RESEARCH ARTICLE

Purple Rice Extract Supplemented Diet Reduces DMH-Induced Aberrant Crypt Foci in the Rat Colon by Inhibition of Bacterial β-Glucuronidase

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Abstract

Background: Purple rice has become a natural product of interest which is widely used for health promotion. This study investigated the preventive effect of purple rice extract (PRE) mixed diet on DMH initiation of colon carcinogenesis. Materials and Methods: Rats were fed with PRE mixed diet one week before injection of DMH (40 mg/kg of body weight once a week for 2 weeks). They were killed 12 hrs after a second DMH injection to measure the level of O^6-methylguanine and xenobiotic metabolizing enzyme activities. Results: In rats that received PRE, guanine methylation was reduced in the colonic mucosa, but not in the liver, whereas PRE did not affect xenobiotic conjugation, with reference to glutathione-S-transferase or UDP-glucuronyl transferase. After 5 weeks, rats that received PRE with DMH injection had fewer ACF in the colon than those treated with DMH alone. Interestingly, a PRE mixed diet inhibited the activity of bacterial β-glucuronidase in rat feces, a critical enzyme for free methylazoxymethanol (MAM) release in the rat colon. These results indicated that purple rice extract inhibited β-glucuronidase activity in the colonic lumen, causing a reduction of MAM-induced colonic mucosa DNA methylation, leaded to decelerated formation of aberrant crypt foci in the rat colon. Conclusions: The supplemented purple rice extract might thus prevent colon carcinogenesis by the alteration of the colonic environment, and thus could be further developed for neutraceutical products for colon cancer prevention.

Keywords: Colon cancer - purple rice extract - aberrant crypt foci - β-glucuronidase - dimethyl hydrazine

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Introduction

In Thailand, colorectal cancer is the third most prevalent form of cancer in males and the fifth in females (Khuhaprema and Srivatanakul, 2008). Aside from genetic predisposition, age and behavioral risk factors include high intake of fats and low fiber. Therefore, improving diet and life style or receiving food supplements with natural chemopreventive agents may help prevent colon cancer (Hemminki, 1993). Some natural phytochemicals have been shown tohave preventative effects on colon cancer, such as curcumin, which could induce apoptosis in HCT-116 human colon cancer cells (Watson et al., 2008). Cranberry extracts and cranberry juice demonstated antiproliferative activity against two colon cancer cell lines; HT-29 and LS-513 (Vu et al., 2012). Moreover, red wine inhibited colon adenocarcinoma cell proliferation by G2/M cell cycle block and reduction in cyclin D1 expression (Gomez-Alonso et al., 2012)

The most common forms of rice consumed in Thailand are white and brown rice. Rice genotypes with red, purple or black bran layers have been traditionally cultivated in southeast Asia as well (Sutharut and Sudarat, 2012). Purple rice (*Oryza sativa* L. *indica*) is cultivated widely in the lower northern area of Thailand. This variety of rice has been used as a traditional Thai medicine as well. Kum Doi Saket , a variety of northern purple rice contains several bioactive compounds, including anthocyanins, gamma-oryzanol and vitamin E derivatives (Boonsit et al., 2010). Punyatong (2008) reported that anthocyanin from purple rice extract inhibited the growth and induced apoptosis on myeloma cells (×63). Purple rice extract (*Oryza sativa* L.) and the anthocyanin aglycones in PRE inhibited VEGF-induced angiogenesis of HUVECs mediated by inhibition of both cell proliferation and migration (Tanaka et al., 2012). In a previous study, we had found that purple rice extract could inhibit the progression of DMH-induced ACF and the growth of colon cancer cell lines by inducing aberrant cells to undergo apoptosis (Unpublished data). Therefore, in the present study we investigated the effect of a purple rice extract supplemented rat diet on the initiation stage of DMH-induced colon carcinogenesis, focusing on DMH metabolism.

Dimethyhydrazine (DMH) is a carcinogen that can induce colon cancer (Povey et al., 2002). Humans may receive hydrazine compounds via environmental pollution

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and contaminated food. In mammals, DMH is oxidized by liver xenobiotic metabolism phase I enzymes (cytochrome P450 2E1; CYP2E1). The product, methylazoxymethanol (MAM) is then conjugated with UDP-glucuronic acid by the phase II enzyme (Uridine diphospho(UDP)-glucuronyl transferase; UDPGT) and is transported in glucuronide conjugated form to the colon via the bile. Bacterial β-glucuronidase has been suggested to play a role in human colon carcinogenesis (Manoj et al., 2001), as it is a key enzyme for the final activation of DMH metabolites to free reactive MAM in the colonic lumen. MAM could generate methylating adducts at guanine bases to form O^{6}-methylguanine, which is believed to be of primary importance as a precarcinogenic lesion for colon carcinogenesis (Godschalk et al., 2003).

