INTRODUCTION

INFLAMMATION

Inflammation is a localized, protective response to injury and arises from the resultant cell damage. Agencies and means of provoking the response include mechanical trauma (especially crushing), radiation (thermal, UV, radioactive emanations), direct chemical damage (caustic and corrosive chemicals), secondary chemical or biochemical damage (metabolic inhibitors, anoxia), invading organisms (viruses, bacteria, parasites) and last but by no means least antibody-antigen reactions (Bowman and Rand, 1980; Guyton and Hall, 1996; Gallin and Snyderman, 1999).

Commonly, inflammation occurs as a defensive response to invasion of the host. Deficiencies of inflammation compromise the host. Excessive inflammation caused by abnormal recognition of host tissue as foreign or prolongation of the inflammatory process may lead to inflammatory diseases as diverse as diabetes, atherosclerosis, Alzheimer's disease, cataracts, reperfusion injury and cancer; to postinfectious syndromes such as in infectious meningitis and rheumatic fever; and to rheumatic diseases such as systemic lupus erythematosus and rheumatoid arthritis. The centrality of the inflammatory response in these varied disease processes makes its regulation a major element in the prevention, control or cure of human disease. Diseases characterized by inflammation are an important cause of morbidity and mortality in humans (Gallin and Snyderman, 1999).

Inflammatory reactions are generally divided into two types: acute and chronic inflammation (Bowman and Rand, 1980). Acute inflammation is the initial response to tissue injury; it is mediated by the release of autacoids.

Some of the autacoids involved are histamine, serotonin, bradykinin, prostaglandins and leukotrienes (Katzung and Furst, 1998). Acute inflammation is characterized by the classic signs of pain, heat, redness, swelling and loss of function. Microscopically, it involves a complex series of events including dilation of arterioles, capillaries and venules with increased permeability and blood flow; exudation of fluids, including plasma proteins; and leukocytic migration into the inflammatory focus (Gallin and Snyderman, 1999). If the initiating stimuli for an inflammatory reaction are not eliminated by the reaction or controlled adequately, a continuing state of inflammation persists (Bowman and Rand, 1980). Chronic inflammation is a long lived reaction, the reaction persisting for weeks or months after the initial exposure to the damaging agent (Hurley, 1983). Characteristically, there is an abundance of exudate, granulomatous tissue, monocytosis with many multi-nuclear giant cells formed by their fusion, lymphocytosis and accumulation of plasma cells. The connective tissue invasion results in the formation of much fibrous tissues (fibrosis) (Bowman and Rand, 1980). Chronic inflammation involves the release of a number of mediators that are not prominent in the acute response. Some of these are interleukins 1, 2 and 3, TNF-α and interferons. 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 (Katzung and Furst, 1998).

Mediators of Inflammation

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

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

Histamine and serotonin (5-Hydroxytryptamine)

Histamine, which is present throughout body tissues, is formed by decarboxylation of the natural amino acid, histidine. Histamine is stored primarily in mast cells, which are usually found along side blood vessels, and also in circulating basophils especially at site of potential tissue injury (Owen, 1987; Burkhallter *et al.*, 1998). Histamine release is based on acute injury

caused by each of a wide variety of noxious stimuli ranging from anaphylaxis through to both chemical and physical insults. It is clearly important in acute inflammation associated with mast cell degranulation in non-rodent species including man (Owen, 1987), whereas in certain rodents, serotonin may be of equal or greater importance because these substances have in common the structural features of a amine group and share common functional effects on blood vessels (Abbas *et al.*, 1997). The acute inflammatory response to histamine comprises vasodilatation, an increase in microvascular permeability and edema formation. Pharmacological analysis of the receptor involvement in these component parts of the inflammatory response has shown that the vasodilatation involves both H_1- and H_2- receptors. Histamine can also cause pain and itching. An alternative role for histamine might be as a co-mediator of inflammation. In acute inflammation, histamine could both act as the vasodilator and increase vascular permeability but in chronic inflammation would only fulfil the vasodilator role, perhaps serving to potentiate the increase in microvascular permeability caused by second mediators such as prostaglandins. Histamine is relatively unimportant in the later stages of the response. Thus, inhibition of histamine responses delays but does not prevent the inflammatory response (Owen, 1987).

