

Brewers' rice attenuated aberrant crypt foci developing in colon of azoxymethane-treated rats

Bee Ling Tan¹, Mohd Esa Norhaizan^{1,2,3*}, Ashok Kumar Pandurangan¹,
Hamzah Hazilawati⁴ and Karim Roselina⁵

¹Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia

²Research Centre of Excellent, Nutrition and Non-Communicable Diseases (NNCD), Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia

³Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, Serdang, Selangor, Malaysia

⁴Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, Serdang, Selangor, Malaysia

⁵Department of Food Technology, Faculty of Food Science and Technology, University Putra Malaysia, Serdang, Selangor, Malaysia

Abstract: Brewers' rice is one of abundant agricultural waste products in the rice industry. The present study is designed to investigate the potential of brewers' rice to inhibit the development of aberrant crypt foci (ACF) in colon of azoxymethane (AOM)-treated rats. The effects on the attenuation of hepatic toxicity and kidney function enzymes were also evaluated. Male Sprague-Dawley rats were randomly divided into five groups: (G1) normal; (G2) AOM alone; and (G3), (G4), and (G5), which were AOM fed with 10%, 20%, and 40% (w/w) of brewers' rice, respectively. The rats in group 2-5 were injected intraperitoneally with AOM (15 mg/kg body weight) once weekly for two weeks. After 8 weeks of treatment, the total number of ACF/colon and the number of ACF in the distal and middle colon were significantly reduced in all treatment groups compared to G2 ($p < 0.05$). Brewers' rice decreased the number of ACF with dysplastic morphology in a dose-dependent manner. Alkaline phosphatase (ALP) level in G5 was significantly lower compared to the G2 ($p < 0.05$). In conclusion, this study found the potential value of brewers' rice in reducing the risk of cancer susceptibility in colon.

Keywords: Brewers' rice, colon cancer, azoxymethane, aberrant crypt foci.

INTRODUCTION

Colorectal cancer (CRC) has become the third leading cancer in men and the second most common cancer in women worldwide (Ferlay *et al.*, 2010). Aberrant crypt foci (ACF) is a pre-neoplastic lesion that is characterised by a clustering of the mucosal cells with an enlarged- and thicker layer of epithelia compared to the surrounding normal crypts. ACF may progress into polyps, which may later develop into adenomas and then into invasive carcinomas (Cappell, 2007).

The cancer treatment is highly expensive and involves drugs that have adverse side effects or toxicity complications. Cheaper alternative methods using natural by-products may have better prospects in terms of economics in the prevention and therapy of cancer (Rayaprolu *et al.*, 2013). Brewers' rice is also known locally as *temukut* and contains approximately 1% of total rice, consisting of broken rice, rice bran, and rice germ. Brewers' rice is a by-product in the rice milling process, which loses many of its nutrients (Sabine, 2010). It is the last and the smallest milling fraction that is removed

during the rice milling process and is usually separated from larger kernels of milled rice (Association of American Feed Control Officials, 2011). The size of the brewers' rice is less than one quarter of the size of a full kernel and is usually used as animal feed and as a brewing adjunct (Glatthar *et al.*, 2003). The production of brewers' rice in rice milling process is explained in more detail by Esa *et al.* (2013).

Our earlier study reported that water extract of brewers' rice induced apoptosis in a human colorectal cancer (HT-29) cell line (Tan *et al.*, 2015) and is speculated due to the presence of beneficial health components such as phytic acid, vitamin E, γ -oryzanol, and antioxidant polyphenolic compounds (Tan *et al.*, 2013). Several studies as reported by Esa *et al.* (2013) also shown that rice germ can prevent azoxymethane (AOM)-induced colonic ACF in rats. Unlike rice bran, the health benefit of brewers' rice has yet to be fully studied. Therefore, the aim of this study was to investigate the beneficial effects of brewers' rice on CRC in AOM-induced ACF formation in rats. Furthermore, the effects on the attenuation of hepatic toxicity and kidney function enzymes were also evaluated.

*Corresponding author: e-mail: nhaizan@upm.edu.my

MATERIALS AND METHODS

Chemicals and reagents

AOM and 10% (v/v) neutral buffered formalin were bought from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Sample preparation

Brewers' rice (from rice variety MR 219) was obtained from a local milling factory, BERNAS in Seri Tiram Jaya, Selangor, Malaysia. Stabilisation was carried out to prevent oxidative rancidity during storage. Immediately after reaching the lab, 500 grams of brewers' rice was stabilised by heating for 2 minutes in a microwave oven at 2450 MHz. Then, the sample was mixed homogeneously and microwaved again for another 2 minutes. Stabilised samples were cooled at room temperature before storage at -20 °C.

