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Preventive effects of rice bran oil on 1,2-dimethylhydrazine/dextran sodium sulphate-induced colon carcinogenesis in rats

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ABSTRACT

This study included F344 rats which were fed AIN-93G-based 14% high-fat diets and were divided into the following six groups: Groups B and N, 14% soybean oil (SO); group P, 14% SO containing 0.04% piroxicam; group L, 5% rice bran oil (RBO) and 9% SO; group M, 9% RBO and 5% SO; and group H, 14% RBO. All the rats—except those in group B—were administered 1,2-dimethylhydrazine/dextran sodium sulphate to induce colitis-related colon carcinogenesis. The rats were sacrificed, and their colons were removed to examine aberrant crypt foci (ACF) and mucin-depleted foci (MDF). The results revealed that the rats from all the RBO group rats exhibited significantly reduced colon tumour formation, MDF, and ACF, especially sialomucin (SIM)-producing ACF. The hepatic antioxidant status, including the glutathione (GSH) and thiobarbituric acid reactive substance levels as well as superoxide dismutase and catalase activities, was superior among the RBO groups, which might contribute to the potential of RBO with respect to delaying colon carcinogenesis.

1. Introduction

Colon cancer is a major cause of morbidity and mortality worldwide (Gellad & Provenzale, 2010). A high-fat diet is associated with the risk of colon cancer (Endo et al., 2009). Colon carcinogenesis is a multistep process that involves stepwise accumulation of molecular and genetic defects in colonic epithelial cells, i.e., changes in the normal epithelium, followed by hyperproliferation, and eventually, detectable carcinoma. Aberrant crypt foci (ACF) are the preneoplastic lesions of colon cancer (Bird, 1987), and different ACF parameters are used as indicators of the degree of colon cancer risk. Jenab, Chen, and Thompson (2001) suggested that sialomucin (SIM)-producing ACF may be more advanced than sulfomucin (SUM)-producing ACF. Mucin-depleted foci (MDF) were observed as an early focal abnormality in the colonic mucosa of rats treated with azoxymethane (AOM) and were found to be closely linked to the inflammatory process (Femia, Dolara, Luceri, Salvadori, & Caderni, 2009).

Recently, dextran sodium sulphate (DSS) was reported to enhance the development of colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) or AOM in animal models (Tanaka et al., 2003). DMH was reported to be a colon procarcinogen that is metabolically activated to the active form in the liver and subsequently transported into the colon *via* bile and blood (Rosenberg, Giardinal, & Tanaka, 2009). DMH also produced free radicals that induced oxidative DNA damage in the liver and colon (No et al., 2007). The DMH/DSS-induced colon cancer animal model was developed to better understand the pathogenesis of colitis-related colon cancer (Tanaka et al., 2003). Animal tissue cultures that may be useful for cancer chemopreventive studies are in the process of development; however, primary intestinal epithelial cells are difficult to obtain and culture (Fenton & Hord, 2006). Therefore, animal models are still included in the standard protocol for colon cancer chemoprevention studies.

Rice (*Oryza sativa*) is a staple food of over half the world's population—especially Asians—who have a markedly lower incidence of colorectal cancer than that in Western populations (Hudson, Dinh, Kokubun, Simmonds, & Gescher, 2000). A recent epidemiological study showed that rice consumption was associated with a decreased risk of distal colorectal cancer (Uchida et al., 2010). Rice bran is the outer layer of brown rice and is obtained as a by-product of the rice milling industry. The polishing process used for rice involves removal of the bran, which is a valuable part of rice grains. Rice bran contains large amounts of bioactive phytochemicals, such as tocopherols, tocotrienols, oryzanols, and phenolic compounds (Zhang, Zhang, Zhang, & Liu, 2010), which

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are lipophilic fractions. Rice bran oil (RBO), which comprises 20–25% of rice bran, is a unique and rich source of bioactive phytochemicals (Lerma-García, Herrero-Martínez, Simó-Alfonso, Mendonça, & Ramis-Ramos, 2009). RBO is reported to have hypolipidemic, antiatherogenic, and antidiabetic properties (Chen & Cheng, 2006). In addition, RBO is a rich source of bioactive phytochemicals with antioxidative and cancer chemopreventive properties. For example, a tocotrienol-rich fraction isolated from RBO suppressed chemically induced hepatocarcinogenesis in rats because of its antioxidative activity (Iqbal, Minhajuddin, & Beg, 2004).

