Chapter 4
Discussion

Colorectal cancer is one of the most common human malignancies. The risk of development of cancer may be due to the combined actions of genotoxic agents and promoting agents in environment. (Ames, 1983; Wattenberg, 1992). An attempt to identify naturally occurring dietary carcinogens and anticarcinogens should lead to new strategies for cancer prevention.

AOM-induced aberrant crypt foci in mice and rats colon are putative precancerous lesion that have been purposed to be a biomarker for a short-term screening assay for potential carcinogens and chemopreventive agents of colon cancer (Pereira *et al.*, 1994). Inhibitory effects of 80% ethanolic extract of *M. loriformis* on AOM-induced DNA adducts and ACF formations in rats were observed in this study. It was found that *M. loriformis* extract inhibited AOM-induced ACF formation both at initiation and promotion stages. (Tables 3 and 4) At initiation stage, *M. loriformis* extract showed protective effect on AOM-induced ACF formation about 20.9% and 39% at doses 1.0 and 0.1 g/kg bw., respectively. Interestingly, feeding of *M. loriformis* extract for only 1 week before first AOM injection showed a reduction of ACF formation 56.0%. ACF is putative marker for colon cancer development, but only in the distal colon where tumors follow the adenomas-carcinomas sequence (Park, Goodlad and Wright, 1997). Table 3 has shown that *M. loriformis* extract at the dose of 1.0 and 0.1 g/kg bw. reduced ACF formation in rat colon, including proximal and distal part, from 115.4 crypts (positive control group) to 94.2 and 70.5 crypts, respectively with the most effective at the lower dose. At promotion stage, *M. loriformis* extract decreased AOM-induced ACF formation. Interestingly, the larger ACF, i.e., four or more crypts per focus was significantly inhibited about 27.0% at dose 0.1g/kg bw. (Table 4). Evidence has been shown that ACF with four or more crypts per focus correlated with colon tumor incidence (Premoselli *et al.*, 1996). These inhibitory effects suggest that *M. loriformis* extract is a promising

chemopreventive agents for protection of colon cancer development. However, the inhibitory effects of *M. loriformis* extract on AOM-induced ACF formation was less than 40%, it seem that the extract may be weakly chemopreventive agents for colon cancer.

AOM, a methylating agent of which its mutagenic and carcinogenic effect caused by forming various DNA adducts, among which O^6-methylguanine adduct (O^6-meG) is considered to be the most important. During DNA replication, O^6-meG mispairs with thymine, resulting in a G to A point mutation. This mutation has been implicated in activation of oncogene such as *K-ras* and inactivation of tumor supressor genes (Saffhill, Margison and O'Conner, 1985). Activation of the *K-ras* oncogene has been noted both in established tumors and in premalignantcy foci in humans (Pretlow *et al.*, 1993). About 50% of human colon cancers have been activated, mutant *K-ras* oncogene, and the most common site is a G to A point mutation at the second position of codon 12 (Bos *et al.*, 1987; Forrester *et al.*, 1987). *K-ras* activating GGT to GAT mutations have also been demonstrated in a proportion of DMH- or AOM-treated rat colon ACF (7-32%), adenoma (4%) and carcinoma (30%)(Vinona *et al.*, 1993). *M. loriformis* extract caused a slightly reduced DNA adduct on AOM-treated rats both in colonic mucosa and muscular layer. The extract significantly reduced O^6-meG adduct formation in muscular layer at a higher dose (0.1 g/kg bw., Table 5). The correlation between treated and/or non treated the extract on AOM-induced ACF formation and DNA adducts were obsreved (Figure 18). Its suggested that the extract decrease AOM-induced ACF formation because of inhibitory effect on DNA adducts. AOM metabolites were transported from the liver to the colon *via* bile or blood. The result showed that AOM-induced DNA adduct formation in colonic mucosa on *M. loriformis* extract treated rat was not significantly difference from positive control group. It is therefore suggested that *M. loriformis* extract has no effect on AOM-metabolite transported *via* the bile. The result also showed the body weight of rat were not different between the extract and/or AOM treated rat. It means that the extract exposure or AOM treated has non toxic to the rat.