An Aberrant Crypt Focus (ACF) is preneoplastic lesion of the rat or human colon. They are used as relevant biomarkers to investigate the initiation step of colon carcinogenesis and are thus used in chemoprevention studies. (Alrawi et al., 2006; Bird, 1995) Therefore, this study investigated effect of purple rice extract mixed diet on DMH-induced aberrant crypt foci formation in regard to the molecular level mechanism.

Materials and Methods

Chemicals

The carcinogen 1, 2-dimethylhydrazine (DMH) was obtained from Tokyo Chemical Industry. P-nitrophenyl-β-D-glucuronide, *E coli* β-glucuronidase, glutathione, uridine diphosphate glucuronic acid (UDPGA), 1-chloro-2,4dinitro benzene (CDNB), glycogen, guanine and O^{6}-MeG were purchased from Sigma-Aldrich Chemical Co. Tris-HCl and sucrose were obtained from Thermo Fisher Scientific Inc. Phenol-chloroform-isoamylalcohol was obtained from Research Organics Inc. Other reagent grade chemicals were obtained from Merck Millipore Bioscience (Thailand).

Rice samples extract and mixed diet preparation

Sticky purple rice variety Kum Doisaket used in this study was supplied by the purple rice research unit, Chiangmai University. Purple rice was ground and stirred in 80% ethanol. An ethanolic extract solution was then prepared, dried to obtain crude extract, and then cyanidine-3-glucoside level measured by HPLC for batch extraction control. Purple rice extract (PRE) was mixed with rat's commercial diet (CP 082, Perfect companion group co., Ltd) at both a low (0.2%w/w) and high dose (2%w/w). Mixed diets were stored at 4ºC before feeding.

Animal experimental protocol

The male Wistar rats (4 weeks of age) 80-100 g body weights used in this study were provided by the national laboratory center, Mahidol University. Rats were individually housed in cages in an air conditioned room (25-30°C) with exposure to 12/12-h light/dark cycle. The animal treatment protocol was approved by the Animal Ethics Committee, Faculty of Medicine, Chiangmai University, Thiland (9/2555).

Rats were divided randomly into 5 groups of 6 rats for ACF determination and 4 groups of 5 rats for examination of xenobiotic metabolism and DNA adducts. In the control groups rats received 0.9% NSS with normal diet (negative control) or 0.2% PRE mixed diet (treatment control). In experimental groups, rats received normal diet or diet mixed with 0.2% or 2% PRE for one week before administration of DMH (40 mg/kg of body weight; s.c.) until the end of the experiment. Rats were injected DMH again one week after the first injection. Body weights and food intake were recorded daily. Rat feces were collected from three rats randomly selected from each group at indicated times and stored at -80ºC for β-glucuronidase activity determination. For studying xenobiotic metabolism and DNA adducts, rats were sacrificed 12 hours after the second DMH injection. The colons and livers of rats in each group were then removed immediately, flushed with 0.9% NaCl solution, and stored at -80°C.

ACF analysis

To evaluate the number of ACF, all rats were sacrificed 3 weeks after the second DMH administration. The colons were removed, filled with 10% formaldehyde in PBS (pH 7.4) to expand colonic mucosa, and stored on ice for 15 min. The colon pieces were fixed in 10% formaldehyde for at least 24 hr. The flattened colons were stained with 2% methylene blue and the size and numbers of ACF were then scored under a light microscope at 40x magnification and recorded according to the criteria of Bird (Bird, 1987).

Bacterial β-glucuronidase activity

This assay was according to the modified method of Deeptha et al (Deeptha et al., 2006). Weighed rat feces from each group were suspended in 0.1M cold PBS (1:5 w/v) and ground. Fecal suspensions were centrifuged at 2,000×g for 5 min and then the enzyme supernatant was collected to determine β-glucuronidase activity by *p*-nitrophenyl-β-D-glucuronide hydrolysis. The feces supernatant was added to a reaction mixture of 0.01 M PBS (pH 7.0), 0.1 mM EDTA, 3.0 mM *p*-nitrophenyl-β-D-glucuronide and distilled water. The reaction was started by adding 100 µl of fecal supernatant, then run at 37 ºC for 15 min and stopped with 4.0 ml of 0.2 M glycine buffer (pH 10.4) in 0.2 M NaCl. The absorbance was measured at 450 nm and the enzyme activity was calculated.

Phase II enzyme activity determination

The preparation of cytosolic and microsomal fractions:The liver tissues were excised and rinsed with cold 0.154 M KCl. Two grams of tissue were minced and homogenated using Ultra-Turrax disperser (IKA® T10 basic) in an ice bath. The homogenate was centrifuged at 15000×g for 20 min, and the supernatant was continuously centrifuged at 100,000×g for one hour. The cytosolic supernatant was carefully transferred into small vials and was then used to measure glutathione-s-transferase (GST) activity. The microsomal pellet was gently suspended in ice cold 0.154 M KCl for measuring uridinediphosphoglucuronyl transferase (UDPGT) activity.

