CHAPTER 1
INTRODUCTION

1.1 The pathophysiology of inflammation

Inflammation is a complex reaction to injurious agents such as microbes and damaged, usually necrotic cells that consists of vascular responses, migration and activation of leukocytes, and systemic reactions. Invertebrates with no vascular system, and even single-celled organisms, are able to get rid of injurious agents such as microbes by a variety of mechanisms. These mechanisms include entrapment and phagocytosis of the offending agent, sometimes by specialized cells (hemocytes), and neutralization of noxious stimuli by hypertrophy of the host cell or one of its organelles. These cellular reactions have been retained through evolution, and the more potent defensive reaction of inflammation has been added in higher species. The unique feature of the inflammatory process is the reaction of blood vessels, leading to the accumulation of fluid and leukocytes in extravascular tissues [1].

Inflammation is divided into acute and chronic patterns. Acute inflammation is rapid in onset (seconds or minutes) and is of relatively short duration, lasting for minutes, several hours, or a few days; its main characteristics are the exudation of fluid and plasma proteins (edema) and the emigration of leukocytes, predominantly neutrophils. The major local manifestations of acute inflammation are shown in Figure 1. Chronic inflammation is of longer duration and is associated histologically with the presence of lymphocytes and macrophages, the proliferation of blood vessels, fibrosis, and tissue necrosis.

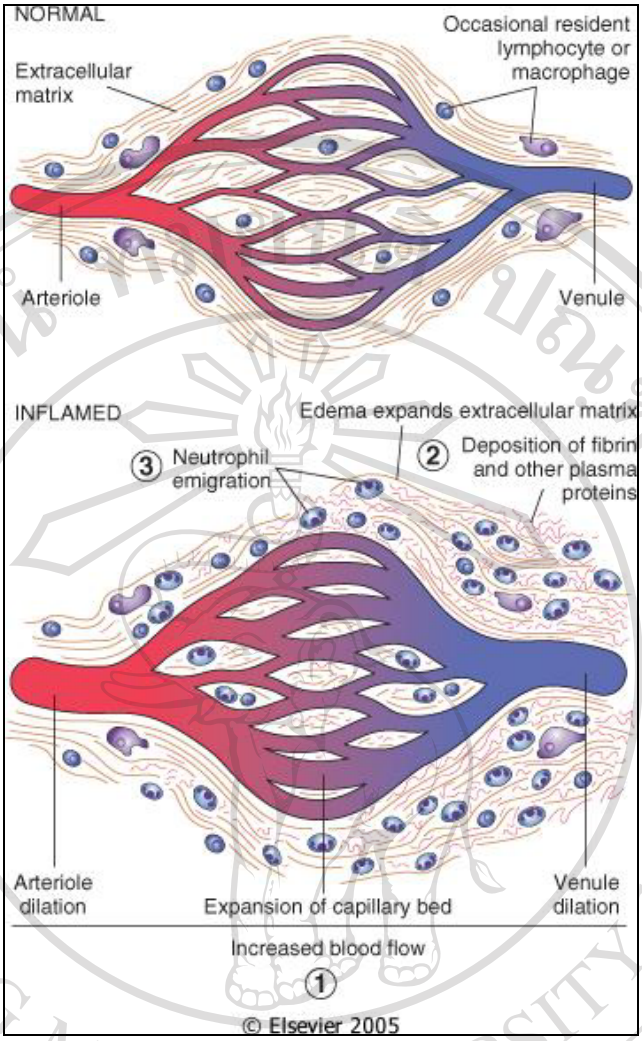

Figure 1. The major local manifestations of acute inflammation, compared to normal. (1) Vascular dilation and increased blood flow (causing erythema and warmth), (2) extravasation and deposition of plasma fluid and proteins (edema), and (3) leukocyte emigration and accumulation in the site of injury [1].

