CHAPTER ONE

1.1. INTRODUCTION

1.1.1. The Colorectal Cancer

Colorectal cancer, also called colon cancer or large bowel cancer includes cancerous growths in the colon, rectum and appendix. With 655,000 deaths worldwide per year, it is the fourth most common form of cancer in the United States and the third leading cause of cancer-related death in the Western world (WHO, 2006). Colorectal cancer is the third commonest cause of cancer deaths in Malaysia. Data from the Malaysian Ministry of Health confirms an increase in colorectal cancer admission rates from 8.1% in 1987 to 11.9% in 1995 (MOH, 1995). Colorectal cancers arise from adenomatous polyps in the colon. These mushroom-shaped growths are usually benign, but some develop into cancer over time. Localized colon cancer is usually diagnosed through colonoscopy.

1.1.2. Aberrant Crypt Foci (ACF)

Aberrant crypt foci (ACF) are clusters of abnormal glands that look like tubes. They lined the colon of the rectum. Aberrant crypt foci form is among the observed earliest changes in the colon that may lead to cancer, as they are formed before colorectal polyps. ACF are, as opposed to normal epithelial cells, apoptosis resistant (Takayama et al., 2005). They are putative precursors of colon cancer (Adler, 2005). It was first described in 1987 by Dr. Ranjana Bird at the University of Toronto. She observed that in experimental animals given a synthetic colon

carcinogen, certain changes occur in the topography of the colon in a few weeks, long before any tumor develops. When the colon was removed, cut open lengthwise, stained with a blue dye, and viewed under the microscope, small clusters or "foci: of darkly stained crypts with thickened walls could be seen. Importantly, the ACF has also been observed in resected human colons, and some contain mutations that are found commonly in human colon tumors (Roderick, 1998). Twenty years later, more than 900 articles deal with ACF on Pub Med data base. Many indirect evidences support the hypothesis that ACF are precancerous lesions (Corpet, 2010). Corpet (2010) reported the correlation between tumor incidence and number of large ACF ($r = 0.76$, $p<0.001$, $N = 36$), which was viewed as a major piece of evidence to support the above mentioned hypothesis.

The crypts in ACF are easy to score on whole mount colon. ACF are observed to be two to three times larger than normal crypts, microscopically elevated, have a slit-like opening, have a thick epithelial lining that stains darker than normal crypts, with a large per-cryptal zone (Corpet and Tache, 2002). When looking for aberrant crypt foci with microscopy, methylene blue is used as a staining agent (Takayama et al., 2005). The resulting figure (Figure 1.1.) is fairly easy to detect under the microscope at low magnification (4x) (Corpet and Tache, 2002).

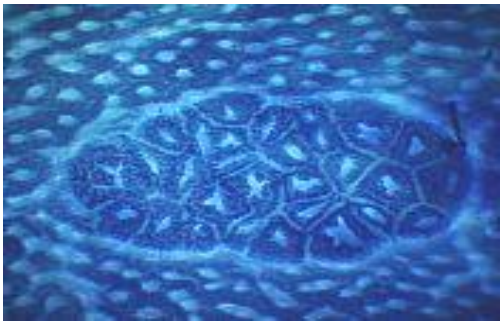

Figure 1.1. Aberrant crypt foci lesions (Corpet and Tache, 2002)

It has been elucidated that ACF were induced by all colon carcinogens in a dose- and species-dependent manner (Bruce and Corpet, 1996). ACF number and growth were modified by the modulators of colon carcinogenesis, and they predicted the tumor outcome in several rodent studies; it was also found to correlate with colon cancer risk, and adenoma size and number in humans; in fact the morphological and genotypic features of ACF in human colons were similar to those in animal colons (Bruce and Corpet, 1996). Many alterations in ACF were observed to be similar to that in tumors; with some ACF showing dysplasia, and carcinoma in rodents and humans' ACF (Bruce and Corpet, 1996).

Chemically induced ACF in rodents have been used extensively to test chemicals and diets that might prevent colorectal cancer, in fact this was reported in more than 400 scientific articles (Corpet and Tache, 2002). The "chemoprevention database" shows the results of all published scientific studies of chemopreventive agents, in people and in animals (Corpet, 2002).