Glutathione-s-transferase activity: GST activity was

measured by monitoring the conjugation of 1-chloro-2,4dinitro benzene (CDNB) with glutathione according to methods of Habig et al (Habig et al., 1974). The 100 mM Phosphate buffer and 0.5 mM CDNB mixture were pre-incubated for 10 min at 37°C and the reaction was started by adding 5.0 mM GSH, followed immediately by 0.1 ml of the supernatant fraction. The absorbance at 340 nm was measured at 5 minutes and the value for the reaction mixture without GSH subtracted, and the enzyme activity was then calculated from the absorbance.

Uridine diphosphoglucuronyl transferase activity: This assay was done using *p*-nitrophenyl phosphate (PNP) as the substrate according to the modified method of Woodcock and Wood (Woodcock and Wood, 1971). The reaction mixture had 0.4 ml total volume containing 0.1 M Tris Hcl (pH 8.5), 4 mM magnesium chloride, 0.5 mM PNP, 2 mM UDPGA and 50 μl of the microsomal fraction. The assay was carried out at 37°C in a shaking water bath for 30 min. The reaction was stopped by adding an equal volume of 0.2 N trichloroacetic acid (TCA). After centrifugation at 10,000 g for 5 min, 0.5 ml of the supernatant was added to 1.0 ml of 0.5 N NaOH. The rate of PNP disappearance was measured at 405 nm, and enzyme activity was then calculated from the absorbance.

Measurement of O^6-Methylguanine DNA adducts by HPLC

The procedure for detection of DNA adducts was based on the method described by Iwitzki et al. (Iwitzki et al., 1998). Frozen liver and colon were defrosted on ice. Total DNA was isolated by phenol-chloroform and DNA in aqueous phase was precipitated with cold ethanol. One milligram of DNA was hydrolyzed in 1M HCl at 95°C for 1 hour (acid hydrolysis). An aliquot of the mild acid hydrolysate was filtered and analyzed for guanine and O^6-MeG content by high-pressure liquid chromatography (HPLC) using an Agilent PL-SCX1000A cation exchange column (250mm×4.6 mm). After 3 min. isocratic separation with buffer A (10% methanol in 20 mM ammonium formate, pH 4.0), a linear gradient was applied ranging from buffer A to 25% buffer B (5% methanol in 200 mM ammonium formate, pH 4.0) for 15 minutes, followed by 25% buffer B for 2 minutes at a flow rate of 1.5 ml/min. The nucleotide bases were detected using a UV detector at 275 nm, and the amount of hydrolysed DNA was quantified by the area of the guanine and O^6-methylguanine.

Results

Effect of purple rice extract on DMH-induced ACF formation in the initiation stage

To determine the effects of PRE on DMH- induced colon carcinogenesis in the initiation stage, the total numbers of ACF/rat and number of aberrant crypts were scored. During the experiment period, the body weights of rats were not significantly different between control and experimental groups. The mean number of ACF and crypt/focus in rat colon are shown in Table 1. All rats treated with DMH exhibited ACF distributed throughout the colon, (Groups 3-5) while no ACF were observed in the NSS-treated group (Group 1-2). Interestingly, rats treated with purple rice extract in the diet with DMH administration showed a significantly lower total number of ACF compared to DMH-treated alone ($p<0.05$), but there were no significant differences between the two dosage groups. Moreover the multiplicity of ACF (number of AC/focus) was not significantly different among groups. These results suggest that PRE in the diet modulated DMH metabolism causing ACF formation in the initiation stage.

Effect of purple rice extract on DNA adducts in DMH-treated rats

To determine the effect of PRE on the DMH generated DNA adducts in colonic epithelial cells, the level of O^6-MeG was determined. O^6-MeG levels in the colon over 12

Table 1. The Effect of Purple Rice Extract on DMH-induced Aberrant Crypt focus Formation in Wistar rats in the Pre-initiation Stage

Treatment	n	ACF/Rat[a]			Total ACF[a]	AC/F
		Proximal	Distal	Rectum	(% inhibition)	
Negative (NSS)	6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
NSS+PRE 2%	6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Positive (DMH)	6	49±14	94±16	29±10	172±15	2.2±0.3
DMH+0.2% PRE	6	23±10	41±4	13±1	77±10 (55.23)[b]	2.3±0.1
DMH+2% PRE	6	30±14	45±18	18±12	93±22 (45.93)[b]	2.1±0.3