Many factors modify the course and morphologic appearance of both acute and chronic inflammation. In acute inflammation, fluid loss from vessels with increased permeability occurs in distinct phases: (1) An immediate transient response lasting for 30 minutes or less, mediated mainly by the actions of histamine and leukotrienes on

endothelium; (2) a delayed response starting at about 2 hours and lasting for about 8 hours, mediated by kinins, complement products, and other factors (prostaglandin, prostacyclin, thromboxane A_2); and (3) a prolonged response that is most noticeable after direct endothelial injury, for example, after burns. The type of emigrating leukocyte varies with the age of the inflammatory response and with the type of stimulus. In most forms of acute inflammation, neutrophils predominate in the inflammatory infiltrate during the first 6 to 24 hours, then are replaced by monocytes in 24 to 48 hours [1]. They destroy invading pathogens and compounds by phagocytosis or opsonisation and these processes involve the production of reactive oxygen species and release of tissue-damaging enzymes sush as proteases and myeloperoxidases [2].

Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid (5, 8, 11, 14-eicosatetraenoic acid) that is derived from dietary sources or by conversion from the essential fatty acid linoleic acid [1]. It is released from membrane phospholipids through the activation of cellular phospholipases (e.g., phospholipase A_2) by mechanical, chemical, and physical stimuli or by other mediators (e.g., C5a). AA metabolites, also called eicosanoids, are synthesized by two principal enzyme pathways, the cyclooxygenase (COX) and the lipoxygenase (LOX) pathways [3, 4]. COX exists in two main isoforms, COX-1 and COX-2. COX-1 is expressed in gastric mucosa and mediates a “housekeeping” function [4]. It regulates several homeostatic processes such as renal blood flow, gastric cytoprotection and platelet aggregation, whereas 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 prostaglandin (PGs) [8, 9]. Comparison of the property of COX-1 and

COX-2 is present in Table 1. The COX pathway products are PGE_2, PGD_2, PGF_{2_α}, PGI_2 (prostacyclin), and thromboxane A_2 (TXA_2). TXA_2, a potent platelet aggregating agent and vasoconstrictor, is the major PGs product from platelets. PGD_2 is the major metabolite of the COX pathway in mast cells, along with PGE_2 and PGF_{2_α}. It causes vasodilation and edema formation. The PGs are also involved in the pathogenesis of pain and fever in inflammation [4, 7]. 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 [1]. The LOX pathway utilizes AA by 5-LOX to produce the LOX products which are also involved in inflammatory reactions as pro-inflammatory mediators [5, 10]. These are also involved in the inflammatory processes by enhancing vascular permeability and through chemotactic attraction of leukocytes [11]. The initial products of the LOX pathway are generated by three different types of LOX, which are present in only a few types of cells: 5-LOX is the predominant enzyme in neutrophils, it gives rise first to the main product, 5-hydroxyeicosatetrenoic acid (HETES), and then to peptide-leukotrienes and leukotrienes (LTs), i.e., LTB_4, LTC_4, LTD_4 and LTE_4. LTB_4 is a very potent chemotactic agent for neutrophils, eosinophils and macrophages, it causes the accumulation of polymorphonuclear leukocyte (PMN), generation of oxygen free radicals and release of lysosomal enzymes [12, 13]. In human skin, LTC_4, LTD_4 and LTE_4 cause transient wheal and flare responses by direct action or through the release of other endogenous mediators such as PGs [3, 14]. Owing to the contribution of LTs to the pathogenesis of many inflammatory processes, they also represent an important target for therapeutic regulation [10]. Generation of AA metabolites and their roles in inflammation are shown in Figure 2.

Table 1. Comparison of COX-1 and COX-2 [21].