Animals, diets, and carcinogen

All experimental protocols involving animals were performed according to the guidelines approved by the Animal Care and Use Committee (ACUC) of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) Serdang, Selangor with approval number UPM/FPSK/PADS/BR-UUH/00461. Male Sprague-Dawley rats were housed in plastic cages (two rats per cage) with wood-chip bedding in a well-ventilated room under controlled conditions: approximately 25-27°C, 50±10% relative humidity, and a 12-hour light/dark cycle. Hygienic conditions were maintained by weekly changes of the woodchip beds. The rats were fed with the American Institute of Nutrition (AIN)-93G diet and tap water, *ad libitum*. The components of the AIN-93G diet were modified based on the nutrient composition of brewers' rice with moisture (11.36±0.12%), ash (1.56±0.26%), protein (9.01±0.27%), fat (1.95±0.11%), total available carbohydrate (72.42±1.25%), and total dietary fiber (5.32±0.04%) (Tan *et al.*, 2014) (table 1). The diets were prepared weekly and kept in a chiller.

Experimental procedures

Following one week of acclimatisation, a total of 30 four-week-old male Sprague-Dawley rats, weighing approximately 90-100 grams were randomly divided into five groups (n = 6 rats for each group), which were (G1): normal, (G2): AOM alone, (G3): AOM + 10% (w/w) of brewers' rice, (G4): AOM + 20% (w/w) of brewers' rice, and (G5): AOM + 40% (w/w) of brewers' rice. At 6 weeks of age, the animals in G2-G5 were intraperitoneally injected with AOM (15 mg/kg body weight) once weekly for two weeks. The control group (G1 and G2) received an AIN-93G diet. G3, G4 and G5 received an AIN-93G diet containing 10%, 20% and 40% (w/w) of brewers' rice, respectively. The amounts of feed intake were measured daily, while body weights were recorded once a week until end of the study. After eight weeks of

treatment, the animals were sacrificed after anaesthetised with diethyl ether. Blood was collected; and the colon was removed. The colon tissue was rinsed with phosphate-buffered saline, fixed in 10% (v/v) neutral buffered formalin before hematoxylin and eosin (H&E) staining.

Analysis of ACF and histological classification of ACF

ACF's were characterised as round, defined clusters of crypts that were polymorphic and larger than normal, and had polymorphic luminal shapes. The classification of ACF in each group was assessed for the presence and degree of dysplasia following to the criteria reported by Roncucci *et al.* (1991; 2000) and Siu *et al.* (1997).

Toxicity by clinical biochemical evaluation

After eight weeks of treatment, blood from the experimental groups was collected, allowed to clot, and centrifuged at 3500 rpm for 10 minutes. The serum was separated and transferred to a microcentrifuge tube. Clinical biochemical evaluation was analysed using methods designed for each instrument. The markers for testing liver function were alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST); while markers for testing kidney function were creatinine and urea. These enzymes were measured spectrophotometrically using Hitachi Analytical Instrument (Roche Diagnostic GmbH, Mannheim, Germany) after analysed using the Roche kit (Penzberg, Germany).

STATISTICAL ANALYSIS

The results obtained are presented as the mean ± standard error of the mean (SEM). The results were analysed using a one-way analysis of variance (ANOVA) with Tukey's test. A value of $p < 0.05$ was considered significant. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.

RESULTS

Body weight

The body weight of all rats was compared between all groups throughout the experiment (data not shown). The results demonstrated that the body weights of all rats increased throughout the experiment. There is a slightly lower body weight in the AOM-alone group after induce with carcinogen, compared to the other groups. At the time of termination (after eight weeks of treatment with brewers' rice), no significant difference was observed in body weight among all groups ($p \geq 0.05$).

Effect of brewers' rice on the incidence of ACF induced by AOM

The effect of brewers' rice on AOM-induced development of ACF in rats is shown in table 2. The results

demonstrated that no ACF was found in the normal group. However, colonic ACF developed in all rats induced with carcinogen. In the AOM-alone group, the total number of ACF/colon was 171.82 ± 6.08 . The analysis showed that feeding of 10%, 20%, and 40% (w/w) of brewers' rice, respectively, significantly decreased the total number of ACF/colon compared to AOM alone ($p < 0.05$). Administration of 40% (w/w) of brewers' rice gave marked reduction (81.2%) of the total number of ACF/colon, followed by 20% (w/w) brewers' rice (63.9%), and 10% (w/w) brewers' rice (56.7%). The number of ACF consisting of one or two crypts was consistently higher, and treatment with 40% (w/w) brewers' rice was significantly reduced compared to AOM alone ($p < 0.05$). Furthermore, number of ACFs containing two crypts in the treatment with 20% (w/w) brewers' rice was also significantly reduced compared to AOM-alone group ($p < 0.05$). The number of ACF containing 3 crypts or 4 or more crypts were consistently lower in groups treated with 10%, 20%, and 40% (w/w) brewers' rice. They shared significant reduction of ACF as compared to AOM-alone group ($p < 0.05$). The results showed that in all brewers' rice-fed groups (AOM + 10%, 20%, and 40% (w/w) brewers' rice), the number of ACF containing one or two crypts was higher compared to ACFs with three crypts or four or more crypts. When looking at crypt multiplicity (four or more crypts) as a parameter, instead of the total number of ACF, the number of ACF consisting four or more crypts was significantly decreased in a dose-dependent manner after administration of brewers' rice in AOM-induced rat CRC, as compared to the AOM-alone group ($p < 0.05$).