[a]Mean± SD of 6 rats in each group, [b]Significantly difference from positive, $p<0.05$

Table 2. Effect of Purple Rice Extract on Bacterial β-Glucuronidase Activity in rat Feces

Groups	Day 1	Fecal beta-glucuronidase activity (nmol $min^{-1}mg^{-1}$)[a]			
		Before inject DMH (1)	After inject DMH (1)	Before inject DMH (2)	After inject DMH (2)
Negative (NSS)	14.7±0.8	20.8±2.3	26.1±0.7	20.9±1.1	38.6±4.3
Positive (DMH)	13.7±0.7	12.2±1.8	79.4±10.7[b]	50.2±18.2[b]	81.1±6.7[b]
Diet control (2% PRE)	15.4±2.5	15.0±5.6	30.4±5.7[c]	18.7±3.5[c]	19.8±0.9c
0.2% PRE + DMH	14.9±2.1	14.4±3.3	27.2±5.5[c]	13.4±3.0[c]	21.7±3.7[c]
2% PRE + DMH	9.8±2.7	19.4±5.3	24.9±4.4[c]	27.2±6.8[c]	16.6±1.0[c]

[a]Mean±SD of 3 rats in each group, [b]Significantly different from negative control group in the same collecting time; $p<0.05$, [c]Significantly different from positive DMH group in the same collecting time; $p<0.05$

h after the DMH injection are shown in Figure 2. In the colon, the DMH treated groups had significantly increased levels of O^{6}-MeG ($p<0.05$) compared to saline-treated group. In purple rice diet treated groups both 0.2 and 2% significantly reduced the level of O^{6}-MeG in the colon ($p<0.05$) when compared to the DMH treatment alone. However there was no difference in the level of O^{6}-MeG between high and low doses. Moreover, the levels of DNA adducts in the livers of DMH treated rats were similar in both PRE and normal diet groups. These results suggested that PRE in the diet could inhibit DMH-induced O^{6}-methyl DNA adduct formation in the colonic mucosa related to ACF formation.

Effect of purple rice extract on phase II xenobiotic metabolism enzyme

Enzymatic activities of GST and UDPGT are shown in Figures 3 respectively. In the DMH treated groups, GST and UDPGT activity were significantly elevated compared to non-treated ($p<0.05$). However in the purple rice extract treated together with DMH administration, the GST and UDPGT activities were not significantly different compared to DMH treatment alone. The results suggest that the stimulation of liver GST and UDPGT enzymatic activity respond to eliminate reactive MAM via bile secretion. The purple rice extract had no effect on phase II enzymatic activity, was similar to the DNA methyltion levels in the parallel livers of treated rats.

Effect of purple rice extract on bacterial β-glucuronidase activity in rat feces

Bacterial β-glucuronidase enzyme has been considered as a key enzyme for the final activation of DMH metabolites to carcinogens in the colonic lumen. The effect of PRE on bacterial β-glucuronidase activity is shown in Table 2. The activities of fecal β-glucuronidase in each group were comparable on the first day and pre-injected DMH in all groups. After DMH administration, rat feces exhibited significantly higher β-glucuronidase activity in both weeks 1 and 2 ($p<0.05$) compared to the non-treated group. On the other hand, purple rice extract-treated rats, both 0.2% and 2% doses of DMH showed lessened fecal β-glucuronidase activity compared to the DMH-treated alone group, both after 1st and 2nd DMH injections (Figure 4). The results indicated that purple rice extract exhibited a preventive effect on DMH-induced ACF formation via the inhibition of β-glucuronidase activity, which might alter the metabolism of the carcinogens in the colon.

The inhibitory effect of PRE on *E coli* β-glucuronidase *in vitro* was also investigated. The enzymatic activities of β-glucuronidase treated with 50, 100 and 200 μg/ml PRE were decreased at all doses (Figure 5a). Figure

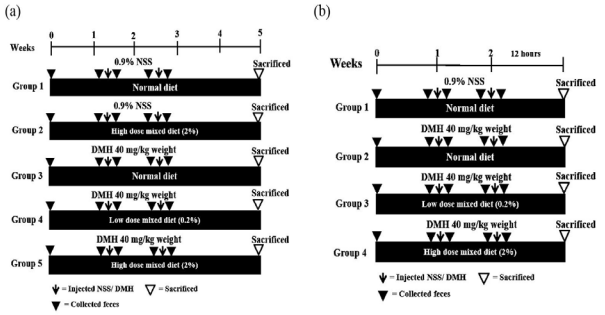

Figure 1. Experimental Protocols for Animal Treatment. **a)** DMH+PRE for ACF experimental; **b)** DMH+PRE for xenobiotic metabolism and DNA adduct experimental

