CHAPTER 1

INTRODUCTION

Plants have been used for health and medical purposes for several thousand years. 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 (1). Herbal medicines, as the major remedy in traditional medical systems, have made a great contribution to maintaining human health. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs (2). Herbal medicines are widely used in many countries because they come from natural sources and are cheap. Since herbal medicines are from nature, most people consider that they are safe. But this is not always true because some herbs are capable of causing adverse effects (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 (4).

Many tropical plants show interesting biological activities with potential therapeutic applications, for example mangosteen or *Garcinia mangostana* Linn (GM), family Guttiferae, grown in the tropical rainforest such as Indonesia, Malaysia, Philippines and Thailand. People in those countries use GM as traditional medicine for the treatment of abdominal pain, diarrhea, dysentery, infected wound, suppuration, chronic ulcer and as an astringent (5), antitumour and antioxidant (6). In the year 2003, Nwafor and Okawuasaba reported that *Asparagus pubescens* showed anti-nociceptive and anti-inflammatory activity in rats (7). Others reported the anti-inflammatory effect of *Hypericum triquetrifolium* Turra which inhibited paw swelling induced by 1% carrageenin in rats (8). Another plant with anti-inflammatory activity is *Curcuma amada* an ethanol extract of rhizome which showed inhibitory activity on various acute phases of inflammation and on the formation of granuloma tissue (9). Some studies in Thailand

reported that the methanol extract of *Clerodendrum petasites* S. Moore shows potent antipyretic and moderate anti-inflammatory activities without ulcerogenic effect (10). The ethanol extract of this plant dose-dependently causes relaxation of tracheal smooth muscle, and may be beneficial in the treatment of asthma (11). Another study reported that *Garcinia hanburyi* Hook f. shows anti-inflammatory activity in both acute and chronic inflammatory models (12).

1.1 INFLAMMATION

Inflammation is the body reaction to invasion by infectious agent, antigen challenge or even just physical, chemical or traumatic damage (13-15). It is a protective response intended to eliminate the initial cause of cell injury. Inflammation accomplishes its protective mission by diluting, destroying or otherwise neutralizing harmful agents. It then sets into motion the events that eventually heal and reconstitute the sites of injury. Damage tissue is replaced by the generation of parenchymal cells, and/or by filling of any residual defect with fibrous scar tissue (13, 14). Although inflammation helps clear infections and, along with repair, makes wound healing possible, both inflammation and repair have considerable potential to cause harm to host such as anaphylactic reactions from insect bites or drugs, as well as certain chronic diseases such as rheumatoid arthritis and atherosclerosis (16). Another harmful example is inflammation in the peritoneum leading to fibrous bands that cause intestinal obstruction (13). Inflammation is divided into two basic patterns, acute and chronic inflammation.

Acute inflammation is of relatively short duration, lasting from few minutes up to few days. It is characterized by 5 signs: rubor (redness), calor (increased heat), tumor (swelling), dolor (pain), and functiolaesa (loss of function) (13, 14, 17). It has two major components, vascular changes and cellular events. The vascular changes begin relatively quickly after injury. Alterations in vessel caliber result in increased blood flow (vasodilation) and cause the redness (erythema) and warmth. The structural changes that permit plasma proteins to leave the circulation (increased vascular permeability), result in the movement of protein rich fluid into the extravascular tissues (13, 14, 17).

Those 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. In cellular events, leukocytes (principally neutrophils) begin to settle out of the flowing blood and accumulate along the vascular endothelial surface; after adhering to endothelial cells, the leukocytes squeeze through an intracellular junction and migrate toward chemoattractants released from a source of injury, then starting phagocytosis, killing and followed by degradation of offending agent (13, 14, 18, 19).

Chronic inflammation is longer in duration, days to years, and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and scarring (13, 14, 20). It is the sum of the responses mounted by the tissues against a persistent injurious agent such as bacterial, viral, chemical, immunologic, etc. (19, 20). The tissues affected by chronic inflammation commonly show evidence of the following pathologic process: (1) Immune response; manifestations of the immune response in injured tissue include the presence of lymphocytes, plasma cells and macrophages, plasma immunoglobulin levels may be elevated. (2) Phagocytosis; Immune phagocytosis is mediated by macrophages that have been activated by T-cell lymphokines and involves antigens that have opsonins attached to their surfaces; Nonimmune phagocytosis is directed against foreign non antigenic particles. (3) Necrosis: commonly there is some degree of necrosis that may affect only scattered individual cells or may be extensive. (4) Repair; repair of tissue damaged by persistent injury is characterized by new blood vessel formation, fibrotic proliferation, and collagen deposition (20). It is distinguished from acute inflammation by the absence of cardinal signs such as redness, swelling, pain, and increased temperature (17, 20). It may progress from acute inflammation, occurs when the acute response cannot be resolved, either because of the persistence of the injurious agent or because of interference in normal process of healing (13-15, 17, 20).

