RESEARCH ARTICLE

Unpolished Thai Rice Prevents Aberrant Crypt Foci Formation through the Invovement of β-catenin and COX-2 Expression in Azoxymethane-Treated Rats

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Abstract

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world, with chronic inflammation and diet as major causes in its development. Chemopreventive effects of natural dietary products have been the focus of studies for prevention over the past decade. This study was conducted to determine the effects of unpolished Thai rice during precancerous stage through the involvement of β-catenin, cyclooxygenase-2 (COX-2) expression and inflammatory cytokines focusing on azoxymethane (AOM)-induced aberrant crypt foci (ACF)-related to CRC. Male Sprague Dawley rats received two injections of AOM (15 mg/kg body weight) at weeks 4 and 5 while rats were treated with 20% or 70% unpolished Thai rice. The rats were sacrificed at week 38 and the colons removed for aberrant crypt foci (ACF) identification. Histopathologic changes, immunohistochemical analysis of β-catenin and COX-2 expression, and cytokine expression of proinflammatory and anti-inflammatory markers were determined. The administration of unpolished Thai rice significantly and dose dependently decreased the total number of ACF and the percentages of ACF with high-grade dysplasia. Interestingly, unpolished Thai rice suppressed the expression of β-catenin and COX-2. In addition, it also altered proinflammatory (IL-6 and IFN-γ) and anti-inflammatory (IL- 10) markers. The results suggested that unpolished Thai rice may provide a promising dietary intake for prevention during precancerous stage of CRC development, through the involvement of β-catenin and COX-2 expression, and also modulate inflammatory cytokines-related to CRC.

Keywords: Colorectal cancer - unpolished rice - aberrant crypt foci - β-catenin - COX-2 - inflammation

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Introduction

Colorectal cancer (CRC) is a common malignancy associated with significant morbidity and mortality worldwide (Jemal et al., 2011). The etiology of CRC is complicated and may be attributed to combined actions of inherited and environmental factors (Pandurangan and Esa, 2013). Both chronic intestinal inflammation and diet are the environmental factors that have been reported to be the major causes of CRC development (Pandurangan, 2013; Candela et al., 2014). CRC develops from normal colonic epithelium through an early adenoma which is arisen by disruption of the Wnt signaling pathway.

Mutations in the adenomatous polyposis coli (APC) gene play an important role in the initial events of CRC development through the Wnt/β-catenin pathway (Oving and Clevers, 2002; Sillars-Hardebol et al., 2010). Aberrant crypt foci (ACF) are the one of the earliest histopathological manifestations of CRC (Takayama et al., 1998). ACF were originally discovered and described by Bird in 1987 as the lesions of colonic crypts from mice that treated with azoxymethane (AOM). They were larger, thicker, and darker staining than normal crypts when visualized with methylene blue. In addition, ACF in rodents colon treated with AOM closely resembled those seen in human colon. Several studies have been investigated ACF as a biomarker for cancer risk in AOM-treated rodents (Bird, 1987; Wargovich et al., 2010).

The role of chronic inflammation in carcinogenesis appears to be multifaceted through a variety of possible mechanisms. Cyclooxygenase-2 (COX-2) expression plays a role to enhance cancer development in the situation of chronic inflammation. The relationship between inflammation and cancer has been made on the basis of various observations, for instance, tumors arise at the sites of chronic inflammation, inflammatory cells are present in tumors, and over-expression of inflammatory cytokines can induce cancer (Kraus and Arber, 2009).

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Inflammatory cytokines can be modulated tumor growth and tumor microenvironment through the interaction between cancer cells and infiltrating inflammatory cells (Kantola et al., 2012).

