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Fucoxanthin-Rich Brown Algae Extract Decreases Inflammation and Attenuates Colitis-associated Colon Cancer in Mice

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Abstract Fucoxanthin is a natural carotenoid that is isolated from seaweed. We evaluated the effects of fucoxanthin-rich brown algae extract (FX-BAE) on the development of dextran sulfate sodium (DSS)-induced colitis, and colitis-associated colon cancer (CACC) in BALB/c mice. Colitis mice were given drinking water containing 3% DSS for 14 days, and fed with or without FX-BAE (1, 2.5, or 5 g/kg bodyweight/day) from day 8 to day 14. Another way, CACC mice were treated with azoxymethane (AOM) and 2% DSS, and fed with or without FX-BAE at 0.5, 1, or 2.5 g/kg every 2 days. Results revealed the disease activity index (DAI), nitric oxide (NO), malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) were all significantly less in FX-BAE treated mice. Additionally, FX-BAE not only decreased the incidence of colonic neoplasm, but also increased superoxide dismutase (SOD) production, lymphocyte proliferation and survival rate in CACC mice.

Keywords: *fucoxanthin, colitis, colon cancer, inflammation, dextran sulfate sodium*

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1. Introduction

Inflammatory bowel disease (IBD), which is comprised of two main types, ulcerative colitis (UC) and Crohn's disease (CD), affects approximately 3.6 million people in the United States and Europe. An alarming rise in previous low-incidence areas, such as Asia, is currently being observed [7,15,27]. IBD is characterized by chronic or relapsing immune activation and inflammation within the gastrointestinal (GI) tract that markedly alters GI function. Accumulated evidence suggests that IBD have a significantly increased risk of developing colitis-associated colorectal cancer (CACC) [16]. The etiology of IBD is often unclear, but manifestations of the disease include severe disruption of the epithelium and its associated mucosal layer by which neutrophils activating, and how they migrate across mucosal epithelia. Activated neutrophils produce excess reactive oxygen spices (ROS) within intestinal mucosa, where many intestinal microbes reside, suggesting that nitrogen species from intestinal microflora and ROS may involve with a complex linkage in initiating and perpetuating colonic inflammation [8,22,35]. These consecutive responses result in pro-inflammatory cytokines overexpression such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [38].

Present-day medical therapy of IBD consists of salicylates, corticosteroids, immune-suppressants and immunomodulators. However, their use may result in severe side effects and complications, such as an increased rate of malignancies or infectious diseases [4,23,42]. Accordingly, we considered it worthwhile to investigate the contribution that diet can make towards preventing the genesis of disease by improving antioxidative system. Carotenoids are a family of natural pigments; they have several biological functions, including radical scavenging, singlet-oxygen quenching activity, and immunomodulation that offer chemopreventive effects against carcinogenesis [26]. The marine carotenoid, fucoxanthin (FX), is present in edible brown seaweeds, and is one of the major xanthophyll components in brown algae. Its structure differs from common carotenoids, and features an allenic bond, and a 5,6-monoepoxide [29]. FX has several medicinal applications such as in treatments for hypertension, obesity, and inflammation, it also has anti-carcinogenic properties [41]. Fucoxanthin's potent activity may result from inhibition of certain signal transduction pathways that are critical to tumor growth and inflammatory responses, such as the AP-1, PPARγ, and NF-κB pathways [20,25,44]. Previous workers have reported that FX not only scavenges ROS including superoxide, hydroxyl radical, and reactive nitrogen species (RNS), but also improves the endogenous antioxidative system by superoxide dismutase (SOD) activity increasing [11,33]. SOD and malondialdehyde (MDA) are two important parameters to assess antioxidation and oxidation levels related to ROS. SOD is an essential enzyme in a network of biological antioxidants that is endogenously induced in order to eliminate superoxide radicals by conversion to hydrogen peroxide and oxygen [6,37].

In the present study, we evaluated the effects of fucoxanthin-rich brown algae extract (FX-BAE) on the development of dextran sulfate sodium (DSS)-induced colitis and colitis-associated colorectal cancer in mice. This study's findings support FX-BAE might decrease the intestinal injury that results from chronic inflammatory conditions such as IBD and associated cancers by reducing oxidative stress.

2. Materials and Methods

2.1. Reagents

Brown algae (*Sargassum muticum*) was purchased from Everyone Excellent Algae Bio. Tec. Co. LTD. (Penghu, Taiwan). Dulbecco's modified eagle medium (DMEM), Roswell park memorial institute medium (RPMI 1640), fetal bovine serum (FBS), and antibiotics were purchased from Invitrogen (Grand Island, NY, USA). Ficoll-Paque was purchased from Pharmacia (WI, USA). The Total Nitric oxide (NO) and Nitrate/Nitrite Assay Kit and Enzyme Linked Immunosorbent Assay (ELISA) kit were purchased from R&D Systems, Inc. (Quantikine, R&D Systems, Inc., USA). The Bicinchoninic Acid (BCA) Protein Assay Kit was obtained from Pierce (Rockford, IL, USA). Other chemicals were purchased from Sigma - Aldrich (St. Louis, MO, USA).

2.2. Brown Alga Extraction

S. muticum, was washed three times with distilled water, then stored in a medical refrigerator at -20°C. Frozen samples were lyophilized and homogenized in a grinder before extraction. To the powdered *S. muticum* was added 3 volumes of extraction buffer I (acetone: ethanol, 7: 3, v/v) with careful stirring. The solution was then filtered and evaporated in vacuo at 35°C. The crude extract was dissolved in 90% ethanol and partitioned with extraction buffer II (ethanol, water, hexane, 9:1:10, v/v/v). The hexane phase was discarded, and FX-BAE was extracted from the aqueous phase using ethyl acetate following the procedure as described by Haugan et al [18]. FX-BAE was purified by flash column chromatography on a LiChroprep RP-18 column (40-63 μm, 11 × 240 mm; Merck) with acetonitrile/methanol/water (67.5:13.5:19) eluent containing 0.1% ammonium acetate [39].

