical mediators produce pain by activating peripheral nociceptors, sensitizing peripheral endings of nociceptors, or stimulating the release of pain-producing substances. Nociceptors are abundant in the skin and underlying soft tissue, joint surfaces, arterial walls, and periosteum; most internal organs, such as lung and uterine tissue, contain few nociceptors (73). It is commonly held that pain is detected by free nerve endings in the skin, tissues, and organs. These nociceptors are located on afferent fibers. The nociceptors respond to mechanical, thermal, and chemical stimuli. These axons vary from very small diameter unmyelinated (C) fibers to larger myelinated (A-delta) fibers. The quality of the pain evoked by A-delta fibers is a "pricking" pain while a "burning" pain is the result of stimulating C fibers. When these fibers are stimulated by noxious stimuli, the information is sent to the dorsal horn of the spinal cord. Several layers of the dorsal horn are directed to higher centers in the brain. In the past, the spinothalamic pathway was considered the major pain pathway. Although this is an important pain pathway, it is now believed that

several ascending pathways are involved in the role of pain. In addition to these pathways, specific nuclei of the brain, such as the reticular formation, periaqueductal gray matter, and thalamus, also are components of pain pathways. Finally, the axons projection from the thalamus to the cerebral cortex are the last component of the pain pathways. This projection system is involved in sensory-discriminative processing and motivational-affective processing, therefore refining the perception of pain (76).

1.3 Fever

Fever is an elevation of body temperature above the normal range (77). It is an almost universal phenomenon of illness, particularly of inflammation and infection. Its purpose is unknown, but up to a certain point it is considered a defence against disease. The hypothalamus set the point at which body temperature is maintained, but body temperature regulation depends on a balance between the heat production and loss (78). Fever can be caused by a number of substances that collectively incite the production of a fever-producing mediator called pyrogen. Pyrogen producing substances include viruses, bacteria, and other microorganisms, products of inflammation, antigen antibody complexes, drugs and chemicals. These substances are ingested by macrophages which then become activated and then release a substance that was formerly called endogenous pyrogen. Recently, endogenous pyrogen has been found to be the same as IL-1, an inflammatory mediator that produces other signs of inflammation such as leukocytosis, anorexia, and malaise (79). These pyrogens act on the temperature regulating center in the hypothalamus to elevate the thermostat set-point. It has been suggested that IL-1 causes fever by inducing the hypothalamus to release PGE_2, which acts on the hypothalamus to evoke the fever response. When the set-point increases to a higher level than the normal, mechanisms for raising the body temperature are activated. The body initiates heat-conservation measures, including vasoconstriction, piloerection (gooseflesh), and shivering, to drive the body temperature up to a new level (19). Drugs that inhibit the synthesis of PGs in E series have antipyretic activity. For example, salicylates reduce raised body temperatures by causing the hypothalamic center to restablish a normal set point. Heat production will not be inhibited, although heat loss will be increased by an increase in cutaneous blood flow

and sweating, caused by the lowered thermostat. Antibiotics indirectly reduce temperature by destroying the bacteria causing the fever (78). Normal body temperature is not affected by aspirin and NSAIDs (80).

1.4 Experimental models used in the present study

1.4.1 Inflammatory models

1.4.1.1 Carrageenin-induced hind paw edema in rats

The rat hind paw edema still remains the most commonly used test for anti-inflammatory activity. The edema is produced in hind paw of rats by injection of phlogistic agents such as brewer's yeast, formalin, dextran, egg albumin, carrageenin, AA. The most commonly used phlogistic agent is carrageenin (81, 82). Carrageenin is a sulphate polysaccharide which has been fractionated with potassium chloride into two separate components, kappa and lambda carrageenin (83). The lambda carrageenin is more active in eliciting either acute or chronic inflammatory responses. Swelling of the paw reaches a peak in 3-5 h, then retains about the same degree of edema for several hours. For routine drug testing, increase of foot volume 3 h after phlogistic agent has been adopted as the measure of effect (81). In this model, the edema is produced by a sequential release of physiological mediators; histamine, 5-HT, kinins and PGs (84). It is important when using this model to assess the effect of the potential anti-inflammatory agent at the appropriate time during the swelling of the hind paw. This test is excellent for detecting inhibitors of COX (85).

1.4.1.2 AA-induced hind paw edema in rats

AA-induced hind paw edema in rats provides a valuable tool for evaluating the *in vivo* anti-inflammatory activity of LOX inhibitors and other agents with a mechanism of action different from COX inhibition. LTs, 5-LOX products of AA, are involved as inflammatory mediators. LTC_4, LTD_4, and LTE_4 cause edema together with increased microvascular permeability (86). The subplantar injection of AA into the hind paw of rats produces edema within 5 min and reaches peak response by 1 h after injection. One of the unique aspects of AA-induced rat paw edema is its

sensitivity to dual inhibitors of AA metabolism (such as phenidone), LOX inhibitors and corticosteroids but is insensitive to selective COX inhibitors (87).

1.4.1.3 Cotton pellet-induced granuloma formation in rats

Cotton pellet-induced granuloma formation in rats was introduced at first by Meier *et al* (1950). This procedure is generally considered to be a measure of the capacity of such agents to interfere with the proliferative components of the inflammatory process. The response to subcutaneously implanted cotton pellet in rat can be divided into at least three phases. These consist of 1) transudative phase, defined as the increase in wet weight of the pellet which occurs during the first 3h after implantation; 2) exudative phase, defined as leakage of fluid from bloodstream around the granuloma and occurring between 3 and 72 h after implanting the pellet; and 3) proliferative phase, measured as the increase in dry weight of the granuloma which occurs between 3 and 6 days after implantation (88).

