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Research article

The inhibition of colon cancer development by black rice bran on BALB/C Mice

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Abstract: Black rice bran (BRB) is well-known for its high antioxidant activity and its pivotal role in preventing colon cancer. The present study aims to investigate the effects of BRB administration on BALB/C mice induced with azoxymethane (AOM) and dextran sodium sulphate (DSS). The 24 mice were divided into three groups: the group induced by colon cancer (C+), the group induced by cancer and given the BRB diet (C+BRB), and the normal group (C−). Both the C− and C+ groups were given a standard AIN-93 M diet containing cellulose fiber. After 16 weeks, the mice were anesthetized, and the colonic tissue was identified for nodule distribution, histopathological observation, and mRNA expression analysis of proliferating cell nuclear antigen (PCNA), caspase-3, and caspase-8 genes using

qRT PCR technique. Nodule distribution in the C+BRB group showed a significant decrease compared to the C+ group, with 1.65 ± 0.71 nodule/cm2 vs. 5.73 ± 2.93 nodule/cm^2. Then, the colon weight was significantly decreased in the C+BRB group, at 0.19 ± 0.04 (g) compared to 0.25 ± 0.03 (g) in the C+ group. Also, the BRB diet in the C+BRB group significantly decreased PCNA mRNA expression compared to the C+ group, with values of 0.58 ± 0.09-fold change vs. 5.22 ± 0.80-fold change. Conversely, increased the mRNA expression of caspase-3 (0.91 ± 0.20-fold change vs. 0.36 ± 0.15-fold change) and caspase-8 (0.51 ± 0.18-fold change vs. 0.13 ± 0.31-fold change). In conclusion, administration of BRB inhibited the rate of cancer development by suppressing cancer cell proliferation and inducing apoptosis.

Keywords: AOM/DSS, black rice bran, caspase-3, caspase-8, colon cancer, PCNA

1. Introduction

Colon cancer is the third leading cause of cancer-induced deaths in both women and men globally, after lung and breast cancer [1]. According to the American Cancer Society, in 2017, 95,520 people contracted colon cancer and 50,260 people died from it in the United States [2]. Several studies have reported that dietary intervention, such as the consumption of functional foods, is one of the main factors for colon cancer prevention. Results of research on the effects of functional food components on colon cancer prevention have been widely reported [3]. Dietary bioactive components, such as phenolic compounds and fibers, have been reported to be potential chemopreventive agents.

Black rice bran (BRB) is well-known for its high composition of antioxidants and fiber. Many bioactive components in BRB contribute to preventing colon cancer. Compared to brown and white rice, BRB has higher levels of anthocyanin and ferulic acid [4]. In addition, the dietary fiber in BRB is known to increase caspase enzyme activity, which marks the occurrence of apoptosis in cancer and induces changes in the morphological characteristics of cells undergoing apoptosis [5,6]. The dietary fiber in the digestive tract traps carcinogens that cause colon cancer and increases the fecal period, facilitating the dissolution of carcinogens in the stool [7].

Colon cancer cell proliferation can be detected by proliferating cell nuclear antigen (PCNA) marker expression. PCNA is a proliferation marker that is an effector in DNA synthesis and is involved in the DNA replication process [8]. During the development of cancer in a tissue, cells undergo a biochemical mechanism aimed at killing cancer cells (apoptosis). The enzymes involved in this apoptotic process in colon cancer are caspase-3, which acts as an effector, and caspase-8z which acts as an initiator. The qRT-PCR analysis will assess PCNA, caspase-3, and caspase-8 markers.

The potential of BRB in inhibiting colon cancer has not been studied in detail. Therefore, an in vivo research study using animal experiments with azoxymethane (AOM) and dextran sodium sulphate (DSS)-induced mice is needed. Studies aim to evaluate the effect of BRB on inhibiting colon cancer development by assessing body weight, organ weights (colon, liver, kidney, and spleen), nodule distribution in the colon, and generally expression of PCNA, caspase-8, and caspase-3.

2. Materials and methods

2.1. Preparation of BRB

BRB, the main ingredient used in this research, was prepared based on our previous study [9]. The "Cempo Ireng" varieties of BRB were sourced from farmers in Cigudeg, Bogor, Indonesia. It was obtained as paddy and then hulled through milling using the Yanmar Rice Huller type HW-60A (Japan) to obtain brown-black rice. The rice was then polished using Satake polisher type N-70-F (Japan), yielding two products: polished rice and BRB. Thereafter, the BRB was sieved using 20 mesh sieves, dried using a FreeZone 4.5 Liter Benchtop Freeze Dryers Labconco (USA) at −50 °C for 24 hours, and stored at −20 °C until use.