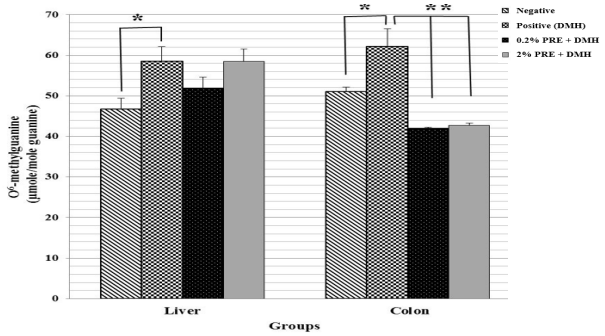

Figure 2. Effect of Purple Rice Extract on the Level of O6-MeG DNA Adduct in DMH-Treated Rats. Each bar represent the mean±SD. Significantly different from negative control;*p<0.05; **p<0.01

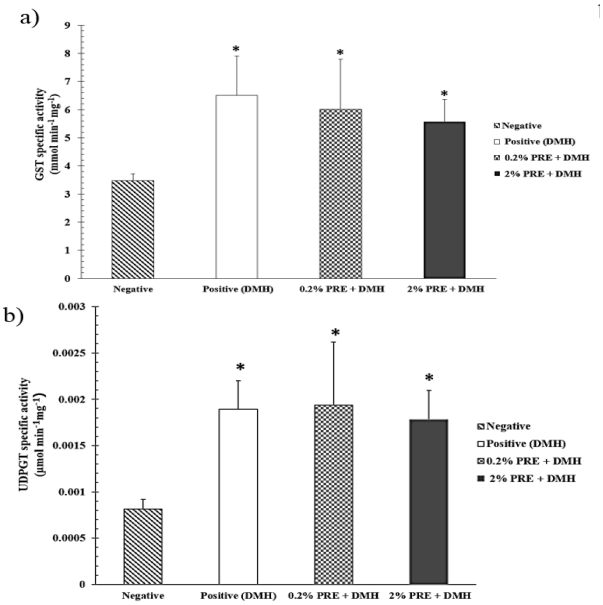

Figure 3. Effect of Purple Rice Extract on Phase II Xenobiotic Metabolism Enzyme. Rat liver homogenate was determined for **a)** Glutathione-S-transferase; **b)** Uridine-diphosphoglucuronyl transferase. Each bar represent the mean±SD of duplicated value of 5 rats. Significantly different from negative control; *p<0.05

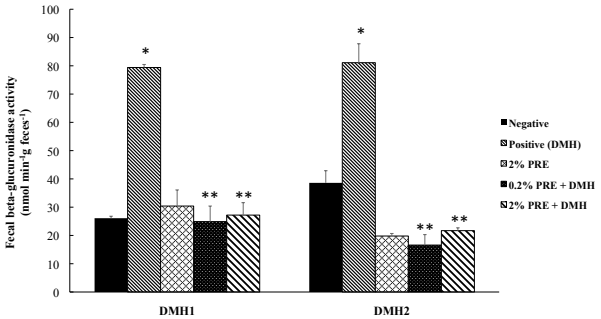

Figure 4. Effect of Purple Rice Extract on Bacterial β-Glucuronidase Activity in Rat Feces. Each bar represent the mean±SD. *Significantly different from negative control; p<0.05,** Significantly different from DMH treatment group; p<0.05

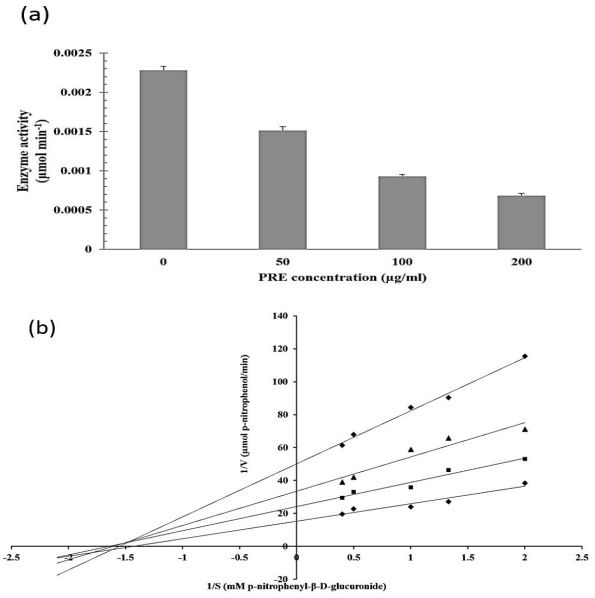

Figure 5. The Inhibitory Effect of PRE on *E coli* β-glucuronidase *in vitro*. PRE inhibited the enzyme activity dose-dependently (a) and non-competitively (b). Amounts of PRE are shown as concentration in 1 ml of the reaction mixture

5bshows the kinetics of β-glucuronidase inhibition by PRE indicating a non-competitive manner, including the reduction of maximum velocity (y-axis) without Km alteration (x-axis). This result confirmed that PRE could inhibit bacterial β-glucuronidase activity in the rat colon.