PROPERTY	COX-1	COX-2
Expression	Constitutive	Inducible; not normally present in most tissues
Tissue location	Ubiquitous expression	Constitutive in parts of nervous system
Cellular localization	Endoplasmic reticulum (ER)	Inflamed and activated tissues , endoplasmic reticulum (ER) and nuclear membrane
Substrate selectivity	Arachidonic acid, eicosapentaenoic acids	Arachidonic acid, γ-linolenate, α-linolenate, linoleate, eicosapentaenoic acids
Role	Protection and maintenance functions	Pro-inflammatory and mitogenic functions
Induction	Generally no induction Human chorionic gonadotropin (hCG) can up-regulate COX-1 in amnion	Induced by bacterial lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), interleukin (IL-1, IL-2), epidermal growth factor (EGF), interferon-γ (IFN-γ)
Inhibition	In vivo: Anti-inflammatory glucocorticoids Pharmacologic: Non-steroidal anti-inflammatory drugs (NSAIDs)	In vivo: Anti-inflammatory glucocorticoids Pharmacologic: Non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 selective inhibitors

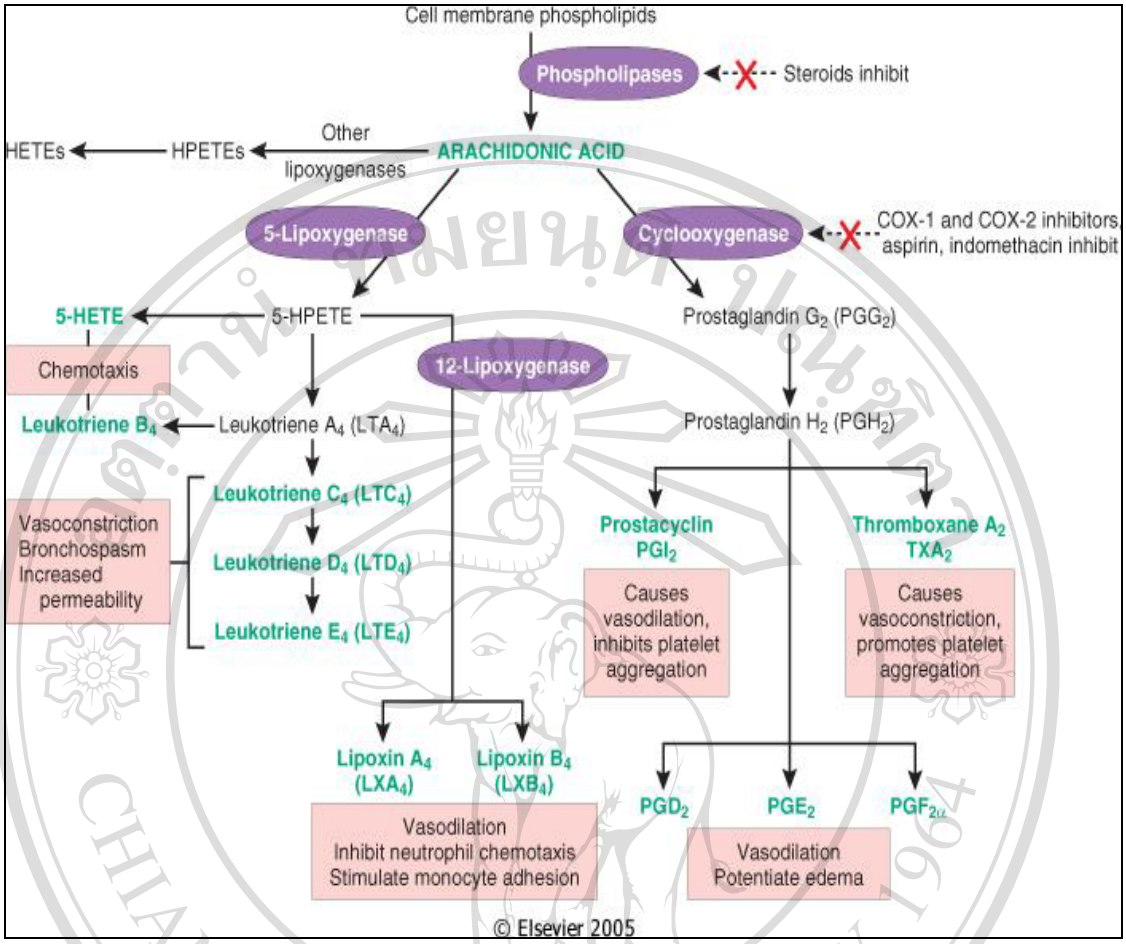

Figure 2. Generation of AA metabolites and their roles in inflammation and the molecular targets of action of some anti-inflammatory drugs [1].