2.3. Animal Studies

Six- to eight-week-old male BALB/c mice were purchased from National laboratory animal center (Taipei, Taiwan). They were acclimatised for 1 week before the experiment began, and were individually housed in a room maintained at 25°C, under a 12 hr day/night cycle throughout the experiments. The colitis mouse model was established according to the methodology reported by Deguchi et al [12]. Briefly, mice were divided into six groups that included the control, and either 3% DSS-induced colitis, or 3% DSS-induced colitis and FX-BAE were administered (FX-BAE 1, 2, or 5 g/kg/day). The positive control group mice were treated with curcumin (0.8 g/kg) (Nakarai Tesk, Kyoto, Japan). CACC induction was performed using the procedure reported by Cui et al. [9] with minor modifications. Mice were given a single intraperitoneal injection of AOM (azoxymethane, 12 mg/kg) at the start of the experiment (week 0), followed by administration of 2% DSS (wt/wt) in drinking water for 7 consecutive days at weeks 3, 6, and 9. CACC mice were divided into 5 groups; group 1 was fed with curcumin (75 mg/kg) as the positive control; group 2, 3, and 4 were fed with FX-BAE at 0.5, 1, and 2.5 g/kg body weight, respectively. The process was terminated at week 11; Details of the colitis and CACC mouse models are showed in Figure 1A and Figure 1B.

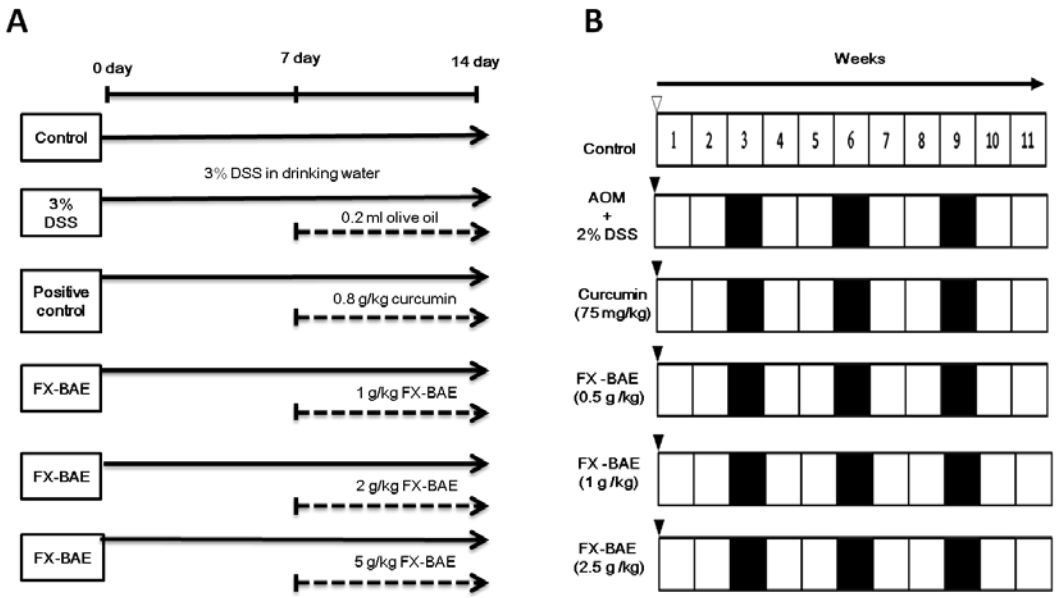

Figure 1. Procedure for administration of fucoxanthin-rich brown algae extract to establish DSS-induced colitis and CACC mouse models. The colitis mouse model was established by treatment with 3% DSS in drinking water for 14 days. (A)Animals were separated into five experimental groups and one positive control group. The control group mice were fed with curcumin (0.8 g/kg/day). Animals in experimental groups were individually fed with FX-BAE at dosages of 1, 2, or 5 g/kg/day from day 8 to day 14. (B) Mice were given a single intraperitoneal injection of AOM (12 mg/kg body weight) at the start of the experiment (week 0) and thereafter, received 2% DSS (wt/wt) in drinking water for 7 days at weeks 3, 6, and 9. For the experiment, CACC induced mice were divided into 5 groups; group 1 was fed with curcumin (75 mg/kg/day) as the positive control; groups 2, 3, and 4 were fed with FX-BAE (0.5, 1, and 2.5 g/kg/per day, respectively. Data are reported as means ±SD (n = 10 mice/group)

2.4. Assessment of DSS-induced Colitis

Daily clinical assessment of DSS-induced colitis was performed, including measurement of food intake and body weight. Colitis severity was assessed using a disease activity index (DAI), which is based on a scoring system as previously reported [17]. A validated clinical DAI ranging from 0 to 4 was calculated based on stool consistency, presence of fecal blood, and decrease in body weight. The DAI evaluation standard is shown in Table 1. Mice were sacrificed at 14 days, and the lengths and weights of their colons were recorded [6].

Table 1. Scoring of disease activity index (DIA) in DSS-induced colitis

Score	Occult/gross bleeding	Weight loss (% of initial wt.)	Stool consistency
0	None	<1	Normal stools
1	Small spots of blood in stool; dry anal region	1~4.99	Soft pellets not adhering to the anus
2	Large spots of blood in stool; blood appears through anal orifice	5~10	Very soft pellets adhering to the anus
3	Deep red stool; blood spreads largely around the anus	>10	Liquid stool on long streams;wet anus
4	Gross bleeding	>20	Diarrhea

Criteria from the work of Gommeaux *et al*., 2007 [17].

2.5. Assessment of Colitis-associated Colon Cancer

Colons were removed and washed in cold 1X phosphate buffered saline (PBS) solution immediately after sacrifice, then cut longitudinally, fixed in 5% buffered formalin, and embedded in paraffin. Five millimeters sections were stained with hematoxylin and eosin (H&E) for examination by light microscopy and the colonic neoplasms determined according to previously reported criteria [2,5].