2.2. Animal studies

All procedures involving the care and use of laboratory animals were performed following approval from the Bimana Indomedical Institutional Animal Care and Use Committee, Bogor, Indonesia (approval number R.02-17-IR). Twenty-four male BALB/C mice (5–8 weeks old) were obtained from Indoani Lab (Bogor, Indonesia), classified into three groups (n = 8 per group), and housed individually in rooms with regulated air and light cycles. During a one-week acclimation, all the mice were fed a standard diet. Afterward, the BRB group alone was further adapted to a modified diet with BRB as a fiber source. Following the third week (pre-induction), the carcinogen-induced group (C+) and BRB mice were injected intraperitoneally with azoxymethane (AOM) and then with dextran sodium sulphate (DSS) (Sigma Aldrich, Merck, St. Louis, MO, USA) at a dose of 10 mg/kg body weight.

Table 1. Diet composition of the study.

Ingredient (g/100g)	C− group	C+ group	C+BRB group
Black rice bran	0.00	0.00	21.68
Casein	14.00	14.00	11.48
Soybean oil	4.00	4.00	1.67
Cellulose powder	5.00	5.00	0.00
Mineral mixture	3.00	3.00	1.81
Vitamin Mixture	1.00	1.00	1.00
Sucrose	10.00	10.00	10.00
Corn starch	63.00	63.00	52.35

Note: C− group: AIN-93M standard diet + 0.9% physiological NaCl (intraperitoneal, once); C+ group: AIN-93M standard diet + AOM 10 mg/kg g body weight (intraperitoneal, once) + DSS 1% (as drinking water, for four days); C+BRB group: BRB enriched diet + AOM 10 mg/1000 g body weight (intraperitoneal, once) + DSS 1% (as drinking water, for four days).

The standard diet based on the AIN-93 M standard (Table 1), should contain the following: 14% protein, 4% fat, 5% food fiber, 3.5% minerals, 1% vitamins, 10% sucrose, and carbohydrates [10]. The proximate analysis findings of sodium caseinate and BRB, which include water, lipid, protein, ash, dietary fiber, and carbohydrate content by difference, were used to calculate the ratio.

The normal (C−) group was intraperitoneally administered Isotonic NaCl 0.9% (Ecosol NaCl,

Braun Pharmaceutical, Karawang, Indonesia) to reduce stress levels. One week after induction, the C+ and BRB groups were given 1% dextran sulfate sodium (DSS) (MP-Bio, Solon, Ohio, USA) in their drinking water ad libitum for four consecutive days to accelerate the occurrence of colon cancer. The cancer formation protocol can be seen in Figure 1. The diet was supplied according to the daily nutrition of mice, which was 15 g/100 g of body weight with diet composition, as shown in Table 1.

Cage cleaning and weighing of mice were done twice a week. At the end of the 20-week study, mice in all groups were euthanized for colon, liver, kidney, and spleen tissue collection. Colonic tissue was visually observed for the presence of masses or nodules. Furthermore, the tissue was frozen at −80 °C for molecular evaluation. The analyses included nodule distribution evaluation using ImageJ, histopathology with lesion neoplasia analysis, and evaluation of caspase-3, caspase-8, and PCNA using real-time PCR techniques [11].