When considered the distribution pattern of ACFs in the colon, the results showed that the ACF were distributed in the distal end of AOM-alone group. This result showed that ACF developed mainly in the distal end of the colon (42.6%), followed by 41.0% in the middle, and the fewest number (16.4%) in the proximal colon. As shown in table 3, administration of brewers' rice not only decreased the number of ACF but also altered the distribution of ACF throughout the entire colon. Administration of brewers' rice significantly inhibited the ACF formation in the distal and middle colon of all brewers' rice-fed groups (AOM + 10%, 20%, and 40% (w/w) brewers' rice) compared to the AOM alone ($p < 0.05$). In the proximal colon, 20% and 40% (w/w) brewers' rice showed a significant reduction compared to AOM-alone group ($p < 0.05$).

Histological classification of ACF

The ACF morphology at eight weeks after administration of brewers' rice was examined to assess the severity of dysplasia. Fig. 1 shows the histological examination of ACF by H&E staining. The number of ACF with varying degrees of dysplasia after eight weeks of treatment with brewers' rice is shown in table 4. More than half of the ACFs were detected with dysplastic morphology, while the others were non-dysplastic. The highest number of

dysplastic ACF was found in the AOM-alone group compared to all brewers' rice-fed groups. The ACF with moderate to severe dysplasia showed a significant reduction in all brewers' rice-fed groups compared to the AOM-alone group ($p < 0.05$), but not significantly different in the mild to moderate dysplasia across all groups ($p > 0.05$). Therefore, brewers' rice suppressed the number of dysplastic ACF in a dose-dependent manner. However, there was no significant difference in the hyperplasia without dysplasia across all brewers' rice-fed groups (AOM + 10%, 20%, and 40% (w/w) brewers' rice) compared to the AOM-alone group ($p > 0.05$). Administration of 40% (w/w) brewers' rice gave the lowest number of ACF with dysplasia, followed by 20% (w/w) brewers' rice and 10% (w/w) brewers' rice.

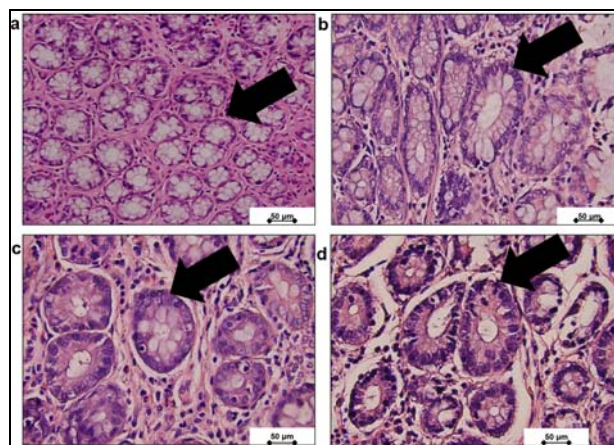


Fig. 1: Morphological evaluations of ACF based on the degree of dysplasia by using H&E staining. Magnification (400 \times). Black arrow indicates (a) normal crypts, (b) histological section involving 2 crypts of ACF indicated hyperplasia without dysplasia, (c) 1 crypt indicates a mild to moderate dysplasia, and (d) 2 crypts indicate a severely dysplastic morphology.

Toxicity by clinical biochemical evaluation

The ALT level in all groups injected with AOM was significantly higher ($p < 0.05$) than normal group (table 5). For ALP level, no significant difference was observed for all brewers' rice-fed groups, compared to the normal group ($p > 0.05$). The ALP level however, was significantly lower in AOM + 40% (w/w) brewers' rice compared to the AOM-alone group ($p < 0.05$). Similar with ALP, there was no significant difference in AST level among all treatment groups compared to the normal group ($p \square 0.05$). And surprisingly, the level of AST in AOM-alone group was significantly lower than the other groups ($p \square 0.05$). Based on the kidney function tests presented in table 5, the results show that after eight weeks of treatment there was no significant difference in creatinine levels ($p > 0.05$) in all experimental groups. The urea level for the normal group and all brewers' rice-fed groups were also not significantly different compared to the AOM-alone group ($p > 0.05$).