However, only a limited number of studies have been performed on the effect of RBO on colon carcinogenesis, despite the fact that the components or extracts of RBO may have protective effects *in vitro* (Hudson et al., 2000; Kong et al., 2009) and *in vivo* (Sunagawa et al., 2009). The aims of the present study were to determine the effects of the RBO components in a modified American Institute of Nutrition (AIN)-93G-based 14% high-fat diet on DMH/DSS-induced colitis-related colon carcinogenesis in rats and to investigate the possible mechanism.

2. Materials and methods

2.1. Materials

Ferric chloride, methylene blue, and acetic acid were purchased from Nacalai Tesque Inc. (Tokyo, Japan), Showa Chemicals Co. (Tokyo, Japan), and Shimakyu Pure Chemicals (Osaka, Japan), respectively. Alcian blue, *N*,*N*′-dimethyl-*p*-phenylenediamine, *N*,*N*′-dimethyl-*m*-phenylenediamine, DMH, and the remaining chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. RBO preparation

Rice (*O. sativa* var. Taikeng No. 9) seeds were purchased from Union Rice Co. Ltd. (Taipei, Taiwan). RBO was extracted by the supercritical carbon dioxide fluid extraction method. Briefly, rice bran (15 kg) was placed in the extraction vessel of the supercritical fluid extractor. RBO was extracted at a pressure of 270 bars and a temperature of 50 °C. The pressure within the extraction vessel was generated with a constant flow rate of carbon dioxide of 1.75 kg/min and was controlled by an automated back-pressure regulator. The extraction process lasted for 2 h, and RBO was collected from the collection vessel after depressurisation of the supercritical carbon dioxide extraction system.

2.3. Experimental design

The animal study protocol was approved by the Institutional Animal Care and Use Committee of Taipei Medical University. The study population included male F344 rats (age, 3–5 weeks; National Laboratory Animal Center, Taipei) that were individually housed in steel cages maintained at 21 °C, with a 12-h light-dark cycle. The rats had *ad libitum* access to the modified AIN-93G-based 14% high-fat diet and to water. After 2 weeks of acclimatisation, the animals were randomly divided into six groups (10 or 16 rats per group) and fed different AIN-93G-based 14% high-fat diets: group B (blank, 14% soybean oil [SO]), group N (negative control, 14% SO), group P (positive control, 14% SO containing 0.04% piroxicam), group L (low dose of RBO [5% RBO] and 9% SO), group M (medium dose of RBO [9% RBO] and 5% SO), and group H (high dose of RBO [14% RBO]). The rats were fed these diets 1 week before the beginning of the experiment, after which all rats, except for those in group B, were intraperitoneally (i.p.) injected with DMH (40 mg/kg body weight) twice a week. The rats received 1% DSS in their drinking water for 1 week after the second DMH injection, and then received distilled water until the end of the experiment (Tanaka et al., 2003). All the rats were sacrified after being fed for 13 weeks, and their colons and livers were obtained for further studies. After excision, the colons were flushed with phosphate-buffered saline (PBS) and examined for colonic neoplasms in order to calculate tumour incidence during the 13-week experiment. The body weights and food intake of the rats were recorded every week.

2.4. ACF counts

The ACF were counted using the method developed by Bird (1987). The colons were cut along the longitudinal axis and flushed with PBS. Each colon was cut into three segments (proximal, middle, and distal) of equal length and fixed flat between filter papers in 10% buffered formalin for at least 24 h. The fixed sections were stained with 0.2% methylene blue solution for 5 min and placed on microscopic slides with the mucosal side facing up. The ACF were counted under a light microscope (Nikon Corp., Tokyo, Japan) at 40× magnification and distinguished from normal crypts by their increased size, irregular and dilated luminal opening, thicker epithelial lining, and pericryptal zone. The total number of ACF per colon, the total number of aberrant crypts (ACs) observed in each focus, and the location of each focus was recorded.

