CHAPTER 1
INTRODUCTION

1.1 Herbal medicines

Herbal medicines, as the major remedy in traditional medical systems, have made a great contribution to maintaining human health [1]. They are readily available, affordable, effective and culturally-acceptable health care modalities [2]. A majority of the world's population still relies on herbal medicines to meet their health needs [1].

Diseases characterized by inflammation, such as rheumatoid arthritis, are one of the important causes of morbidity and disability in human [3]. Patients affected by these diseases need medicines chronically to control their inflammatory-associated symptoms and to arrest or slow progression of diseases. However, many patients do not respond to the conventional used drugs and/or cannot tolerate to their side effects. Therefore, they are turning increasingly to the use of herbal medicines as an alternative treatment. In addition, the relatively very high cost of some newer drugs with lower side effects may be the potential reason for these patients to turn into this alternative therapy, especially in developing countries, including Thailand. The Thai government has also set up a national plan for the development of medicinal plants in order to build-up self-reliance on herbal medicine supplies [4].

1.2 Inflammation

Inflammation normally is a localized protective response to an injurious stimulus evoked by a wide variety of noxious stimuli (e.g., microorganisms, foreign particles, or physical injuries). The primary functions of the inflammatory response are the elimination of the pathogenic insult and the removal of injured tissue components by phagocytosis, and this process involves the reactive oxygen species (ROS) production and the release of tissue-damaging enzymes, including protease

and myeloperoxidases. Historically, inflammation has been referred to as either acute or chronic, depending on the persistence of the injury, clinical symptoms and the nature of inflammatory response. Acute inflammation, which is characterized by the classic signs of pain, heat, redness, swelling and loss of function [3, 5], has rapid onset (seconds or minutes) and short duration (minutes, hours or few days). The hallmarks of acute inflammation include (1) accumulation of fluid and plasma components in the affected tissue (edema), (2) intravascular stimulation of platelets, and (3) the presence of polymorphonuclear leukocytes (PMNs). Acute inflammation normally resolves by mechanisms that have remained somewhat elusive. The resolution initiates in the first few hours after an inflammatory response begins. After entering tissues, granulocytes promote the switch of arachidonic acid (AA)–derived prostaglandins (PGs) and leukotrienes (LTs) to lipoxins (LXs), which initiate the termination sequence. Neutrophil recruitment thus ceases and programmed death by apoptosis is engaged. These events coincide with the biosynthesis of resolvins and protectins, which critically shorten the period of neutrophil infiltration by initiating apoptosis. Consequently, apoptotic neutrophils undergo phagocytosis by macrophages, leading to neutrophil clearance and release of anti-inflammatory and reparative cytokines such as transforming growth factor-β1 (TGF-β1) [6]. By contrast, chronic inflammation has longer duration with persistence of inflammatory cells and tissue damage, and often results in aberrant repair. The characteristic cell components in chronic inflammation are lymphocytes, plasma cells, and macrophages [5, 7].

Several mechanisms are involved in the promotion of the inflammatory process. Recent work has focused on adhesive molecules, including the E-, P-, and L-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and leukocyte integrins, in the adhesion of leukocytes and platelets to endothelium at sites of inflammation. Activated endothelial cells play a key role in targeting circulating cells to inflammatory sites. Cell adhesion occurs by recognition of cell-surface glycoproteins and carbohydrates on circulating cells due to the augmented expression of adhesion molecules on resident cells. Thus, endothelial activation results in leukocyte adhesion as the leukocytes recognize newly expressed L-, and P-selectin. The scheme of interactions between cell-adhesion molecules

during the initial binding and tight binding of leukocytes to activated endothelial cells is shown in Figure 1. Recruitment of inflammatory cells to sites of injury involves the concerted interactions of several types of soluble mediators in regulation of inflammatory response [8]. Specific inflammatory mediators [e.g., histamine, serotonin, bradykinin, anaphylatoxins, LTs, PGs, platelet activating factor (PAF), and nitric oxide (NO)] produced at the sites of injury regulate this response of the vasculature to injury. Among these mediators are vasoactive molecules that act directly on the vasculature to increase vascular permeability [5].