CHEMICAL MEDIATORS OF INFLAMMATION

Vasoactive amines; Histamine (β-aminoethylimidazole) is a potent bioamine with multiple activities in various pathological and physiological conditions (21). Histamine exerts its physiologic effects by interacting with target cell receptors, designated H_1, H_2, H_3 (22, 23); the most recently described H_4 receptor is more widely distributed, especially in organs associated with the immune system (24, 25). Its receptors are functionally coupled to G-proteins (26-29). Histamine is widely distributed in tissue, particularly in mast cells adjacent to vessels, although it is also present in circulating basophils and platelets (13, 30). Histamine is released in response to a variety of stimuli including interaction of antigen with IgE antibodies on the mast cell surface (31). Stimulation of IgE receptors also activates phospholipase A_2, leading to the production of mediators, including platelet-activating factor (PAF), metabolites of arachidonic acid and kinins also are generated during some allergic responses (31). Thus, the mast cell secretes a variety of inflammatory compounds in addition to histamine and each contributes to varying extents to the major symptoms of the allergic response such as constriction of bronchi, decrease in blood pressure, increased vascular permeability, edema formation (30-32) and stimulant of nociceptive itch nerves (31, 33).

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 gastrointestinal tract, platelets and specific regions of the CNS (34-36). It is released after tissue injury, and it exerts algesic and analgesic effects depending on the site of action and the receptor subtype (37). 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 (38), 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 (37, 38). 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 receptor (NK-1) (39). SP is thought to transmit nociceptive information and contribute to occurrence of pathological pain states such as inflammation and nerve injury (40); it regulates vessel tone and moderates vascular permeability (41).

Many of the effects of inflammation are mediated by three interrelated plasma-derived factors, the kinins, the clotting system and the complement system, all linked by the initial activation of Hageman factor or factor XII of the intrinsic coagulation cascade (32). Kinins are a group of potent vasodilator peptides (42). Kinins, including bradykinin and kallidin, that are produced and act at the site of tissue injury or inflammation. In the periphery the actions of kinins include vasodilatation, increased vascular permeability and the stimulation of immune cells and peptide-containing sensory neurones to induce pain (43). Bradykinin receptor 1 (B_1) and bradykinin receptor 2 (B_2) are G-protein coupled receptors which mediate kinin effects (44, 45). In normal tissue bradykinin causes an acute sensation of pain by an action at B_2 receptors, but in inflamed tissue the pharmacology of the response changes to that of B_1 receptors (46). Kinins are potent stimulators of neural and neuroglial tissues inducing the synthesis and release of other pro-inflammatory mediators such as prostanoids and cytotoxins (cytokines, free radicals, nitric oxide) (43).

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 concurrently 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 (47, 48).

The complement system plays an important role in both immunity and inflammation. Complement components, C3a and C5a also called anaphylatoxins increase vascular permeability and cause vasodilation by inducing mast cells to release histamine. C5a also activates the lipoxygenase pathway of arachidonic acid metabolism in neutrophils and monocytes causing further release of inflammatory mediator. C5a activates leukocytes and increases the affinity of their integrins, thereby increasing adhesion to endothelium. It is also a potent chemotactic agent for neutrophils, monocytes, eosinophils and basophils. C3b and C3bi when fixed to a microbial surface act as opsonins, augmenting phagocytosis by cells bearing C3b receptors (neutrophils and macrophages) (48).

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 secretion is stimulated by endotoxin, immune complexes, toxins, physical injury, or a variety of inflammatory mediators (32). IL-1 and TNF induce endothelial activation with increased expression of adhesion molecules, secretion of additional cytokines and growth factors, production of eicosanoids and nitric oxide (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 (13). 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 importants role in mediating the hypotensive effects of septic shock, including diminished myocardial contractility and vascular smooth muscle relaxation (49).