Serum alkaline phosphatase activity can also be assessed in cotton pellet-induced granuloma formation in the rats. Alkaline phosphatase is a lysosomal enzyme. It is widely distributed in many tissues, including the osteoblasts (the bone building cells), the cell lining the sinusoids and bile canaliculi in liver. It is reported that the activity of alkaline phosphatase in serum is markedly increased during inflammation (89). Alkaline phosphatase activity in pouch wall is elevated during cotton pellet granuloma formation on the seventh day and decreased on the fourteenth day, when healing occurred. Measurement of alkaline phosphatase activity in serum of rats implanted with the cotton pellet indicates the activity of agents on the production and release of alkaline phosphatase in chronic inflammation (90).

1.4.2 Algesic model

1.4.2.1 Formalin test

The formalin test is often considered as an appropriate model of clinical pain because the nociceptive stimulus, tissue injury by injection of the irritating chemical reagent, induces continuous pain (91, 92). The formalin test in mice is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. The two distinct periods of high licking activity can be identified, an early phase

lasting the first 5 min and a late phase lasting from 20 to 30 min after the injection of formalin (93). The two phases may have different nociceptive mechanisms. The early phase is due to a direct chemical stimulation effect on nociceptors and PGs do not play an important role during this phase. The late phase is dependent on the combination of an inflammatory reaction mediated by PGs in the peripheral tissue and functional changes in the dorsal horn of the spinal cord; this phase can be inhibited by NSAIDs and steroids. Centrally acting analgesics inhibit both phases (93-96).

1.4.3 Pyretic model

1.4.3.1 Yeast-induced hyperthermia in rats

This method appears to be reproducible and accurate for assaying non-narcotic antipyretic compounds (97). Toxins from bacteria such as endotoxin act on monocytes, macrophages, and Kupffer cells to produce cytokines that act as endogenous pyrogens. These pyrogens stimulate PG synthesis. PGs act on the thermoreceptive region in the preoptic anterior hypothalamus and raise the set point of the temperature regulating center, which leads to increased body temperature. IL-1B, IL-6, β-IFN, γ-IFN and TNF-α can act independently to produce fever (98). Yeasts are capable of stimulating the release of endogenous pyrogens from polymorphoneuclear leukocytes and monocytes and TNF from other cells. Antipyretic drugs appear to reduce fever by inhibiting the synthesis or release of PGs in the thermoregulatory center (61, 98).

1.5 Historical background of *Dasymaschalon lomentaceum*

Dasymaschalon lomentaceum is a herb in the family Annonaceae, known in Thai as “Prong kiu”, “Titto”, “Teen kai” and “Deuy kai” (99, 100, 101). It is a plant indigenous to Thailand. It has been found in the Northeast of Thailand (102). It is a moderate sized deciduous tree, 2 to 4 m high, with square stem. The leaf is lanceolate or oblong shape, 3 to 5 cm wide and 7 to 12 cm long, dark green upper side, creamy white lower side, mostly alternate and simple leaf. The flower is simple, light green or yellow petal, cone shaped. The fruit is aggregate, 6 to 12 fruits, cylinder shaped, bright red when ripe (101).

The people in the Northeastern part of Thailand use this plant as a remedy for pain and muscle sprain. In Thai folklore medicine, preparation of stem of *D. lomentaceum* with stems of "Phee pai" and "Khan sang" are prepared by boiling in water. The decoction of these plants is used for the treatment of pain and muscle sprain (102). In some formulae of Thai folklore medicine, root of *D. lomentaceum* is traditionally used for the treatment of pain (100).

Annonaceae is a large family comprising 120 genera and more than 2000 species; many members of this family are used in folk medicine for various purposes. The phytochemical studies of the Annonaceae have shown that alkaloidal and non-alkaloidal constituents are present. Moreover, some of the alkaloidal and non-alkaloidal compounds are pharmacologically important. For example, C-benzylated flavanones exert cytotoxic and antimicrobial properties, diterpenes exert antitumor activities, liveroline exerts antiparkinsonian properties, and liriodenine exerts antitumor, antibacterial, and antifungal activities (103). In the studies of other plants in the genus *Dasymaschalon*, the hexane extract from the leaves of *D. sootepense* shows strong cytotoxic activity against the L1210 tumor cell line (104). However, phytochemical, biological, and pharmacological studies of *D. lomentaceum* have not yet been performed. The preliminary research proved by screening of anti-inflammatoy property, showed that the methanol extract of *D. lomentaceum* inhibits ear edema in rats induced by ethyl phenyl propiolate (EPP).

1.6 Hypothesis

D. lomentaceum, in a preliminary screen was proved to be effective as an anti-inflammatory agent on ear edema in rats induced by EPP. The hypothesis of this study was therefore that, *D. lomentaceum* possessed anti-inflammatory, analgesic and antipyretic effects

1.7 Purposes of the study

The purpose of the present study were to evaluate the anti-inflammatory, analgesic and antipyretic activities of the methanol extract of *D. lomentaceum* in various animal models. The mechanism of action of the methanol extract from *D.*

lomentaceum on the inflammatory process, pain pathway and its effect on the gastric mucosa were also examined in comparison with diclofenac and prednisolone.

Figure 2. *Dasymaschalon lomentaceum* Finet et Gagnep.