Several natural dietary compounds including phenolics, flavonoids, carotenoids, and alkaloids have been well known to delay, prevent, or reverse the development of CRC. Their phytochemicals have been known to block the initiation or reverse the promotion stage in CRC carcinogenesis (Surh, 2003; Nishino et al., 2007). β-catenin over-expressions and positive COX-2 have been shown to be associated with a more aggressive behavior of CRC. The potential targets of β-catenin and COX-2 have been more studied and identified as useful targets for chemopreventive agents to prevent CRC (Hegazy et al., 2013). Unpolished rice is a major source of phytophenolics, which exhibits high level of antioxidant activity and high potential to reduce the risk of CRC. Previously, unpolished Thai rice has been revealed to inhibit ACF formation in AOM-induced rats (Katyama et al., 2002; Suwannalert and Rattanachitthawat, 2011). Although the consumption of unpolished Thai rice has been predicted to prevent ACF-related to CRC, the effects of unpolished Thai rice on β-catenin, COX-2 and inflammatory response in AOM-induced CRC rats were not be investigated. In this study, we determined the effects of unpolished Thai rice on precancerous stage through the involvement of β-catenin, COX-2 expression, and inflammatory cytokines in AOM-induced CRC rats. The results of this study may provide a promising dietary intake for the prevention or suppression of CRC development. In addition, of β-catenin and COX-2 expression may be used for tumor biomarkers in CRC progression.

Materials and Methods

Animals

Male Sprague Dawley rats, 4 weeks old were purchased from National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom, Thailand. Prior to start the experiment, the rats were acclimatized to the animal room conditions for 4 week. Ethical approval of this study was obtained from the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Chiang Mai University, Thailand (CMU-ACUC:R3/2554).

Experimental design

Thirty six male Sprague Dawley rats were randomly divided into 6 groups, 6 rats for each group. Group 1 (untreated rats), the rats were fed with standard commercial diet. Group 2 (L-UTR) and Group 3 (H-UTR), the rats were fed with 20% and 70% of red-colored unpolished Thai rice in standard commercial diet, respectively. Group 4 (AOM-treated rats), the rats were induced with AOM and fed with standard commercial diet. Group 5 (AOM + L-UTR) and Group 6 (AOM + H-UTR), the rats were induced with AOM and fed with 20% and 70% of red-colored unpolished Thai rice in standard commercial diet, respectively. At week 4 and 5 after fed with unpolished Thai rice, the rats in Group 4-6 were injected subcutaneously with AOM at a dose of 15 mg/kg body weight. At week 38, all rats were sacrificed and the entire colons were cut longitudinally along the taenia coli to expose the lumen of both ascending and descending regions. All colon tissues were fixed in 10% buffered formalin for at least 24 h before ACF identification. Whole blood of each rat was collected by allowed the blood to clot without disturbance at room temperature. The clot was removed by centrifugation at 1500 g for 10 min. The serum was carefully collected and stored at -20°C until use.

Identification of ACF

The identification of ACF was slightly modified according to the method that originally described by Bird in 1987. The formalin-fixed colonic tissues were cut and stained in 0.1% methylene blue solution for 3-5 min. The total number of ACF and the number of aberrant crypts (AC) were counted under light microscope. ACF were identified with the following morphological characteristics: (i) the enlarged and elevated crypts than normal mucosa, and (ii) increased pericryptal space and irregular lumens (Xiao et al., 2008).

Histological analysis of ACF

The formalin-fixed colonic tissues were processed and appropriately oriented in paraffin blocks for longitudinal sectioning serially at 4 μM thickness. All sections were stained with hematoxylin and eosin (H&E) for histological evaluation. Five fields of ACF were randomly observed under light microscopy from each section sample. ACF were classified into three categories based on nuclear to cytoplasmic ratio, cell polarity, chromatin pattern, mitotic figures, and mucin secretion (Xiao et al., 2008).

(1). ACF with hyperplasia showed only hyperplastic cells, no dysplasia

(2). ACF with low-grade dysplasia

- Elongated, slightly crowded and pseudostratified nuclei
- Polarity well preserved
- Normal or slightly reduced number of goblet cells

(3). ACF with high-grade dysplasia

- Elongated, crowded and pseudostratified nuclei
- Markedly increased nucleus to cytoplasm ratio
- Significantly reduced number of goblet cells