The use of the ACF system to study modulators of carcinogenesis has been accelerated for the last 10 years; this was owed to its advantage of being simple and economical tool for preliminary screening of potential chemopreventive agents, while allowing a quantitative assessment of the mechanisms of colon

carcinogenesis. Though, the role of ACF as preneoplastic lesions was however recently challenged, viewing Mucin Depleted Foci (MDF) as a possible candidate for better endpoints (or MDF, BCAC, flat ACF, ACF min). Still some researchers like Corpet and Tache (2002) argue that ACF are not to be left, because they afford a relatively quick and simple way to screen for compounds in the diet that might either cause or inhibit colon cancer (Corpet and Tache, 2002).

1.1.3. Colon Cancer Chemopreventive Effect of *Phyllanthus niruri*

Phyllanthus niruri belong to the plant family of Euphorbiaceae. It is herbaceous plant that inhabits the tropical and subtropical regions. It has been reported to be popularly used in traditional medicine either as whole plant, fresh leaves or as fruits in the treatment of various diseases, particularly hepatitis and other viral infection (Wang, 2000). This plant is reported to have medicinal importance for numerous ailments like dysentery, influenza, vaginitis, tumors, diabetes, diuretics, jaundice, kidney stone, dyspepsia, antihepatotoxic, antihepatitis-B, antihyperglycemic and also as antiviral and antibacterial (Abdulla *et al.*, 2010, Chopra *et al.*, 1986). Extract of this plant contains several bioactive molecules such as lignans, phyllanthin, hypophyllanthin, flavonoids, glycosides and tannins. *P. niruri* was reported to possess anticarcinogenic (Rajeshkumar *et al.*, 2002) and antioxidant activities (Abdulla *et al.*, 2010).

1.1.4. Antioxidant Activity of Plant Extracts

Antioxidant activity is popularly used to indicate the ability of an antioxidant to scavenge some radicals. Free radical colorimetry relies on the reaction of an antioxidant with the stable free radical 2, 2-diphenyl-l-picrylhydrazyl (DPPH) dissolved in methanol (Buijnsters, 2001). DPPH is a relatively stable paramagnetic free radical that accepts electrons or H^+ radicals to become a stable diamagnetic molecule (Brand-Williams, 1995). The reduction of DPPH by an antioxidant results in the formation of a purple-blue colored solution which gives the measure of the activity by spectrophotometer at 541 nm (Buijnsters, 2001).On the other hand, Ferric-reducing antioxidant power (FRAP) assay is a well known method for measuring the ferric reducing ability (antioxidant power) of plasma. Reduction of ferric to ferrous ion causes a development of blue color complex which gives maximum absorption at 593nm. This color development indicates that a reductant (antioxidant) is present in the sample (Benzie and Strain, 1996).

Several studies reported that the antioxidant activity of plants extract has a beneficial effect of chemoprotection against AOM-induced cell oxidative stress in several human diseases and experimental models of colon cancer (Abdulla *et al.*, 2010). Plant such as *Phyllanthus niruri* (Abdulla *et al.*, 2010), *P. amarus* (Rajeshkumar *et al.*, 2002), *Vaccinium macrocarpon* Ait (Sunkara *et al.*, 2008), *Aloe arborescens* Miller var. (Shimpo *et al.*, 2001), were reported to prevent

AOM-induced ACF in experimental model animals due to their free radical scavenging property.

Therefore, this study aimed to evaluation of chemoprotective effects of *Phyllanthus niruri* against AOM-induced foci of aberrant crypts in rats

1.2. Objectives of the Study

1.2.1. General Objective

The main objective of this study is to evaluate chemoprotective effects of *Phyllanthus niruri* against AOM-induced foci of aberrant crypts in rats using rats as experimental model animal. The understanding of these effects would pave a way to an alternative therapy to this disease.

1.2.2. Specific Objectives

1. To determine the acute toxicity of *P. niruri* whole plant ethanolic extract *in vivo*.
2. To evaluate the chemoprotective effect of *P. niruri* against AOM-induced foci of aberrant crypts in rats grossly.
3. To evaluate the antioxidant properties of *P. niruri* ethanolic extract *in vitro*.