# CHAPTER 2

# LITERATURE REVIEWS

## 2.1 Inflammation

Inflammation [16] is a dynamic process of body that responses to mechanical injuries, burns, microbial infections, toxic chemical substances and physical damage stimuli. The inflammation is generally divided into two types, acute and chronic inflammation, based on time to be occurred [17].

Acute inflammation will occur in a short time which takes a few minutes until 1-2 days. The main features of the inflammatory response are vascular change; vasodilatation, i.e. widening of the blood vessels to increase the blood flow to the inflammatory area; increased vascular permeability which allows the components to diffuse into the inflamed site; plasma cascade systems such as complement system, kinin system, coagulation system and fibrinolysis system; extravasation, i.e. leukocytes normally reside in blood and must move into the inflamed area to aid in inflammation as shown in Figure 1. The major signs of inflammation are pain, swelling, redness, heat and loosed of function [18-20].

Chronic inflammation is most appropriately defined in terms of the process, in which continuing inflammation and attempted tissue healing by repair occur simultaneously. It is an inflammatory response of prolonged duration; weeks, months, or even indefinitely. Chronic inflammation can evolve from acute inflammation or occur without an acute phase. The key features and cellular components of chronic inflammation include: tissue destruction; infiltration by macrophages, lymphocytes and plasma cells (occasional eosinophils); repair of injured tissue by granulation tissue (capillaries & fibroblasts) laying down fibrous tissue (mostly collagen) [16, 17, 20, 21].

### 2.1.1 Inflammatory mediators

Inducers of inflammation can stimulate to produce numerous inflammatory mediators. Many of these inflammatory mediators have effects in common on the circulatory system and on the recruitment of leukocytes. These mediators can be derived from plasma proteins or secreted by cells. Inflammatory mediators can be classified into ten groups according to their biochemical properties as following

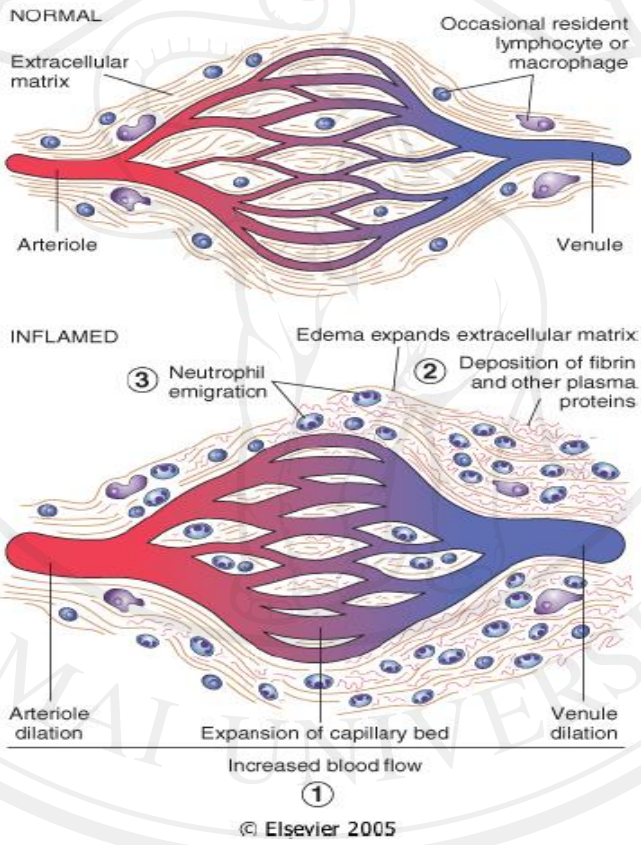


**Figure 1** Comparing of vascular change in acute inflamed and normal tissue [22]

#### 2.1.1.1 Vasoactive amines

Histamine and serotonin play a role in the inflammation especially histamine, which is firstly released when the inflammation has been occurred. They are produced and stored within granules of mast cell and platelets. These mediators

have complex effects on the vasculature, causing increased vascular permeability and vasodilatation or vasoconstriction, depending on the environment [18, 23-25, 26].

#### 2.1.1.2 Vasoactive peptides

Vasoactive peptides can be stored in active form of secretory vesicles such as substance P or generated by proteolytic process of inactive precursors in the extracellular fluids for example kinins, fibrinopeptide A, fibrinopeptide B and fibrin degradation products. Substance P is released by sensory neurons and can itself cause mast cell degranulation. Other vasoactive peptides are generated through proteolysis the Hageman factor, thrombin or plasmin and cause vasodilation and increased vascular permeability [18, 19, 25].

