CHAPTER 1

INTRODUCTION

1.1 Introduction

Hemorrhoid is a chronic disease in which bulging blood vessels in and about the anus and lower rectum are enlarged. Types of hemorrhoids are external and internal depending on the location; for example external hemorrhoids are near the anus but are not painful. Nevertheless, if the thrombosis is developed, it will cause suffering. Internal hemorrhoids are in the anus under the lining. Typical symptoms are painless bleeding and protrusion. The factors which cause hemorrhoids are: chronic constipation, diarrhea, frequent defecation, sitting for long periods of time and pregnancy may lead to hypertension and increased strain during bowel movements (Taweechaikarn 2001). These factors lead to stretching tissues under support vessels. Then the vessels dilate, the membrane of the vessels become thin and then bleed. Effects of hemorrhoids cause inflammation of anus tissues (Cataldo et al. 2005) and lead to pain . Hemorrhoids can be treated in many ways depending on the symptoms and level of disease. Alternative treatments have been used to treat hemorrhoid more than modern medicine such as Thai folk doctors using many plants and preparations to treat or relieve pain from inflammation and wounds from hemorrhoids.

Inflammation is a biochemical and cellular process that occurs in vascularized tissues. It is a process that defends against infections and promotes tissue repair and healing. The process of inflammation is characterized by the contribution of several mediators such as prostaglandins (PGs), nitric oxide (NO), tumor necrosis factor-α (TNF$-\alpha$), and interleukin-2 (IL-2) (Sautebin 2000 and Mehrotra et al. 2003). 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 et al., 2003).

A complex process of inflammation which was responsible for the sensation of pain was prostaglandins (PGs) (Fennel et al. 2004). The enzyme that converted arachidonic acid to PGs is prostaglandin endoperoxide synthase or cyclooxygenase.

There were two discovered isoforms of cyclooxygenase as follows: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 was found in most tissues and is associated with many physiological functions through the release of PGs. Contradictory, COX-2 was rarely found in most tissues and strongly associated with inflammation process because it was used in order to inhibit inflammation (Sautebin 2000).

Nitric oxide (NO) is a mediator that is produced by inducible nitric oxide synthase (iNOS) during inflammatory reactions (Tezuka et al. 2000). NO is a free radical produced in mammalian cells constitutively or induced by various cell activators. NO contributes to the immune defense against tumor cells but excess production of NO is the cause of various diseases, such as acute and chronic inflammation or autoimmune diseases. Thus, inhibition of NO accumulation is a beneficial therapeutic strategy (Aktan, 2004; Tewtrakul & Itharat, 2007).

Tumor necrosis factor-α (TNF- α) is a potent pro-inflammatory cytokine that has an important role in the inflammation process. TNF- α activates vascular endothelium and increases vascular permeability, which leads to increased entry of IgG. It also has systemic effects, such as fever and systemic edema (Tewtrakul et al., 2008).

Cancer are developed from genetic changed in cell called “cancer cells” with many hallmarks of cancer, for examples, resistance to anti-growth, self-sufficient growth and pro-death signals. Nevertheless, only the genetic changes occur within cancer cells themselves such activated oncogenes or dysfunctional tumor suppressors are not sufficient for responsibility for many aspect of cancer development. Inflammation has been associated with this development of cancer (Rakoff-Nahoum, 2006).

There are several hallmarks of cancer, one of hallmark is inflammation. Inflammatory mediators can induce genetic instability which is a mechanism relate to cancer. There are two relevant pathways; intrinsic pathway and extrinsic pathway. Intrinsic pathway relates to activate of various classes of oncogenes development from inflammation and extrinsic pathway relates to conditions which promote cancer development (Colotta et al. 2009).

Although hemorrhoids do not relate to rectal cancer directly, the cause of two these diseases is chronic constipation. Nevertheless, in 2008, the first common cancer found in male was colon and the third found in female is rectum cancer (Hospital-Base cancer registry, 2008). However, factors of both diseases are closely related (Cataldo et al. 2005). Hence, it is beneficial if we can discover or develop a Thai traditional medicine preparation for hemorrhoid treatment which could also be used to treat colon and rectum cancer.

Use of Ridsiduangmahakan preparation was originated from the internists in many hospitals use this preparation as herbal treatment for hemorrhoid patients. The preparation of the recipe is as follow: *Anacyclus pyrethrum* L., *Anethum graveolens* L., *Angelica sylvestris* L., *Artemisia vulgaris* L., *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, *Cinnamomum zeylanicum* L., *Commiphora abyssinica* Berg., *Cuminum cyminum* L., *Foeniculum vulgare* Mill., *Lepidium sativum* L., *Myristica fragrans* L., *Nigella sativa* L., *Picrorrhiza kurroa* Royle. Ex Benth. *Piper chaba* Hunt, *Piper nigrum* L., *Piper ribesioides* Wall., *Platycladus orientalis* (L.) Franco, *Pouzolzia pentandra* J.J. Bennett, *Terminalia chebula* Retz gall, *Zingiber officinale* Roscoe.

Thus, this study is aimed to investigate anti-inflammation and cytotoxic activities against colon and rectum cancer cell lines (LS174T and SW480) of Ridsidaungmahakan preparation and its plant ingredients. These results will support the use of this preparation by Thai folk doctors for reducing inflammation from hemorrhoids. In addition, it may be developed to treat colon and rectum cancer.

1.2 Research Problems

1. Do Ridsidaungmahakan preparation and their plant ingredients exhibit anti-inflammatory activity against mouse leukemic monocyte macrophage (RAW 264.7) cell line?

2. Do Ridsidaungmahakan preparation and their plant ingredients exhibit cytotoxic activity against human colon adenocarcinoma cell line (LS174T and SW480) cell line?

3. Which isolated compounds from Ridsidaungmahakan preparation exhibit anti-inflammatory activitiy?

4. Are Ridsidaungmahakan preparation extracts stable in stressed or accelerated conditions?

1.3 Overall and specific aims

1. Overall aims

Overall aims of this research are to study anti-inflammation and cytotoxicity of ethanolic extracts of Ridsidaungmahakan preparation and their ingredients.

2. Specific aims

2.1 To study the anti-inflammatory activity such as Nitric oxide, Tumor necrosis factor-α and cyclooxygenase-2 inhibitory effect of ethanolic extract of Ridsidaungmahakan preparation and its ingredients against mouse leukemic monocyte macrophage (RAW 264.7) cell line.

2.2 To study the cytotoxicity of ethanolic extract of Ridsidaungmahakan preparation and their ingredients against human colon adenocarcinoma cell line (LS174T and SW480).

2.3 To study the bioassay-guided fractionation to find active compounds in Ridsidaungmahakan preparation.

2.4 To study the stability of markers and their compounds in Ridsidaungmahakan preparation.

1.4 Outcome and Benefit

The research findings will provide:

1. Knowledge on the ethanolic extracts of Ridsidaungmahakan preparation and their ingredients that exhibit anti-inflammatory effects against mouse leukemic monocyte macrophage (RAW 264.7) cell line.
2. Knowledge on the ethanolic extracts of Ridsidaungmahakan preparation and their ingredients that exhibit cytotoxicity against Human colon adenocarcinoma cell line (LS174T and SW480).
3. Knowledge on active compounds or markers of this preparation and stability of preparation extracts.