Discussion

This study has demonstrated the preventive effect of purple rice extract (PRE) supplemented diet on the initiation stage of colon carcinogenesis in rats that received colon carcinogens. The phytochemicals in purple rice include high amounts of hydrophilic phenolic compounds; anthocyanins and proanthocyanidins (Sompong et al., 2011). There are several research reports on the biological activity of these phenolic compounds. The bran extracts of riceberry contain bioactive constituents that have putative cancer chemopreventive properties. Both the dichloromethane and the methanolic extracts significantly inhibited colon, breast and blood cell cancers by suppressing cell growth and proliferation and also via inducing cell cycle arrest and apoptosis (Leardkamolkarn et al., 2011). In 2013, anthocyanins purified from blueberry extracts were found to inhibit melanoma tumor cell proliferation and induce apoptosis (Bunea et al., 2013). Kum doisaket contains several active compounds such as proanthocyanidin and cyanidin 3-glucoside. The cyanidin 3-glucoside was a major source of anticancer activity and was found to be a major compound in proanthocynadin (Chen et al., 2005). Beside C-3-G, another active ingredient also found in purple rice is gamma-oryzanol (Boonsit et al., 2010). Both these ingredients have pharmacological effects, including antioxidant, anticancer and other beneficial effects (Sutharut and Sudarat, 2012). Therefore, purple rice may be valuable for its anticancer properties as well as a raw material for functional food types. In our previous studies, rats treated with crude ethanolic extracts of purple rice displayed inhibition of the progression of DMH-initiated ACF by induction of aberrant colonic epithelial cells undergoing apoptosis (Unpublished data). In this study rats were fed PRE in the form of a diet mixture. PRE was absorbed continually depending on diet consumption, which maintained bioavailability of the effective substance in the blood.

In this protocol rats were treated with PRE mixed diet for 1 week before induction of ACF by DMH in order to study the modulating effect of xenobiotic metabolism in rat liver and the final outcome marker, ACF at 5 weeks of the experiment (Raju, 2008). Purple rice extract reduced the total number of ACF when compared to DMH-treated rats but the effects of the dosage were not significant. These results suggest that 0.2% PRE was a sufficient dose to inhibit ACF formation.

There are a number of possible mechanisms for preventing DMH induced ACF formation. To clarify the mechanism of PRE on DMH-induced ACF formation, the modulating effect of PRE on DMH metabolism was investigated. Methylazoxymethanol (MAM), which is an electrophilic methylating agent (Samanta et al., 2008), can methylate at a number of guanine bases of DNA in colonic mucosa cells. O^{6}-methylguanine is believed to be proportional to the number of ACF. Our results showed that the PRE had an inhibitory effect on the formation of O^{6}-MeG in rat colonic epithelial cells ($p<0.05$), which suggests that the PRE may decrease DNA adducts, resulting in the reduction of ACF formation in the colonic mucosa. However, O^{6}-MeG in the liver had no significant difference. Pfohl-Leszkowicz reported that O^{6}-MeG in colonic mucosa was more correlated to ACF in colonic mucosa (Pfohl-Leszkowicz et al., 1995) than in the liver. Suaeyun el. al. also reported that the reduction of O^{6}-MeG in colonic mucosa was correlated with the reduction of ACF or adenocarcinoma in the rat colon. The lemon grass extract inhibited the release of activated aglycon methylazoxymethanol, from a glucuronide conjugate in the colon and decreased both DNA adducts and ACF in the rat colon (Suaeyun et al., 1997).

The inhibition of ACF formation by alteration of DNA methylation might be one effect of PRE on xenobiotic metabolism of DMH in the liver or on the MAM-glucuronide deconjugation in the colonic lumen. There are several reports showing that chemopreventive agents have stimulating effects on phase II xenobiotic metabolism enzymes. Caffeic acid, garlic oil, sinigrin and propyl gallate all showed an ability to induce phase II enzymes (Manson et al., 1997). As expected, after DMH administration, both phase II enzymes (GST and UDPGT) were elevated due to the xenobiotic conjugation process. Nirmala et al. reported that the enzyme activity levels of both GST and UDPGT were significantly higher in all the carcinogen treated animals compared to controls (Nirmala et al., 2010). Bock et al. explained that UDP-GT activity increase together with the decrease of cytochrome P-450-dependent monooxygenase may contribute to the resistance of preneoplastic hepatocytes to the cytotoxic actions of chemical carcinogens (Bock et al., 1982). Unfortunately, PRE did not stimulate hepatic