2.6. Determination of Nitric Oxide, Lipid Peroxidation, and Expression of pro-Inflammatory Cytokines

Colonic tissue samples were obtained from the mid-colons of mice, washed with DMEM containing 2% FBS (v/v), and 1% (v/v) antibiotics (Invitrogen, Grand Island, NY, USA), then cut into small sections. Tissue samples (0.5 cm) were placed in a 48-well plate with 1 ml of DMEM medium containing 0.2% FBS (v/v), incubated for 24 h at 37°C under 5% CO_2. The supernatants of the cell-free colonic tissue culture were determined for nitrite content using a commercial nitrite/nitrate colorimetric assay kit, and for TNF-α and IL-6 production using an ELISA kit. The absorbance at 540 nm was measured with a 96-well plate reader [32]. Plasma obtained from mice was centrifuged at 3,000 rpm at 10°C and then assessed for nitrite and cytokine production. All procedures were conducted according to the kit manufacturer's instructions.

2.7. In Vitro Proliferation Assay to Assess Spleen Lymphocyte Function

Spleens from colitis and CACC mice were weighed after washed 3 times with cold 1X PBS, and then placed into glass tubes, each with 3 ml RPMI medium containing 5% FCS and 1% antibiotics. The splenocyte mixture was prepared by gently grinding spleen tissue with a glass tissue-grinder. Ficoll-Paque was added to splenocyte mixture to an equivalent volume (v/v), and centrifuged at 2000 rpm for 30 minutes at 4°C. Monocytes obtained from spleen tissue were placed into a 96-well culture plate in RPMI medium and either concanavalin A (Con A) (10 μg/ml), or lipopolysaccharide (LPS) (20 μg/ml). After culturing under 5% CO_2 for 48 h at 37°C, the proliferations of T cells and B cells were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [10,31].

2.8. Statistical Analysis

Data are reported as means ±SEM. We used one-way analysis of variance and the Student's Newman-Keuls test for post hoc comparisons to determine differences between the control and experimental groups. The Student's *t* test was performed for paired samples. A p value < 0.05 was considered significant.

3. Results

3.1. Fucoxanthin -rich Brown Algae Extract

The yield and fucoxanthin content of *Sargassum muticum* extract were shown in Table 2. FX-BAE was compared with the fucoxanthin standard by using HPLC that included Colum RP-18, mobile phase (acetontrile: methanol: water; 67.5: 13.5: 19) containing 0.1% (w/v) ammonium acetate and flow rate of 1 mL/1 min. The chromatography analysis of fucoxanthina standard and FX-BAE was shown in Figure 2A-B.

Table 2. The yield and fucoxanthin content of Sargassum muticum extracts*

	FX-BAE (g)	Content of fucoxanthin (mg)	Yield (%)**
First extract	6.830(±0.85)	1120.96(±378.25)	17.1
Second extract	4.80(±0.55)	600.34(±100.56)	12.5
Total	11.63(±0.96)	1721.31(±454.69)	14.8

* Extraction from Brown algae powder.
**Yield (%) = content of fucoxanthin (g) /extract (g).
Each data represents the mean ± S.E. of three independent experiments.

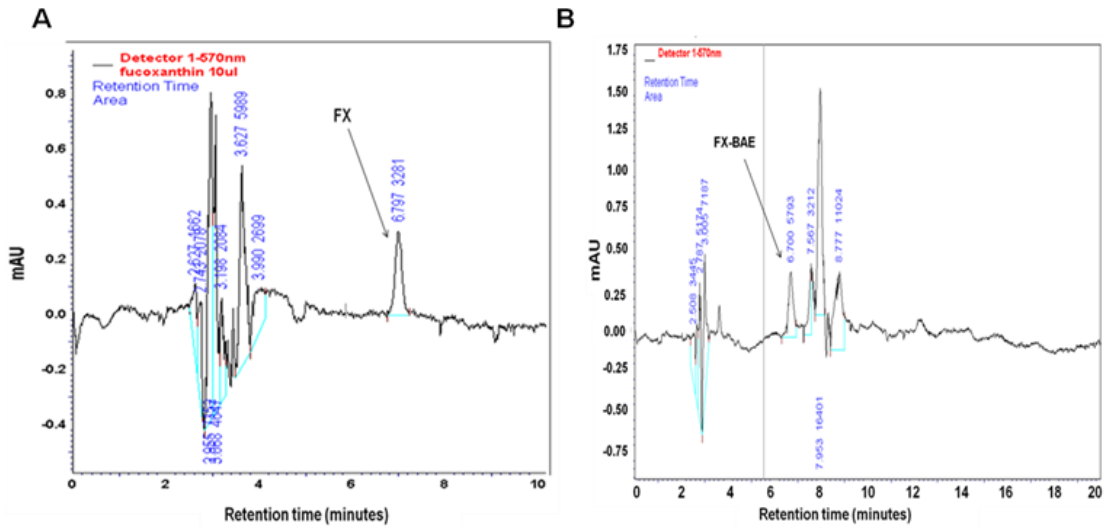

Figure 2. Chromatograms analysis of fucoxanthin-rich brown algae extract using HPLC. FX-BAE was obtained and compared with fucoxanthin standard by using HPLC. The condition of operation included: Colum RP-18, mobile phase (acetontrile : methanol : water; 67.5: 13.5 :19) containing 0.1% (w/v) ammonium acetate and flow rate of 1 mL/1 min. Chromatograms of fucoxanthin standard (Figure 2A) and X-BAE (Figure 2B) were indicated by the black arrow

3.2. Treatment with Fucoxanthin-rich Brown Algae Extract Alleviated Intestinal Injury in DSS-induced Colitis Mice

We examined the effects of FX-BAE treatment on the DSS-induced colitis mice. As shown in Table 3, on days 8, 9, and 10 after initiation of DSS-induced colitis, the DAI was significantly lower in the DSS only treatment group than it was in the DSS and curcumin, or FX-BAE treatment groups. There were significant reductions in DAI on days 11 and 14 for animals treated with FX-BAE (2 and 5 g/kg/day) compared with the curcumin treated group ($p < 0.05$). The colon weight/length ratio, a marker for tissue edema, was significantly greater in DSS-treated mice than it was in DSS and curcumin, or FX-BAE-treated mice ($P < 0.05$; Figure 3A-B). These observations indicate that FX-BAE suppressed the development of DSS- and curcumin-induced colitis. The effect was most significant in the 2 and 5 g/kg FX-BAE treatment groups.