Table 1: Composition of experimental diets

Ingredients (g/1000 g diet)	Group				
	G1	G2	G3	G4	G5
Brewers' rice	-	-	100.0	200.0	400.0
Corn starch	397.5	397.5	315.3	233.2	68.9
Casein	200.0	200.0	191.0	182.0	164.0
Maltodextrin	132.0	132.0	132.0	132.0	132.0
Sucrose	100.0	100.0	100.0	100.0	100.0
Soybean oil	70.0	70.0	68.1	66.1	62.2
Powdered cellulose	50.0	50.0	44.7	39.4	28.7
AIN-93G mineral mix	35.0	35.0	33.4	31.9	28.8
AIN-93G vitamin mix	10.0	10.0	10.0	10.0	10.0
L-cystine	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
tert-butylhydroquinone	0.014	0.014	0.014	0.014	0.014

AIN - American Institute of Nutrition. G1 and G2, AIN-93G diet; G3, AIN-93G diet containing 10% (w/w) of brewers' rice; G4, AIN-93G diet containing 20% (w/w) of brewers' rice; G5, AIN-93G diet containing 40% (w/w) of brewers' rice.

Table 2: The effect of brewers' rice on AOM-induced ACF development in rats

Treatment	No. of crypts per ACF				Total no. of ACF/colon
	1 crypt	2 crypts	3 crypts	4 or more crypts	
Normal	0	0	0	0	0
AOM alone	57.48±3.37 ^a	40.24±5.94 ^a	28.22±6.94 ^b	45.88±8.05 ^b	171.82±6.08 ^b
AOM + 10% (w/w) brewers' rice	36.92±11.46 ^{ab}	20.94±1.16 ^{ab}	8.88±1.71 ^a	7.68±1.11 ^a	74.42±6.80 ^a
AOM + 20% (w/w) brewers' rice	36.76±4.87 ^{ab}	11.32±1.79 ^b	7.62±3.78 ^a	6.36±3.23 ^a	62.06±7.16 ^a
AOM + 40% (w/w) brewers' rice	21.08±4.98 ^b	7.53±1.90 ^b	2.17±0.67 ^a	1.48±0.75 ^a	32.26±4.54 ^a

Table 3: Regional distribution of colonic ACF in rats treated with AOM and fed with brewers' rice

Treatment	Distribution of ACF		
	Distal	Middle	Proximal
Normal	0	0	0
AOM alone	73.24±15.13 ^b	70.44±6.48 ^b	28.14±2.81 ^a
AOM + 10 % (w/w) brewers' rice	20.44±5.43 ^a	12.44±3.57 ^a	41.54±3.07 ^{ab}
AOM + 20 % (w/w) brewers' rice	22.96±4.25 ^a	23.02±7.97 ^a	16.08±4.23 ^{bc}
AOM + 40 % (w/w) brewers' rice	14.80±2.26 ^a	12.23±6.67 ^a	5.23±1.46 ^c

Table 4: Histological classification of ACF

Morphology	Group treatment				
	Normal	AOM alone	AOM + 10 % (w/w) brewers' rice	AOM + 20 % (w/w) brewers' rice	AOM + 40 % (w/w) brewers' rice
Hyperplasia without dysplasia	0	1.42±0.77 ^a	2.04±0.47 ^a	2.44±0.67 ^a	1.70±0.42 ^a
Mild to moderate dysplasia	0	34.24±8.85 ^a	27.36±1.17 ^a	26.66±5.04 ^a	13.61±4.25 ^a
Moderate to severe dysplasia	0	136.16±19.36 ^b	45.02±22.80 ^a	32.96±10.23 ^a	16.95±3.17 ^a

AOM - azoxymethane, ACF - aberrant crypt foci. Values are expressed as mean ± SEM (n = 6). Values in the same column (Tables 2 and 3) or same row (Table 4) with different superscript letter indicate significant difference by Tukey's test (p<0.05).

Table 5: Hepatic toxicity and kidney function enzymes after eight weeks treatment with brewers' rice

	ALT (U/L)	ALP (U/L)	AST (U/L)	Creatinine (umol/L)	Urea (mmol/L)
Normal	27.80±2.01 ^a	100.00±10.72 ^{ab}	155.30±10.37 ^a	48.00±2.48 ^a	5.50±0.44 ^b
AOM alone	40.50±4.02 ^b	155.00±28.91 ^a	98.70±5.66 ^b	47.00±2.35 ^a	5.10±0.28 ^{ab}
AOM + 10 % (w/w) brewers' rice	42.20±2.23 ^b	97.00±10.05 ^{ab}	156.80±39.61 ^a	43.00±2.48 ^a	4.00±0.20 ^a
AOM + 20 % (w/w) brewers' rice	48.60±4.81 ^b	106.00±11.11 ^a	152.40±6.72 ^a	47.00±1.89 ^a	4.90±0.25 ^{ab}
AOM + 40 % (w/w) brewers' rice	48.40±6.06 ^b	75.00±4.38 ^b	145.50±13.42 ^a	47.00±1.18 ^a	4.60±0.19 ^{ab}

AOM - azoxymethane, ALT - alanine aminotransferase, ALP - alkaline phosphatase, AST - aspartate transaminase. Values are expressed as mean ± SEM ($n = 6$). Values in the same column with different superscript letter indicate significant difference by Tukey's test ($p < 0.05$).