Cytokines are polypeptide products of many cell types (but principally activated lymphocytes and macrophages) that modulate the function of other cell types. Long known to be involved in cellular immune response, these products have additional effects that play important roles in both acute and chronic inflammation [1]. Tumor necrosis factor (TNF) and interleukin-1 (IL-1) are the most relevant of cytokines, which exercise an influence on the inflammatory process [1, 15]. They produce many of the same pro-inflammatory responses which include mobilization and activation of PMNs; induction of COX and LOX enzymes; increasing in adhesion molecule expression; activation of B-cells, T-cells, and natural killer cells; and stimulation of production of other cytokines. Clearly, many of the events associated with acute inflammatory reaction can be mediated by TNF and IL-1. Other actions of these agents likely contribute to the fibrosis and tissue degeneration of chronic proliferation phase of inflammation; stimulation of fibroblast proliferation, induction of collagenase and activation of osteoblasts and osteoclasts [16]. As well as their important local effects, the cytokines produced by macrophages and neutrophils have long range effects that contribute the host defense. One of these is the elevation of body temperature, which is caused by IL-1, IL-6, TNF-ζ, and other cytokines. These are termed “endogenous pyrogens” [17]. Other cytokines, including IL-8 and interferon-γ (IFN-γ), exert additional effects such as increased chemotaxis for leukocytes and increased phagocytosis. All these effects result in the accumulation of fluid (edema) and leukocytic cells in the injured areas [16]. Chemical mediators of inflammatory response are demonstrated in Table 2.

Inflammation is terminated when the offending agent is eliminated and the secreted mediators are broken down or dissipated. In addition, there are active anti-

inflammatory mechanisms that serve to control the response and prevent it from causing excessive damage to the host [1].

Table 2. Chemical mediators of the inflammatory response [21].

Action	Mediators
Vasodilation	Prostaglandins (PGs): PGI_2, PGE_1, PGE_2, PGD_2 Nitric oxide (NO)
Increased vascular permeability	Histamine C3a, C5a (complement components) Bradykinin Leukotrienes (LTs), especially LTC_4, LTD_4, LTE_4 Platelet-activating factor (PAF) Calcitonin gene-related peptide (CGRP) Substance P
Chemotaxis, leukocyte recruitment and activation	C5a LTB_4, lipoxins (LX) LXA_4, LXB_4 Bacterial products
Tissue damage	Neutrophil and macrophage lysosomal products Oxygen radicals NO
Fever	IL-1, IL-6, TNF-α LTB_4, LXA_4, LXB_4
Pain	PGE_2, PGI_2 Bradykinin CGRP

1.2 Drugs for treatment of inflammation

The treatment of patients with inflammatory diseases involves two primary goals: first, the relief of pain which is often the presenting symptom and the major continuing complaint of the patients; and second, slowing of tissue-damaging processes [18]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used for inflammation therapy [19, 20].

NSAIDs are important because of their combined anti-inflammatory, antipyretic, and analgesic properties. The ultimate goal of most NSAID therapies is to inhibit the COX-mediated generation of proinflammatory eicosanoids and to limit the extent of inflammation, fever, and pain [21]. The anti-inflammatory activity of NSAIDs is mediated chiefly through inhibition of biosynthesis of PGs by inhibition of COX-2 and COX-1 activities [22]. Analgesic effect of NSAIDs is due to a decreasing of PGs generation resulting in less sensitization of nociceptive nerve endings to inflammatory mediators. 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 [23]. Side effects of these drugs, that resulted from blockage of the synthesis of endogenous PGs and TXA_2 include disturbances in gastrointestinal (i.e. dyspepsia, gastric irritation and gastric ulceration), renal (i.e. salt water retention leading to edema) and platelet function (i.e. increase the bleeding time) [9, 16, 24]. 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 [9, 22]. Despite the benefits of current NSAIDs, these drugs only suppress the signs of the underlying inflammatory response [21].