Immunohistochemical analysis of β-catenin and COX-2

Prior to immunohistochemical staining, the slides of paraffin embedded sections were heated at 80°C for 15-20 min, deparaffinized in xylene, rehydrated through graded alcohol series and soaked in water for 10 min at room temperature. Antigen retrieval was performed in a microwave oven using citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in methanol for 30 min. Slides were then rinsed in phosphate-buffered saline (pH 7.6) and blocked non-specific antigen sites by incubation with normal goat serum for 15 min. The sections were immunostained with the primary antibody, anti-β-catenin (1:1000) or anti-COX-2 (1:100), shaken for 60 min in moist chamber and then left overnight in refrigerator. After the primary antibody incubation step, the slides were rinsed in phosphate-buffered saline and incubated

with polyclonal goat anti-rabbit Immunoglobulin (Ig)/ Horseradish Peroxidase (HRP) (1:100), for 45 min. Color was developed with the substrate 3,3'-diaminobenzidine (DAB) for 10 min. The slides were then rinsed gently with distilled water and counterstained with Meyer's hematoxylin, dehydrated, and mounted with permount. Negative controls were prepared simultaneously for all samples by replacing the primary antibody with phosphate-buffered saline (Hong et al., 2004). In this study, ACF were counted and scored for β-catenin and COX-2. Five fields of ACF were randomly selected from each section sample. The immunoreactive score of each area was determined by the summation of the % positive cells and intensity of the staining. The positivity for β-catenin and COX-2 were calculated based on the percentage of positive cells and localized pattern. The scoring system for β-catenin and COX-2 immunohistochemistry staining was shown as below.

β-catenin Immunohistochemical Scoring

Evaluation of % positive cells		Evaluation of stain intensity	
		Membranous staining	Cytoplasmic staining
0	no staining	0 no staining	0 no staining
1+	≤ 25% positive	1+ mild staining	1+ mild staining
2+	> 25-50% positive	2+ moderate staining	2+ moderate staining
3+	> 50-75% positive	3+ intense staining	3+ intense staining
4+	> 75% positive		

COX-2 Immunohistochemical Scoring

Evaluation of % positive cells		Evaluation of stain intensity	
0	no staining	0	no staining
1+	≤ 25% positive	1+	mild staining
2+	> 25-50% positive	2+	moderate staining
3+	> 50-75% positive	3+	intense staining
4+	> 75% positive		

Inflammatory cytokines analysis

All inflammatory cytokines were determined by enzyme-linked immunosorbent assay (ELISA) kits (Quantikine® IL-6, IL-10, and IFN-γ Immunoassay, USA). Cytokine assay was performed according to the manufacturer's recommendation.

Statistical analysis

The statistical software package, SPSS for windows version 18.0 was used for all statistical analysis. Differences in ACF and AC numbers in each group, the levels of β-catenin and COX-2 expression, and the markers of inflammatory cytokines were compared by the one-way Analysis of Variance (ANOVA). Histological evaluation was analyzed by Chi-square test. The data were presented as Mean±SEM. The level of significance was accepted at $p \leq 0.05$.

Results

Effect of unpolished Thai rice on ACF formation in AOM-induced rats

The morphological study of normal colonic mucosa and ACF were identified under light microscope after methylene blue staining and shown in Figure 1A-B. In this study, AOM-induced ACF formation in rats was found only in the descending colon. Table 1 summarized the total number of AC per rat (AC/square centimeter) and ACF per rat (ACF/square centimeter). No ACF formation was detected in untreated rats, L-UTR, and H-UTR. The total density of AC in AOM + H-UTR (18.13±5.58 AC/square centimeter, p=0.01) group showed significantly decreased when compared with the AOM treated group (77.79±18.02 AC/square centimeter). Total density of ACF in AOM + L-UTR (11.64±3.87 ACF/square centimeter, p<0.01) and AOM + H-UTR (5.28±1.78 ACF/square centimeter, p<0.01) groups showed significantly decreased in dose dependent when compared with the AOM-treated group (21.33±5.36 AC/square centimeter).