DISCUSSION

The weight loss that accompanies the tumour is related to a reduction not only in body fat stores but also in body protein mass (Alfin-Slater and Kritchevsky, 1991). There is increasing evidence of a direct tumour effect on host protein metabolism as reported by Brennan (1977). Protein turnover is altered in the cancer-bearing host, and there is a net loss of nitrogen in non-malignant tissues when protein degradation exceeds synthesis. The absence of significant differences in body weight in the present study shows that dietary intake of brewers' rice has no apparent effect on the results. Furthermore, the fact that the rats fed with the brewers' rice diet were able to thrive and gain body weight in a manner similar to the rats that consuming the control diet also reveals that the highest dosage of brewers' rice diet up to 40% is well tolerated by rats (Tan *et al.*, 2014). The dosage of 10% (w/w) of brewers' rice was selected following study by Boateng *et al.* (2009) on rice bran and germ. This concentration was considered the dosage that will give rise to inhibit the ACF. Moreover, higher concentration (20% and 40% (w/w) of brewers' rice) was used to examine the dose-dependent effect of brewers' rice as chemopreventive agent in rat experimental colon cancer model.

ACFs are regarded as the gold standard biomarker in colon cancer and are used extensively in chemopreventive studies (Corpet and Tache, 2002). The total number of ACF per colon was significantly reduced in a dose-dependent manner after administration of brewers' rice in AOM-induced rat CRC. This finding was consistent with a study reported by Norazalina *et al.* (2010), who revealed that the ACF was reduced after administration with rice bran phytic acid. Treatment with brewers' rice leads to the inhibition of ACF formation, which may or may not have developed into invasive carcinomas. Aberrant crypts appeared larger, with a thicker epithelial lining; stained darker compared to normal crypts; and usually clustered into a focus, containing one to hundreds of aberrant crypts

when observed under microscope (Takayama *et al.*, 1998). According to Bird (1995), ACF with multiplicity of four or more crypts are more highly correlated with tumour incidence, compared to total number of ACF. Increasing number of ACF in the distal colon, compared to the middle and proximal colon, in 40% (w/w) brewers' rice-fed group does not indicate that brewers' rice promoted the ACF formation in the distal colon. On the contrary, it is because of the ability of brewers' rice to inhibit the development of ACF in the middle and proximal colon. Our finding was consistent with those found by Cheng and Lai (2003) and van Beelen *et al.* (2009) who revealed that the density of ACF in the distal colon was higher than the proximal colon.

The data show that all rats with AOM-induced colon carcinogenesis developed ACF. These results indicate that feeding of brewers' rice did not affect the number of ACF containing of one or two crypts, but actually suppressed the number of ACF containing of three crypts, four or more crypts, and the total number of ACF/colon. These results further support our earlier *in vitro* study, who showed that brewers' rice extracts can inhibit the growth of a human colon cancer (HT-29) cell line (Tan *et al.*, 2013). Previous studies have also found that rice bran (Li *et al.*, 2011) and germinated brown rice (Latifah *et al.*, 2010) suppressed the colon carcinogenesis. The morphological classification of ACF provides information on changes in the crypt. These changes are the key features of precancerous lesions in the development of colon cancer (Di Gregorio *et al.*, 1997). In general, the morphology of ACF was classified according to the established histological criteria: hyperplasia without dysplasia, mild to moderate dysplasia, and moderate to severe dysplasia.

Most of the previous studies reported that the protective effects of additive and/or synergistic effects of several phytochemicals (Wedge *et al.*, 2001; Liu, 2003; Tsuda *et al.*, 2004). Hence, in the present study, brewers' rice

rather than individual compounds were administered to the rats. We found a greater suppression on ACF administration with brewers' rice. The marked suppression of ACF may be possibly due to the dietary constituents, which act synergistically. Dietary chemoprevention studies have indicated that the magnitude of anticancer effect in the whole food or whole food extract is greater than its individual phytochemicals (Ricciardiello *et al.*, 2011; Shukla and George, 2011). In addition, whole rice bran consumption also has been reported to provide protective effect against cancerous cells compared to the protection given by isolated phytochemicals (Phutthaphadoong *et al.*, 2009).

Furthermore, the attenuation effect of brewers' rice on oxidative stress may be explained partly by its antioxidant properties. Our previous study showed that the high antioxidant activity of the brewers' rice, which is measured by the β -carotene bleaching test, the DPPH radical scavenging assay, and the ferric reducing antioxidant power may further supporting these findings (Tan *et al.*, 2013). These results suggest that the attenuation effect on AOM-induced oxidative stress may be mediated at least partly through free radicals scavenging capacity of brewers' rice.