Kinins

Kinins are a group of potent vasodilator peptides. They are formed enzymatically by the action of enzymes known as kallikreins or kininogenases acting on protein substrates called kininogens. Three kinins have been identified in mammals: bradykinins, kallidin (lysylbradykinin) and methionyllysylbradykinin. Note that each kinin contains bradykinin in its structure (Reid, 1998). A variety of factors including tissue damage, allergic reactions, viral infections and other inflammatory events activate a series of

proteolytic reactions that generate bradykinin and kallidin in the tissue (Babe and Serafin, 1996). The kinins generated locally contribute to the acute and possibly the chronic phase of the inflammatory reaction by producing vasodilation, local edema, pain and synthesis of prostaglandins (Regoli, 1987; Babe and Serafin, 1996). Kinins may also modulate migration of white blood and tissue cells that take part to the inflammatory process. Several of the biological effects of bradykinins are mediated by endogenous agent such as prostaglandins and histamine and/or 5-hydroxytryptamine (Regoli, 1987). There are at least two distinct receptors for kinins, which have been designated B_1 and B_2 (Babe and Serafin, 1996). B_2 receptors mediate a large number of rapidly occurring biological effects, particularly the symptoms and signs of inflammation, while B_1 receptors appear to be involved in some retarded, long lasting effects of kinins such as collagen synthesis and cell multiplication (Regoli, 1987). Considerable effort has been directed toward developing kinin receptor antagonists, since such drugs have considerable therapeutic potential as anti-inflammatory and antinociceptive agents. Actions of kinins mediated by prostaglandin generation can be blocked nonspecifically by inhibitors of prostagladin synthesis (Reid, 1998).

Platelet-activating factor (PAF)

PAF is a phospholipid mediator formed by different cells including eosinophils, macrophages, neutrophils and vascular endothelium. Its biosynthesis involves the acetylation of a precursor released from membrane phospholipids by activated phospholipase A_2. PAF activates most inflammatory cells and induces a variety of *in vivo* effects related to inflammation, particularly to immediate hypersensitivity and accordingly to bronchial asthma (Vargaftig and Braquet, 1987). Like the eicosanoids, PAF

is not stored in cell but is synthesized in response to stimulation. It is elaborated by leukocytes and mast cells and exerts proinflammatory effects. Signs and symptoms of inflammation include increased vascular permeability, hyperalgesia, edema and infiltration of neutrophils (Campbell and Halushka, 1996). PAF may be of particular importance in late phase reactions, in which it can activate inflammatory leukocytes. In this situation, the major source of PAF may be basophils or the surface of vascular endothelial cells (stimulated by histamine or leukotrienes) rather than mast cell (Abbas *et al.*, 1997). It is still impossible to determine clearly the role of PAF as a potential mediator in inflammation. The possibility that it plays an important role is nevertheless as likely, if not more so than in case of the eicosanoids (Vargaftig and Braquet, 1987).

Eicosanoids

Eicosanoids are formed from certain polyunsaturated fatty acids (principally, arachidonic acid), these include the prostaglandins (PGs), prostacyclin (PGI_2), thromboxane A_2 (TXA_2) and the leukotrienes (LTs) (Campbell and Halushka, 1996). There are two principal enzyme pathways of arachidonic acid oxygenation involved in inflammatory processes, the cyclooxygenase which produces PGs and the 5-lipoxygenase which produces LTs (Salmon and Higgs, 1987). PGs and LTs are released by a host of mechanical, thermal, chemical, bacterial and other insults and they contribute importantly to genesis of signs and symptoms of inflammation (Campbell and Halushka, 1996). The scheme of the major metabolic transformations of arachidonic acid are shown in Figure 1.