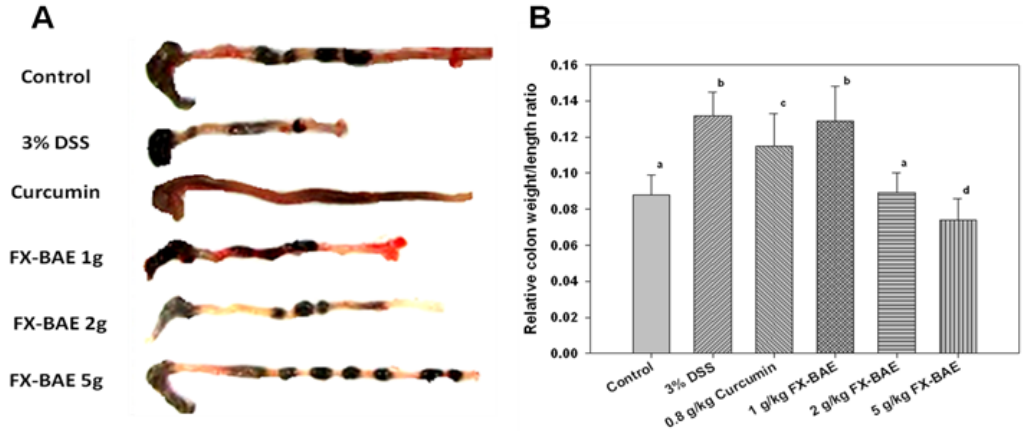

Figure 3. Colonic tissue changes in DSS-induced mouse model after treatment with fucoxanthin-rich brown algae extract. Mice were administered 3% DSS in drinking water for 14 days, and treated with FX-BAE (0, 1, 2, or 5 g/kg/day). Curcumin (0.8 g/kg/day) provided the positive control. The colon length and weight: length ratio was significantly greater in DSS-treated mice than it was in DSS and curcumin, or FX-BAE- treated mice ($p < 0.05$; Figure A-B). These observations indicate that effect of FX-BAE to suppress the development of DSS-induced colitis is similar to curcumin. This was particularly the case for 2 and 5 g/kg FX-BAE treatment groups. Data are reported as means ±SD (n = 10 mice/group)

Table 3. Effect of fucoxanthin-rich brown algae extract on Disease activity indexin colonic tissue of IBD mice*

Period (day)	Control		3% DSS		Curcumin (0.8g)		FX-BAE (1g)		FX-BAE (2g)		FX-BAE (5g)	
8	0	(±0.00)^{a}	2.94	(±0.13)^{b}	2.98	(±0.03)^{b}	2.91	(±0.05)^{b}	2.97	(±0.03)^{b}	2.89	(±0.11)^{c}
9	0	(±0.00)^{a}	2.82	(±0.09)^{b}	2.94	(±0.16)^{c}	2.97	(±0.11)^{c}	2.91	(±0.06)^{c}	2.94	(±0.16)^{c}
10	0	(±0.00)^{a}	3.21	(±0.06)^{b}	3.11	(±0.11)^{c}	3.25	(±0.12)^{b}	3.15	(±0.07)^{c}	3.12	(±0.13)^{c}
11	0	(±0.00)^{a}	3.29	(±0.09)^{b}	3.15	(±0.16)^{c}	3.28	(±0.07)^{b}	3.28	(±0.06)^{b}	3.08	(±0.07)^{d}
12	0	(±0.00)^{a}	3.42	(±0.10)^{b}	3.28	(±0.10)^{c}	3.41	(±0.05)^{b}	3.35	(±0.11)^{bc}	3.18	(±0.09)^{d}
13	0	(±0.00)^{a}	3.73	(±0.08)^{b}	3.44	(±0.08)^{c}	3.68	(±0.06)^{bc}	3.57	(±0.07)^{bc}	3.38	(±0.11)^{d}
14	0	(±0.00)^{a}	3.91	(±0.07)^{b}	3.45	(±0.11)^{c}	3.87	(±0.15)^{bc}	3.79	(±0.12)^{bc}	3.39	(±0.13)^{d}

FX-BAE, fucoxanthin-rich brown algae extract. ^{bc}Means in the row not sharing a common letter are significantly different between groups at $p < 0.05$. * mean ± SE.

3.3. Fucoxanthin-rich Brown Algae Extract Reduces Oxidative Stress and Pro-inflammatory Cytokine Expression in DSS-induced Colitis Mice

DSS-induced colitis is characterized by edema, infiltration of inflammatory cells into the mucosa and submucosa, destruction of epithelial cells, and mucosal thickening. Increasing oxidative stresses involving ROS and NO accompany the unregulated expression of pro-inflammatory cytokines such as TNF-α and IL-6. FX-BAE reduced the production of malondialdehyde (MDA) (Figure 4A), and reduced the total NO content in colonic tissue of DSS-induced colitis mice (Figure 4B). FX-BAE incubated with DMEM medium containing 2% FBS induced reductions in NO release, and in TNF-α and IL-6 secretion from colonic tissue (Figure 4C-E). These results are similar to observations made during the examination of plasma from DSS-induced colitis animals. MDA concentrations were not decreased significantly in groups treated with curcumin or FX-BAE (Figure 5A), however, there was an increase in superoxide dismutase (SOD) in the FX-BAE treatment group (Figure 5B). The expressions of pro-inflammatory cytokines TNF-α and IL-6 were downregulated (Figure 5C-D), suggesting that FX-BAE may lessen colonic damage and reduce inflammation.