Nitric oxide (NO) is a short half-life, soluble, free radical gas produced by a variety of cells. NO plays multiple roles in inflammation including relaxation of vascular smooth muscle, promotes edema and vascular permeability. NO stimulates the synthesis of inflammatory prostaglandins by activation of COX-2. Thus, inhibition of the NO pathway may have a beneficial effect on inflammatory diseases (50).

Arachidonic acid (AA) metabolites also called eicosanoids. This products derived from the metabolism of AA affect a variety of biologic processes, including inflammation and hemostasis (51). 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 two major pathways, cyclooxygenase (COX) and lipoxygenase (LOX) (49). The scheme of the major metabolic transformations of AA is shown in Figure 1.

In the COX pathway, products are prostaglandin E_2 (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 prostaglandin product from platelets. Endothelium possesses prostacyclin synthase, 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 prostaglandins are also involved in the pathogenesis of pain and fever in inflammation (51, 52). COX exists in two main isoforms, COX-1 and COX-2. COX-1 is expressed in gastric mucosa and mediates a “housekeeping” function (41, 51). 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 (53, 54). There are several lines of evidence to support the notion that COX-derived products are important mediators of inflammation, i.e., PGs synthesis is increased at sites of inflammation (32).

COX is an important enzyme that is inhibited by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Recently, an alternative splice variant of COX-1 that is selectively inhibited by acetaminophen has been identified and called COX-3 (55, 56). 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 (55).

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 (57). 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-AS) (58), which cause vasoconstriction, bronchospasm, and increased vascular permeability (32, 58).

Platelet-activating factor (PAF) 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. PAF enhances leukocyte adhesion, chemotaxis, leukocyte degranulation and the oxidative burst and also stimulates the synthesis of other mediators, particularly arachidonic acid (AA) metabolites (51). The summary of the major biological activities of chemical mediators of inflammation is shown in Table 1.