M. loriformis also showed antioxidant activity (Figure 19). The extract inhibit *t*-BHP-induced lipid peroxide, malondialdehyde (MDA), formation by dose

dependent manner. Many different antioxidants have been shown to inhibit induction of cancer by wide variety of chemical carcinogens and/or radiation at many target sites in mice, rats, hamsters and human. The detailed mechanism(s) for AOM-induced carcinogenesis is not known. Possible mechanisms have been purposed including direct interaction of carcinogens or one of its metabolites, decreased enzyme activities or altered enzyme pathway responsible for carcinogenic activation and increased activities of enzyme pathway responsible for detoxifying carcinogens (Slaga, 1995). A number of antioxidants is effective inhibitors of either tumor initiation, promotion and/or progression such as the phenolic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), antagonized the carcinogenic action of 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (B(a)P) on the forestomach of mice and mammary gland of rats (Slaga, 1995). The antioxidant activity of *M. loriformis* extract might be a possible mechanism to inhibit ACF formation, at either initiation or promotion stage of colon carcinogenesis. The extract showed inhibitory effect on *t*-BHP-induced lipid peroxide formation less than 40%. It suggested that the extract had mild antioxidant activity. However, there are many interference on *t*-BHP-induced lipid peroxide assay such as DMSO. Further study should include positive control and control amount DMSO. In addition, new method which less interference and high accuracy should be used in the further study.

Chemoprotectors may alter the metabolism of carcinogens through induction of the xenobiotic metabolizing enzymes response in carcinogen metabolism. Many types of chemical agents that provide protection against the toxic and neoplastic effects of chemical carcinogen may contain common biological property; such as they are inducers of enzymes involved in the metabolism of carcinogens and other xenobiotics in animal tissues and their cell in culture (Prochaska and Talalay, 1988; Talalay, der Long and Prochaska, 1988). The enzymes involved in the metabolism of xenobiotics may devide into two broad classes: Phase I enzymes (eg. cytochrome P450 dependent) functionalize generally hydrophobic xenobiotics and endogenous compounds by oxidation or reduction and convert them to more polar products; and Phase II enzymes (e.g. GST and UDP-GT) carry out conjugation of these functionalized compounds with endogenous ligands, thereby increasing their

solubility in water and facilitating their excretion. There are two types of chemopreventive enzyme inducers, designated as monofunctional and bifunctional inducers. Monofunctional inducers elevate the activities of phase II enzymes without significantly affecting the activities of the phase I, where as bifunctional inducers increase the activities of certain cytochromes P450, as well as phase II enzymes.

Possible mechanisms of *M. loriformis* extract mediate an inhibition of ACF formation may involve the induction of xenbiotic-metabolizing enzymes. *M. loriformis* extract showed effects on xenobiotic-metabolizing enzyme both in phase I and phase II enzymes (Tables 7,8,9 and 10). In rats treated with the extract for 10 days, phase I enzyme both P450 and APD activities were slightly increased by dose dependent manner. Continuation treatment with the extract for thirty days, both P450 and APD activities were reduced. The extract showed different effects on phase II enzyme (GST, GT and DT-diaphorase) activities. GST activities was reduced after ten days exposure but increased after thirty days without dose dependent manner. DT-diaphorase is a flavoprotein that catalyses two-electron reduction of quinones, quinone imines and nitrogen oxides. In previous study, *M. loriformis* extract had DT-diaphorase inducing activity on murine hepatoma cell line (Hepa 1c1c7) (Vinitketkumnuen *et al.*, 1996). Inducers of DT-diaphorase may play an important role in cancer chemoprevention programs and may also be useful in enhancing the antitumor efficacy of bioreductive agents. However from this study, the extract has no effect on DT-diaphorase activities in rat liver either after ten or thirty days exposure. The differentiation may cause of cell biodiversity. The extract also altered UDP-GT activities. After ten and thirty days exposure, UDP-GT activity in rat livers was decreased. The UDP-GT is a family of enzymes that catalyse the covalent addition of glucuronic acid to a wide range of lipophilic chemicals. They play a major role in the detoxification of many endogenous compounds by generating products that are more polar and, thus more readily excreted in bile or urine (Meech and Mackenzie, 1997). From these results, the extract reduced phase I enzyme both P450 content and APD activity. It was suggested that the small amount of AOM-metabolite, MAM, were generated (Weisburger, 1971). Furthermore, the extract reduced UDP-GT activities therefore the conjugation reaction between MOM and glucuronide may decrease. The

lower amount of MAM conjugated may be formed and small amount of AOM may be regenerated at colonic mucosa, resulted in reduction of O^6-meG adduct formation in colonic mucosa.

In conclusion, *M. loriformis* extract reduced AOM-induced ACF and DNA adducts formation. The inhibitory effect may due to the antioxidant and enzyme modulatory activities presented in the extract. Although the extract slightly reduced phase I enzyme in short-term treated rats (10 days) but at long-term treated rats (30 days) the extract show slightly increased phase I enzyme. It was suggested that *M. loriformis* extract affect at the initiation phase of colon carcinogenesis, but not the growth of differentiation in promotion stage. The possible mechanism of chemoprotection of *M. loriformis* extract at initiation stage may be through selective alteration of enzymes in xenobiotic-metabolizing system. Its antioxidant activity may involve at promotion stage. However, the exact mechanism of inhibitory effect on AOM-induced ACF and DNA adducts formation should be further determined.