2.3. Quantitative RT-PCR analysis

RNA was extracted from colonic tissue that had been frozen at −80 °C. The colonic tissue was homogenized using a grinding mortar. Then, about 30 mg of the homogenate was transferred into a sterile 1.5 mL micro-centrifugation tube containing Tryzol. The extraction procedure was performed using Direct-Zol RNA Miniprep Plus following standard protocols (Zymo Research, CA, USA). The RNA concentration was measured using Nanodrop spectrophotometer (Thermo Scientific, USA). Reverse transcription of RNA to cDNA was conducted using a standard procedure from SuperScript III Reverse Transcriptase (Invitrogen, Thermo Scientific, USA). Analysis of the relative quantification of gene expression was performed with Real-Time PCR Detection System (iQ5, Bio-Rad Laboratories Inc., Hercules, CA, USA) using SsoFast EvaGreen Supermix (Bio-Rad, CA, USA). The master mix for real-time PCR was prepared for the expression analysis of caspase-3 (CASP3), caspase-8 (CASP8), and PCNA genes, normalized toward the GAPDH gene (Glyceraldehyde 3-phosphate dehydrogenase). The primers used in this study were: CASP3 (Forward 5'-TGGGCCTGAAATACCAAGTC-3'; Reverse 5'-AAATGACCCCTTCAT CACCA-3'), CASP8 (Forward 5'-GATGAGGCAGACTTTCTGCT-3'; Reverse 5'-CATAGTTCAC GCCAGTCAGGAT-3'), PCNA (Forward 5'-CTCACGTCTCCTTGGTACAGCTTAC-3'; Reverse 5'CTTCCTCATCT TCAATCTTGGAG-3'), and GAPDH (Forward 5'GCCATCTACAAGCAGTCACA-3'; Reverse 5'CCCTTTCTTGCGGAG ATTCT-3'). The protocol for real-time PCR amplification included 42 cycles comprising enzyme activation at 98 °C for 2 minutes, denaturation at 98 °C for 5 seconds, and annealing, extension, and data collection at 55 °C for 10 seconds. Evaluating of gene expression was performed using the Livak method or comparison of delta threshold (ΔCT) [12].

2.4. Data analysis

This study, which included eight replications, used a completely randomized design (CRD) in three treatments. Data are reported as averages ± SD. Experimental data were processed using MiniTab 17.0 software and displayed in one-way ANOVA to identify differences between treatment levels. Tukey's HSD test continued different results between treatment levels, and the significance value was determined based on the 5% level.

3. Results

3.1. Animal conditions during cancer development

The study found that the C−, C+, and C+BRB groups exhibited good clinical conditions and behaviors. This finding was evidenced by data showing that the body weight of these groups remained stable. According to the data (Table 2), an increase in body weight was found in the C+BRB group. All three groups were given the same feed intake, meeting the daily nutritional requirement of 15 g/100 g of body weight on average.

The analysis of the mice's body weight revealed significant differences ($p < 0.05$) in the C+BRB group. The mean body weight of the C+ group (25.66 ± 1.28) was significantly lower than that of the C+BRB group (29.10 ± 3.31) ($p < 0.05$). The weight of colon organs in the C+ group was significantly higher than in the other groups ($p < 0.05$). There were no significant differences in the average weight of the liver, kidney, and spleen across all groups of mice, indicating that the induction of AOM/DSS in the test group did not significantly affect the weight of these organs.

Table 2. Average body weight and organ weight of BALB/C mice.

Groups	Body (g)	Colon (g)	Liver (g)	Lymph (g)	Kidney (g)
C−	26.06 ± 1.96^{a}	0.21 ± 0.03^{b}	1.23 ± 0.14	0.09 ± 0.01	0.21 ± 0.02
C+	25.66 ± 1.28^{a}	0.25 ± 0.03^{c}	1.24 ± 0.15	0.09 ± 0.01	0.20 ± 0.02
C+BRB	29.10 ± 3.31^{b}	0.19 ± 0.04^{a}	1.37 ± 0.24	0.09 ± 0.03	0.22 ± 0.01

Note: All data were presented as mean±SD. Different superscripts on the same column showed a significant difference ($p < 0.05$).

3.2. Cancers distribution in colon tissue of BALB/C mice

Macroscopic evaluation of nodules in the colon tissue was performed. Visual analysis using the ImageJ application revealed no nodules or cancers in the C− group, whereas these were found in the C+ and C+BRB groups (Figure 1).