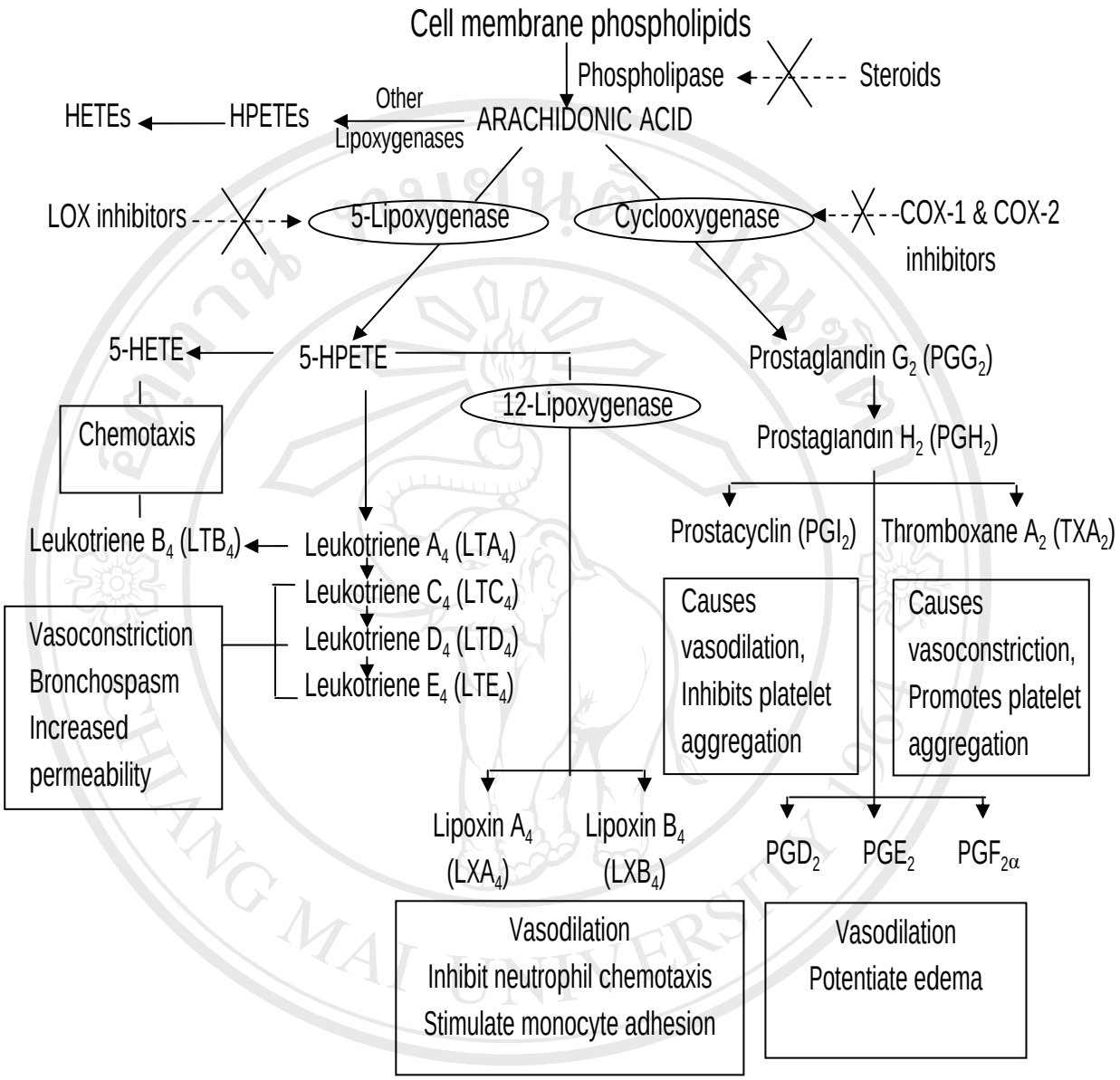

Figure 1. Scheme of the major metabolic transformation of arachidonic acid (32).

Table 1. Effect of inflammation and their major mediators (13).

Major Biological Activities	Chemical mediator
Vasodilation	Prostaglandins Nitric oxide
Increased vascular permeability	Vasoactive amines (histamine, serotonin) C3a and C5a (By release of vasoactive amines) Bradykinin Leukotrienes C_4, D_4, E_4 Platelet-activating factor
Chemotaxis, Leukocyte activation	C5a Leukotrienes B_4 Chemokines(e.g. interleukin 8 [IL-8].)
Fever	IL-1, IL-6, Tumor necrosis factor Protaglandins
Pain	Prostaglandins Bradykinin
Tissue damage	Neutrophil and macrophage lysosomal enzymes Oxygen metabolites Nitric oxide

NON STEROIDAL ANTI-INFLAMMATORY DRUGS

Non steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used for inflammation therapy (57, 59). The mechanism of action of NSAIDs involves inhibition of COX. NSAIDs have three major actions which are anti-inflammatory action, analgesia and antipyrexia (60). The anti-inflammatory action is exerted via the decrease in vasodilator PGs (PGE_2 and PGI_2), thus inhibiting their effects and resulting in less vasodilation and edema. This antiprostaglandin effect is considered to be the primary mechanism by which these drugs produce both therapeutic and adverse effects. All NSAIDs have side effects i.e. NSAID related gastropathy (the most common serious side effect), reversible NSAID nephropathy, dermal complications, hematologic complications, hepatic complications, central nervous system complications, pulmonary complications, etc. (61). Aspirin and traditional NSAIDs are nonselective COX inhibitors because they inhibit both COX-1 and COX-2. COX-1 is expressed in the gastric mucosa, and the mucosal prostaglandins generated by COX-1 are protective against acid-induced damage (49). Thus inhibition of cyclooxygenase by aspirin or NSAIDs also causes gastric ulceration (62). To preserve the anti-inflammatory effects of COX inhibition but prevent the harmful effect on gastric mucosa, highly selective COX-2 inhibitors are now available (63). They were design to relieve pain, fever, and inflammation as effectively as older NSAIDs, but with fewer adverse effects, especially stomach damage (64). Analgesic effect of NSAIDs is due to a decrease of PGs generation resulting in less sensitization of nociceptive nerve endings to inflammatory mediators. 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 hypothalmic 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 pathways (62). Anti-inflammatory steroids such as cortisol inhibit the release by phospholipase A_2 of arachidonic acid from phospholipids and thus inhibit formation of all arachidonic acid derivatives (65).

1.2 EXPERIMENTAL MODELS USED IN THE PRESENT STUDY

1.2.1 INFLAMMATORY MODELS

1.2.1.1 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 produced in hind paw of rats by injection of phlogistic agents such as brewer's yeast, formalin, dextran, egg albumin, carrageenin, arachidonic acid. The most commonly used phlogistic agent is carrageenin (66, 67). Carrageenin is a sulphate polysaccharide which has been fractionated with potassium chloride into two separate components, kappa and lambda carrageenin (68). 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 (66). In this model, the edema is produced by a sequential release of pharmacological mediators; histamine, 5-HT, kinins and PGs (69). 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 (70).