Phytic acid (inositol hexaphosphate, IP₆) is found in rice bran, most cereals, nuts, oilseeds, and legumes. Phytic acid inhibits the growth of transplanted tumours (Jariwalla *et al.*, 1988), through the chelation of iron, which is associated with the inhibition of iron-related initiation and promotion of carcinogenesis (Norhaizan *et al.*, 2011). Rice bran phytic acid has been shown to suppress CRC via inhibition of β -catenin and cyclooxygenase (COX-2) (Nurul Husna *et al.*, 2013).

Vitamin E has been reported to reduce the number of AOM-induced colon tumours (Shivapurkar *et al.*, 1995). Tocotrienols induce apoptosis and inhibit the proliferation of human breast cancer cell (MCF7), related to cytotoxic activity against tumours cells (Nesaretnam *et al.*, 2002). Several findings also reported that administration of high vitamin E diets at 600 and 100 mg/kg, respectively, reduced the colorectal tumours and incidence of carcinoma in 1,2-dimethylhydrazine (DMH)-induced rats (Cook and McNamara, 1980; Sumiyoshi, 1985).

Gamma-oryzanol, a component present in the rice bran, particularly consists of phytosterols including cycloartenol, β -sitosterol, 24-methylenecycloartenol, and campesterol (Lerma-Garcia *et al.*, 2009). Gamma-oryzanol in rice bran has been suggested to inhibit tumour growth in tumour-bearing mice by induction of natural killer (NK) cell activity and the activation of macrophages. Gamma-oryzanol is known to exert a strong antioxidative activities (Xu *et al.*, 2001), it may interfere intracellular signaling pathways of reactive oxygen radicals. It also has been demonstrated to display

numerous biological effects such as anti-inflammation (Islam *et al.*, 2008). A study by Ward *et al.* (1973) has been reported that AOM is a metabolite of DMH which shows a strong carcinogenic in the intestinal tract but to a lesser extent in the liver and kidney. Therefore, the potential attenuation effect of brewers' rice on hepatic toxicity and kidney function enzymes were also evaluated in AOM-induced rats after eight weeks treated with brewers' rice.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most common markers that measure hepatocellular injury (Bürger *et al.*, 2005). Liver damage can be divided into direct hepatocyte destruction or bile flow impairment (Chin *et al.*, 2008). Cytoplasmic enzymes in the hepatocytes may leak from cells into the blood during the early stage of liver damage, when the permeability of membranes increases (Sallie *et al.*, 1991). The ALT level in all groups injected with AOM was significantly higher ($p < 0.05$) than normal group. This is agreed with finding by Matkowskyj *et al.* (1999) that demonstrated AOM can increase the liver toxicity risk via increasing the level of ALT activity.

Serum urea and creatinine were used as indicators of kidney function (Williams, 1999). The creatinine and urea levels were normal in all groups. Thus, there is no direct adverse effect on kidney function in AOM-treated rats eight weeks after administration of brewers' rice. AOM-treated histopathological liver and kidney lesions might be occurred more frequently compared to the changes of the level in biochemical. Therefore, further histological changes of liver and kidney damage in AOM-induced after eight weeks treated with brewers' rice are recommended to confirm the findings.

CONCLUSION

Our study suggests that brewers' rice is a potential chemopreventive agent that can inhibit the ACF formation that lead to colon cancer. The finding presented in this study also reveals that brewers' rice has the potential to reduce ALP, creatinine, and urea. However, further study are needed to elucidate the underlying mechanisms involved in reducing ACF development in AOM-treated rats after eight weeks administration of brewers' rice.

ACKNOWLEDGEMENTS

Special thanks to BERNAS, Seri Tiram Jaya, Selangor, Malaysia for supplying the brewers' rice sample. We also acknowledge the Ministry of Education, Malaysia for providing the research funding under RUGS-2 project no. 05-02-12-1862RU to implement the project.