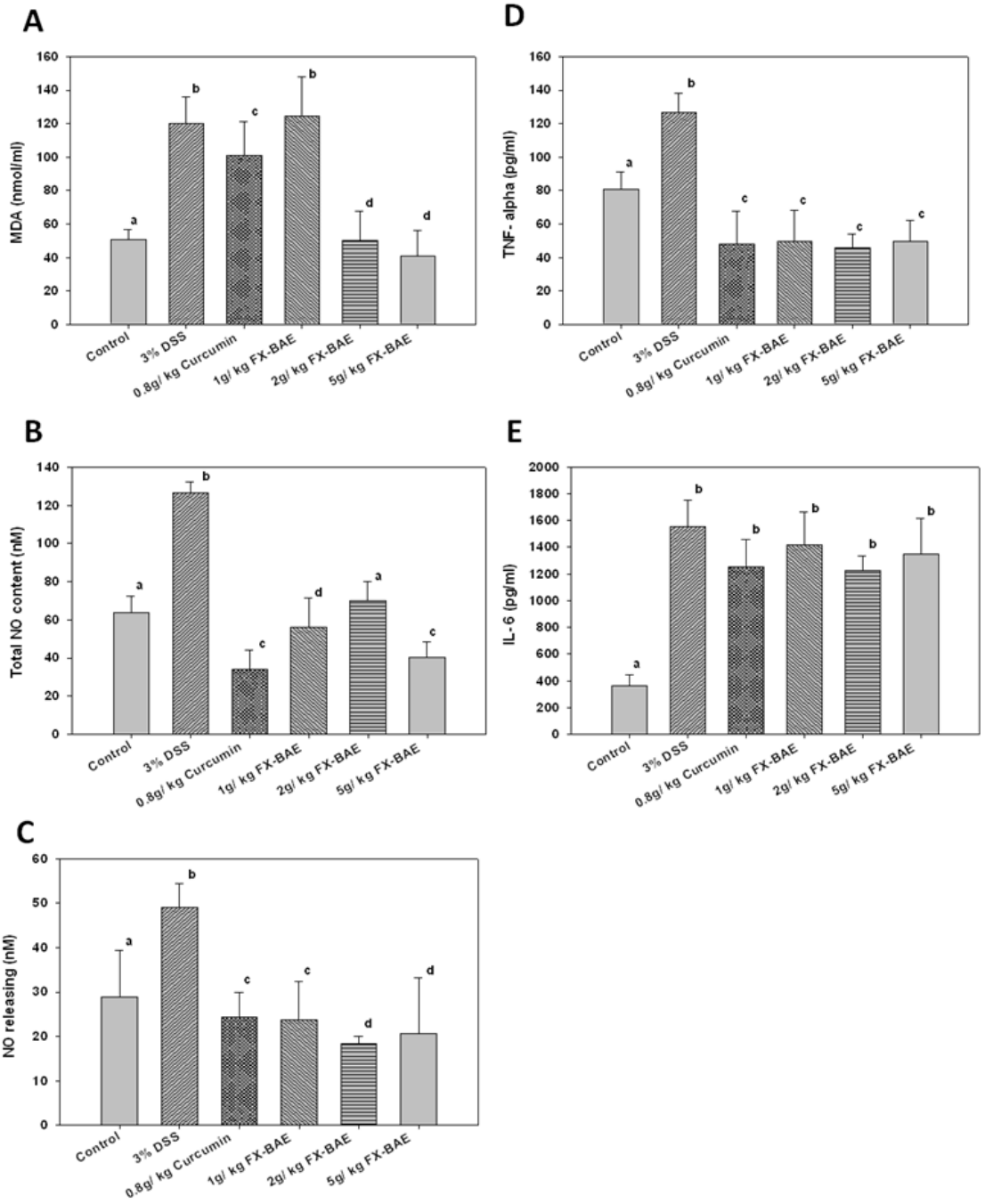

Figure 4. Fucoxanthin-rich brown algae extract reduces oxidative stress and pro-inflammatory cytokine expression in DSS-induced colitis mouse model. Colonic tissue samples were obtained from DSS-induced colitis mouse models and dissected. Blotting tissue samples dry with filter paper, the samples were homogenized in ice-cold KCl (0.15 M) for detection of the effects of FX-BAE on oxidative stress and pro-inflammatory cytokine expression. (A) FX-BAE reduced production of malondialdehyde (MDA), and (B) reduced total NO content in colonic tissue. The reductions were most significant for 5 g/kg/day FX-BAE treatment group. (C) The same FX-BAE dosage of 5 g/kg/day also diminished NO release, and (D) TNF-α and (E) IL-6 secretion from colonic tissue. Data are reported as means ±SD (n = 10 mice/group). Different letters indicate significantly differences, with $p < 0.05$