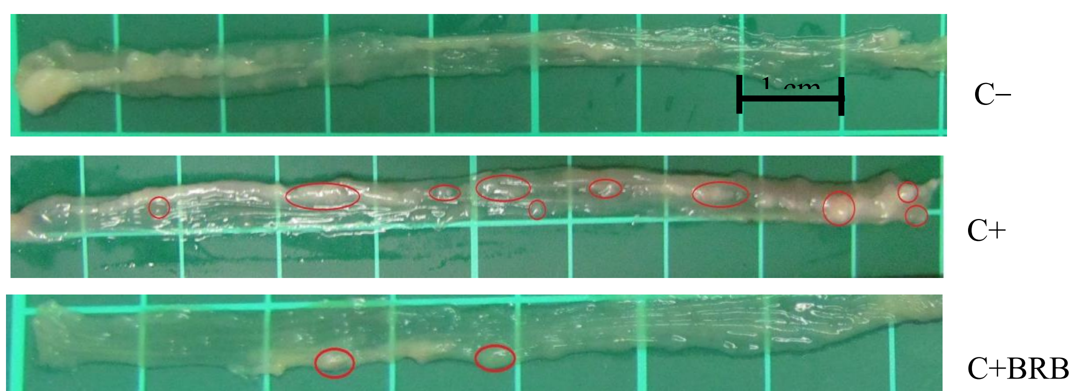


Figure 1. Macroscopic photographs of colon in groups C−, C+, and C+BRB. Scale = 1 cm. ○ : Shape of nodules in the colon tissue which is marked by the formation of a bulge or lump.

The distribution of cancers in the C+ group was significantly higher ($p < 0.05$) compared to the C+BRB group (Figure 2), with more than a two-fold difference: 5.73 ± 2.93 and 1.65 ± 0.72, respectively. There was no significant difference between the C− group and the C+ BRB group. Cancer nodules in the C+ group spread to almost all parts of the colon tissue, exhibiting various shapes.

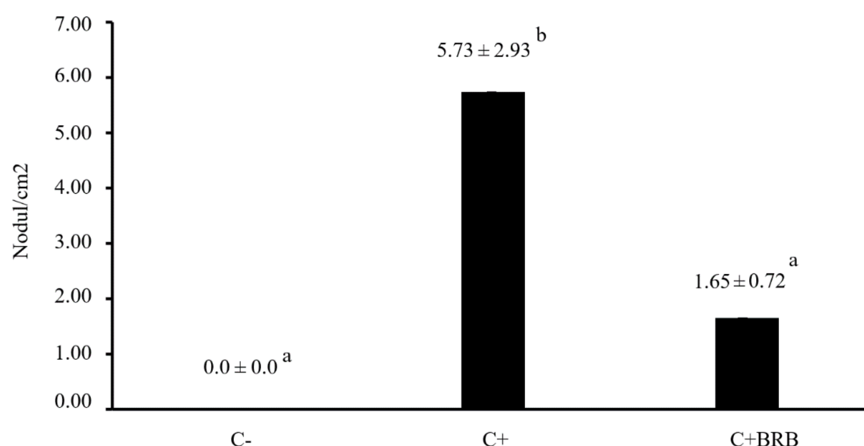
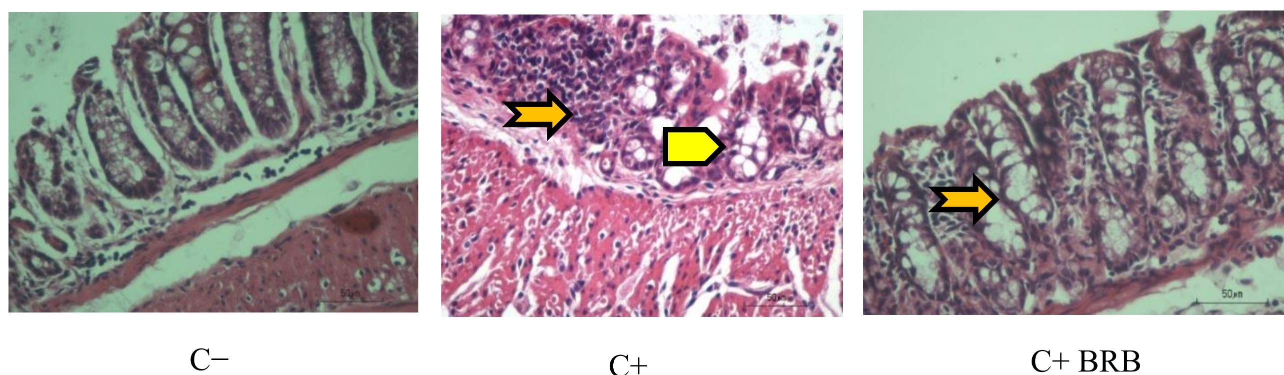


Figure 2. Distribution of nodules in the colon tissue of BALB/C mice in each test group. Data are shown in average (n = 8). Different superscripts on the same line showed a significantly different level of 5% ($p < 0.05$).



C− C+ C+ BRB

Figure 3. Microscopic images of BALB/C mice colon tissue. C− (negative control group (normal mice), C+ (group without black rice bran + AOM/DSS), C+BRB (black rice bran group + AOM/DSS). ⇨: Inflammation of inflammatory cells: ⇨: damaged cell.