1.2.1.2 Arachidonic acid-induced hind paw edema in rats

Arachidonic acid-induced hind paw edema in rats provides a valuable tool for evaluating the in vivo anti-inflammatory activity of lipoxygenase inhibitors and other agents with a mechanism of action different from COX inhibition. Leukotrienes (LTs), 5-lipoxygenase products of AA, are involved as inflammatory mediators. Leukotriene C_4, LTD_4, and LTE_4 cause edema together with increase microvascular permeability (71). 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 arachidonic acid-induced rat paw edema is its sensitivity to dual inhibitors of arachidonic acid metabolism (such as phenidone), lipoxygenase inhibitors and corticosteroids but is insensitive to selective cyclooxygenase inhibitors (72).

1.2.1.3 Cotton pellet-induced granuloma formation in rats

Cotton pellet-induced granuloma formation in rat 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 component 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, define as the increase in wet weight of the pellet which occurs during the first three hour 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. 3) proliferative phase, measured as the increase in dry weight of the granuloma which occurs between three and six days after implantation (73).

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 was markedly increased during inflammation (74). Alkaline phosphatase activity in pouch wall was 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 indicates the activity of agents on the production and release of alkaline phosphatase in chronic inflammation (75).

1.2.2 ALGESIC MODEL

1.2.2.1 Formalin test

The formalin test is often considered an appropriate model of clinical pain because the nociceptive stimulus, tissue injury by injection of the irritating chemical reagent, induces continuous pain (76, 77). The formalin test in mice is a valid and reliable model of nociception and it 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 (78). 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 steroid. Centrally acting analgesics inhibited both phases (78-81).

1.2.3 PYRETIC MODEL

1.2.3.1 Yeast-induced hyperthermia in rats

This method appears to be reproducible and accurate for assaying non-narcotic antipyretic compound (82). 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. PG act on the thermoreceptive region in the preoptic anterior hypothalamus and raise the set point of the temperature regulating center, which leads to increase body temperature. IL-1B, IL-6, β-IFN, γ-IFN and TNF-α can act independently to produced fever (83). 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 (54, 83).

1.2.4 ACUTE TOXICITY

Acute toxicity is the model used for evaluation the toxic characteristics of substance. Acute oral toxicity is the adverse effects occurring a short time of oral administration of a high single dose of substance or multiple doses given within 24 h. The study is conducted to provide information about the mode of toxic action of a substance and to confirm the mortality. If the one dose level of at least 5000 mg/kg body weight, using the procedures described for the study, produces no compound-related mortality, then a full study using three dose levels may not be necessary (84, 85).

1.3 HISTORICAL BACKGROUND OF PLANTS

Murdannia loriformis (Hassk) Rolla Rao et Kammathy is a herb in the family Commelinaceae (86). Many plants in the family Commelinaceae have also been used as folk medicines in Central and South America, for wound healing, diuretic and astringent properties (87). Thai name of *M. loriformis* is “Ya Pak King”. It has 1.5 cm. width and 10 cm. length leaf. The flower shows on the stem in blue or purple petal. It needs just a small patch of land, thrives in loose and sandy soil and under the shade, such as a large tree. It is originated in southern China and is abundant in northern Thailand. Chinese practitioners use it as a remedy for cancers in early state, as well as for treating other diseases including colds, throat infections, pneumonia (88), flu (88, 89), and for facilitating the wound healing (88). In 1984, *M. loriformis* became famous in Thailand when a cancer patient recovered after drinking its fresh juice. It is claimed to have a potential for treating, healing or preventing cancer by strengthening the immune system (88, 90). Now many cancer patients also take it to reduce side effects from radiotherapy and chemotherapy (88). It is also used by patients with diabetes mellitus (88). The study of the hypoglycemic effect of *M. loriformis* was investigated in glucose-induced hyperglycemic mice. The result showed that, the hot water and ethanol extracts significantly reduced blood glucose of the mice (91). For using this plant, it is prepared by grinding about 100-120 g of fresh aerial plant part, then adding 4 tablespoons (60 ml)

of clean water. The extract is filtered through thin cloth; for best results the patient takes the filtrate orally twice a day in the morning and evening, before meals (88, 92).