Effect of unpolished Thai rice on histopathological changes in AOM-induced rats

In the present study, total ACF from AOM, AOM +

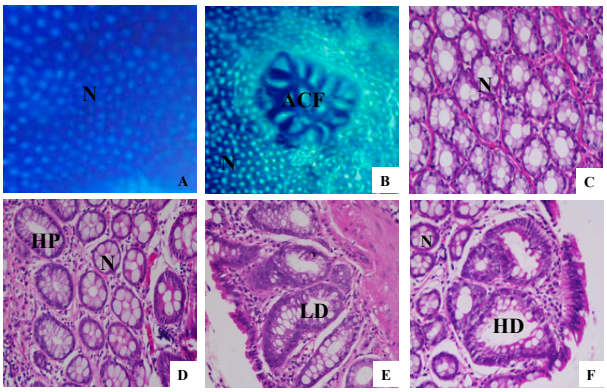

Figure 1. Methylene Blue and H&E Staining of Colonic Mucosa. Methylene blue staining exhibited normal colonic mucosa in untreated rats (A) and ACF formation in AOM-treated group (B) (Original magnification 40x). H&E staining for histopathological evaluation exhibited normal colonic mucosa in untreated rats (C). In AOM-treated group exhibited ACF with hyperplasia (D), low-grade dysplasia (E), and high-grade dysplasia (F), respectively (Original magnification 400x). N = normal crypt, HP = ACF with hyperplasia, LD = ACF with low-grade dysplasia, HD = ACF with high-grade dysplasia

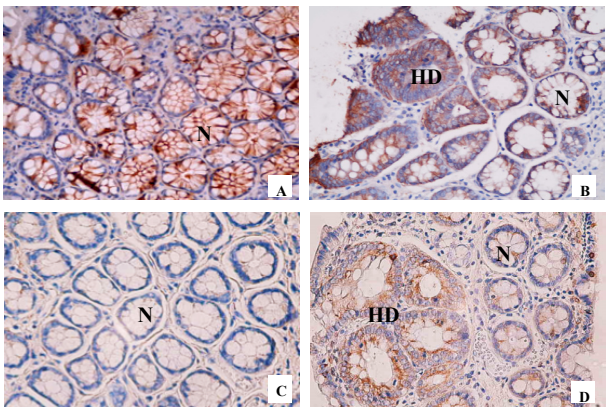

Figure 2. Immunohistochemical staining of β-catenin and COX-2 expression. High intensity of membranous β-catenin staining in colonic tissue from untreated rats (A). Membranous and cytoplasmic β-catenin staining in AOM-treated group (B). No COX-2 expression in colonic tissue from untreated rats (C). COX-2 positive staining in AOM-treated group (D) (Original magnification 400x). N = normal crypt, HD = ACF with high-grade dysplasia

Table 1. Effect of Unpolished Thai Rice on ACF Formation and Histopathological Changes in AOM-Treated Rats

Group	Total number/cm²		Histopathological changes			p
			Hyperplasia	Dysplasia (%)		
	AC	ACF	(%)	Low-grade	High-grade	
Untreated rats	0.00 ± 0.00	0.00 ± 0.00	0	0	0	
L-UTR	0.00 ± 0.00	0.00 ± 0.00	0	0	0	
H-UTR	0.00 ± 0.00	0.00 ± 0.00	0	0	0	
AOM	77.79 ± 18.02	21.33 ± 5.36	20.00	30.00	50.00	
AOM + L-UTR	39.70 ± 12.38	11.64 ± 3.87a**	23.33	46.67	30.00	a*
AOM + H-UTR	18.13 ± 5.58b**	5.28 ± 1.78b**	50.00	33.33	16.67	b**, c**

ANOVA was used to test the difference of ACF formation in each group. Data were expressed as mean ± SEM; Chi-squre test was used to test the difference of histopatholigical changes in each group; *, **Statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; [a, b] The p-values of AOM + L-UTR and AOM + H-UTR groups when compared with AOM group, respectively; [c] The p-value of AOM + H-UTR group when compared with AOM + L-UTR group; AOM = azoxymethane, L-UTR = low dose of unpolished Thai rice, H-UTR = high dose of unpolished Thai rice, AC = aberrant crypt, ACF = aberrant crypt foci

Table 2. Effect of Unpolished Thai Rice on β-catenin Expression in AOM-Treated Rats