3.3. Histopathology of BALB/C mice colon

Histopathological analysis of the colon tissue revealed morphological differences between the C− group and the AOM/DSS treatment groups (C+ and C+BRB groups) (Figure 3). Histologically, the C− group exhibited normal colonic mucosa characterized by elongated crypts filled with goblet or epithelial cells. In contrast, the C+ group exhibited severe inflammatory cell infiltration in the lamina propia. The crypts in the C+BRB group were no longer apparent or were missing in the multifocal area, but the damaged epithelial cells were still visible on the tissue surface.

3.4. mRNA expression level of PCNA, caspase-3, and caspase-8

Further observation of the effect of BRB on inhibiting the development of colon cancer in BALB/C mice was performed by evaluating PCNA, caspase-3, and caspase-8 markers using qRT-PCR (Table 3). The higher the value of caspase-3 and caspase-8, the higher the level of apoptotic activity in the colon tissue, indicating lower colon cancer activity. PCNA is widely used to detect cancer cells due to its involvement with enzymes in DNA replication.

Table 3. Colonic mRNA expression level by quantitative RT-PCR*.

Groups	PCNA	Caspase-3	Caspase-8
C−	1.00 ± 0.21^a	1.00 ± 0.18^a	1.00 ± 0.51^a
C+	5.22 ± 0.80^b	0.36 ± 0.15^b	0.13 ± 0.03^b
C+BRB	0.58 ± 0.09^a	0.91 ± 0.20^a	0.51 ± 0.18^a

*C− (normal group + standard feed); C+ (induction of AOM/DSS + standard feed); and C+BRB (induction of AOM/DSS + feed modified black rice bran). Data are shown in average + SD (n = 8). Different superscripts on the same line showed a significantly different level of 5% ($p < 0.05$).

The observation showed that the C+BRB group had lower PCNA gene expression (0.58 ± 0.09) than the C+ group (5.22 ± 0.80). The administration of fermented BRB extracts up to 0.2% was reported to inhibit colon cancer occurrence through a mechanism that reduces cell proliferation and lesion numbers [11]. At the levels of caspase-3 and caspase-8 apoptosis gene expression, the C+BRB group showed higher levels (0.91 ± 0.20 for caspase-3 and 0.51 ± 0.18 for caspase-8) compared to the C+ group. The C+ group, which received standard feed, was unable to suppress cancer development in colon tissue.

4. Discussion

4.1. Animal conditions during cancer development

The body weight result shows that the C− and C+BRB groups tend to have higher weights compared to the C+ group. This may be due to the effects of cancer development in the digestive system. This can be seen in the rate of cancer spread in the C+ group, which is more than in the C+BRB group (Figure 1). The presence of cancer in the digestive system, such as in the colon, will inhibit the digestive system from absorbing nutrients into the body, resulting in a decrease in body weight in animals with cancer [13]. This condition is often called cachexia—A condition of malnutrition characterized by weight loss; anemia; metabolic disorders; depression; and changes in acid-base balance, vitamin, and electrolyte levels in the body [14].

The higher average body weight in the C+BRB group is also attributable to the absence of a decrease in their feed intake. Feed modified with BRB can potentially inhibit the development of colon cancer in the digestive system. Therefore, the clinical conditions and body weight of the C+BRB group tend to be better. Some bioactive components in BRB actively contribute to preventing colon cancer, namely ferulic acid, anthocyanin, γ-oryzanol, β-sitosterol, tocotrienol/tocopherol, tricin, ρ-coumaric, sinapic, syringic acid, and phytic acid [15]. These bioactive components can potentially repair cells damaged by the induction of AOM/DSS carcinogens and suppress cancer development. They can also

suppress cell damage in the cytoplasm and cancer cell proliferation [16]. This result is consistent with the study on Sprague-Dawley rats conditioned for colon cancer and fed BRB [15].

The greater weight of the colon in the C+ group is due to the distribution of nodules that spread to colon tissue. Besides being influenced by the original morphological conditions, the weight of an organ can also be affected by abnormalities such as lumps or nodules, as well as the presence of fluid or solids in the organ [17]. Cancer development due to AOM/DSS induction can be observed macroscopically by nodules or lumps in colon organs [11].

