Chapter 1

Introduction

1. Introduction

The reactive oxygen species (ROS), reactive nitrogen species (RNS), and free radicals are implicated in mediating various pathological processes including cancer, aging, atherosclerosis, and inflammatory diseases (Bor, Chen, & Yen, 2006). Oxidative stress is thought to result from an imbalance between the generation of ROS and free radicals and the antioxidants that scavenge them. Antioxidants may act by preventing first-chain initiation or breaking chain promotion by scavenging free radicals. The overproduction of RNS is associated with oxidative stress and with chronic inflammation, which are involved in the pathophysiology of various diseases such as arthritis, diabetes, atherosclerosis, and carcinogenesis. Nitric oxide (NO) and its derivative peroxynitrite ($ONOO^-$), produced from NO and superoxide, are the main sources of RNS *in vivo*. Nitric oxide is an astounding range of biological roles including modulation of vascular tone, memory formation and inflammation (Darley-Usmar, Wiseman, & Halliwell, 1995).

Inflammation is associated with many different types of tissue injury. Causes include allergy reaction, and infection such as bacteria, fungi and virus. In addition, physical agents (heat, cold) or injury are other causes of inflammation (Sae-Wong, 2007), which is a defense reaction of the organism when tissue injury occurs. It will stimulate the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be included, maintained or aggravated by many diseases (Gupta, Mazumder, Kumar, & Kumar, 2003). The aim is to repair the damage or at least to limit it and also to remove the cause, for example, bacteria or foreign bodies (Silbernagl & Lang, 2000).

The inflammatory response is a complex process with several characteristic features that include the activation of monocytes, granulocytes, and lymphocytes and the release and activation of inflammatory mediators, the

complement system, and humoral mediators. The inflammatory process begins with a stimulus that causes the release of prostaglandins from cells. Stimuli such as lipopolysaccharides (membrane component of bacteria) can induce inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Once activated by an injurious agent (infection, burn, etc.), the restoration of homeostasis after an inflammatory insult leads to interaction between lymphocyte and macrophage and provides activation and release inflammatory mediators. Phagocytic cells, especially macrophages, are implicated in immunopathological disorders related to oxidative stress, including inflammation and diseases. Macrophages are sensitive to changes in the oxidant-antioxidant balance because of the production of ROS and RNS as part of their normal function. Therefore, macrophages offer an excellent model system to study NO inhibitory activity (Bor, Chen, & Yen, 2006).

An allergy is one of the immune dysfunctions, and is a serious health problem worldwide. In many countries such as Australia, United States of America, Canada, Sweden, and United Kingdom, the incidence rate for allergic disorders are still increasing every year. In Thailand, the incidence rate for allergic disorders has increased steadily since 1987. Allergic disorders affect a large percentage of the population, and allergic rhinitis and asthma are two of the most common chronic medical conditions (Blaiss, 1999). Allergies, especially immediate hypersensitivity disorders such as asthma, allergic rhinoconjunctivitis, atopic dermatitis and eczema, are a principal health problem, and the prevalence of allergy has increased over the past two decades (Choi & Yan, 2009). Nowadays, the pathogenesis of allergic asthma is believed to be atopic in more than 50% of adults and at least 80% of affected children (World Health Organization [WHO], 2002). More than 10 million patients, including children and adults, have been diagnosed with allergic rhinitis. The prevalence rate of allergic disorders in children was estimated to be as high as 38% (7 million people), and around 20% in adults. About 15% and 7% of allergic rhinitis patients were children and adults, respectively. About 80% of allergic rhinitis patients could be related to allergic asthma, whereas about 40% of allergic asthma patients could be related to allergic rhinitis (Bunnak, 2007).

Allergy rhinitis, asthma, atopic dermatitis, and atopic eczema are among the common causes of chronic ill health. It is well known that IgE-dependent mast

cell activation is associated with the allergic diseases. Upon IgE-dependent activation, mast cells rapidly secrete the preformed and de novo synthesized allergic mediators such as histamine, cytokines, proteases, and arachidonic derivatives. Consequently, various acute and chronic allergic responses are induced by these mediators (Lee et al., 2007).

The mediators are released from mast cells and basophils such as histamine, prostaglandins, leukotrienes, proteases and platelet activating factors (PAF). These mediators induce smooth muscle contraction, vasodilation, increase vascular permeability, mucous secretion and bronchoconstriction. Therefore, these mediators lead to hypersensitivity and inflammation (Matsuda, Tewtrakul, Morikawa, Nakamura, & Yoshikawa, 2004; Matsuda, Tewtrakul, Morikawa, & Yoshikawa, 2004).