Group	Number of ACF/field	Score summation of % positive cells	Score of stain intensity	
			Membranous staining	Cytoplasmic staining
AOM	7.73 ± 0.90	29.31 ± 0.21	1.53 ± 0.31	1.33 ± 0.35
AOM + L-UTR	6.67 ± 0.79	26.00 ± 0.18	1.80 ± 0.35	1.07 ± 0.25
AOM + H-UTR	4.00 ± 0.66	25.00 ± 0.17a**, b*	2.20 ± 0.35a**, b**	0.87 ± 0.34

ANOVA was used to test the difference of β-catenin expression in each group. Data were expressed as mean ± SEM. *, **Statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. [a] The p-value of AOM + H-UTR group when compared with AOM group, [b] The p-value of AOM + H-UTR group when compared with AOM + L-UTR group, AOM = azoxymethane, L-UTR = low dose of unpolished Thai rice, H-UTR = high dose of unpolished Thai rice

Table 3. Effect of Unpolished Thai Rce on COX-2 Expression in AOM-Treated Rats

Group	Number of ACF/field	Score summation of % positive cells	Score of stain intensity
AOM	3.60 ± 0.69	33.33 ± 0.26	2.40 ± 0.16
AOM + L-UTR	2.00 ± 0.53	30.00 ± 0.16	1.40 ± 0.13a**
AOM + H-UTR	1.87 ± 0.73	25.00 ± 0.19b*	1.33 ± 0.19b**

ANOVA was used to test the difference of COX-2 expression in each group. Data were expressed as mean ± SEM. *, **Statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. a The p-value of AOM + H-UTR group when compared with AOM group, b The p-value of AOM + H-UTR group when compared with AOM + L-UTR group, AOM = azoxymethane, L-UTR = low dose of unpolished Thai rice, H-UTR = high dose of unpolished Thai rice

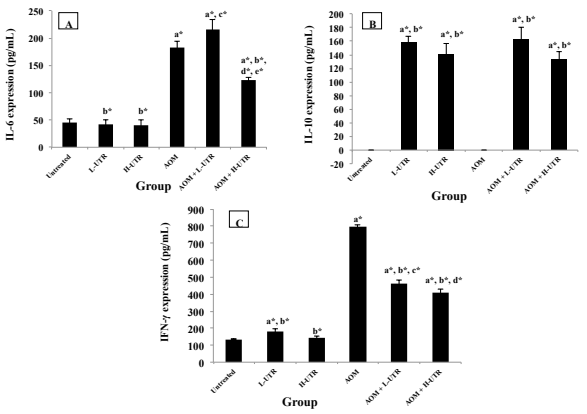

Figure 3. Inflammatory Cytokine Expression (pg/mL). *Statistically significant at $p \leq 0.01$, a) Significant difference from untreated rats. b) Significant difference from AOM-treated group. c) Significant difference between AOM + L-UTR and L-UTR. d) Significant difference between AOM + H-UTR and H-UTR. e) Significant difference between AOM + H-UTR and AOM + L-UTR

L-UTR, and AOM + H-UTR groups were subjected to histological analysis. As shown in Figure 1C-F, ACF were examined and classified according to the criteria as ACF with hyperplasia, ACF with low-grade dysplasia, and ACF with high-grade dysplasia. The effect of unpolished Thai rice on histopathological changes in AOM-induced colorectal ACF is shown in Table 1. The percentages of ACF with high-grade dysplasia in AOM + L-UTR (30.00%, p=0.01) and AOM + H-UTR (16.67%, p<0.01) groups showed statistically significant reduced in dose dependent when compared with the AOM-treated group (50.00%). It is indicated that unpolished Thai rice attenuated the progressing ACF with high-grade dysplasia in AOM-induced rats.