2.5. Identification of MDF and mucin-producing ACF

The mucin-producing ACF in the distal colon segment and the MDF were stained with high-iron diamine alcian blue (HIDAB) (Caderni et al., 1995). Briefly, 20 mg *N*,*N*′-dimethyl-*p*-phenylene diamine and 120 mg *N*,*N*′-dimethyl-*m*-phenylene diamine were dissolved in 50 ml distilled water and 1.4 ml of 60% ferric chloride. The methylene blue-stained colons were immersed in high-iron diamine solution in petri dishes for 50 min at room temperature; these petri dishes were protected from light. Next, the colons were rinsed with distilled water, stained with 1% alcian blue in 3% acetic acid for 30 min, rinsed in 80% ethanol, and then rinsed with distilled water; the colons were then observed under a light microscope (Nikon Corp., Tokyo, Japan) at 40× magnification. The MDF were identified using the criteria established by Caderni et al. (2003), i.e., they had no or very little mucin production and also fulfilled at least 2 of the following criteria: (1) distortion of the opening of the lumen compared with normal surrounding crypts, (2) elevation of the lesion above the surface of the colon, and (3) a crypt multiplicity of >3. The distal colon segments were assessed for mucin-producing ACF by using the criteria established by Jenab et al. (2001): if the ACF were stained dark brown by HIDAB, it indicated SUM production, whereas if they were stained bright or dark blue, it indicated SIM production. ACF with greater than 85% SUM-producing cells were considered as SUM-producing ACF (SUM-ACF), those with greater than 85% SIM-producing cells were considered as SIM-producing ACF (SIM-ACF), and those with a smaller percentage of both were considered as mixed-type ACF (MIX-ACF).

2.6. Lipid peroxidation measurement

The liver and colon samples were homogenised with 10 mM Tris–HCl buffer (pH 7.4) containing 250 mM sucrose and 1 mM ethylenediaminetetraacetic acid (EDTA) (Shih, Chang, Yang, Chou, & Cheng, 2008). After centrifugation at 4500 *g* for 10 min, the supernatants were collected and lipid peroxidation was evaluated using thiobarbituric acid-reactive substances (TBARSs) as indicators. The homogenates were mixed with a solution containing

0.22% sulphuric acid, 0.67% thiobarbituric acid, and 10% phosphotungstic acid, and the mixture was incubated at 95 °C for 1 h. Next, the reaction mixture was incubated on ice for 10 min. The reactants were extracted using *n*-butanol and centrifuged at 1600 *g* for 10 min; their fluorescence was measured at an emission wavelength of 555 nm and an excitation wavelength of 515 nm.

2.7. Measurement of antioxidant enzyme activity and glutathione content

The superoxide dismutase (SOD) activity assay was performed using a commercial kit (RANSOD Kit; Randox Laboratories, Crumlin, Antrim, UK). After centrifugation, the liver and colon homogenates were mixed with 0.05 mM xanthine, 0.025 mM 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT), and 80 U/L xanthine oxidase. The absorbance was monitored at 505 nm. One unit of SOD is defined as the amount of enzyme that inhibits the rate of INT reduction per minute by 50%. The catalase activity was assayed using a commercial kit (Catalase Assay Kit; Cayman Chemical Company, Ann Arbor, MI, USA). The liver and colon samples were homogenised with 50 mM potassium phosphate (pH 7.0) containing 1 mM EDTA and centrifuged at 10,000 *g* at 4 °C for 15 min. Catalase activity was initiated in the supernatants by adding hydrogen peroxide and incubating the reaction mixture on a shaker for 20 min; the reaction was subsequently terminated by adding potassium hydroxide. A solution of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole and potassium periodate was added to the mixture, and the absorbance was monitored at 540 nm. One unit of catalase is defined as the amount of enzyme that causes the formation of 1.0 nmol of formaldehyde per minute. The glutathione (GSH) content of the liver and colon samples was assayed using a commercial glutathione assay kit (Cayman Chemical Company). The supernatants were mixed with metaphosphoric acid and centrifuged at 3000 *g* at 4 °C for 10 min. The upper layer was incubated with freshly prepared assay reagents on a shaker for 25 min, and the absorbance was monitored at 405 nm.

2.8. Statistical analysis

Data were expressed as mean (standard deviation [SD]). The differences in data between the experimental groups were assessed using one-way analysis of variance (ANOVA) and the SAS software (SAS Institute, Cary, NC, USA). The mean values were considered significantly different at $P < 0.05$, as determined by Duncan's multiple-range test.