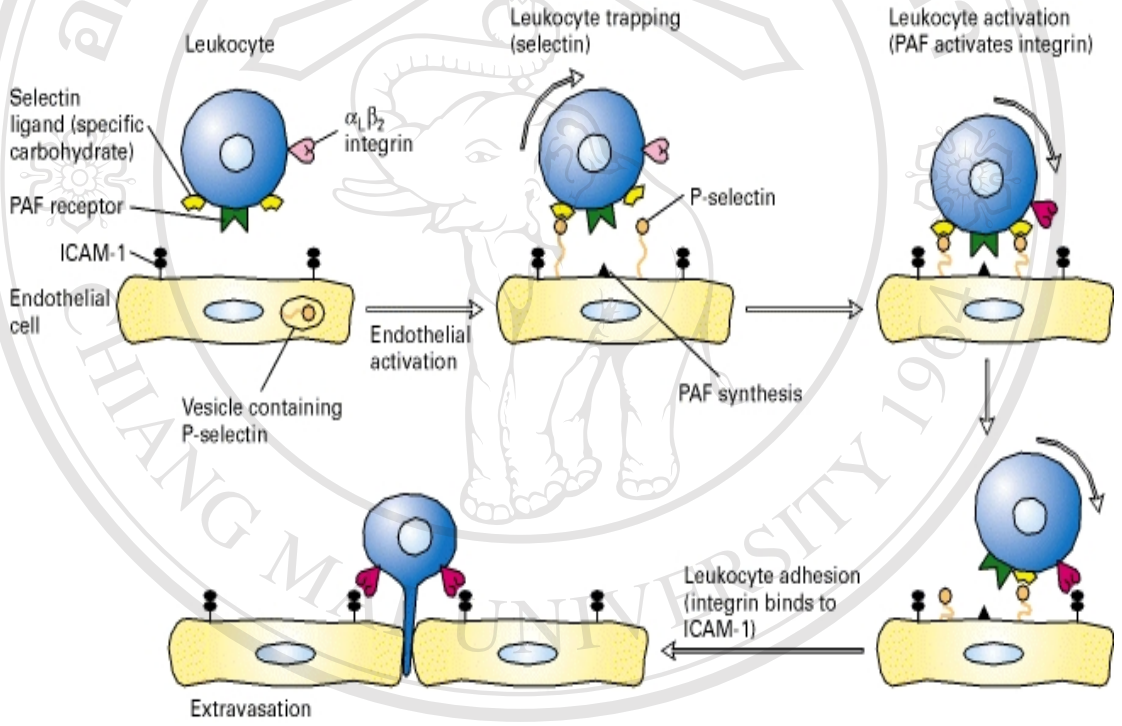

Figure 1 Interactions between cell-adhesion molecules during the initial binding and tight binding of leukocytes to activated endothelial cells [9].

Phospholipids and fatty acid derivatives released from plasma membranes are metabolized into mediators and homeostatic regulators by inflammatory cells and injured tissues. As part of a complex regulatory network, prostanoids, LTs and LXs, which are derivatives of AA that is metabolized from phospholipids by phospholipase A_2 (PLA_2), can promote and/or inhibit inflammation. AA is further metabolized by

cyclooxygenase-1 (COX-1) and COX-2 to generate prostanoids including PGs and thromboxane A_2 (TXA_2). Various prostanoids also play key roles in the inflammatory process, such as PGD_2, PGE_2, and prostacyclin (PGI_2) induce vasodilatation, inhibit inflammatory cell function and bronchodilatation; $PGF_{2\alpha}$ induces vasodilatation and bronchoconstriction; and TXA_2 induces vasoconstriction, enhances inflammatory cell function (especially platelets) and bronchoconstriction. COX-1 is constitutively expressed by most cells, although some studies suggest it may increase upon cell activation. It is a key enzyme in the synthesis of PGs, which protect the gastrointestinal mucosal lining, regulate water/electrolyte balance, stimulate platelet aggregation to maintain normal hemostasis, and maintain resistance to thrombosis on vascular endothelial cell surfaces. COX-2 expression is generally low but increase substantially upon stimulation, generating metabolites important in the induction of pain and inflammation. The early inflammatory prostanoid response is COX-1 dependent, then COX-2 becomes the major source of prostanoids as inflammation progresses. Both COX isoforms generate PGH_2, which is then the substrate for the production of prostanoids. The profile of PG production depends in part on the cells present and their activation state. Thus mast cells produce predominantly PGD_2; macrophages generate PGE_2 and TXA_2; platelets are the major source of TXA_2; and endothelial cells produce PGI_2. Prostanoids affect immune cell function by binding to G protein coupled cell surface receptors, leading to the activation of a range of intracellular signaling pathways in immune cells and resident tissue cells. PGF_2, PGI_2, and TXA_2 bind individual receptors, whereas PGD_2 and PGE_2 bind multiple receptors and receptor subtypes. The repertoire of prostanoid receptors expressed by various immune cells differs, and the functional responses of these cells are, therefore, modified differently according to the prostanoids present. LTs, the second major family of AA derivatives, are metabolized by 5-lipoxygenase (5-LOX). LTA_4 is metabolized to LTB_4, a compound with potent chemotactic activity for neutrophils, monocytes, and macrophages. In other cell types, especially mast cells, basophils, and macrophages, LTA_4 is converted to LTC_4 followed by LTD_4 and LTE_4. The mixture of these LTs is called cysteinyl-LTs or slow-reacting substances of anaphylaxis (SRS-A). The SRS-A stimulate the contraction of smooth muscle, enhance vascular permeability and are responsible for the development of many

