CHAPTER 4

Discussion and conclusion

In this study, the dichloromethane and methanol extracts of the purple rice bran variety Phayao did not exhibit clastogenic effect but presented anticlastogenicity against AFB_1-inducing micronucleus formation in rat liver. The applicable cancer chemopreventive dose in human was approximately 500 mg/day of purple rice bran for individual consumption.

Our group found that the antimutagenicity in Ames test and anticarcinogenic enzyme-inducing properties in Hepa1c1c7 cells of the rice variety Kum Phayao were stronger than those of other purple rice varieties including Kum Doisaket and Kum Nan (Chewonarin, 2012). Rice bran is an outer layer coating the endosperm of whole rice grain. Due to nourishment in bran, this part was selected for the present study. Furthermore, the dichloromethane and methanol were used for extraction of both lipophilic and hydrophilic ingredients in purple rice bran, respectively.

In this animal study, the short-period treatment of purple rice bran extract by either dichloromethane or methanol did not enhance the number of rat liver micronuclei. This feature might describe that the components in extracts did not cause liver DNA damage or mutation. According to the results of the extracts did not alter cell proliferative markers including binucleated and mitotic hepatocytes. The agents which are able to modify chromosomal structure are called clastogens which probably cause of carcinogenesis. While, the substances which can promote cell division or result in mitosis are defined as mitogens. The coincidence of clastogenic and mitogenic effects relates to cancer-supporting factor. It might suggest that purple rice bran extracts are neither clastogen nor mitogen in rat hepatocarcinogenesis. Nonetheless, the low dose effect of dichloromethane extract as shown by enhanced the activities of phase I and II enzymes

might be a cellular adaptation for homeostasis. Hence, the lower concentration was authorized and evaluated the optimal dose for used.

AFB_1 is metabolized by CYP1A2 and 3A2 in rats producing AFB_1-8, 9-epoxide. This ultimate metabolite can covalently bind with DNA in the liver forming AFB_1-N^7-guanine DNA adduct leading to genetic changes and mutation in target cells (Hamid, 2013). These modifications of genetic materials such as DNA strand breakage and DNA base damage were shown as fragmented chromosome or interference to intact chromosome during mitosis, called micronuclei. The initiation process of AFB_1-induced hepatocarcinogenesis occurs rapidly and preserves when DNA repairing system cannot conquer. The DNA adduct was subsequently interact with guanine base of p53 gene which is the most effective gene in human cancer. After cell replication or stimulation by partial hepatectomy associated with out of cell cycle controlling from mutated p53 gene, the mutant cells with micronuclei were amplified and easily detected. Thus, the micronucleus formation in the liver was designed as the end-point marker of mutagenicity in this study. The intraperitoneal administration of AFB_1 increased the frequency of micronucleated hepatocytes in a dose-dependent manner. It indicated that the active AFB_1 disturbed DNA in not only point mutation but also a large region. Furthermore, the repeated injection of AFB_1 at dose of 200 ug/kg bw significantly perturbed in AFB_1 metabolism by induction of metabolizing enzyme, CYP1A2, activity and reduction of detoxifying enzyme, GST, activity. From these data, the double injection of AFB_1 at dose of 200 ug/kg bw was designated as the standard protocol for anticlastogenic study.

During xenobiotics including toxic substances enter to the body, the detoxifying system especially in the liver tends to remove them by either phase I or phase II metabolizing enzymes upon their structures (Shimada, 2006). The purple rice bran extracts decreased activities and protein expression of some phase I xenobiotic metabolizing enzymes including CYP1A1, CYP1A2, CYP3A2 and NADPH-cytochrome P450 reductase in AFB_1-induced rats. This indicated that the purple rice bran extracts might influence on either pre- or post-translational level. The effect of purple rice bran extracts on mRNA expression of cytochrome P450 needs to be further investigated. Likewise, the methanol extract of purple rice bran also enhanced activities of GST and UGT which are phase II xenobiotic metabolizing enzymes in AFB_1-induced rats. It might cause from the