3. Results

3.1. Effect of RBO on the number of DMH/DSS-induced ACFs in rat colons

During the 13-week experiment, colonic ACF were observed in all the DMH/DSS-induced rats (Table 1). The rats from all the RBO-treated groups had significantly lower ($P < 0.05$) total numbers of ACF and ACs than the rats from group N. The rats from group P had the lowest total ACF and ACs among the DMH/DSS-induced rats, and these numbers were significantly different ($P < 0.05$) from those for the other DMH/DSS-induced rats. However, the crypt multiplicity (total number of ACs/total number of ACF, i.e., number of aberrant crypts per focus) did not differ between the groups. The ACF were divided into two groups on the basis of the number of crypts: (1) the small ACF group where each focus contained 1, 2, or 3 ACs and (2) the large ACF group, where each focus contained 4 or more ACs. The rats from all the RBO-treated groups had a significantly lower ($P < 0.05$) number of 1-, 2-, 3-, and 4-crypt ACF as well as small and large ACF than did the rats from group N (Table 2). The rats from group P had the lowest number of small and large ACF among the DMH/DSS-induced rats, and this number was significantly different ($P < 0.05$) from that for the other DMH/DSS-induced rats.

3.2. Effects of RBO on the distribution of DMH/DSS-induced ACF in rat colons

ACF were mainly found in the middle and distal colon in all DMH/DSS-induced rats; these ACF accounted for approximately 90% of the total ACF (Table 3). The rats from all the RBO-treated groups showed a significant reduction ($P < 0.05$) in the number of ACF in each colon segment. The rats from group P exhibited significantly different ($P < 0.05$) and the lowest number of ACF in the proximal, middle, and distal colons.

3.3. Effects of RBO on the types of mucin-producing ACF and MDF observed in DMH/DSS-induced rats

HIDAB staining revealed that the ACF in the distal colons of the rats from group N were SUM-ACF (35% of the total ACF) and MIX-ACF (38% of the total ACF), as shown in Table 4. The rats from the RBO-treated groups—especially groups M and H—had higher percentages of SUM-ACF (~51%) than of MIX-ACF (32–35%) and SIM-ACF (14–17%). The rats from the RBO-treated groups had a significantly lower ($P < 0.05$) number of MIX-ACF and SIM-ACF than the rats from group N (Table 4). The rats from group P exhibited significantly different ($P < 0.05$) and the lowest numbers of SUM-,

Table 1
Effect of RBO on DMH/DSS-induced ACF in the colons of male F344 rats.[a,b]

Group[c]	ACF incidence (ACF rats/total rats)	Total ACF/colon	Total AC/colon	Crypt multiplicity (total AC/total ACF)
N	100% (8/8)	287 ± 70^{A}	619 ± 116^{A}	2.2 ± 0.2
P	100% (8/8)	94 ± 40^{C}	194 ± 71^{C}	2.1 ± 0.2
L	100% (8/8)	192 ± 26^{B}	424 ± 63^{B}	2.2 ± 0.2
M	100% (8/8)	167 ± 41^{B}	363 ± 77^{B}	2.2 ± 0.2
H	100% (8/8)	191 ± 32^{B}	426 ± 87^{B}	2.2 ± 0.1

[a] All values are mean ± SD ($n = 8$).
[b] Values with the same letter in a column are not significantly different from one another as determined by Duncan's multiple range test, $P < 0.05$.
[c] N: high-fat AIN-93G diet; P: high-fat AIN-93G diet containing 0.04% piroxicam; L: high-fat AIN-93G diet containing 5% rice bran oil and 10% soybean oil; M: high-fat AIN-93G diet containing 10% rice bran oil and 5% soybean oil; and H: high-fat AIN-93G diet containing 15% rice bran oil. The groups of N, P, L, M, and H were treated with DMH/DSS to induce colon cancers.