4.2. Cancer distribution in colon tissue of BALB/C mice

Nodules or cancers are tissue lumps smaller than 0.5 cm. A nodule may contain inflammation or a mixture of tissue and fluid [18]. Morphologically, in patients with colon cancer, the distribution of nodules/cancers in the colon will be apparent. Generally, nodules or cancers are an early sign of cancerous tissue growth. C+ group cancer nodules spread across almost all parts of the colonic tissue, exhibiting various shapes such as oval or round. BALB/C mice induced by AOM/DSS carcinogen compounds exhibited the presence of nodules or cancers in the colon [11]. The distribution of colonic diarrhea nodules indicates the development of colorectal cancer caused by AOM/DSS carcinogens. The spread of colonic diarrhea nodules is caused by the accumulation of inflammatory cells, resulting in clots or fluid in the colon tissue.

The data obtained from this study shows that the AIN-93M control diet was unable to suppress inflammation and cancer formation induced by the presence of AOM/DSS carcinogens. The cellulose powder component, which is the source of fiber in AIN-93M diet, is believed to be unable to suppress cancer development in mouse colon tissue. This inability may be due to cellulose's relatively low fermentation by bacteria in the colon [19], resulting in lower SCFA production. SCFAs are produced by the fermentation of food fiber by lactic acid bacteria (LAB) in the colon, and they can suppress the development of cancer in the colon [13,20].

On the other hand, this study showed that feed modified with BRB can inhibit the development of colon cancer by suppressing nodule formation in colon tissue, thus inhibiting cancer cell proliferation. Furthermore, bioactive components such as phenolic compounds in BRB can also suppress cancer cell proliferation and DNA damage in colon tissue [21].

4.3. Histopathology of BALB/C mice colon

Histopathological examination of colon tissue in the C+ group reveals cancer development at the dysplasia stage. Dysplasia is a stage of carcinoma characterized by abnormal cell growth, stacked cell nuclei reaching half the thickness of the epithelium, and loss of cell polarity [10]. The dysplasia stage is also characterized by loss of crypts and goblet cells, spread of inflammatory cells in the tissue area, visible damage to epithelial cells, and solidification of the tissue by the cell nucleus [18].

Histopathological examination of the BRB group showed that the cancer development rate was inhibited by substituting fiber with BRB. This is evidenced by the number of nodules in the colon area, indicating that the BRB group has a lower distribution of nodules compared to the C + group. BRB contains dietary fibers, such as β-glucans, that can be fermented by intestinal microbes, such as LAB. This fermentation can later produce SCFA compounds known to be chemopreventive agents in colon cancer. These SCFAs induce several proapoptotic genes such as caspase-3, caspase-9, and bid, while

also inhibiting DNA damage in the cell nucleus [22]. It also plays a role in stimulating intestinal epithelial cell multiplication, as these fatty acids are a component of cell membrane phospholipids [21]. In addition to fiber, BRB contains some bioactive components that prevent colon cancer. Anthocyanin, the most significant antioxidant in BRB, binds to the active side of the proliferation protein, PCNA, by stopping the progression of cyclin and CDK in the cell cycle [23]. Furthermore, ferulic acid and tocopherol in BRB reportedly inhibit the proliferation of cancer cells by inducing apoptotic genes [24].

4.4. Proliferation marker (PCNA) and apoptosis markers (caspase-3 and caspase-8)

The result indicates that BRB could reduce the expression of PCNA genes during cancer development. Feed modified with BRB is believed to have an antiproliferative ability by influencing the formation of enzymes that contribute to replication and transcription. This leads to an inhibition of transcription activity in the S phase, leading to cell cycle repair and, ultimately, control of excessive proliferation [25]. Some bioactive components, such as ferulic acid, phytate, and oryzanol, which have the potential for antiproliferative effects in BRB, can be increased in number by fermentation. During fermentation, microbes synthesize enzymes that can break down ester bonds and release bound phenolics, thereby increasing the number of free phenolic compounds [26].