Effect of unpolished Thai rice on β-catenin expression in AOM-induced rats

In this study, immunohistochemistry staining of β-catenin localization patterns was analyzed. The expression and localization of β-catenin are shown in Figure 2A-B. In normal colonic mucosa, β-catenin was mainly localized in the membrane of cell-to-cell border both colonic epithelium and goblet cells (Figure 2A). Dysplastic ACF in AOM-treated group showed β-catenin localization from cell membrane into cytoplasm and also showed more intensity (Figure 2B), as compared with the normal mucosa. In this study, we analyzed β-catenin expression in ACF of AOM + L-UTR and AOM + H-UTR

groups when compared to that of AOM-treated group. The effect of unpolished Thai rice on β-catenin expression evaluated as % positive cells and stain intensity is shown in Table 2. In AOM-treated group, high score summation of positive cells with β-catenin staining was 29.31 ±0.21. In the treatment groups have a tendency to decrease the β-catenin positive cells with statistical significance in AOM + H-UTR group (25.00 ±0.17, p<0.01) when compared with AOM-treated group. Cytoplasmic β-catenin expression showed a tendency to decrease the intensity with dose dependent manner of AOM + L-UTR and AOM + H-UTR groups. In contrast, membranous β-catenin expression showed significantly increased in AOM + H-UTR group (2.20 ±0.35, p<0.01) when compared with AOM-treated group (1.53 ±0.31). These results demonstrated that unpolished Thai rice attenuated the abnormal β-catenin expression and translocation in AOM-induced rats.

Effect of unpolished Thai rice on COX-2 expression in AOM-induced rats

The staining of COX-2 expression in colonic mucosa is shown in Figure 2C-D. COX-2 was not expressed in normal colonic mucosa whereas dysplastic ACF in AOM-treated group showed COX-2 expression mainly in cytoplasm. The effect of unpolished Thai rice on COX-2 expression evaluated as % positive cells and stain intensity is shown in Table 3. In AOM-treated group, ACF showed high score summation of positive cells COX-2 staining (33.33±0.26). In the treatment groups, positive COX-2 staining were significantly decreased in AOM + H-UTR group (25.00 ±0.19, p=0.03) when compared with AOM-treated group. The stain intensity of COX-2 expression was significantly lower in dose dependent of AOM + L-UTR (1.40±0.13, p<0.01) and AOM + H-UTR groups (1.33±0.19, p<0.01) when compared with the AOM-treated group (2.40±0.16). These results demonstrated that unpolished Thai rice suppressed COX-2 expression-related to dysplastic progression in AOM-induced rats.

Effect of unpolished Thai rice on inflammatory cytokines expression in AOM-induced rats

The levels of serum cytokine concentrations (pg/mL) including interleukin (IL)-6, IL-10, and interferon gamma (IFN-γ) are showed in Figure 3. Serum levels of IL-6 and IFN-γ in untreated rats and unpolished Thai rice-treated groups without AOM induction showed significantly lower values at p<0.01 when compared with AOM induction group. The high values of IL-6 and IFN-γ were observed in AOM-treated group at 182.38±12.04 pg/mL and 794.72±15.23 pg/mL, respectively. Interestingly, serum level of IL-6 in AOM + H-UTR (123.26±5.18 pg/mL) was significantly decreased when compared with AOM-treated group at p<0.01. In addition, serum level of IFN-γ was significantly decreased with dose dependent manner at 460.49±23.29 pg/mL (p<0.01) and 409.51±21.34 pg/mL (p<0.01) in AOM + L-UTR and AOM + H-UTR groups, respectively when compared with AOM-treated group. On the contrary, serum IL-10 concentration was not detected in both untreated rats and AOM-treated groups. The high values of IL-10 were observed in unpolished Thai rice-treated groups (Figure 3B). No significant difference was observed in IL-10 level between unpolished Thai rice-treated groups of with- and without-AOM induction. ed in both untreated rats and AOM-treated groups. The high values of IL-10 were observed in unpolished Thai rice-treated groups (Figure 3B). No significant difference was observed in IL-10 level between unpolished Thai rice-treated groups of with- and without-AOM induction. These results implicated that unpolished Thai rice induced anti-inflammatory marker through the stimulation of IL-10 (anti-inflammatory cytokine) expression which is responsible to suppress certain proinflammatory cytokines expression, e.g., IL-6 and IFN-γ in AOM-induced rats.