REFERENCES

- Alfin-Slater RB and Kritchevsky D (1991). Cancer and Nutrition. Plenum Press, New York.
- Association of American Feed Control Officials (2011). Brewers rice. <http://www.aafco.org/>. Accessed 4 Jan 2011.
- Bird RP (1995). Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Cancer Lett.*, **93**: 55-71.
- Boateng J, Verghese M, Panala V, Walker LT and Shackelford L (2009). Protective effects of rice bran on chemically induced colon tumorigenesis may be due to synergistic/additive properties of bioactive components. *Int. J. Cancer Res.*, **5**: 153-166.
- Brennan MF (1977). Uncomplicated starvation versus cancer cachexia. *Cancer Res.*, **37**: 2359-2364.
- Bürger C, Fischer DR, Cordenuzzi DA, Batschauer APB, Filho VC and Soares ARS (2005). Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (Acmela brasiliensis) (Asteraceae) in mice. *J. Pharm. Sci.*, **8**: 370-373.
- Cappell MS (2007). From colonic polyps to colon cancer: pathophysiology, clinical presentation, screening and colonoscopic therapy. *Minerva Gastroenterol. Dietol.*, **53**: 351-373.
- Cheng L and Lai MD (2003). Aberrant crypt foci as microscopic precursors of colorectal cancer. *World J. Gastroenterol.*, **9**: 2642-2649.
- Chin JH, Abas HH and Sabariah I (2008). Toxicity study of *Orthosiphon stamineus* Benth (Misai Kucing) on Sprague Dawley rats. *Trop. Biomed.*, **25**: 9-16.
- Cook MG and McNamara P (1980). Effect of dietary vitamin E on dimethylhydrazine-induced colonic tumors in mice. *Cancer Res.*, **40**: 1329-1331.
- Corpet DE and Tache S (2002). Most effective colon cancer chemo preventive agents in rats: A review of aberrant crypt foci and tumour data, ranked by potency. *Nutr. Cancer*, **43**: 1-21.
- Di Gregorio C, Losi L, Fante R, Modica S, Ghidoni M, Pedroni M, Tamassia MG, Gafà L, Ponz de Leon M and Roncucci L (1997). Histology of aberrant crypt foci in the human colon. *Histopathol.*, **30**: 328-334.
- Esa NM, Ling TB and Peng LS (2013). By-products of rice processing: An overview of health benefits and applications. *J. Rice Res.*, **1**: 107.
- Ferlay J, Shin HR and Bray F (2010). Estimates of worldwide burden of cancer in 2008: Globocan 2008. *Int. J. Cancer*, **127**: 2893-2917.
- Glatthar J, Heinisch J and Senn T (2003). The use of unmalted triticale in brewing and its effect on wort and beer quality. *J. Am. Soc. Brew. Chem.*, **61**: 182-190.
- Islam MS, Murata T, Fujisawa M, Nagasaka R, Ushio H, Bari AM, Hori M and Ozaki H (2008). Anti-inflammatory effects of phytosteryl ferulates in colitis induced by dextran sulphate sodium in mice. *Br. J. Pharmacol.*, **154**: 812-824.
- Jariwalla RJ, Sabin R and Laeson S (1988). Effects of dietary phytic acid (phytate) on incidence and growth rate of tumor prompted in Fischer rats by a magnesium supplement. *Nutr. Res.*, **8**: 813.
- Latifah SY, Armania N, Tze TH, Azhar Y, Nordiana AH, Norazalina S, Hairuszah I, Saidi M and Maznah I (2010). Germinated brown rice (GBR) reduces the incidence of aberrant crypt foci with the involvement of β -catenin and COX-2 in azoxymethane-induced colon cancer in rats. *Nutr. J.*, **9**: 16.
- Lerma-Garcia MJ, Herrero-Martinez JM, Simo-Alfonso EF, Mendonca CRB and Ramis-Ramos G (2009). Composition, industrial processing and applications of rice bran gamma-oryzanol. *Food Chem.*, **115**: 389-404.
- Li SC, Chou TC and Shih CK (2011). Effects of brown rice, rice bran and polished rice on colon carcinogenesis in rats. *Food Res. Int.*, **44**: 209-216.
- Liu RH (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemical. *Am. J. Clin. Nutr.*, **3**: 517-520.
- Matkowskyj KA, Marrero JA, Carroll RE, Danilkovich AV, Green RM and Benya RV (1999). Azoxymethane-induced fulminant hepatic failure in C57BL/6J mice: characterization of a new animal model. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **277**: 455-462.
- Nesaretnam K, Radhakrishnan A, Selvaduray KR, Reimann K, Pailoor J, Razak, G, Mahmood MM and Dahliwal JS (2002). Effect of palm oil carotene on breast tumorigenicity in nude mice. *Lipids*, **37**: 557-560.
- Norazalina S, Norhaizan ME, Hairuszah I and Norashareena MS (2010). Anticarcinogenic efficacy of phytic acid extracted from rice bran on azoxymethane-induced colon carcinogenesis in rats. *Exp. Toxicol. Pathol.*, **62**: 259-268.
- Norhaizan ME, Ng SK, Norashareena MS and Abdah MA (2011). Antioxidant and cytotoxicity effect of rice bran phytic acid as an anticancer agent on ovarian, breast and liver cancer cell lines. *Mal. J. Nutr.*, **17**: 367-375.
- Nurul Husna S, Norhaizan ME, Hairuszah I, Abdah Md A, Norazalina S and Ashok KP (2013). Preventive Inositol Hexaphosphate extracted from rice bran inhibits colorectal cancer through involvement of Wnt/ β -catenin and COX-2 pathways. *BioMed. Res. Int.*, **2013**: 681027.
- Phutthaphadoong S, Yamada Y, Hirata A, Tomita H, Taguchi A, Hara A, Limtrakul PN, Iwasaki T, Kobayashi H and Mori H (2009). Chemo preventive effects of fermented brown rice and rice bran against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in female A/J mice. *Oncol. Rep.*, **21**: 321-327.
- Rayaprolu SJ, Hettiarachchy NS, Chen P, Kannan A and Mauromostakos A (2013). Peptides derived from high oleic acid soybean meals inhibit colon, liver and lung cancer cell growth. *Food Res. Int.*, **50**: 282-288.
- Ricciardiello L, Bazzoli F and Fogliano V (2011). Phytochemicals and colorectal cancer prevention-myth