Caspase-3 and caspase-8 genes have not been maximally induced in the metabolism of C+ group mice, preventing suppression of inflammation and DNA damage caused by carcinogens. BRB can potentially suppress cancer occurrence and increase the expression of caspase-3 and caspase-8 apoptotic genes during cancer development in mice. The presence of phytic acid and ferulic acid in BRB has been shown to increase apoptotic genes such as caspase-3 and inhibit cancer proliferation in TRAP mice [27]. Also, it can induce other apoptotic genes, such as pro-caspase 8. Caspase-8, an active form of pro-caspase-8, proteolytically activates caspase-3. Caspase-3 is a caspase effector gene that can alter cell morphology, such as DNA fragmentation, thereby suppressing cancer development. Anthocyanin and ferulic acid in BRB also act as antioxidants that can neutralize free radicals, repairing DNA damage, inducing apoptosis, and suppressing transcription and translation of cancer cells in the proliferation mechanism caused by cancer-forming carcinogens [28]. Phenolic compounds promote apoptosis in cancer cells induced by carcinogenic compounds through intrinsic pathways (mitochondria) [29]. In addition, butyric acid produced from fiber-soluble food fermentation is reported to be one of the agents that can induce apoptosis in cancer cells. Butyric acid can activate apoptotic genes in the intrinsic pathway through mitochondria by activating caspase-9 and caspase-3 enzymes [30].

5. Conclusions

BRB-based feed can potentially inhibit colon cancer development in BALB/C mice induced with AOM/DSS. The fiber and bioactive components of BRB have been shown to inhibit cancer development by reducing cancer distribution in colon tissue, suppressing cancer cell proliferation by decreasing PCNA gene expression, and inducing apoptosis by increasing caspase-3 and caspase-8 gene expression. In vivo testing is advised on BALB/C mice fed with fermented BRB to inhibit colon cancer, alongside further confirmation of indications of decreased cancer spread and proliferation, as well as induction of apoptosis in the intrinsic pathway. Moreover, some apoptotic genes that work on intrinsic pathways, such as Bcl-2 family members, Apaf-1, and caspase 9, can be further investigated to determine their gene expression levels during colon cancer development inhibition.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest in this research.

Authors contributions

SB, LN, A, and HS: Conceptualization; YK, FNAD, LN, BPP, and US: Methodology; YK: Research; FNAD, US, NDY, S and SB: Analysis and Data Curation; SB, LN, and BPP: Supervision; SB and YK: Writing Draft Preparation; SB, A, and HS: Writing, Review and Editing. All authors have read and agreed to the published version of the manuscript.

References

1. World Health Organization (2017) Cancer. Available from: http://www.who.int/mediacentre/.
2. American Cancer Society (2017) Cancer Facts and Figures in 2017. American Cancer Society. Inc. Surveillance Research.
3. Datsis A, Tsoga A, Langouretos V (2010) The role of functional foods in the prevention of colorectal cancer. *Hellenic J Surg* 82: 224–232. https://doi.org/10.1007/s13126-010-0035-5
4. Moongngarm A, Daomukda N, Khumpika S (2012) Chemical compositions, phytochemicals, and antioxidant capacity of rice bran, rice bran layer, and rice germ. *Apcbee Proc* 2: 73–79. https://doi.org/10.1016/j.apcbee.2012.06.014
5. Antczak C, Takagi T, Ramirez CN, et al. (2009) Live cell imaging of caspase activation for high content screening. *J Biomol Screen* 14: 956–969. https://doi.org/10.1177/1087057109343207
6. Shi Y (2002) Mechanisms of caspase activation and inhibition during apoptosis. *Mol Cell* 9: 459–470. https://doi.org/10.1016/S1097-2765(02)00482-3
7. Zheng Z, Shetty K (2000) Solid-state bioconversion of phenolics from cranberry pomace and role of Lentinus edodes beta-glucosidase. *J Agric Food Chem* 48: 895–900. https://doi.org/10.1021/jf990972u
8. Bravo R, Macdonald-Bravo H (1987) Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites. *J Cell Biol* 105: 1549–1554. https://doi.org/10.1083/jcb.105.4.1549