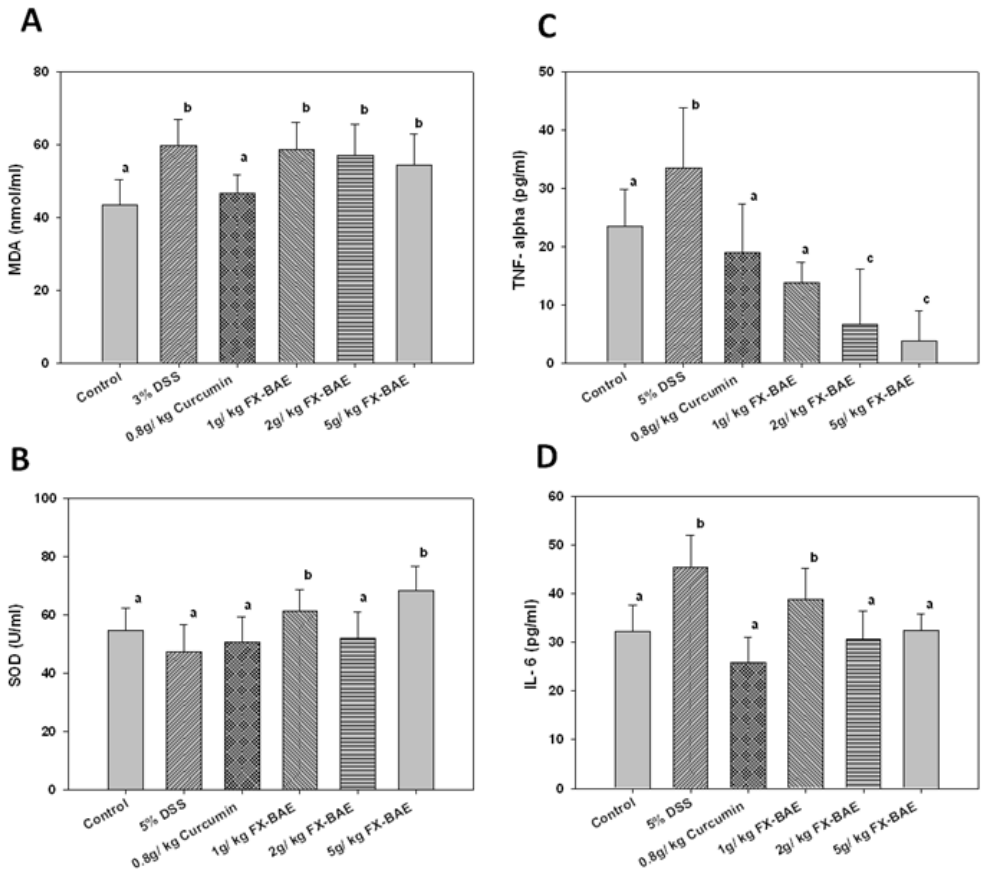

Figure 5. Fucoxanthin-rich brown algae extract decreases oxidative stress and pro-inflammatory cytokine expression in DSS-induced colitis plasma. Plasma samples were collected from DSS-induced colitis mice and assessed for oxidative stress and pro-inflammatory cytokine expression. (A) MDA concentration was not significantly decreased in groups treated with FX-BAE, (B) SOD levels were increased after treatment with FX-BAE. (C) FX-BAE also caused significant decreases in TNF-α expression in the dosage range 1- 5 g/kg/day, and IL-6 production was lessened. Curcumin (0.8 g/kg/day) was used as a positive control. Data are reported as means ±SD (n = 10 mice/group). Different letters indicate significantly differences with $p < 0.05$

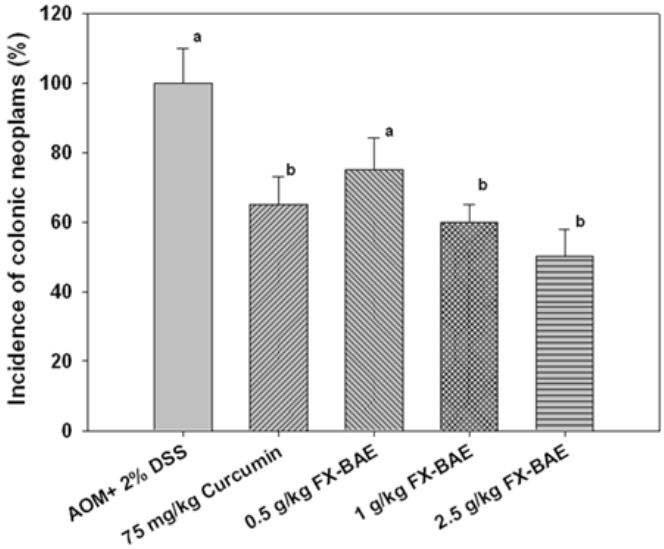

Figure 6. Incidence of colonic neoplasm change in CACC mouse model after treatment with fucoxanthin-rich brown algae extract. CACC mice were grouped and fed with FX-BAE at dosages of 0, 0.5, 1, or 2.5 g/kg every 2 days. (A) There was a significant reduction of DAI score of disease animals that received FX-BAE at 7 weeks. The data shows 2.5 g/kg/day dosage of FX-BAE produced greater efficacy than CACC mice fed with 75 mg/kg curcumin. Data are reported as means ±SD (n = 10 mice/group). (B) The incidence of AOM and DSS-induced colonic neoplasm was reduced by nearly 50% after treatment with FX-BAE, similar to the effects of curcumin treatment. Data are reported as means ±SD (n = 10 mice/group). Different letters indicate significantly differences, with $p < 0.05$

3.4. Fucoxanthin-rich Brown Algae Extract Ameliorates CACC Progression

Chronic inflammation of colon can induce colitis-associated colon cancer. Several studies reported natural compounds that possess anti-inflammatory properties, and that may have application in the prevention of carcinogenesis. Accordingly, we examined the effects of FX-BAE on the CACC animal model. As shown in Table 4, the results revealed significant reductions in DAI levels

following treatment with curcumin. Interestingly, at 7-weeks, administration of FX-BAE at 2.5 g/kg dosage was more effective for treatment of AOM and DSS-induced CACC than treatment with 75 mg/kg curcumin. Another way, treatment with FX-BAE reduced the incidence of AOM and DSS-induced colonic neoplasm by nearly 50%, similar to the rates seen for the curcumin treatment group (Figure 6).

Table 4. Effect of fucoxanthin-rich brown algae extract on Disease activity indexin in colonic tissue of CACC mice*

Period (week)	Control		AOM + 2% DSS		Curcumin (75 mg)		FX-BAE (1g)		FX-BAE (2g)		FX-BAE (5g)	
1	0	(±0.00)^{a}	0.11	(±0.16)^{b}	0.23	(±0.13)^{b}	0.11	(±0.15)^{b}	0.16	(±0.13)^{b}	0.13	(±0.11)^{b}
2	0	(±0.00)^{a}	0.31	(±0.11)^{b}	0.32	(±0.16)^{b}	0.31	(±0.19)^{b}	0.37	(±0.26)^{b}	0.34	(±0.17)^{b}
3	0	(±0.00)^{a}	0.88	(±0.09)^{b}	0.83	(±0.14)^{b}	0.87	(±0.21)^{b}	0.84	(±0.27)^{b}	0.79	(±0.13)^{b}
4	0	(±0.00)^{a}	1.68	(±0.31)^{b}	1.42	(±0.19)^{c}	1.59	(±0.27)^{b}	1.55	(±0.36)^{b}	1.41	(±0.19)^{c}
5	0	(±0.01)^{a}	1.97	(±0.15)^{b}	1.66	(±0.16)^{c}	1.88	(±0.23)^{bc}	1.81	(±0.31)^{bc}	1.71	(±0.12)^{bc}
6	0	(±0.00)^{a}	2.48	(±0.21)^{b}	1.78	(±0.23)^{c}	2.44	(±0.36)^{b}	2.38	(±0.47)^{b}	1.75	(±0.28)^{c}
7	0	(±0.00)^{a}	2.71	(±0.18)^{b}	1.99	(±0.21)^{c}	2.49	(±0.26)^{bc}	2.48	(±0.27)^{bc}	1.62	(±0.22)^{d}
8	0	(±0.02)^{a}	2.94	(±0.16)^{b}	2.17	(±0.17)^{c}	2.88	(±0.31)^{b}	2.47	(±0.19)^{bc}	1.99	(±0.21)^{d}
9	0	(±0.00)^{a}	3.11	(±0.22)^{b}	2.28	(±0.29)^{c}	2.71	(±0.29)^{bc}	2.51	(±0.19)^{bc}	1.87	(±0.33)^{d}
10	0	(±0.00)^{a}	3.32	(±0.24)^{b}	2.37	(±0.21)^{c}	3.12	(±0.36)^{c}	2.84	(±0.17)^{bc}	1.71	(±0.33)^{d}
11	0	(±0.00)^{a}	3.56	(±0.19)^{b}	2.48	(±0.19)^{c}	3.21	(±0.23)^{bc}	2.94	(±0.32)^{bc}	1.64	(±0.19)^{d}
12	0	(±0.00)^{a}	3.78	(±0.16)^{b}	2.41	(±0.26)^{c}	3.67	(±0.27)^{b}	3.42	(±0.21)^{bc}	1.62	(±0.24)^{d}