#### 2.1.1.3 Fragments of complement components

The complement fragment C3a, C4a and C5a are produced by several pathways of complement activation. C5a promotes granulocyte and monocyte recruitment and induces mast cell degranulation, thereby affecting the vasculature [18, 19].

#### 2.1.1.4 Lipid mediators

Lipid mediators, which are group of eicosanoids and platelet-activating factors (PAF), are derived from phospholipids that are present in the inner leaflet of cellular membranes. Cytosolic phospholipase $A_2$, which is activated by intracellular $Ca^{2+}$, generates arachidonic acid (AA) and lysophiphatidyl acid from phosphatidlycholine. AA is metabolized to form eicosanoids either by cyclooxygenase; COX-1 and COX-2, which generate prostaglandins and thrombroxanes, or lipoxygenases, which generate leukotrienes and lipoxines. The prostaglandins $E_2$ ($PGE_2$) and prostacyclin $I_2$ ($PGI_2$) cause vasodilatation. Lipoxins inhibit inflammation and promote resolution of inflammation and tissue repair. PAF belongs to acetylglycerol ether phosphocholine group. They are generated by the acetylation of lysophosphatidic acid and activate several processes that occur during

the inflammatory response, including recruitment of leukocytes, vasodilation and vasoconstriction, increased vascular permeability and platelet activation [18, 23-25].

#### 2.1.1.5 Cytokines and chemokines

Inflammatory cytokines are small proteinaceous molecule and produced by many cell types, especially by macrophage and mast cells. They have several roles in the inflammatory response including activation of endothelium and leukocytes and induction of the acute-phase response [18, 25].