Table 2
Effects of RBO on the numbers of crypts per focus in DMH/DSS-induced ACF in colons of male F344 rats.[a,b]

Group[c]	Number of foci containing					Small ACF[d]	Large ACF[e]
	1 Crypt	2 Crypts	3 Crypts	4 Crypts	≧5 Crypts		
N	91 ± 33^{A}	108 ± 32^{A}	52 ± 10^{A}	26 ± 4^{A}	10 ± 4^{A}	252 ± 71^{A}	36 ± 5^{A}
P	32 ± 19^{B}	33 ± 14^{C}	18 ± 7^{C}	9 ± 5^{D}	1 ± 1^{B}	84 ± 39^{C}	10 ± 5^{C}
L	53 ± 17^{B}	79 ± 14^{B}	36 ± 7^{B}	17 ± 6^{BC}	7 ± 4^{A}	168 ± 26^{B}	24 ± 9^{B}
M	52 ± 23^{B}	63 ± 13^{B}	31 ± 11^{B}	13 ± 3^{CD}	8 ± 4^{A}	146 ± 40^{B}	20 ± 6^{B}
H	51 ± 12^{B}	79 ± 15^{B}	37 ± 8^{B}	18 ± 6^{B}	7 ± 5^{A}	167 ± 24^{B}	25 ± 10^{B}

[a] All values are mean ± SD (n = 8).
[b] Values with the same letter in a column are not significantly different from one another as determined by Duncan's multiple range test, $P < 0.05$.
[c] N: high-fat AIN-93G diet; P: high-fat AIN-93G diet containing 0.04% piroxicam; L: high-fat AIN-93G diet containing 5% rice bran oil and 10% soybean oil; M: high-fat AIN-93G diet containing 10% rice bran oil and 5% soybean oil; and H: high-fat AIN-93G diet containing 15% rice bran oil. The groups of N, P, L, M, and H were treated with DMH/DSS to induce colon cancers.
[d] Small ACF: ACF containing 1, 2 or 3 crypts.
[e] Large ACF: ACF containing 4 or more crypts.

Table 3
Effect of RBO on the distributions of DMH/DSS-induced ACF in the colons of male F344 rats.[a,b]

Group[d]	ACF distributions at the colon segments		
	Proximal	Middle	Distal
N	32 ± 13^{A} (11%)[c]	128 ± 46^{A} (44%)	128 ± 37^{A} (45%)
P	9 ± 5^{C} (10%)	40 ± 18^{C} (42%)	45 ± 23^{C} (48%)
L	20 ± 9^{B} (11%)	87 ± 18^{B} (45%)	85 ± 21^{B} (44%)
M	20 ± 7^{B} (12%)	75 ± 31^{B} (45%)	72 ± 24^{B} (43%)
H	18 ± 14^{BC} (9%)	90 ± 31^{B} (47%)	83 ± 14^{B} (44%)

[a] All values except for incidence are mean ± SD (n = 8).
[b] Values with the same letter in a column are not significantly different from one another as determined by Duncan's multiple range test, $P < 0.05$.
[c] Values in parentheses are percentages of a segmented ACF/total numbers of ACF.
[d] N: high-fat AIN-93G diet; P: high-fat AIN-93G diet containing 0.04% piroxicam; L: high-fat AIN-93G diet containing 5% rice bran oil and 10% soybean oil; M: high-fat AIN-93G diet containing 10% rice bran oil and 5% soybean oil; and H: high-fat AIN-93G diet containing 15% rice bran oil. The groups of N, P, L, M, and H were treated with DMH/DSS to induce colon cancers.

Table 4
Effects of RBO on the different types of ACF producing mucins in DMH/DSS-induced ACF in the distal colon of male F344 rats.[a,b]

Group[c]	Numbers of different types of ACF producing mucins[d]		
	SUM	MIX	SIM
N	45 ± 17^{A} (35%)[e]	48 ± 19^{A} (38%)	35 ± 14^{A} (27%)
P	24 ± 11^{C} (54%)	14 ± 7^{C} (31%)	7 ± 5^{C} (15%)
L	32 ± 7^{BC} (38%)	34 ± 10^{B} (40%)	19 ± 7^{B} (22%)
M	37 ± 13$^{A–C}$ (51%)	23 ± 7^{BC} (32%)	12 ± 8^{BC} (17%)
H	43 ± 7^{AB} (51%)	29 ± 5^{C} (35%)	12 ± 5^{BC} (14%)

[a] All values are mean ± SD (n = 8).
[b] Values with the same letter in a column are not significantly different from one another as determined by Duncan's multiple range test, $P < 0.05$.
[c] N: high-fat AIN-93G diet; P: high-fat AIN-93G diet containing 0.04% piroxicam; L: high-fat AIN-93G diet containing 5% rice bran oil and 10% soybean oil; M: high-fat AIN-93G diet containing 10% rice bran oil and 5% soybean oil; and H: high-fat AIN-93G diet containing 15% rice bran oil. The groups of N, P, L, M, and H were treated with DMH/DSS to induce colon cancers.
[d] SUM: sulfomucin; MIX: mixed sulfomucin and sialomucin; and SIM: sialomucin.
[e] Values in parentheses are percentages of a fixed type of ACF producing mucins/total numbers of ACF producing mucins.