FX-BAE, fucoxanthin-rich brown algae extract. ^{bc}Means in the row not sharing a common letter are significantly different between groups at $p < 0.05$. *mean ± SE.

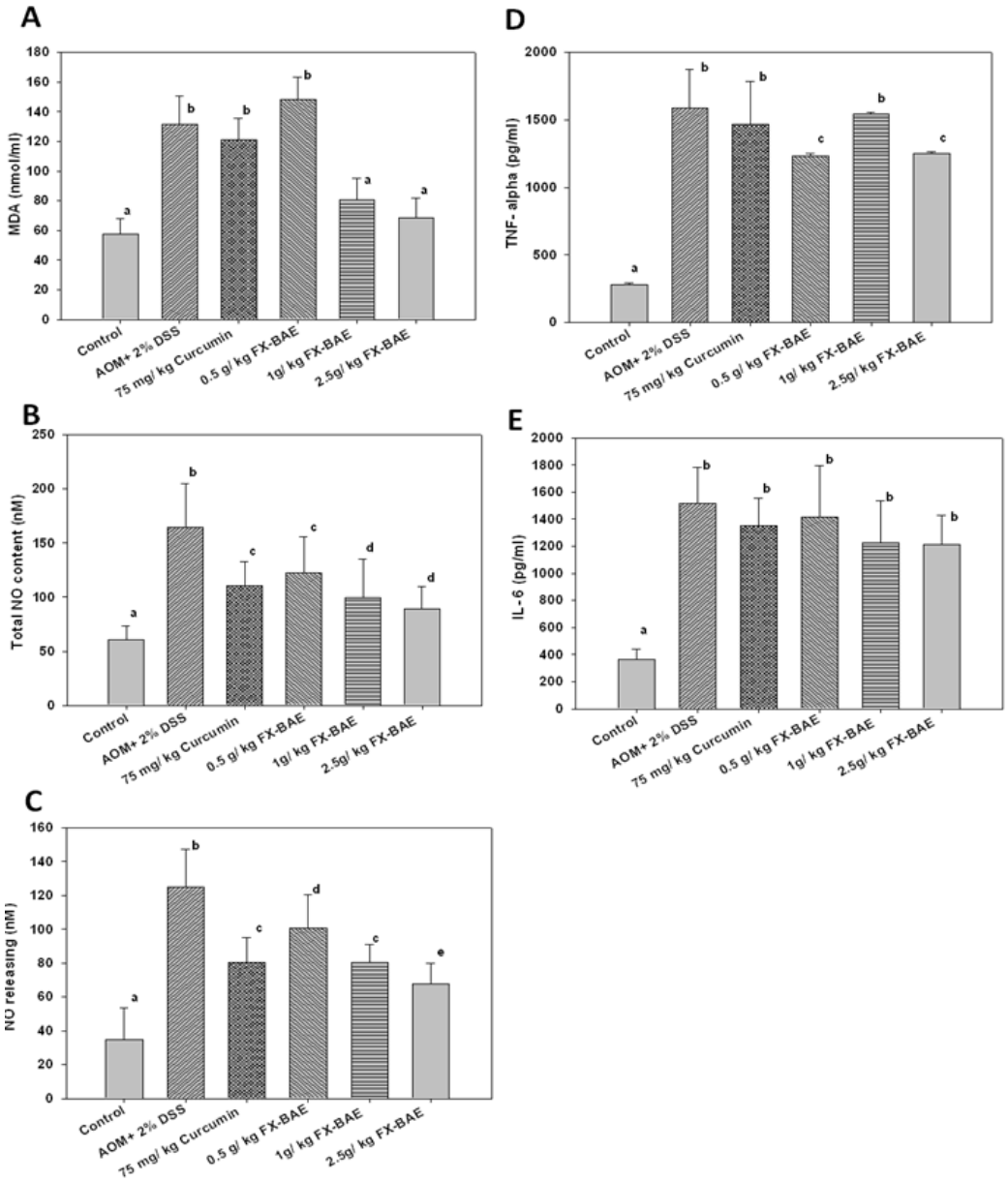

Figure 7. Fucoxanthin-rich brown algae extract decreases oxidative stress and pro-inflammatory cytokine expression in CACC mice. Mice were grouped and fed with FX-BAE at dosages of 0.5, 1, or 2.5 g/kg every two days. (A) Total NO and (B) MDA production of colonic tissue was decreased in the 1 and 2.5 mg/kg FX-BAE treatment groups ($p < 0.05$; Figure 5A-B). (C) FX-BAE decreased the release of NO, and lessened the secretion of (D) TNF-α and (E) IL-6 in colonic tissue. Curcumin (75 mg/kg every 2 days) was used as a positive control. Data are reported as means ±SD (n = 10 mice/group). Different letters indicate significant differences, with $p < 0.05$