clinical symptoms associated with allergic-type reactions. LXs, the third family of AA products, are generated within the vascular lumen by cell-cell interactions. LXs are proinflammatory mediators and expressed during inflammation, atherosclerosis and thrombosis. Several cell types can synthesize LXs from LTs. LTA_4, released by activated leukocytes is available for transcellular enzymatic conversion by neighboring cell types. When platelets are adherent to neutrophils, LTA_4 from neutrophils is converted by platelet 12-lipoxygenase, resulting in the formation of LXA_4 and LXB_4. Monocytes, eosinophils and airway epithelial cells generate 15*S*-hydroxyeicosatetraenoic acid (15*S*-HETE) which is taken up by neutrophils and converted to LXs via 5-LOX. Activation of this pathway can also inhibit LTs biosynthesis, thereby providing a regulatory pathway. Moreover, aspirin initiates the transcellular biosynthesis of a group of LXs termed aspirin-triggered lipoxins, or 15-epimeric-lipoxins (15-epi-LXs). When aspirin is administered in the presence of inflammatory mediators, 15*R*-HETE is generated by COX-2. Activated neutrophil then converts 15*R*-HETE to 15-epi-LXs which are anti-inflammatory lipid mediators [5].

In addition, several cytokines also play essential roles in orchestrating the inflammatory process including the increase of body temperature. These cytokines, especially interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α) and interferons (IFNs), are called endogenous pyrogens. IL-1 stimulates PGE_2 synthesis in the hypothalamus thermoregulatory centers, thereby altering the thermostat that controls body temperature, whereas TNF-α and IL-6 also increase body temperature by a direct action on the hypothalamus [5, 10]. IL-1 and TNF-α are considered principal mediators of the biological responses to bacterial lipopolysaccharide (LPS, also called endotoxin). They are secreted by monocytes, macrophages, adipocytes and other cells. Working in concert with each other and various cytokines, including IL-8 and IFN-γ, they induce gene expression and protein synthesis in a variety of cells to mediate and promote inflammation [8].

1.3 Drugs for the treatment of inflammation

The treatment of inflammation involves two primary aims: first, the improvement of pain which is often the major symptom of patients; and second, the

delay of the tissue-damaging process [11]. At present, the anti-inflammatory drugs commonly used are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids.

NSAIDs are anti-inflammatory, analgesic, and antipyretic drugs. The exception is acetaminophen which has antipyretic and analgesic activities but is largely devoid of anti-inflammatory activity. When employed as analgesics, these drugs usually are effective only against pain of low to moderate intensity, such as dental pain, myalgia and arthralgia. It seems logical to select NSAIDs with rapid onset for the management of fever associated with minor illness. NSAIDs find their clinical application as anti-inflammatory agents in the treatment of musculoskeletal disorders, such as rheumatoid arthritis and osteoarthritis. In general, NSAIDs provide only symptomatic relief from pain and inflammation associated with the disease but do not arrest the progression of pathological injury to tissue [8]. NSAIDs inhibit PGs biosynthesis by blocking COX, both COX-1 and COX-2. The inhibition of COX-1 is directly related to many adverse effects, particularly gastrointestinal bleeding, hypertension, and renal insufficiency. At present, this problem leads to the development of specific COX-2 inhibitors [5]. All of the selective COX-2 inhibitors have been shown to be less prone than equally efficacious doses of traditional NSAIDs to induce endoscopically visualized gastric ulcers and this has provided the basis of Food and Drug Administration approval. However, selective inhibitors of COX-2 are thought to be problematic in cardiovascular system. They depress PGI_2 formation by endothelial cells without concomitant inhibition of platelet TX. PGI_2 restrains the cardiovascular effects of TXA_2, affording a mechanism by which selective inhibitors might increase the risk of thrombosis; consistent with results from postmarketing trials of rofecoxib. Placebo-controlled trials have now revealed an increased incidence of myocardial infarction and stroke in patients treated with rofecoxib, valdecoxib and celecoxib, consistent with a mechanism-based cardiovascular hazard for the class. Regulatory agencies in the United States, Europe and Australia have concluded that all three drugs increase the risk of heart attack and stroke and will be labeled accordingly and restricted with respect to marketing directly to consumers. Only celecoxib remains on the market in the United States [8].