It is now clear that there are two isozymes of cyclooxygenase (COX) called COX-1 and COX-2. Both isozymes catalyze the conversion of arachidonic acid to PGH_2, the most important step in the formation of both

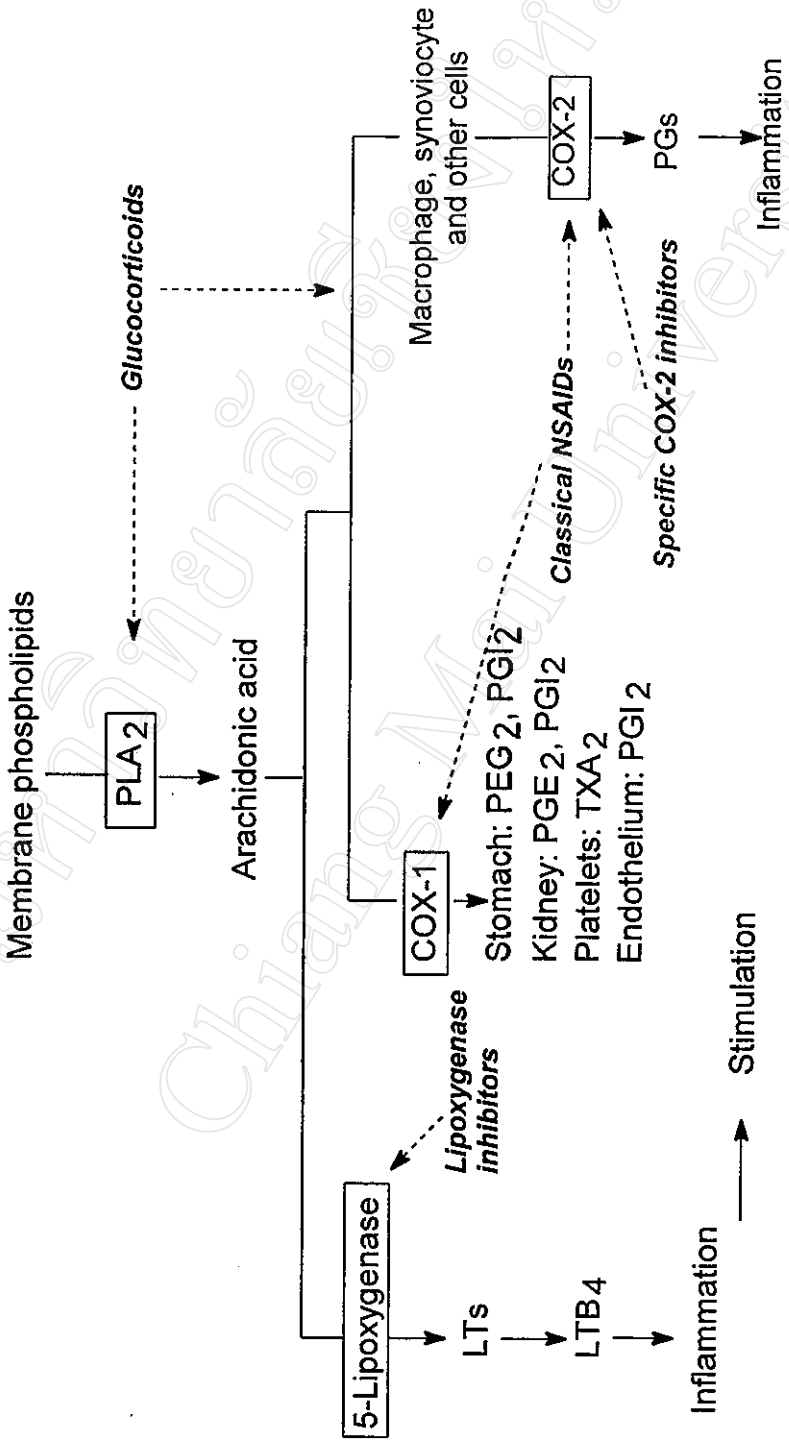

Figure 1. Scheme of the major metabolic transformations of arachidonic acid.