Anti-inflammatory corticosteroids such as dexamethasone and prednisolone, have powerful anti-inflammatory effect. They block all the known pathway of eicosanoid synthesis, perhaps by stimulating the synthesis of several inhibitory proteins collectively called anexins or lipocortins. They inhibit phospholipase A_2 activity, probably by interfering with phospholipid binding and thus preventing the release of AA. Although the use of corticosteroids as anti-inflammatory agents does not address the underlying cause of the disease, the suppression of inflammation is of enormous clinical utility and has made these drugs among the most frequently prescribed agents [24].

The side effects of the anti-inflammatory drugs are one of the major problems in developing medicine today [25]. Therefore, new anti-inflammatory drugs lacking those effects are being searched for all over the world as alternatives to NSAIDs [26]. Traditional medicine, therefore, has a major role in health care delivery systems in terms of numbers of people served by the health care systems throughout the world. Recognition of this fact, many countries of the world has currently started paying due attention to promote it. Recent years, herbal medicines are becoming popular alternative medicine and have been used by many people. Herbal medicine is readily available, affordable, effective and culturally acceptable health care modality [27, 28].

1.3 Anti-inflammatory activity of plants

There have been many reports of compounds being found in plants which showed interesting biological activities with anti-inflammatory. In addition, many plants with a medicinal reputation for treating inflammatory diseases have shown anti-inflammatory action in animal models used by pharmacologists to screen compounds

for anti-inflammatory activity. For example, G. Amresh, *et al.* (2007) reported that the *Cissampelos pareira* roots extracts showed anti-inflammatory activity against histamine-, 5-HT-and PGE_2- induced hind paw edema, which indicates that the extract exhibits its anti-inflammatory action by means of inhibiting the synthesis, release or action of inflammatory mediators [29]. The other plant, the methanol extract of *Solanum trilobatum* showed anti-inflammatory activity against carrageenan-induced paw edema in rat. Another study reported that a chloroform extract of *Salvia triloba* shows anti-inflammatory activity in both acute and chronic models and the ulcerogenic effect of extracts were found to be less than that of acetyl salicylic acid [30]. Some studies in Thailand reported that the methanol extract of *Clerodendrum petasites* show potent antipyretic and moderate anti-inflammatory activities without ulcerogenic effect [31]. The ethyl acetate extract from *Garcinia hanburyi* showed anti-inflammatory, analgesic and antipyretic activities. Inhibition of the synthesis and/or release of inflammatory mediators may be the main mechanism(s) of action of this extract [32].

1.4 Background of *Garcinia wallichii* Choisy

Garcinia wallichii Choisy belongs to the family Guttiferae which includes some economically important species e.g. mangosteen (*Garcinia mangostana* Linn.), chamuang (*Garcinia cowa* Roxb.) and somkhag (*Gacinia atroviridis* Griff.). The Thai name of *G. wallichii* is "Pawa Som" and has been found in humid mixed or evergreen forests [33].

1.5 Description of *Garcinia wallichii*

Garcinia wallichii is a perennial plant with small-sized , 8-12 m high, the trunk is straight. Bark is dark brown mix grey colour. The leaf is oblong shape, 4.5 to 7.5 cm wide and 9.5 to 21 cm long, mostly doesn't shed leaves and simple leaf. The flowers have light-colored white, light fragrance. Mature fruit yellowish, globose about 3-4.5 cm in diameter (Figure 3). The interior of the fruit is divided into several segments, the meat is yellow and sweet, the seeds are brown [33].