Corticosteroids, especially glucocorticoids (e.g., prednisolone, dexamethasone), potently suppress inflammation and tissue destruction in a variety of inflammatory and autoimmune diseases through various mechanisms. They can block pathway of eicosanoid synthesis, stimulate the synthesis of inhibitory protein lipocortin-1 (annexin-1) that inhibits PLA_2 leading to the reduction of PGs and LTs synthesis [12]. Moreover, they can modulate cytokine and chemokine production, inhibit accumulation of leukocytes and decrease vascular permeability as well. However, since they exert effects on almost every organ system, the clinical use of and withdrawal from corticosteroids can be complicated by a number of serious side effects, such as gastric and intestinal ulcers, infections, bone resorption, fluid retention, electrolyte imbalance and atrophy of the adrenal glands, and some of which are life-threatening [8]. The scheme of steps in the biosynthesis of eicosanoids and sites of drug action is shown in Figure 2.

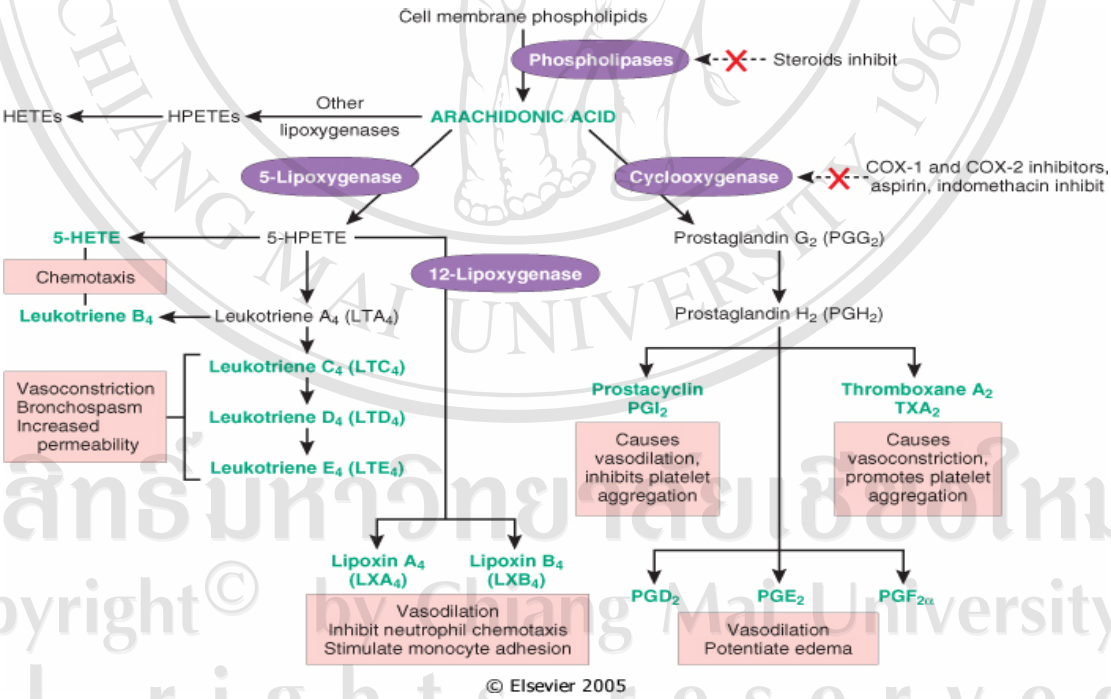

Figure 2 Steps in the biosynthesis of eicosanoids and sites of drug action [7].