PGI_2 and TXA_2. COX-1, also called constitutive COX, is present in the platelets, endothelium, kidney and stomach mucosa whereas COX-2, also called inducible COX, may be induced by an inflammatory stimulus in macrophages or other cells (Antonio and Souza Brito, 1998). Of the various cyclooxygenase products formed during inflammation, PGE_2 and PGI_2 are the most important. These products are both potent vasodilator and hyperalgesic agents and since they have been detected at sites of inflammation, it is believed that they contribute to the erythema, edema and pain which are characteristics of the inflammatory response (Salmon and Higgs, 1987). PGE_2 is also a powerful pyrogenic substance and its production is thought to account for the fever induced by interleukin-1 (IL-1), an endogenous pyrogen (Bernheim *et al.*, 1980). Although PGs do not appear to have direct effects on vascular permeability, both PGE_2 and PGI_2 markedly enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed region in combination with mediators such as bradykinin and histamine. Moreover, they potentiate the pain-producing activity of bradykinin and other autacoids. Similarly, the combination of PGE_2 or PGI_2 with chemotactic factors results in plasma leakage from the microcirculation by a mechanism dependent on circulating polymorphonuclear leukocytes (PMNs) (Salmon and Higgs, 1987; Campbell and Halushka, 1996). TXA_2 is a major product of arachidonic acid metabolism in platelets which promotes platelet aggregation and vasoconstriction (Campbell and Halushka, 1996). COX-1 has long been thought to be the site of action of NSAIDs. Ideal anti-inflammatory drugs should have an inhibitory action on PG synthesis mediated by COX-2 but not by COX-1. Thus, an inhibitor of COX-2 may be anti-inflammatory drug without the side effects of reducing renal function or producing gastric ulcerations (Antonio and Souza Brito, 1998).

The LTs can be divided, on the basis of their chemical structures and pharmacological actions into LTB_4, LTC_4, LTD_4, LTE_4 and LTF_4 (Piper and Samhoun, 1987). LTB_4 is the strongest candidate as an inflammatory mediator. It has powerful effects on PMN function; it is a potent chemokinetic, chemotactic and degranulating agent for PMNs. The actions of LTB_4 on PMNs are stereospecific, are not shared by other LTs. In human skin, LTB_4, LTC_4 and LTD_4 cause transient wheal and flare responses either by a direct action or through the release of other endogenous mediators (Salmon and Higgs, 1987). LTC_4 and LTD_4 appear to act on the endothelial lining of postcapillary venules to cause exudation of plasma. They also are bronchoconstrictors in man (Campbell and Halushka, 1996).

Cytokines

Interleukins-1 (IL-1) and tumor necrosis factor (TNF) are the most relevant of polypeptide mediators which exercise an influence on the inflammatory process. They are collectively termed cytokines (Billingham, 1987). 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 TNF-α, IL-1, IL-6 and other cytokines. These are termed "endogenous pyrogens" (Janeway *et al.*, 1999). IL-1 and TNF produce many of same proinflammatory responses which include mobilization and activation of PMNs; induction of cyclooxygenase and lipoxygenase enzymes; increase in adhesion molecule expression; activation of B-cells, T-cells, and natural killer cells; and stimulation of production of other cytokines (Insel, 1999). TNF is capable of inducing IL-1 release. Clearly, many of the events associated with acute inflammatory reaction can be mediated by IL-1 and TNF (Billingham, 1987). 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 (Insel, 1999). On occasions, IL-1 or TNF may be the sole mediators; it seems more likely that the cytokines act in concert with other classes of inflammatory mediators in defense of the host. Where the cytokines differ from so many other classes of inflammatory mediators, however, is in their potential to mediate the tissue destruction of chronic diseases such as rheumatoid arthritis (Billingham, 1987).