The chemical constituents of *M. loriformis* were reported to be phytosteryl glucoside, glycosphingolipid, amino acid, flavonoids, and plant membrane lipid (93-95). Phytochemical study of *M. loriformis* by Jiratchariyakul (1996) revealed that, ethanol extract contains phytosterylglycoside (G1a) and glycosphingolipid (G1b). The structures were identified as 3-βO-D-glycopyranosyl-24 ξ-ethyl-cholest-5-ene (G1a), 1-β -O-D-glycopyranosyl-2(2′-hydroxy-6′-ene-cosamide)-sphingosine (G1b) (96). G1b showed a moderate cytotoxicity with ED_{50} of 16 μg/ml against human breast ATCC HTB20 (BT474) and colon (SW620) cancer cell lines, and also stimulated the proliferation of lymphocyte (97). In 1999 Narintorn was first reported new component, ceramide (MC1). MC1 structures was identified as 3β -hydroxyl-3-(1′-hydroxy-hexadecane)-2-(2′′-hydroxy-tetracosanamide)-propane-1-ol (98).

The study on antimutagenicity reported that ethanol extract of *M. loriformis* had non-mutagenic effect in *salmonella typhimurium* TA 98 and TA 100 with and without metabolic activation and exerts antimutagenicity toward various kinds of mutagens (99-101). It inhibits azoxymethane (AOM)-induced aberrant crypt focus (ACF) formation in the initiation stage in rat colon but not growth or differentiation in promotion stage (102). The possible mechanism of chemoprotection of *M. loriformis* extract in initiation stage may be through selective alteration of enzymes in xenobiotic-metabolizing systems i.e. aminopyrine-N-demethylase (APD), UDP-glucuronyl transferase (UDP-GT) and glutathione S-transferase (100). Its ethanol extract shows anticarcinogenic enzyme, DT-diaphorase induction (99, 101). In addition, it shows mild antioxidant activity (100-102). The water and ethanol extracts of *M. loriformis* do not show any cytotoxic and anti-proliferative effects on leukemic cell lines (103). Others reported that the immune response of the pressed juice has no cytotoxic effect to the peripheral blood mononuclear cell (PBMC), but directly stimulates the proliferation of lymphocytes (104). Cancer studies indicate that cancer develops when the body's immune system is weakened, therefore a strengthened immune system is crucial in the fight against cancer (88, 105).

Pharmacological study of pressed juice in rats has not shown any acute toxicity or abnormalities in growth, blood chemistry or other pathology of the internal organs. The LD_{50} in the rats is more than 120 g/kg or about 300 times of human dose (106). Rats which have received pressed juice in the concentration of 2.8, 7.0 and 14 g/kg for 3 months do not show any signs of chronic toxicity (107). Recent study revealed that the ethanol extract of *M. loriformis* shows antinociceptive effect in pain model induced by formalin. The screening of anti-inflammatory property revealed that *M. loriformis* extract inhibits ear edema in rats induced by ethyl phenyl propiolate (EPP) (108).

1.4 HYPOTHESIS

Murdannia loriformis was proved to be effective as an anticancer, antimutagen, anticarcinogenic enzyme inducer and antioxidant agent. There are several lines of evidence about close relationship between cancer and inflammation. Cyclooxygenase-2 (COX-2) is highly express in many types of cancer including breast and colon cancer (109-117). Chronic inflammation may be important in the pathogenesis of colorectal carcinoma. The study in rats suggests that *M. loriformis* inhibits the initial phase of colon cancer, but not the growth or differentiation in promotion stage (102). The component of *M. loriformis*, G1b showed a moderate cytotoxicity against human breast and colon cancer cell lines (93). Therefore this plant may possess anti-inflammatory effect as well. Analgesic and antipyretic effects are related to anti-inflammatory activity and also useful in cancer pain and fever. The hypothesis of this study is therefore, *M. loriformis* possesses anti-inflammation, analgesic and antipyretic effects.

1.5 PURPOSE OF THE STUDY

The purpose of the present study was to evaluate the anti-inflammatory, analgesic and antipyretic activities of the ethanol extract from *M. loriformis* in various animal models in comparison with reference drugs such as indomethacin, phenidone, codeine and prednisolone. The mechanism of action of the ethanol extract from *M. loriformis* on the inflammatory process and its effect on the gastric mucosa were also examined in comparison with indomethacin and prednisolone. The acute toxicity of the ethanol extract of this plant was also determined.

Figure 2. *Murdannia loriformis* (Hassk.) Rolla Rao et Kammathy.