hydrophilic ingredients of purple rice bran prefer to be conjugated with some polar molecules by phase II metabolizing enzymes than oxidized by phase I metabolizing enzymes. Whereas, the lipophilic compounds in dichloromethane extract might require phase I reaction to increase their polarity, it hence increased the activity of phase I enzymes. These data suggested that purple rice bran extracts could reduce chromosomal fragmentation caused by AFB_1 leading to suppression of hepatic micronuclei formation in AFB_1-initiated rats by attenuation of some xenobiotic metabolizing enzymes in metabolism.

Currently, cancer chemoprevention is a valued optional approach due to its provision of the strategy for cancer control by suppressing, delaying or reversing the process of carcinogenesis with non-toxic substances from synthetic or natural sources. According to Wattenberg (1981), the cancer chemopreventive agents are classified into 2 groups depend on their mechanisms as blocking agents which involve in initiation stage and suppressing agents which influence on promotion and progression. It suggests that the candidate antimutagens in purple rice bran might partly act as blocking agents that prevent the reaction between AFB_1 and hepatic DNA in the initiation step through alteration of xenobiotic metabolizing enzymes.

Since the induction of phase II enzymes has been considered as one main protective mechanism against chemical carcinogens and initiation of carcinogenesis (Gerhäuser, 1997), xenobiotic metabolizing enzyme inducers can be divided into 2 types. First, the bifunctional inducer is the agent that is able to elevate both phase I and II enzymes. Some natural anticarcinogens such as sulforaphane and flavones, as well as some toxicants such as dioxins and azo dyes are classified as bifunctional inducers (Zhang, 1992; Kensler, 1997; Miao, 2003). Second, the monofunctional inducer is the agent that can selectively up-regulate the function of phase II enzymes with or without the reduction of phase I enzymes. The monofunctional inducers included isothiocyanates, coumarins, thiocarbamates, isoimperatorin and oltipraz (Talalay, 1989; Pokharel, 2006; Kochar, 2010). Many evidences have suggested that monofunctional inducers are correlated to cancer chemopreventive agents in experimental animals and human (Talalay, 1995). The present work showed the methanol extracts of purple rice bran enhanced phase II enzymes and suppressed phase I enzymes in the livers of AFB_1 -treated rats. These results are in

line with previous studies on oltipraz and curcumin (Ciolino, 1998; Langouet, 2000; Langouet, 1995). It was indicated that some active ingredients in methanol extract of purple rice bran play a role as monofuntional inducer.

It was found that the methanol extract of purple rice bran contained large amounts of hydrophilic components including phenolic acids, flavonoids and anthocyanins. Some reports demonstrated that several flavonoids and phenolic acids act as either cytochrome P450 inhibitor or phase II enzyme enhancer (Krajka-Kuzniak, 2005; Moon, 2006). However, the recent work could not decide which flavonoids in methanol extract of purple rice bran act as monofuntional inducers. Further investigation need to be evaluated. In addition, some lipophilic compounds were detected in dichloromethane extract of purple rice bran including gamm-oryzanol and tocols. This extract particularly inhibited some cytochrome P450 insozymes which correlated to the study of gamma-oryzanol and tocotrienol (Umehara, 2004; Kawakami, 2007). It was suggested that these phytochemicals might perform as blocking agents through the ability of phase I and II enzyme modulations.

In conclusion, the dichloromethane and methanol extracts of purple rice bran did not present genotoxicity but possibly provided a cancer chemoprotective effect on AFB_1-initiated hepatocarcinogenesis in rats. The inhibitory mechanisms might be associated with the modulation of detoxifying enzymes relating AFB_1 metabolic activation. The purple rice bran might be beneficial in prevention of AFB_1-initiated hepatocarcinogenesis.