MIX-, and SIM-ACF in the distal colon. MDF, which are also preneoplastic lesions of colon cancer, were observed in almost all the DMH/DSS-induced rats (Table 5). All the rats from the RBO-treated groups had a significantly lower ($P < 0.05$) total number of MDF than the rats from group N. The inhibitory effect of RBO on the MDF was dose-dependent, and the effects of RBO on groups M and H were compatible with those on group P. MDF almost appeared in the middle and distal colons, and the rats from all the RBO-treated groups had a significantly lower ($P < 0.05$) number of MDF in these colon segments than did the rats from group N (Table 5).

3.4. Effects of RBO on GSH content, antioxidant enzyme activities, and TBARS levels in the livers and colons of DMH/DSS-induced rats

The antioxidant parameters (GSH content, catalase and SOD activities, and TBARS levels) in the livers differed among the groups of DMH/DSS-induced rats (Table 6). The rats from group N had the lowest hepatic GSH content, lowest catalase and SOD activities, and highest TBARS levels among all the groups, and this difference was statistically significant ($P < 0.05$). Compared to the rats in group N, the rats in the RBO-treated groups—especially groups M and H—showed significantly different ($P < 0.05$) and elevated hepatic GSH content, elevated catalase and SOD activities, and reduced TBARS levels; these results were compatible with those for group B. However, no significant differences were observed between these parameters in the colon tissues of DMH/DSS-induced rats (Table 6).

4. Discussion

To the best of our knowledge, this is the first study reporting that RBO can effectively reduce DMH/DSS-induced colon carcinogenesis in a rat model, on the basis of histological analysis of parameters such as ACF, mucin-producing ACF, and MDF. During the experimental period, colonic tumours were observed only in the rats from group N, with an incidence of 31%. These results showed that RBO can suppress the formation of both preneoplastic lesions and neoplasms in the colon tissues of rats.

ACF are considered the standard biomarker of colon carcinogenesis and have been used in many chemopreventive studies; their growth and morphological characteristics have been widely studied. ACF appear predominantly in the distal colon early in carcinogenesis; subsequently, they appear in the proximal colon, with some ACF exhibiting focal expansion (Caderni et al., 1995). In this study, ACF were mainly distributed in the middle and distal colons in all the DMH/DSS-induced rats; these ACF accounted for approximately 90% of the total ACF, and a significant reduction ($P < 0.05$) in the number of ACF was observed in each colon segment with all doses of RBO (Table 3). ACF growth is reported to occur through the mechanism of "crypt fission" (Tsukamoto et al., 1999), and therefore, ACF with more crypts indicate a more advanced cancer stage. In the present study, the rats in the RBO groups showed a significant reduction ($P < 0.05$) in the number of small and large ACF

Table 5
Effect of RBO on numbers of DMH/DSS-induced MDF in the colon of male F344 rats.[a,b]

Group[c]	MDF incidence	MDF numbers/colon	MDF distributions at the colon segments		
			Proximal	Middle	Distal
N	100% (8/8)	5.3 ± 1.16^{A}	0.1 ± 0.4 (5%)[d]	2.3 ± 1^{A} (42%)	2.9 ± 0.8^{A} (53%)
P	87.5% (7/8)	1.5 ± 0.93^{C}	0.1 ± 0.4 (8%)	0.5 ± 0.5^{B} (33%)	0.9 ± 0.6^{C} (59%)
L	100% (8/8)	3.4 ± 1.06^{B}	0.3 ± 0.4 (11%)	1.2 ± 1^{B} (32%)	2.0 ± 0.5^{B} (57%)
M	100% (8/8)	2.1 ± 0.99^{C}	0.1 ± 0.4 (6%)	0.8 ± 0.7^{B} (35%)	1.3 ± 0.7^{BC} (59%)
H	100% (8/8)	1.8 ± 0.88^{C}	0 ± 0 (0%)	1.1 ± 0.6^{B} (60%)	0.6 ± 0.9^{C} (40%)