9. Zulfafamy KE, Ardiansyah, Budijanto S (2018) Antioxidative properties and cytotoxic activity against colon cancer cell WiDr of *Rhizopus oryzae* and *Rhizopus oligosporus*-fermented black rice bran extract. *Curr Res Nutr Food Sci* 6: 23–24. https://doi.org/10.12944/CRNFSJ.6.1.03
10. Glasauer A, Chandel NS (2014) Targeting antioxidants for cancer therapy. *Biochem Pharmacol* 92: 90–101. https://doi.org/10.1016/j.bcp.2014.07.017
11. Suzuki R, Kohno H, Sugie S, et al. (2006) Strain differences in the susceptibility to azoxymethane and dextran sodium sulfate-induced colon carcinogenesis in mice. *Carcinogenesis* 27: 162–169. https://doi.org/10.1093/carcin/bgi205
12. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402–408. https://doi.org/10.1006/meth.2001.1262
13. Phutthaphadoong S, Yamada Y, Hirata A, et al. (2010) Chemopreventive effect of fermented brown rice and rice bran (FBRA) on the inflammation-related colorectal carcinogenesis in ApcMin/+ mice. *Oncol Rep* 23: 53–59. https://doi.org/10.3892/or_00000605
14. Trujillo EB, Bergerson ASL, Graf JC, et al. (2005) Cancer in American society for parenteral and enteral nutrition support practice manual. *Nutr Care Org* 4: 150–170.
15. Norazalina S, Norhaizan ME, Hairuszah I, et al. (2010) Anticarcinogenic efficacy of phytic acid extracted from rice bran on azoxymethane-induced colon carcinogenesis in rats. *Exp Toxicol Pathol* 62: 259–268. https://doi.org/10.1016/j.etp.2009.04.002
16. Tomita H, Kuno T, Yamada Y, et al. (2008) Preventive effect of fermented brown rice and rice bran on N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis in rats. *Oncol Rep* 19: 11–15. https://doi.org/10.3892/or.19.1.11
17. Kampa M, Alexaki VI, Notas G, et al (2004) Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. *Breast Cancer Res* 6: R63–R74. https://doi.org/10.1186/bcr752
18. Rosenberg DW, Giardina C, Tanaka T (2009) Mouse models for the study of colon carcinogenesis. *Carcinogen* 30: 183–196. https://doi.org/10.1093/carcin/bgn267
19. Lupton JR (2004) Microbial degradation products influence colon cancer risk: the butyrate controversy. *J Nutr* 134: 479–482. https://doi.org/10.1093/jn/134.2.479
20. Andoh A, Fujiyama Y (2004) Anti-inflammatory roles of dietary fiber and short-chain fatty acids as regards inflammatory bowel diseases. *Agro Food Ind Hi-Tech* 15: 42–43.
21. Katyama M, Yoshimi N, Yamda Y, et al. (2002) Preventive effect of fermented black rice and rice bran against colon carcinogenesis in male F344 rats. *J Oncol* 9: 7–22. https://doi.org/10.3892/or.9.4.817
22. Yi W, Fischer J, Krewer G, et al. (2005) Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. *J Agric Food Chem* 53: 7320–7329. https://doi.org/10.1021/jf051333o
23. Yin MC, Lin CC, Wu HC, et al. (2009) Apoptotic effects of protocatechuic acid in human breast, lung, liver, cervix, and prostate cancer cells: potential mechanisms of action. *J Agric Food Chem* 57: 6468–6473. https://doi.org/10.1021/jf9004466
24. Muntana N, Prasong S (2010) Study on total phenolic contents and their antioxidant activities of Thai white, red and black rice bran extracts. *Pak J Biol Sci* 13: 170–174. https://doi.org/10.3923/pjbs.2010.170.174

25. Kim HY, Kim JH, Yang SB, et al. (2007) A polysaccharide extracted from rice bran fermented with Lentinus edodes enhances natural killer cell activity and exhibits anticancer effects. *J Med Food* 10: 25–31. https://doi.org/10.1089/jmf.2006.116
26. Zeng H, Lazarova DL, Bordonaro M (2014) Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. *World J Gastrointest Oncol* 6: 41–51. https://doi.org/10.4251/wjgo.v6.i2.41
27. Kuno T, Nagano A, Mori Y et al. (2016) Preventive effect of fermented brown rice and rice bran against Prostate Carcinogenesis in TRAP Rats. *Nutrient* 87: 421. https://doi.org/10.3390/nu8070421
28. Gschwantler M, Kriwanek S, Langner E, et al. (2002) High-grade dysplasia and invasive carcinoma in colorectal adenomas: a multivariate analysis of the impact of adenoma and patient characteristics. *Eur J Gastroenterol Hepatol* 14: 183–188. https://doi.org/10.1097/00042737-200202000-00013
29. Rosa LDS, Silva NJA, Soares NCP, et al. (2016) Anticancer properties of phenolic acids in colon cancer—A review. *J Nutr Food Sci* 6: 1000468.
30. Ruemmele FM, Schwartz S, Seidman EG, et al. (2003) Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. *Gut* 52: 94–100. https://doi.org/10.1136/gut.52.1.94

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