Discussion

Colorectal carcinogenesis is multistep processes that involved with a progressive disruption of homeostatic mechanisms which controlling cell proliferation, cell differentiation, and programmed cell death. This progressive disruption seems to be mediated by several natural dietary compounds that can modulate intestinal epithelial cell signaling pathway (Fredericks et al., 2015). Previously, several studies indicated that increasing consumption of fruits, vegetable or high fiber diets may prevent the initiation and promotion stages of CRC (Chan and Giovannucci, 2010; Pan et al., 2011). ACF are considered as putative precursors for colon cancer. ACF formation accompanies changes in the morphology of colonic crypts in both benign and cancer. Since ACF formation is currently the earliest noted change visible with only a microscope, ACF can be used as a biomarker for colon cancer. Previous studies showed that the density of ACF increases from proximal to distal part of colon, being the highest in the rectosigmoidal region, which corresponds to anatomical location of CRC development. Besides, ACF in both human and rodents are more frequently found to locate in the distal parts than in the proximal parts of colon (Fenoglio-Preiser and Noffsinger, 1999). In this study, all ACF were observed only in the descending region which is similar to the previous reported.

Unpolished Thai rice, a good source of phytophenolics, has been reported to inhibit ACF formation in AOM-induced rats through oxidative stress defense mechanism (Suwannalert and Rattanachitthawat, 2011; Tammasakchai et al., 2012). Moreover, it showed the strongest association with CRC chemoprevention by reducing the colorectal polyp formation in human (Tantamango et al., 2011). In the present study, we investigated the effect of unpolished Thai rice on ACF formation and precancerous stage in AOM-induced rats. The results demonstrated that the administration of unpolished Thai rice significantly and dose dependently reduced the total number of AC and ACF formation in AOM-treated rats. In addition, unpolished Thai rice showed statistically significant and dose dependently reduced the percentages of ACF with high-grade dysplasia. In general, dysplastic ACF have been suggested to be more relevant precancerous lesions for CRC, whereas hyperplastic ACF often lack of the

potential to develop into cancer (Mori et al., 2005).

Mutation in the β-catenin expression has been regarded as early critical events during CRC carcinogenesis and is considered to play a gate keeper role in the development of CRC in both human and animal models (Takahashi et al., 1998). Alteration of β-catenin occurs in approximately 80% of human colon cancer which is known to result in cytoplasmic β-catenin accumulation. Several natural products have been reported to reduce the expression of β-catenin during colon carcinogenesis (Ashokkumar and Sudhandiran, 2011). In this study, we observed the decrease expression of membranous β-catenin accompanied by increase cytoplasmic β-catenin of dysplastic ACF in AOM-treated group whereas the unpolished Thai rice-treated groups clearly showed the decreased in cytoplasmic β-catenin expression. The results of this study demonstrated that unpolished Thai rice has the potential to reduce β-catenin expression in AOM-treated rats. Our results supported previous studies on β-catenin expression which was mainly localized at cell membrane of cell-to-cell borders in normal adjacent colon and increased during treatment with AOM (Xiao et al., 2008; Hegazy et al., 2013). It is indicated that unpolished Thai rice might be involved in the regulation of β-catenin level in the early stage of CRC carcinogenesis.

Over-expression of COX-2 in most cancer cells is found to stimulate cellular proliferation, enhance angiogenesis, enhance tumor invasiveness, and inhibit apoptosis. High expression of COX-2 also associated with inflammation process. Polyphenols are known to be powerful anti-oxidants and free radical scavengers which also have anti-inflammation properties. Polyphenolic compounds such as red wine and black tea have been reported to modulate COX-2 expression in AOM-induced rats (Luceri et al., 2002). In this study, COX-2 expression was not detected in untreated rats and unpolished Thai rice-treated groups which indicated that unpolished Thai rice is not affected to normal colonic cells. On the other hand, the AOM-treated group showed COX-2 over-expression throughout the tissues which was mainly located in cytoplasm. Our results demonstrated that unpolished Thai rice showed significantly decrease COX-2 expression in dose dependent manner. Furthermore, our results revealed the correlation between COX-2 over-expression and cytoplasmic β-catenin expression which has been reported in recent studies, suggesting a local interaction between β-catenin and COX-2 molecules to progress the growth and invasion of CRC (Hegazy et al., 2013; Kazem et al., 2014). Previous studies reported that COX-2 expression may be enhanced by Wnt/β-catenin signaling pathway (Nunez et al., 2011). It is supposed that unpolished Thai rice may down regulate Wnt signaling pathway via the inhibition of β-catenin, then decreasing COX-2 expression. These results were revealed by significantly decreased COX-2 expression in dose dependent after treatment with unpolished Thai rice. Therefore, increased β-catenin and COX-2 expression can be used as biomarkers for tumor progression.