GST and UDPGT activity over the range of DMH used. Therefore, phase II enzyme activities in the DMH treatment with or without PRE were similar in regard to MAM-glucuronide conjugation and excretion. This could indicate that reactive MAM was eliminated from the rat liver regularly, which resulted in an unchanged level of O^{6}-MeG in the liver. Suaeyun et al. also reported that orally administered lemon grass extract inhibited O^{6}-MeG formation in the colonic mucosa and significantly inhibited ACF formation, and that the magnitude of the decrease in DNA-binding paralleled the decrease in ACF formation, but it did not inhibit O^{6}-MeG in the liver (Suaeyun et al., 1997). Therefore, hepatic O^{6}-MeG has not been shown related with colonic ACF formation.

Intestinal bacteria elevated the deconjugating enzyme when presented with xenobiotic conjugates in the colon for carbohydrate utilization by the cleaving process. Beta-glucuronidase enzyme has been considered as a key enzyme for deconjugation of MAM-glucuronide to free MAM in the colonic lumen, resulting in an increase in DNA adduct level and ACF (Gadelle et al., 1985). Some dietary substances had shown inhibitory effects on bacterial β-glucuronidase activity *in vivo*. Manoj et al. reported that dietary fibers isolated from coconut or black gram can reduce the formation of colon tumors induced by the carcinogen DMH by reducing the activity of the intestinal as well as fecal β-glucuronidase (Manoj et al., 2001). Moreover, dietary caraway markedly suppressed ACF development and bacterial enzyme β-glucuronidase activities during the initial stages of colon carcinogenesis induced by DMH (Deeptha et al., 2006). Interestingly, our results showed that fecal β-glucuronidase activity of purple rice extract-treated rats were decreased, caused the reduction of free MAM in the colonic lumen. This result was parallel to the inhibitory effect of PRE on ACF and DNA adducts in the colonic mucosa. PRE also inhibited *E coli* β-glucuronidase *in vitro*. The enzyme kinetics showed that PRE could inhibit β-glucuronidase in a noncompetitive manner (Figure 5b). Various phytochemical substances have inhibitory effects on β-glucuronidase activity. Extracts of *Chondria crassicaulis* showed an inhibitory effect against beta glucuronidase as a mixed competitive (Sekikawa et al., 2002) while Serres et al. also reported that proanthocyanidin-A2 from cranberry could inhibit β-glucuronidase directly in a non-competitive manner (Serres et al., 1997). However, the inhibition mechanisms of phytochemicals on β- glucuronidase were dependent on the active compounds mixed in those extracts.

In conclusion, purple rice extract inhibited β-glucuronidase activity in the colonic lumen, resulting in a decrease of MAM-glucuronide deconjugation. The reduction of free MAM might reduce colonic mucosa DNA adducts and ACF in this DMH-induced colon carcinogenesis model. Therefore, purple rice could be further developed as a neutraceutical product for colon cancer prevention in Thailand.

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References

Alrawi SJ, Schiff M, Carroll RE, et al (2006). Aberrant crypt foci. *Anticancer Res*, **26**, 107-19.

Bird RP (1987). Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. *Cancer Letters*, **37**, 147-51.

Bird RP (1995). Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Cancer Letters*, **93**, 55-71.

Bock KW, Lilienblum W, Pfeil H, Eriksson LC (1982). Increased Uridine Diphosphate-Glucuronyltransferase Activity in Preneoplastic Liver Nodules and Morris Hepatomas. *Cancer Res*, **42**, 3747-52.

Boonsit P, Pongpiachan P, Julsrigival S, Karladee D (2010). Gamma Oryzanol Content in Glutinous Purple Rice Landrace Varieties. Chiang Mai University. *J Nat Sci*, **9**, 151-7.

Bunea A, Rugină D, Sconţa Z, et al (2013). Anthocyanin determination in blueberry extracts from various cultivars and their antiproliferative and apoptotic properties in B16-F10 metastatic murine melanoma cells. *Phytochemistry*. **95**, 436-44

Chen PN, Chu SC, Chiou HL, et al (2005). Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis *in vitro* and suppress tumor growth *in vivo*. *Nutr Cancer*, **53**, 232-43.

Deeptha K, Kamaleeswari M, Sengottuvelan M, and Nalini M (2006). Dose dependent inhibitory effect of dietary caraway on 1,2-dimethylhydrazine induced colonic aberrant crypt foci and bacterial enzyme activity in rats. *Invest New Drugs*. **24**, 479-88.

Gadelle D, Raibaud P, and Sacquet E (1985). beta-Glucuronidase activities of intestinal bacteria determined both *in vitro* and *in vivo* in gnotobiotic rats. *Applied Environmental Microbiology*, **49**, 682-85.