Complement

The complement system of blood plasma and extravascular tissue fluid plays an important role in many immune defense reactions and absence of a functional complement system reduces many inflammatory reactions. Activation of complement is mediated by either of two distinct pathways, known as the classical and alternative pathway. The classical pathway is initiated by antigen-antibody complexes while the alternative pathway is typically activated by bacterial cell wall carbohydrates. Complement activation promotes acute inflammation, recruitment of leukocytes and killing of pathogens by phagocytosis, and lysis or release of toxic products. The central event in complement activation is cleavage of C3 with the liberation of C3a. Similarly, activation of C5 results in the formation of C5a. C3a and C5a are anaphylatoxins because they release histamine and stimulate smooth muscle contraction. The actions of C3a include histamine release and increase vascular permeability in human skin. In addition, C5a may also contribute to inflammatory reactions by stimulating the release of other mediators. Apart from the release of histamine, C5a may also release protein mediators such as degenerative enzymes, cationic proteins and IL-1.

Similarly, C5a can release lipid mediators such as LTs and PAF. PG synthesis has been reported to be stimulated by C5a and vasodilator PGs are well known to potentiate inflammatory reactions. Thus, inflammatory responses begun by complement activation may be prolonged and potentiated by the actions of mediators released by C5a. The C5- derived peptides have potent effects on leukocytes and promote neutrophil endothelial interactions which lead to neutrophil accumulation and associated edema formation (Jose, 1987).

Anti-inflammatory drugs

The treatment of patients with inflammation involves two primary goals; first, to relief of pain which is often the presenting symptom and the major continuing complaint of the patient; and second, the slowing or in theory - arrest of tissue - damaging process (Katzung and Furst, 1998). At present, anti-inflammatory drugs can be divided into nonsteroidal anti-inflammatory drugs (NSAIDs), anti-inflammatory corticosteroids and disease modifying anti-rheumatic drugs (DMARDs).

Aspirin and NSAIDs such as indomethacin, ibuprofen, naproxen, etc. are used to suppress signs and symptoms of inflammation. These drugs also exert antipyretic and analgesic effects, but it is their anti-inflammatory properties that make them most useful in the management of disorders in which pain is related to the intensity of inflammatory process (Katzung and Furst, 1998). Inhibition of COX, the enzyme responsible for the biosynthesis of the PGs and certain related autacoids, generally is thought to be a major facet of the mechanism of NSAIDs (Insel, 1999). Selectivity for COX-1 versus COX-2 is variable and incomplete. During therapy with these drugs, inflammation is reduced by decreasing of release of mediators from granulocytes, basophils and mast cells. The NSAIDs decrease the

sensitivity of vessels to bradykinin and histamine, affect lymphokine production from T lymphocytes and reverse vasodilation (Katzung and Furst, 1998). In addition to sharing many therapeutic activities, NSAIDs share several unwanted side effects. The most common is a propensity to induce gastric or intestinal ulceration. Although local irritation by orally administered drugs allows back diffusion of acid into the gastric mucosa and induces tissue damage, parenteral administration also can cause damage and bleeding, correlated with inhibition of biosynthesis of gastric PGs, especially PGI_2 and PGE_2, that serve as cytoprotective agents in the gastric mucosa (Insel, 1999).

Anti-inflammatory corticosteroids which are given systemically (orally or by injection) for the treatment of inflammatory diseases are prednisolone, dexamethasone, hydrocortisone, etc. In addition, a number of others are applied topically for treatment of local inflammatory reactions (Bowman and Rand, 1980). Corticosteroids block all the known pathways of eicosanoid synthesis, perhaps by stimulating the synthesis of several inhibitory proteins collectively called annexins or lipocortins. They inhibit phospholipase A_2 activity, probably by interfering with phospholipid binding and thus preventing the release of arachidonic acid (Foegh *et al.*, 1998). Corticosteroids have powerful anti-inflammatory effects. Unfortunately, the toxicity associated with chronic corticosteroid therapy inhibits their use except in the control of acute flare-ups of joint diseases (Katzung and Furst, 1998).