COX-2, rate-limiting enzyme, is required for prostaglandin biosynthesis which is induced by broad spectrum of growth factor and proinflammatory cytokines. High expression of COX-2 also associated with inflammation process. Inflammatory cytokines, key regulators of immune responses, modulate tumor growth, and tumor microenvironment through mediating interactions between cancer cells and infiltrating inflammatory cells (Kantola et al., 2012). In the present study, serum levels of interleukin (IL)-6 and interferon gamma (IFN-γ) were low levels in untreated rats and unpolished Thai rice-treated groups indicating no inflammatory reaction occurs in the rats when treated with unpolished Thai rice alone. The serum values of IL-6 and IFN-γ were elevated in all groups with AOM induction implicating inflammation involvement, which are similar to the prior studies. Previously, several studies have been reported to increase IL-6 expression in the serum of patients with CRC and also associated with tumor stage, size, and metastasis of CRC. In addition, increased serum level of IL-6 has been shown in animal models of colitis-associated CRC induced by AOM/DSS (Greten et al., 2004; Knupfer and Preiss, 2010). Recently, serum IL-6 and IFN-γ profiles were found to be significantly higher in CRC patients than in those of healthy controls. These findings suggested that increased levels of proinflammatory cytokines are strongly associated with the risk of CRC (Kantola et al., 2012). Interestingly, our results demonstrated that unpolished Thai rice effectively decreased serum levels of proinflammatory cytokines (IL-6 and IFN-γ). It is strongly indicated that unpolished Thai rice decreased inflammatory reactions in AOM-induced rats by downregulation of proinflammatory cytokines.

In contrast to IL-6, undetectable of IL-10 was found in both untreated rats and AOM-treated groups. It could be suspected that lack of IL-10 expression is related to no inflammation in untreated rats. Concurrently, absence of IL-10 level in AOM-treated group is consistent with previous studies. This undetectable IL-10 level would be due to the counterbalance between proinflammatory stimuli and anti-inflammatory activity. Nevertheless, the elevation of IL-10 serum levels in the unpolished Thai rice treatment groups seems to exert its protective effect as anti-inflammatory cytokine. In previous studies, high level of IL-10 expression was shown to correlate with poor survival of cancer patients (Visco et al., 2004), whereas some other studies showed contrary results (Soria et al., 2003; Toiyama et al., 2010). Therefore, deep insight into the controversial functions of IL-10 in chronic diseases and cancer is important required (Zhao et al., 2015). IL-10 exerted both of anti-inflammatory and anti-tumor effects which inhibited tumor growth. IL-10 deficient mice have been shown to develop colitis and then colitis-associated cancer (Sturlan et al., 2001). Surprisingly, our results showed significantly increased level of IL-10 in unpolished Thai rice pretreated with AOM. It is indicated that IL-10 controls inflammatory reaction by suppressing the expression of proinflammatory cytokines which is similar to previous studies. IL-10 has been reported to inhibit the production of proinflammatory mediators by monocytes and macrophages such as IFN-γ, IL-6, IL-8, and tumor necrosis factor -α (TNF-α). Inhibition of IL-10 is overcome by increasing IFN-γ concentration which was the competitive interaction between two cytokines

pathways (De Waal Malefyt et al., 1991). However, the effect of IL-10 is quite complex which is still considered as anti-inflammatory and immunosuppressive properties.

Our present results suggest that unpolished Thai rice has anti-inflammatory effect which simultaneously decreases levels of certain proinflammatory cytokines and elevates immunosuppressive cytokine.

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