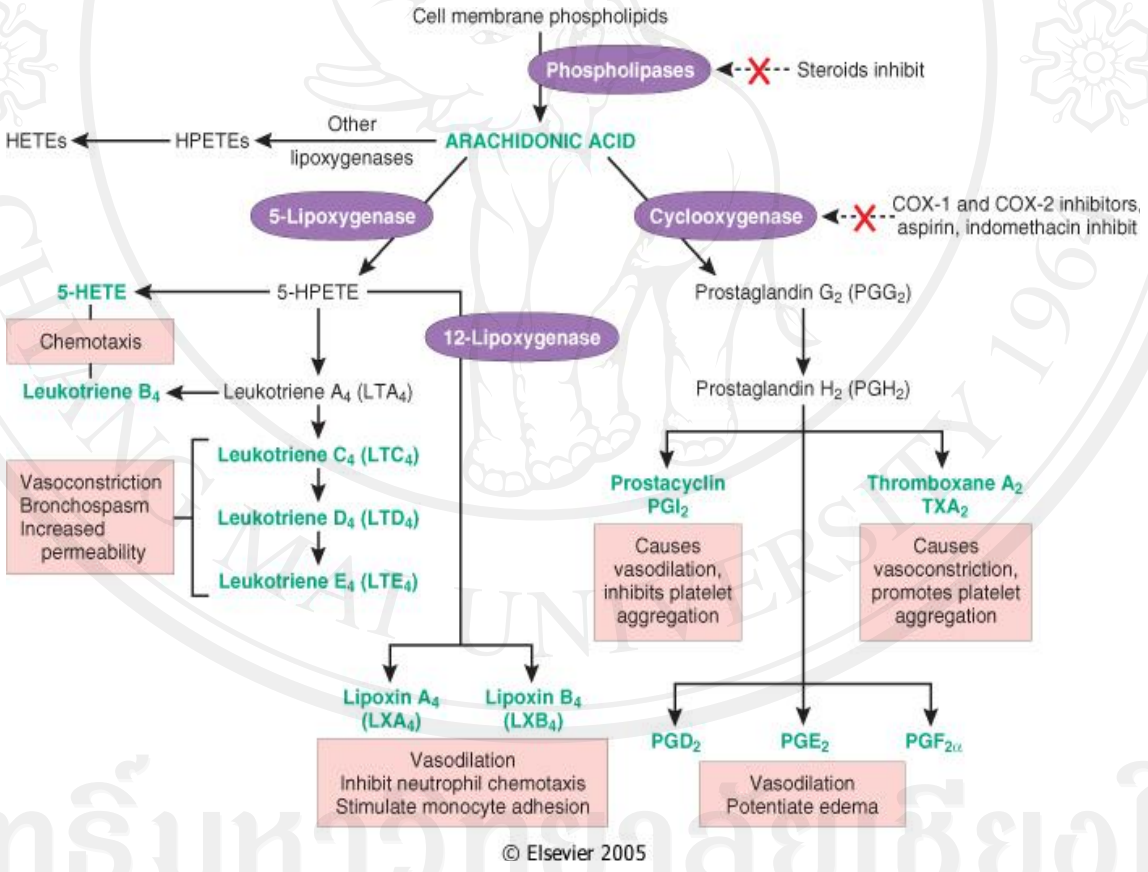


**Figure 2** Synthesis pathway of arachidonic acid metabolite [27]

#### 2.1.1.6 Chemokines

Chemokines are produced by many cell types in response to inducers of inflammation. They play an important role in neutrophil migration and activating the adhesion molecules on surface of neutrophils [18, 25].

#### 2.1.1.7 Proteolytic enzymes

Proteolytic enzymes have diverse roles in inflammation. They have important roles in many processes, including host defense, tissue remodeling and leukocyte migration [18].

#### 2.1.1.8 Nitric oxide

Nitric oxide is a free radical which produced from endothelial vessel cells. It is synthesized from L-arginine as a precursor by nitric oxide syntase enzyme. Nitric oxide plays an important regulatory or modulatory role in variety of inflammation conditions [28].

#### 2.1.1.9 Oxygen-derived free radicals

Oxygen-derived free radicals may be released extracellular from leukocytes after exposure to microbes, chemokines and immune complexes. The superoxide anion and hydrogen peroxide are the major species produced within the cell and these metabolites can combine with nitric oxide to form other reactive nitrogen intermediates [29].

#### 2.1.1.10 Lysosomal constitutes of leukocytes

Neutrophils and momocytes contain lysosomal granules. They are released and may contribute to the inflammation response [30].

Effects of chemical mediators on target site are shown in Table 1

**Table 1** Chemical mediators on the inflammation response [18, 28-30]

| Effects | Mediators |
|---|---|
| Vasodilatation | Prostaglandins: $I_2$, $E_2$, $D_2$, $F_{2\alpha}$ |
| | Nitric oxide |
| | Histamine |
| Increased vascular permeability | Histamine |
| | C3a and C5a (anaphylatoxin) |
| | Bradykinin |
| | PAF |
| | Leukotriene $C_4$, $D_4$, $E_4$ |
| | Substance P |
| Chemotaxis, leukocyte recruitment and activation | C5a |
| | Leukotriene $B_4$ |
| | Chemokine |
| | Tumor necrotic factor (TNF) |
| | Interleukin-1 (IL-1) |
| Fever | TNF |
| | IL-1, IL-6 |
| | Prostaglandins: $I_2$, $E_2$ |
| Pain | Prostaglandins: $I_2$, $E_3$ |
| | Bradykinin |
| Tissue damage | Neutrophil and macrophage lysosomal enzymes |
| | Oxygen radicals |
| | Nitric oxide |

### 2.2 Anti-inflammatory assay

Anti-inflammation study is divided into two methods. First is done by *in vitro* anti-inflammatory assay. To evaluate the anti-inflammatory activity, the chemical reaction of inflammatory enzyme is measured. AA metabolite which is important in the inflammation process is synthesized by two major enzymes, cyclooxygenase and lipoxygenase [31, 32]. Recently, the drugs generally used for relieving the inflammation are involved with cyclooxygenase especially COX-2. Therefore, study of this enzyme may help in quick screening for anti-inflammatory activity of compounds before testing in the animal study. The outcome of *in vitro* study will be percentage of inhibition against COX-2 or the decrease of enzyme activity [33]. Second is *in vivo* anti-inflammation test, it can be tested by various models. The stimulants, for example, croton oil [34], 12-0-Tetradecanoylphorbol 13-acetate (TPA) [35], arachidonic acid (AA) [36], ethyl phenylpropiolate (EPP) [37], are used to induce the inflammation. The principle model used for rapid *in vivo* anti-inflammation screening is substance-induced ear edema in rats [38]. Edema is a parameter to detect whether the agents are active against acute inflammation. The inducer is applied on both ears of each rat. By using EPP, the EEP mechanism involved can be suggested that it causes release of many inflammation mediators such as kinin, serotonin and prostaglandins [39]

### 2.3 *Tabernaemontana divaricata* (Linn.) R. Br.

*T. divaricata* belongs to Apocynaceae. It is commonly known as Clavel De la lndia, East lndian and Rosebay Crape Jasmine. *T. divaricata* is a garden plant in Southeast Asia. It is evergreen shrub forms shaped like symmetrical mounds 6-feet high, horizontal branches having the appearance of an attractive; almost horizontal shrub, leaves; large, shiny, dark green leaves, 6 or more inches in length and 2 inches wide, and waxy blossoms with white which are especially fragrant at night; five-petal pinwheels, gathered in small clusters on the stem tips [1-3] as showed in Figure 3.