Members of the group slow-acting antirheumatic drugs (SAARDs) or DMARDs include methotrexate, azathioprine, penicillamine, hydroxychloroquin and chloroquin, organic gold compounds and sulfasalazine. The effects of DMARDs may take 6 weeks to 6 months to become evident. Very little is known about their mechanism of action, but

they may slow the bone damage associated with rheumatoid arthritis and are thought to affect more basic inflammatory mechanism than do the NSAIDs. Considerable controversy surrounds the long-term efficacy of these drugs. The discovery that numerous cytokines are present in joints affected by the disease process suggests that one or more of these may be useful targets of disease-modifying drug therapy (Katzung and Furst, 1998).

EXPERIMENTAL MODELS

1. Inflammatory models

Ethyl phenylpropiolate (EPP) and arachidonic acid (AA)-induced ear edema in rats: Ear edema induced in rats by AA or EPP was suggested to serve as a more useful model for the testing of anti-inflammatory activity (Yong *et al.*, 1984). This experiment seems to be a useful model for the rapid *in vivo* screening of agents since only small amount of a test substance is needed. By using both edema inducers (EPP and AA), the mechanism involved can be suggested. LTs are involved in the formation of edema when AA is used as inducer whereas kinins, 5-HT and PGs are released in EPP induced ear edema. In the search for new anti-inflammatory compounds, EPP-induced ear edema in rats is a rapid screening test (Brattsand *et al.*, 1982; Yong *et al.*, 1984). AA-induced ear inflammation in mice has been reported to be sensitive in detecting the anti-inflammatory action of lipoxygenase inhibitors (Carlson *et al.*, 1985).

Carrageenin-induced paw edema in rats: The hind paw edema induced in rat by subplantar injection of irritants including formalin, kaolin, dextran, carrageenin, arachidonic acid, etc., has long been known and used for testing substances for anti-inflammatory property. The most commonly used irritant is carrageenin (Winter *et al.*, 1962). Carrageenin is a sulphate

polysaccharide which has been fractionated with potassium chloride into two separate components, kappa and lambda carrageenin (Di Rosa, 1972). The lambda carrageenin is more active in eliciting either acute or chronic inflammatory responses. Carrageenin-induced hind paw edema in rats was first introduced by Winter *et al.* (1962). This model has become common as a test for anti-inflammatory activity. The advantage of carrageenin-induced edema in comparison with the edema elicited by other phlogistic agents, is its responsiveness to doses of all clinical used anti-inflammatory drugs well below the toxic level with the degree of edema inhibition in a dose-related manner (Winter *et al.*, 1962).

Cotton pellet-induced granuloma formation in rats: Meire *et al.* (1950) first introduced the method using cotton pellet to induce granuloma formation. This method is generally employed to measure the interfering capacity of agents on the proliferative phase of inflammatory process. The response to a subcutaneously implanted cotton pellet in rat has been divided into three phases, namely (1) a transudative phase, a fluid that is low in protein and noninflammatory in origin, defined as the increase in wet weight of the pellet which occurred during the first three hours after implantation (2) an exudative phase, defined as a leakage of fluid from the bloodstream around the granuloma and occurring between 3 and 72 hours after implanting the pellet, and (3) a proliferative phase, measured as the increase in dry weight of the granuloma which occurs between three and six days after implantation (Swingle and Shideman, 1972). The net dry weight of granuloma tissue indicates the intensity of the subchronic inflammation.

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 the liver. It is reported that the activity of alkaline phosphatase in serum was markedly increased during

inflammation. 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 this group of rats will indicate the activity of agents on the production and release of alkaline phosphatase (Nishikaze *et al.*, 1980).

Acetic acid-induced writhing response in mice: Most NSAIDs usually possess analgesic activity. Inhibition on PG biosynthesis is considered to be a shared mechanism of anti-inflammatory, analgesic and antipyretic actions of NSAIDs (Milton, 1982). Therefore, it is interesting to investigate the analgesic activity of test drugs possessing anti-inflammatory activity.

