

# CHAPTER I

## INTRODUCTION

### **The rationale for the study**

Colon cancer is the third most common cancer which causes cancer related deaths worldwide. The incidence is high especially in developed countries and it is now increasing in developing countries including Thailand [1, 2]. Increased colon cancer risk is associated with genetic, environment, lifestyle and dietary factors. Epidemiological studies have indicated that diet has a significant influence on the risk of developing colon cancer. Western dietary habits, especially high consumption of fat and red meat increase the colon cancer risk whereas high consumption of fruits, vegetables and dietary fiber probably protects against colon cancer [3]. Because diet plays an important role both in increasing as well as reducing the risk for colon cancer, it is believed that 90% of colon cancer can be prevented by dietary intervention or consumption of supplementary diet that has protective effect against colon cancer [3-5].

Numerous studies in different experimental systems have shown that fruits and vegetables exert their beneficial effects through various mechanisms. The protective effects of diets rich in fruits and vegetables against colon carcinogenesis might be the result of their high content of anti-oxidant vitamins and fibers [6-8]. Apart from fruits and vegetables, dietary proteins such as soy protein, whey protein and buckwheat have also been found to reduce the development of colon cancer in animals [9-11]. Interestingly, sericin which is a non-dietary protein has been reported to suppress colon tumorigenesis in both mice and rats [12, 13].

Sericin is one type of the proteins found in the cocoons of silkworm, *Bombyx mori*. The silk cocoons are composed of two major types of proteins: fibroin and sericin. Fibroin is a fibrous protein, secreted from the posterior part of silk gland. Sericin is a glue-like protein, secreted from the middle part of silk gland and envelops the fibroin that helps the formation of silk cocoon. During textile processing, most of

sericin from the cocoon is removed and discarded as waste product. If sericin is recovered and recycled, it would be economic benefit.

Sericin has been shown to be resistant to digestive protease enzymes. This resistant property might make it be beneficial for the health of digestive tract as well as colon [13, 14]. Moreover, sericin, due to its proteinous nature, is a biocompatible and biodegradable material. There are several lines of evidence showing the chemopreventive effect of sericin against colon cancer in animal studies. However, there is still limited number of studies regarding the effect and mechanism of action of sericin for this issue. So far there are two studies from a group of Japanese researchers. The first study has revealed that sericin protected colon tumorigenesis in mice treated with 1,2-dimethylhydrazine (DMH) [9]. The mechanisms of its chemoprevention are associated with the ability to reduce colonic oxidative stress and cell proliferation [9]. The second study has revealed that consumption of sericin suppressed colonic oxidative stress and aberrant crypt foci (ACF) in rats treated with DMH [10]. This later study has indicated that undigested sericin may play a key role for this particular effect [10].

It can be noted that protein preparation of sericin is generally heterogeneous as it consists of various sizes of polypeptides. Since sericin is a polymeric protein, different extraction techniques for protein preparations give rise to polypeptides of different sizes or molecular weights. It has been reported that small size sericin (5-100 kDa) accelerates cell proliferation in culture better than large size sericin (50-200 kDa) [11]. Thus, the size of sericin appears to be important for its activity. However, there has been no information regarding to the size of sericin on chemopreventive effect against colon cancer. Therefore, it is interesting to investigate such effect of different molecular sizes of sericin. In addition, this study was aimed to investigate the effect and mechanism of action of such sericin preparations that could affect colon cancer cell proliferation by determining cell apoptosis and cell cycle regulation. In the present study, three sericin preparations that are different in size; small, medium and large, are provided from Suranaree University of Technology. These sericin(s) were tested in SW480 human colon cancer cells in comparison with FHC normal colon cells. We postulated that the reduction of cell proliferation could be the result of induction of cell apoptosis and/or arresting cell cycle process. To test this possibility, the effect of



sericin on cell apoptosis, activation of caspase-3 and expression of anti-apoptotic Bcl-2 and pro-apoptotic Bax proteins, as well as cell cycle regulation were investigated.

According to the cell culture study, medium size sericin was selected for further *in vivo* study since it showed the best potential. We attempted to investigate the effect and mechanism of action of this sericin in rat treated with DMH. For this part of study, rats were fed with 4% sericin diet. Casein diet was used as a control. In this study, the protection of sericin was investigated when it was given before and during DMH-treatment. The number and multiplicity of aberrant crypt foci (ACF) in proximal, middle and distal parts of colon were analyzed. The mechanism of action of sericin related to cell proliferation was investigated via the expression of Ki67 protein. The Ki67 is an important biological marker in cell proliferation and its expression can be used objectively to estimate the occurrence and progression of various cancers including colon cancer [12]. Besides the cell proliferation, apoptosis is an important process to eliminate cancer cells and regulate cell proliferation. In this study, the expression of Bcl-2 anti-apoptotic protein related to cell apoptosis was determined. Sericin has been reported to suppress colon tumorigenesis in animal models through, at least in part, the anti-oxidant properties which suppress oxidative stress in colon. In the present study, the level of lipid peroxidation in rat colon was also determined. Since immunomodulatory activity of some compounds is believed to be associated with their anti-cancer effect. Therefore, we monitored the immunological status in the blood of all rats. The effect of sericin consumption on the immune response may provide useful information to support the chemopreventive activity of sericin against colon cancer.

From all above mentioned reasons, the main goal of our research was to investigate the effect of sericin isolated in Thailand in cell culture and animal studies. This finding may encourage the use of sericin as a dietary supplement for colon cancer prevention. In addition, because Thailand is one of silk agriculture countries, successfully recycling or utilizing sericin from cocoon waste may significantly increase the income of silk agriculture or industry.

### **Objectives of the study**

To determine the chemopreventive effect and mechanism of action of sericin against colon cancer in animal and cell culture models.

**Specific aims in cell culture model.** To determine the effect of three different size sericins on;

1. The proliferation of cancer and normal cell lines.
2. Cell apoptosis of colon cancer cell line by flow cytometry, caspase-3 activity, Bcl-2 and Bax protein expression.
3. The pattern of cell cycle of colon cancer cell line.

**Specific aims in animal model.** To determine the effect of sericin diet on;

1. Tumor development and the incidence and multiplicity of ACF.
2. Oxidative stress in rat colons by measuring lipid peroxidation.
3. Proliferation and apoptosis of colonic cells by measuring Ki67 and Bcl-2
4. Immunological status in rat blood.

### **Expected outputs of the study**

1. Sericin shows chemopreventive activity in cell culture by reducing cell proliferation and induction cell apoptosis in colon cancer cell lines.
2. Sericin shows a chemopreventive effect in animal model by reducing the incidence of ACF as well as tumor formation.
3. The immunological status of sericin-fed rats is improved.

### **Expected outcomes**

1. Sericin protein reduces the risk of colon tumor development.
2. Sericin protein has a potential to be used as a dietary supplement for colon cancer prevention.
3. Publications in international journals.