The phytochemicals of *T. divaricata* from leaves, stem and root have been previously reported [2-4, 9]. They are divided into two major groups, the alkaloids and non-alkaloids constituents. The alkaloids are arranged in 11 main classes:

Vincosan, Corynanthean, Vallesiachotaman, Strychnan, Aspidospermatan, Plumeran, Eburan, Ibogan, Tacaman, Bis-indole and Miscellaneous [2-9] as showed in Table 2. In the group of non-alkaloids, the constituents such as terpenoids [3], steroids [3], flavonoids [3], phenyl propanoids [2, 3], phenolic acids [2, 3] and enzymes [3] have been isolated from *T. divaricata*.

**A** **B**

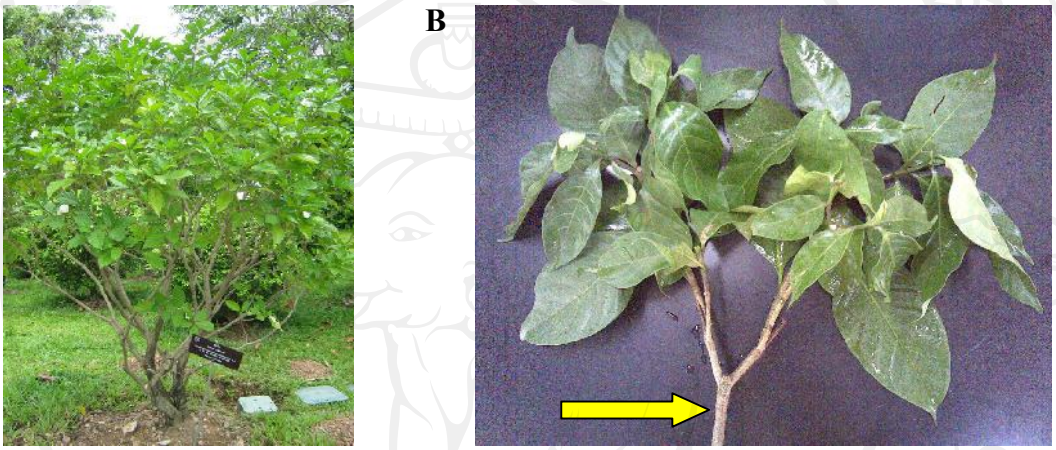

**Figure 3** *T. divaricata* Tree (A) and the stem used (yellow arrow) (B)

**Table 2** Classification of the indole alkaloids occurring in *T.divaricata* [2]

| **Class (abbreviation)** | **Structure characteristics** |
|---|---|
| Vallesiachotaman (V) | C(2)-C(3)-C(14)-unit, N(4)-C(17) bond |
| Corynanthean (C) | C(2)-C(3)-C(14)-unit, N(4)-C(21) bond |
| Vincosan (D) | C(2)-C(3)-C(14)-unit, no N(4)-C(17) or N(4)-C(21) bond |
| Strychnan (S) | C(2)-C(16)-C(15)-unit, C(3)-C(7) bond |
| Aspidospermatan (A) | C(2)-C(16)-C(15) unit, no C(3)-C(7) bond |
| Ibogan (I) | C(2)-C(16)-C(17)-C(14) unit |
| Plumeran (P) | C(2)-C(16)-C(17)-C(20) unit |

**Table 2** (continued)

| Class (abbreviation) | Structure characteristics |
|---|---|
| Tacaman (T) | N(1)-C(16)-C(17)-C(14) unit |
| Euburnan (E) | N(1)-C(16)-C(17)-C(20) unit |
| Bis-indole (B) | Two indole alkaloids attached to each other |
| Miscellaneous (M) | - |