[a] All values are mean ± SD ($n = 8$).
[b] Values with the same letter in a column are not significantly different from one another as determined by Duncan's multiple range test, $P < 0.05$.
[c] N: high-fat AIN-93G diet; P: high-fat AIN-93G diet containing 0.04% piroxicam; L: high-fat AIN-93G diet containing 5% rice bran oil and 10% soybean oil; M: high-fat AIN-93G diet containing 10% rice bran oil and 5% soybean oil; and H: high-fat AIN-93G diet containing 15% rice bran oil. The groups of N, P, L, M, and H were treated with DMH/DSS to induce colon cancers.
[d] Values in parentheses are percentages of a fixed segment of MDF/total numbers of MDF.

Table 6
Effect of RBO on GSH level, antioxidant enzyme activities and TBARS of liver and colon in the DMH/DSS-induced F344 rat.[a,b]

Parameters	B[c]	N	P	L	M	H
Glutathione levels (nmol/μg protein)						
Liver	10.68 ± 3.82^{AB}	5.69 ± 0.94^{C}	11.99 ± 3.36^{A}	7.92 ± 2.91^{BC}	11.01 ± 1.99^{AB}	9.37 ± 2.37^{AB}
Colon	3.82 ± 0.88^{A}	3.40 ± 0.81^{ABC}	3.64 ± 0.66^{AB}	3.25 ± 0.55^{ABC}	2.79 ± 0.47^{C}	2.96 ± 0.77^{BC}
Catalase (U/μg protein)						
Liver	1.10 ± 0.24^{A}	0.70 ± 0.17^{B}	1.05 ± 0.32^{A}	0.88 ± 0.41^{AB}	0.92 ± 0.20^{AB}	1.02 ± 0.30^{A}
Colon	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
Superoxide dismutase (U/mg protein)						
Liver	0.67 ± 0.09^{A}	0.47 ± 0.08^{C}	0.56 ± 0.19^{ABC}	0.54 ± 0.09^{BC}	0.61 ± 0.12^{AB}	0.62 ± 0.08^{AB}
Colon	0.14 ± 0.05	0.13 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.13 ± 0.04	0.11 ± 0.05
Thiobarbituric acid reactive substances (nmol MDA/mg protein)						
Liver	0.47 ± 0.14^{C}	0.92 ± 0.27^{A}	0.73 ± 0.14^{B}	0.61 ± 0.14^{BC}	0.58 ± 0.11^{BC}	0.55 ± 0.10^{C}
Colon	2.91 ± 0.42	2.75 ± 0.30	2.69 ± 0.53	2.74 ± 0.54	2.70 ± 0.28	2.84 ± 0.32

[a] All values are mean ± SD.
[b] Values with the same letter in a row are not significantly different from one another as determined by Duncan's multiple range test, $P < 0.05$.
[c] B: high-fat AIN-93G diet, non-induced by DMH/DSS; N: high-fat AIN-93G diet; P: high-fat AIN-93G diet containing 0.04% piroxicam; L: high-fat AIN-93G diet containing 5% rice bran oil; M: high-fat AIN-93G diet containing 10% rice bran oil; and H: high-fat AIN-93G diet containing 15% rice bran oil. The groups of N, P, L, M, and H were treated with DMH/DSS t.

(Table 2), which suggested that RBO may be effective against colon carcinogenesis at an early stage of ACF development.

Many different ACF parameters have been evaluated as indicators of the degree of colon cancer risk. It has been observed that ACF differ in the type of mucin production, including SIM, SUM, and mixed types (Caderni et al., 1995), and that SIM production in many colonic tumours is correlated with an increased level of dysplasia (Uchida, Kado, Onoue, & Tohyama, 1997). Therefore, it is important to identify the different types of mucin produced by ACF to evaluate the degree of ACF development (Jenab et al., 2001). In the present study, on comparison with the rats from group N, it was found that all doses of RBO significantly reduced ($P < 0.05$) the numbers of both SIM-ACF and MIX-ACF in the distal colon (Table 4). No differences were observed between group N and group M or group H with respect to the number of SUM-ACF. In addition, the RBO-fed rats showed higher percentages of SUM-ACF than MIX-ACF. These results also suggested that RBO may delay ACF development and arrest it at an early stage.