- or reality? *Nature Reviews. Gastroenterol. and Hepatol.*, **8**: 592-596.
- Roncucci I, Pedroni M, Vaccina F, Benatti P and de Pol LM (2000). Aberrant crypt foci in colorectal carcinogenesis. Cell and crypt dynamics. *Cell Prolif.*, **33**: 1-18.
- Roncucci L, Stamp D, Medline CJB and Bruce WR (1991). Identification and quantification of aberrant crypt foci and micro adenomas in the human colon. *Hum. Pathol.*, **22**: 287-295.
- Sabine C (2010). Better dog care, better dog nutrition. <http://www.betterdogcare.com/?page=main>. Accessed 4 Jan 2011.
- Sallie R, Tredger J and William R (1991). Drugs and the liver. *Biopharm. Drug Dispos.*, **12**: 251-259.
- Shivapurkar N, Tang Z, Frost A and Alabaster O (1995). Inhibition of progression of aberrant crypt foci and colon tumor development by vitamin E and β -carotene in rats on a high risk diet. *Cancer Lett.*, **91**: 125-132.
- Shukla Y and George J (2011). Combinatorial strategies employing nutraceuticals for cancer development. *Ann. New York Acad. Sci.*, **1229**: 162-175.
- Siu IM, Pretlow TG, Amini SB and Pretlow TP (1997). Identification of dysplasia in human colonic aberrant crypt foci. *Am. J. Pathol.*, **150**: 1805-1813.
- Sumiyoshi H (1985). Effects of vitamin E deficiency on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats. *Hiroshima J. Med. Sci.*, **34**: 363-369.
- Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, Kato J, Kogawa K, Miyake H and Niitsu Y (1998). Aberrant crypt foci of the colon as precursors of adenoma and cancer. *The New Engl. J. Med.*, **339**: 1277-1284.
- Tan BL, Norhaizan ME, Heshu SR, Hazilawati H and Roselina K (2014). Brewers' rice induces apoptosis in azoxymethane-induced colon carcinogenesis in rats via suppression of cell proliferation and the Wnt signaling pathway. *BMC Complement. Altern. Med.*, **14**: 304.
- Tan BL, Norhaizan ME, Suhaniza HJ, Lai CC, Norazalina S and Roselina K (2013). Antioxidant properties and antiproliferative effect of brewers' rice extract (*temukut*) on selected cancer cell lines. *Int. Food Res. J.*, **20**: 2117-2124.
- Tan BL, Norhaizan ME, Yeap SK and Roselina K (2015). Water extract of brewers' rice induces antiproliferation of human colorectal cancer (HT-29) cell lines via the induction of apoptosis. *Eur. Rev. Med. Pharmacol. Sci.*, **19**: 1022-1029.
- Tsuda H, Ohshima Y, Nomoto H, Fujita K, Matsuda E, Iigo M, Takasuka N and Moore MA (2004). Cancer prevention by natural compounds. *Drug Metab. Pharmacokinet.*, **19**: 245-263.
- van Beelen VA, Spenkelink B, Mooibroek H, Sijtsma L, Bosch D, Rietjens IM and Alink GM (2009). An n-3 PUFA-rich microalgal oil diet protects to a similar extent as a fish oil-rich diet against AOM-induced colonic aberrant crypt foci in F344 rats. *Food Chem. Toxicol.*, **47**: 316-320.
- Ward JM, Yamamoto RS and Brown CA (1973). Pathology of intestinal neoplasms and other lesions in rats exposed to azoxymethane. *J. Natl. Cancer Inst.*, **51**: 1029-1039.
- Wedge DE, Meepagala KM, Magee JB, Smith SH, Huang G and Larcom LL (2001). Anticarcinogenic activity of strawberry, blueberry, and raspberry extracts to breast and cervical cancer cells. *J. Med. Food*, **4**: 49-51.
- Williams MH (1999). Nutrition for health, fitness and sport. McGraw-Hill, Boston.
- Xu Z, Hua N and Godber JS (2001). Antioxidant activity of tocopherols, tocotrienols and α -oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis(2-methylpropionamide) dihydrochloride. *J. Agric. Food Chem.*, **49**: 2077-2081.