The writhing response induced in rat or mice by intraperitoneal injection of a noxious agent is commonly used as a basis for testing analgesic activity. The response consists of a wave of constriction and elongation passing caudally along the abdominal wall, sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs. The latency and duration of writhing response depends on the characteristics of the challenge substances. The substance, which has a long latency, such as acetic acid or phenylbenzoquinone, may be supposed to act indirectly by liberating an endogenous substance that excites pain nerve endings (Collier *et al.*, 1968). The inhibitory effect of a substance on writhing response in this test was found to be well correlated with clinical results in humans (Taber *et al.*, 1969).

2. Ulcerogenic models

Pyrolus ligation: This model has been first described by Shay *et al.* (1945). It is a simple and uniform method for study of gastric secretion in the rat. Since, this model causes accumulation of intraluminal HCl, the total acidity can be measured

Indomethacin induced-gastric lesions: NSAIDs, e.g. acetylsalicylic acid (ASA), phenylbutazone and indomethacin, are known to induce ulcers during the course of their anti-inflammatory action, i.e. prostaglandin synthetase inhibition through the cyclooxygenase pathway (Pal and Nag Chaudhuri, 1991). It has been reported that the reduction of gastric mucus is a possible cause of the lesion induced by indomethacin (Menguy and Desbaillets, 1967).

HISTOLOGICAL BACKGROUND OF *GARCINIA SPECIOSA*

G. speciosa, commonly known in Thailand as "Pava", "Kava" or "Sarapee pa", is a middle size plant in the family Guttiferae. *G. speciosa* is widely grown in the north and northeast of Thailand. It's leaf is dense with frequent lines. The flower's shape looks like Chamuang's flower of about 30 – 50 mm in diameter. *G. speciosa* is used in folk medicine in Thailand. The decoction of dried flower, taken orally, is used for appetite and treatment of fever, and the boiled water extract from dried leaf as a laxative (Tiangburanatham, 1993).

No pharmacological effects of *G. speciosa* have been reported. However, some species of *Garcinia* are widely used for different types of inflammatory diseases. In Thailand, dried stembark of *G. cowa* is used as an antipyretic agent and fresh pericarp of *G. mangostana* is employed as topical anti-inflammatory agent (Chairungsrilerd *et al.*, 1996; Likhitwitayawuid *et al.*, 1997). In Nigeria, dried fruit and root of *G. kola* are indicated to treat