The pharmacological activities of *T. divaricata* have previously been investigated. At concentrations of 150 and 200 mg/kg, the extract from *T. divaricata* show significantly anti-inflammatory activity on the carageenin-induced paw edema compared to vehicle control group [2]. Furthermore, at the concentration of 150 mg/kg, the extract shows better response time than that of the control group without *T. divaricata* treatment when stimulated with the heat in analgesic testing [2]. The extract of *T. divaricata* possess anti-tumor action that can suppress mesangial cell proliferation via the reduction of IL-1, IL-6 and TNF-α expression on Immunoglobulin A nephopathy **(**IgA-N**)** which is the most general pattern in glomerulonephritis [40]. Anti-oxidant activity done by carbon tetrachloride **(**$CCl_4$**)** induced hepatotoxicity model is revealed that *T. divaricata* shows hepatoprotective effect via the lipid peroxidation and increases the anti-oxidant agent [41]. The acetylcholinesterase inhibitory study indicates that at a concentration of 0.1 mg/ml of the root extract from *T. divaricata* showes greater inhibition against acetylcholinesterase than that of the galanthamine. It was found that the active compounds are 19, 20-dihydrotabernamine and 19, 20 dihydroervahanine, which is classified in a group of bisindole alkaloids. Therefore, they are valuable for Alzheimer's disease [8, 9]. The neuropharmacological study indicates that the ethanolic extract from *T. divaricata* causes the dose-related decreased of motor activity, ataxia, loss of righting reflex, decreased respiratory rate and loss of screen grip. From these results, the loss of screen grip and decreased muscle tone in the rat model suggests that *T. divaricata* may act as a skeletal muscle relaxant. Moreover, this *in vivo* study suggests that *T. divaricata* has the depressive effects on both of

peripheral and central nervous systems [10]. Regarding to the toxicity [4], it was found that the mouse received 150-200 mg/kg of both of ethanolic and aqueous extracts from *T. divaricata* show no sign of toxicity. In contrast, the cytotoxic activities were found that voacristine, which is one of the major alkaloids, exhibits the dose-independent on cytostatic and cytotoxic effects on cultures of yeast [42].

## 2.4 Solid lipid nanoparticles (SLN)

Nanotechnology is engineering at the molecular (groups of atoms) level. It is the collective term for a range of technologies, techniques and processes that involve the manipulation of matter at the smallest scale in the level of nanometer ($1/10^9$ cm) or Angstrom unit. Recently, the nanotechnology is widely used for medications and pharmaceutics especially in the drug delivery system [43]. The size of nanoparticles was less than 1 micrometer.

Solid lipid nanopaticles (SLN) [44, 45] are typically spherical with average diameter between 50 to 1000 nm. The SLN was developed from the advantages of solid particles, emulsions and liposomes. Accordingly, the advantages of SLN included protection of incorporated active compound against chemical degradation, consisted of tolerated excipients, ability to load both poorly hydrophilic and lipophilic substances, non-toxic of lipid particles, avoid using the organic solvents in the production and simply to be produced in large scale for industries. Form these views, SLN are used in topical formulation recently, not only pharmaceutics but also for cosmetic products. For example, curcuminoids loaded SLN showed that incorporated curcuminoids in SLN could reduce the sensitivity of curcuminoids on light and oxygen [46] SLN could overcome the chemical unstability of active compound in Tea polyphenols loaded SLN [47]. *Nigella Sativa* L. essential oil was loaded into SLN for improving the topical delivery and improves treatment efficacy as well as patient compliance. Moreover, it showed high physical stability at various storage temperatures during 3 months [48]. Furthermore, the SLN act as occlusive agent to increase the water content of the skin that then support the delivery of drug into the deep skin [15]. However, the commonly disadvantages include low drug-loading

capacities and the presence of alternative colloidal structures (micelles, liposomes, mixed micelles, drug nanocrystals), which cause stability problems during storage.

**2.5 Cream**

Cream is a semisolid dosage form of emulsion containing one or more drug substances dissolved or dispersed in a suitable base and consisting of a two-phase system comprised of at least two immiscible liquids, one of which is dispersed as droplets (internal or dispersed phase) within the other liquid (external or continuous phase), generally stabilized with one or more emulsifying agents. This dosage form is generally used for external application on the skin or mucous membranes [49, 50]. Cream is widely used for skin application especially hydrophilic cream, which is one of the conventional cream types, because it shows a good spreading on the skin, water-washable, increase the skin penetration, exhibit local effect and reduce the side effect from blood circulation. For example, hydrophilic cream is used for incorporation of green tea polyphenols with the aim of prevention of UVB-induced oxidation of lipids and proteins in mouse skin [51], it also provides the highest release of flavonoids such as *Dodonea viscosa* extract [52]. Moreover, hydrophilic cream with *Calendula officinalis* (L.) extract shows the good quality of stability of carotenoids which are used for topical application with anti-oxidant activity [53]. It also increases the skin hydration level and reduces transepidermal water loss [54]. According to above reviews, cream is widely used for being base in field of cosmetics and health pharmaceutical products.