1.4 Historical background of *Stahlianthus involucratus* (King ex Baker) Craib ex Loes.

Stahlianthus is a genus in the Zingiberaceae family, at least 7 species can be found in East and Southeast Asia [13]. The pharmacological and phytochemistry studies of plants in this genus have not been reported yet. *S. involucratus*, one of plants in this genus, has been discovered in the name of *Kaempferia involucrata* in 1890 [14] and then changed to *S. involucratus* in 1930 [15]. Its local names are "Tu tian qi" in Chinese [16] and "Easkine" in Bangladesh [17]. It is distributed in the forest and on the mountains of China, India, Laos, Myanmar, Sikkim, Vietnam, Cambodia, and also Thailand [16]. It is a very small cute plant (about 18 inches height) and grows better in bright shade. Its rhizomes are brown-yellow inside and strongly fragrant. Its leaves are green or purplish and obovate-oblong. White flowers appear among the leaves in late summer [14, 15]. The pictures of *S. involucratus* are shown in Figure 3.

The Zingiberaceae, or the Ginger family, is a large family of perennial herbaceous plants that contains at least 1,200 species of 53 genera in the world [18] and about 1,000 species in tropical regions of Asia, including Thailand [19]. There were variety uses of plants in this family in folk remedies for the treatment of several disorders such as swelling [20], asthma, cough, and pain [21]. These plants are also useful for digestive [22], rheumatic and liver disorders [23], as well as skin diseases [24]. Its rhizomes had been used in Chinese traditional medicine for promoting blood circulation, reducing pain and treating rheumatism [16], whereas, in Bangladesh, they have been used to alleviate fever [17]. Several pharmacological activities of plants in Zingiberaceae family, such as anti-inflammatory and analgesic [25]; antipyretic [26]; antibacterial [27]; antifungal and antiviral [28]; anticancer [29]; antioxidant [30]; vasorelaxant [31]; anti-emetic [32]; and sedative [33] activities, had been shown in many scientific evidences.

The anti-inflammatory effect of Zingiberaceae rhizomes was the most recognized effect that had been found in a variety of species, such as *Alpinia officinarum* [34, 35], *Zingiber officinale* Roscoe [36, 37], *Curcuma aeruginosa* Roxb. [21, 38, 39], and *C. longa* L. [40]. Their scientific evidences of anti-inflammatory activity, *in vivo* and *in vitro*, were as follows. The methanol extract of *Curcuma*

longa L. showed anti-inflammatory effect in carrageenin-induced paw edema and cotton pellet granuloma growth in rats [41]. It can inhibit LT formation in rat peritoneal polymorphonuclear neutrophils [42], and also inhibit the activities of PL, LOX, COX-2, LTs, TXs, PGs, NO, collagenase, elastase, hyaluronidase, TNF-α, and IL-12 [43], *in vitro*. The methanol extract of *A. officinarum* showed inhibitory effects of PGs, LTs [34, 35], and nitric oxide synthetase (iNOS) activities [44]. The hydroalcoholic extract of *Z. officinale* Roscoe can reduce carrageenin-induced paw swelling in rats [45], inhibit the production of pro-inflammatory cytokines from rat peritoneal macrophages stimulation *in vitro* [46], and reduce serum levels of IL-1β, IL-2, IL-6, and TNF-α in collagen-induced arthritic rats [37]. The methanol extract of *C. aeruginosa* Roxb. showed the reduction of acetic acid-induced writhing response in mice, and the chloroform extract can suppress the licking activity in late phase of the formalin test in mice [39]. The biological active compounds of Zingiberaceae family plants that exerted anti-inflammatory activity include, for example, gingerol and shogaol of *Z. officinale* Roscoe extract [28], phenylpropanoid, flavonoid, neolignan and diarylheptanoid of *A. officinarum* extract [34, 35], and curcuminoids especially curcumin of *C. longa* L. extract [47]. Although, in traditional medicine, *S. involucratus* has been used for anti-inflammation, analgesic, and antipyretic [16, 17], however, the scientific evidences for its pharmacological activities have not been reported yet.

Figure 3 *Stahlianthus involucratus* (King ex Baker) Craib ex Loes.

1.5 Hypothesis

S. involucratus rhizomes ethanol extract (SI extract) possesses anti-inflammatory, analgesic, and antipyretic activities.

1.6 Purpose of the study

To verify the anti-inflammatory, analgesic, and antipyretic activities of SI extract in various animal models.