The study of anti-allergic activity by inhibitory effects on the release of histamine from mast cells and basophils found that β-hexosaminidase was stored in the secretory granules and was secreted along with histamine products on mast cell degranulation (Schwartz, Lewis, Seldin, & Austen, 1981). Therefore, when granules in mast cells or basophils degranulate, an enzyme β-hexosaminidase is usually released along with histamine. This enzyme is used as a biomarker for antigen-induced degranulation in rat basophilic leukemia (RBL-2H3) cell line (Tewtrakul, Tansakul, & Panichayupakaranant, 2009).

Prasaprohyai, as a Thai traditional medicine preparation, is commonly used for fever and cold treatment. Prasaprohyai consists of Proh hom (*Kaempferia galanga* L., Zingiberaceae) as active plants and 20 other medicinal plants. The other 20 medicinal plants include Krawan (*Amomum testaceum* Ridl., Zingiberaceae), Thian ta takkataen (*Anethum graveolens* L., Umbelliferae), Kot so (*Angelica dahurica* Benth., Umbelliferae), Kot chiang (*Angelica sinensis* (Oliv.) Diels, Umbelliferae), Kot chula lampha (*Artemisia annua* L., Compositae), Kot kamao (*Atractylodes lancea* (Thunb.) DC., Compositae), Thian khao (*Cuminum cyminum* L., Umbelliferae), Chan daeng (*Dracaena loureiri* Gagnep., Dracaenaceae), Thian khao plueak (*Foeniculum vulgare* Mill. var. *dulce* (Mill.) Thell., Umbelliferea), Thian daeng (*Lepidium sativum* L., Cruciferae), Kot hua bua (*Ligusticum sinense* Oliv. cv. Chuanxiong,

Umbelliferae), Saraphi (*Mammea siamensis* Kosterm., Guttiferae), Bunnak (*Mesua ferrea* L., Guttiferae), Phikul (*Mimusops elengi* L., Sapotaceae), Chan thet, Mace and Nutmeg (*Myristica fragrans* Houtt., Myristicaceae), Kasorn bua luang (*Nelumbo nucifera* Gaertn., Nelumbonaceae), Thian dam (*Nigella sativa* L., Ranunculaceae) and Kan phlu (*Syzygium aromaticum* (L.) Merr. et Perry, Myrtaceae) (Foundation of resuscitate and encourage Thai Traditional Medicine, 2005).

Since the cause of fever and cold are inflammatory and allergic effects, this study aims to investigate the anti-inflammatory and anti-allergic effects of Prasaprohyai and each component plants extracts which were used to reduce fever and cold. In this study, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method was also used to measure the antioxidants property and of the extract of Prasaprohyai preparation and its ingredients. Griess reagent was used to measure the anti-inflammatory activity by inhibitory effects of all extracts on nitric oxide (NO) production activated by lipopolysaccharide (LPS) in RAW 264.7 cell lines. Anti-allergy activity was determined by inhibitory activity of the extracts on antigen-induced β-hexosaminidase release as a marker of degranulation in RBL-2H3 cells. The results from this research will support the use of a Thai traditional medicine called Prasaprohyai for inflammation and allergy treatment in fever and colds.

2. Overall aims

The overall aims of this research are to study the antioxidant, anti-inflammatory and anti-allergy effect of ethanolic and water extracts of Prasaprohyai preparation and its ingredients.

3. Specific aims

1. To study the antioxidant activity of ethanolic extract of Prasaprohyai preparation and its ingredients.

2. To study the antioxidant activity of water extract of Prasaprohyai preparation and its ingredients.

3. To study the anti-inflammatory effect of ethanolic extract of Prasaprohyai preparation and its ingredients against murine macrophage leukemia (RAW 264.7) cell line.

4. To study the anti-inflammatory effect of water extract of Prasaprohyai preparation and its ingredients against murine macrophage leukemia (RAW 264.7) cell line.

5. To study the anti-allergy effect of ethanolic extract of Prasaprohyai preparation and its ingredients against rat basophilic leukemia (RBL-2H3) cell line.

6. To study the anti-allergy effect of water extract of Prasaprohyai preparation and its ingredients against rat basophilic leukemia (RBL-2H3) cell line.