Godschalk RWL, Schooten FJV, Bartsch H (2003). A critical evaluation of DNA adducts as biological markers for human exposure to polycyclic aromatic compounds. *J Biochem Mol Biol*, **36**, 1-11.

Gómez-Alonso S, Collins VJ, Vauzour D, et al (2012). Inhibition of colon adenocarcinoma cell proliferation by flavonols is linked to a G2/M cell cycle block and reduction in cyclin D1 expression. *Food Chem*, **130**, 493-500.

Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem*, **249**, 7130-9.

Hemminki K (1993). DNA adducts, mutations and cancer. *Carcinogenesis*, **14**, 2007-12.

Iwitzki F, Schlepegrell R, Eichhorn U, et al (1998). Nickel(II) inhibits the repair of O6-methylguanine in mammalian cells. *Arch Toxicol*, **72**, 681-9.

Khuhaprema T, Srivatanakul P (2008). Colon and Rectum Cancer in Thailand: An Overview. *Jpn J Clin Oncol*, **38**, 237-43.

Leardkamolkarn V, Thongthep W, Suttiarporn P, et al (2011). Chemopreventive properties of the bran extracted from a newly-developed Thai rice: The Riceberry. *Food Chem*. **125**, 978-85.

Manoj G, Thampi BSH, Leelamma S, Menon PVG (2001). Effect of dietary fiber on the activity of intestinal and fecal beta-glucuronidase activity during 1,2-dimethylhydrazine

induced colon carcinogenesis. *Plant Foods Hum Nutr*, **56**, 13-21.

Manson MM, Ball HW, Barrett MC, et al (1997). Mechanism of action of dietary chemoprotective agents in rat liver: induction of phase I and II drug metabolizing enzymes and aflatoxin B1 metabolism. *Carcinogenesis*, **18**, 1729-38.

Nirmala K, Krishna T, Polasa K (2010). Modulation of xenobiotic metabolism in ginger (*Zingiber officinale* Roscoe) fed rats. *Int J Nutr Metab*, **2**, 56-62.

Pfohl-Leszkowicz A, Grosse Y, Carrière V, et al (1995). High levels of DNA adducts in human colon are associated with colorectal cancer. *Cancer Res*, **55**, 5611-6.

Povey AC, Badawi AF, Cooper DP, et al (2002). DNA alkylation and repair in the large bowel: animal and human studies. *J Nutr*, **132**, 3518-21.

Raju J (2008). Azoxymethane-induced rat aberrant crypt foci: Relevance in studying chemoprevention of colon cancer. *World J Gastr*, **14**, 6632-5.

Samanta S, Swamy V, Suresh D, et al (2008). Protective effects of vanadium against DMH-induced genotoxicity and carcinogenesis in rat colon: Removal of O6-methylguanine DNA adducts, p53 expression, inducible nitric oxide synthase downregulation and apoptotic induction. *Mutat Res-Gen Tox En*, **650**, 123-31.

Sekikawa C, Kurihara H, Goto K, Takahashi K (2002). Inhibition of beta-glucuronidase by extracts of *Chondria crassicaulis*. *Bull Fisheries Sci Hokkaido Univ,* **53**, 27-30.

Serres R, McCown B, Zeldin E (1997). Detectable ,-glucuronidase activity in transgenic cranberry is affected by endogenous inhibitors and plant development. *Plant Cell Rep*, **16**, 641-46.

Sompong R, Siebenhandl-Ehn S, Linsberger-Martin G, Berghofer E (2011). Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chem*, **124**, 132-40.

Suaeyun R, Kinouchi T, Arimochi H, Vinitketkumnuen U, Ohnishi Y (1997). Inhibitory effects of lemon grass (Cymbopogon citratus Stapf) on formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. *Carcinogenesis*, **18**, 949-55.

Sutharut J, Sudarat J (2012). Total anthocyanin content and antioxidant activity of germinated colored rice. *Int Food Res J*, **19**, 215-21.

Tanaka J, Nakamura S, Tsuruma K, et al (2012). Purple rice (*Oryza sativa* L.) extract and its constituents inhibit VEGF-induced angiogenesis. *Phytother Res*, **26**, 214-22.

Vu KD, Carlettini H, Bouvet J, et al (2012). Effect of different cranberry extracts and juices during cranberry juice processing on the antiproliferative activity against two colon cancer cell lines. *Food Chem*, **132**, 959-67.

Watson JL, Hill R, Lee PW et al (2008). Curcumin induces apoptosis in HCT-116 human colon cancer cells in a p21-independent manner. *Exp Mol Pathol*, **84**, 230-33.

Woodcock BG, Wood GC (1971). Effect of protein-free diet on UDP-glucuronyltransferase and sulphotransferase activities in rat liver. *Biochem Pharm*, **20**, 2703-13.