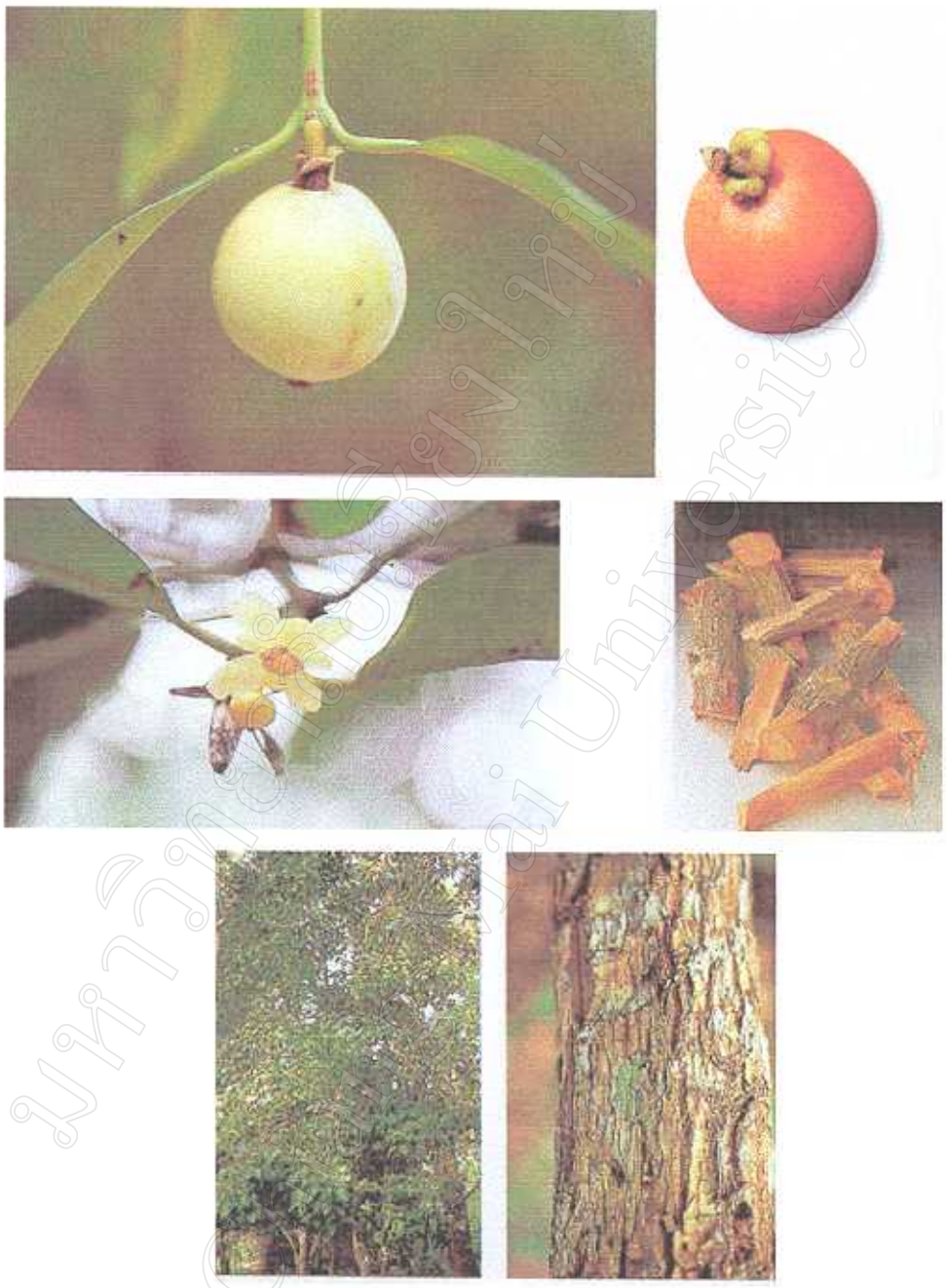

Figure 2. "Pava", "Kava" or "Sarapee pa"

(*Garcenia speciosa*, family Guttiferae)

arthritis and inflammation of the respiratory tract, respectively (Iwu and Anyanwu, 1982; Iwu *et al.*, 1990). The hexane extract of dried seed from *G. kola* showed anti-inflammatory activity on carrageenin-induced pedal edema model in rat and inhibited gastric lesions induced by indomethacin when orally administered as a powder in 25 % of diet (Brade, 1993; Ibironke *et al.*, 1997).

Preliminary screening of the methanol extract from the bark of *G. speciosa* using carrageenin-induced paw edema in rats revealed the anti-inflammatory activity. In addition, it possessed non-ulcerogenic activity when orally given in equal dose of aspirin (0-urai, 1999). It was therefore of interest to study its anti-inflammatory and anti-ulcerogenic effects in detail.

PURPOSE OF THE STUDY

The purpose of the present study was to evaluate the anti-inflammatory, analgesic and anti-ulcerogenic activities of the methanol extract from the bark of *G. speciosa* in many inflammatory and ulcerogenicity models in comparison with reference drugs. Aspirin, phenylbutazone, phenidone, prednisolone and indomethacin were used as reference drugs for inflammatory models whereas misoprostol and cimetidine were used for ulcerogenic models. The mechanism of action of the methanol extract from the bark of *G. speciosa* on the inflammatory process was also examined in comparison with some nonsteroidal and steroidal anti-inflammatory drugs.