1.6 Uses of genus Garcinia in traditional medicine and phytochemical study

Guttiferae plant family consists of about 50 genera and more than 1200 species [34, 35]. The genus Garcinia (Guttiferae) is a group of well known fruit trees in Malaysia. The fruit of many species are edible and serve as a substitute for tamarinds in curries. Many species produce a yellow resin which is used in making varnishes and treating wounds. Some species have been shown to exhibit significant antimicrobial and pharmacological activities [36]. Some species of Garcinia are widely used for different types of inflammatory diseases. In Thailand, dry stembark of *Garcinia cowa* Roxb. is used as an antipyretic agent and fresh pericarp of *Garcinia mangostana* Linn. is employed as a topical anti-inflammatory agent [37, 38]. In Thai floklore medicine, gamboge, the yellow gum-resin secreted in latex-tubes (ducts) in the middle bark of *Garcinia hanburyi* Hook f. is used externally for infected wound and systemically for pain and edema [39, 40]. In Ayurvedic medicine the pericarp of mangosteen-fruit has wide use against inflammation and diarrhea [41]. The phytochemical studies of the Guttiferae have shown that xanthone constituents are present. The xanthones in the pericarp are composed of mangostione, α-mangostin, β-

mangostin, γ-mangostin, gartinin, and garcinone E [42, 43, 44]. The xanthones, α- and γ-mangostins, are major bioactive compounds found in the fruit hulls of the mangosteen [44, 45]. The biological activities of α-mangostin have been confirmed to consist of a competitive antagonism of the histamine (H_1 receptor) [37, 46], antibacterial activity against *Helicobacter pylori*, anti-inflammatory activities, inhibition of oxidative damage by human low-density lipoproteins (LDL) [46], antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) [47], and weak antioxidant activity [37]. The other xanthone derivative, γ-mangostin has also been reported to have several pharmacological activities, such as being a potent inhibitor of animal Cdk-activating kinases (Cak), plant Ca^{2+}-dependent protein kinases (CDPK) [45], and a selective antagonist for 5-HT_{2A} receptors in smooth muscle cells and platelets [48, 49]. Moreover, α- and γ-mangostins can inhibit both human immunodeficiency virus (HIV) infection [50, 51] and anti-inflammatory effects [52]. However, phytochemical, biological and pharmacological studies of *G. wallichii* have not yet been performed.

The preliminary research proved by screening model of anti-inflammatoy property showed that the methanol extract of *G. wallichii* (GW extract) inhibited ear edema formation in rats induced by ethyl phenyl propiolate (EPP). Therefore, it is of interest to prove its effects in detail in other inflammatory models. The mechanisms of anti-inflammatory action are included. Other related properties such as analgesic activity and acute toxicity were tested.

1.7 Hypothesis

The genus Garcinia (Guttiferae) is widely used for different types of inflammatory diseases. In Thailand, dry stembark of *Garcinia cowa* Roxb. is used as an antipyretic agent and fresh pericarp of *Garcinia mangostana* Linn. is employed as a topical anti-inflammatory agent [37, 38]. In Thai floklore medicine, gamboge, the yellow gum-resin secreted in latex-tubes (ducts) in the middle bark of *Garcinia hanburyi* Hook f. is used externally for infected wound and systemically for pain and edema [39, 40]. Therefore, the hypothesis of this study is *G. wallichii* possesses anti-inflammatory and analgesic effects.

1.8 Purposes of the study

The purposes of the present study were to verify anti-inflammatory and analgesic activities of the methanol extract from *G. wallichii* in various animal models in comparison with reference drugs such as diclofenac, prednisolone and morphine. The mechanisms of action of the methanol extract from *G. wallichii* on the inflammatory process and pain pathway were also examined in comparison with reference drugs. Moreover, the acute toxicity of the methanol extract of this plant was also evaluated.

Figure 3. *Garcinia wallichii* Choisy.