Because the changes in the Wnt signalling pathway and the mutations in the beta-catenin, *Apc*, and *K-ras* genes are similar in both MDF and tumours, MDFs are considered to be a promising biomarker for studying the effects of chemopreventive agents on colon carcinogenesis (Femia & Caderni, 2008). In addition, MDF are closely linked with inflammatory processes and tumour formation (Femia et al., 2009). The preneoplastic lesions in the order of increasing malignancy are as follows: SUM-ACF, MIX-ACF, SIM-ACF, and MDF. In the present study, all the doses of RBO (especially the medium and high doses) significantly reduced ($P < 0.05$) the number of MDF and affected the distribution of the MDF within the middle and distal colons (Table 5), suggesting that RBO can suppress the development of advanced preneoplastic lesions of colon cancer.

DMH and its related compounds induced neoplasms specifically in rat colons. These compounds are metabolised in the liver *via* AOM and methylazoxymethanol to form active intermediates and are subsequently transported to the colon *via* bile or blood. Moreover, Weisburger (1971) reported that DMH produced free radicals in the blood, liver, and large bowel in experimental models. Some studies indicated that DMH reduced hepatic antioxidant status and increased TBARS levels in animals (Aranganathan, Selvam, & Nalini, 2009). The results of the present study are consistent with those of the above mentioned studies.

The phytochemicals from RBO, such as tocotrienols, γ-oryzanol, and other plant sterols, are involved in anti-inflammatory and apoptosis-induced processes in colon cancer cell lines (Akihisa et al., 2000; Kong et al., 2009). In the present study, the rats fed with medium and high doses of RBO (groups M and H, respectively) had significantly higher ($P < 0.05$) hepatic GSH levels and SOD activity than the rats from group N. The rats from group H had significantly higher ($P < 0.05$) hepatic catalase activity than the rats from group N (Table 6), which was compatible with the results for the rats from group P. In addition, the TBARS levels in the rats from all the groups that were fed RBO were significantly lower ($P < 0.05$) than those in the rats from group N, i.e., all doses of RBO significantly reduced the TBARS levels (Table 6). Piroxicam, a non-steroidal anti-inflammatory drug with chemopreventive activity,

was used as a positive control; it inhibited the formation of preneoplastic ACF in the colon tissues of rats, reduced the numbers of small and large ACF, and altered the ACF distribution in colon segments. Recent studies indicate that RBO significantly reduces the mitochondrial DNA levels of 8-hydroxy-2′-deoxyguanosine levels, which is a sensitive biomarker of oxidative DNA damage and oxidative stress, in the liver tissue of rats with streptozotocin-induced diabetes (Hsieh et al., 2005). RBO is a rich source of γ-oryzanol; γ-oryzanol consists of a number of phytosteryl ferulates, such as 24-methylenecycloartanyl ferulate, cycloartenyl ferulate, campesteryl ferulate, β-sitosteryl ferulate, and campestanyl ferulate. The results of recent studies indicate that γ-oryzanol and phytosteryl ferulates enhance the levels and radical scavenging activities of antioxidant enzymes (Akiyama, Hori, Takahashi, & Yoshiki, 2005; Islam et al., 2008). Moreover, it was found that the γ-oryzanol-rich fraction extracted from rice bran by using supercritical fluid extraction regulated the expression of antioxidant- and oxidative stress-related genes in the livers of stressed rats (Ismail, Al-Naqeeb, Abd Aziz bin Mamat, & Ahmad, 2010). In the present study, we noted that the rats from group H had lower hepatic TBARS levels than the rats from group P (Table 6). Therefore, we suggest that the ability of RBO to inhibit colon carcinogenesis might stem, in part, from antioxidative and anti-inflammatory processes.

5. Conclusion

RBO can delay DMH/DSS-induced colon carcinogenesis in rats fed with an AIN-93G-based 14% high-fat diet. It can also enhance the antioxidant status and promote antioxidant enzymes in rats. These findings suggest that RBO can be used as a health food for preventing colon cancer.

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