3.5. Fucoxanthin-rich Brown Algae Extract Reduces Oxidative Stress and Downregulates Pro-inflammatory Cytokine Expression in the CACC Mouse Model

FX-BAE induced a reduction in the incidence of colonic neoplasms in AOM and DSS-induced CACC animals, however, the effect of FX-BAE treatment on the immune response in CACC mice is unclear. Further, we examined the efficacy of FX-BAE in the prevention of oxidative stress and pro-inflammatory cytokine expression in CACC mice. The results show that MDA and total NO were decreased in colonic tissue following treatment with FX-BAE at doses of 1 and 2.5 mg/kg every 2 days, respectively (Figure 7A-B). Similar effects were seen for NO release, and for TNF-α and IL-6 secretion in colonic tissue culture (Figure 7C-E). Systemic pro-inflammation markers revealed that MDA, TNF-α, and IL-6 were downregulated in the plasma of CACC animals, while SOD levels were increased (Figure 8A-D).

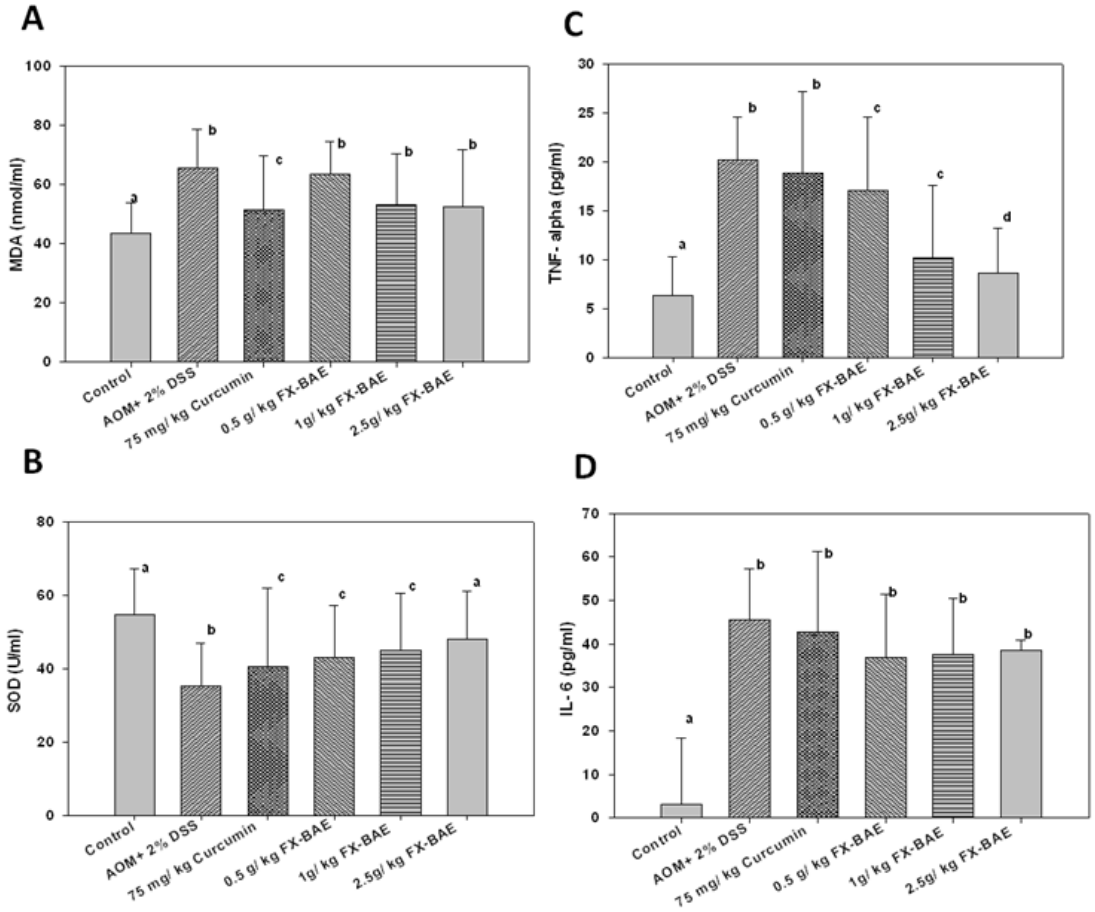

Figure 8. Fucoxanthin-rich brown algae extract decreases oxidative stress and pro-inflammatory cytokine expression in CACC mouse plasma. Plasma was collected from CACC mice and assessed for oxidative stress and pro-inflammatory cytokine expression. (A) MDA production was decreased, whereas (B) SOD levels were increased in CACC mice treated with FX-BAE. Systemic inflammatory cytokines (C) TNF-α and (D) IL-6 were significantly diminished in CACC mouse plasma after treatment with FX-BAE at dosages of 0.5, 1, and 2.5 g/kg every 2 days. Data are reported as means ±SD (n = 10 mice/group). Different letters indicate significant differences, with $p < 0.05$

3.6. Fucoxanthin-rich Brown Algae Extract Induces Lymphocyte Proliferation and Increases Survival Rates in the CACC Mouse Model

Recent clinical studies have demonstrated that anti-carcinoma immunity increases with the rise and progression of a carcinoma; tumor-infiltrating immune cells (TICs), including T, B, and natural killer (NK) cells become activated, and the numbers of these lymphocytes and macrophages positively correlate with cancer-specific survival rates in patients with various carcinomas. Therefore, we examined the effect of FX-BAE on the immunity systems of CACC animals. Compared with DSS-induced colitis group (Figure 9A), FX-BAE reduced the spleen enlargement significantly in CACC mice (Figure 9B). The results also showed that FX-BAE induces proliferation of T cell and B cell lymphocytes in CACC mice, and the optimal dosages for increased immunity were 1.0 and 2.5 g/kg FX-BAE ($P < 0.05$; Figure 9C). CACC mice administered with 1.0 or 2.5g/ kg FX-BAE showed increases in survival rate from 40% to 60% for treatment at 1 g/kg bodyweight, and 40% to 70% increases for treatment at 2.5 g/kg bodyweight, as compared with mice that did not receive curcumin or FX-BAE treatments (Figure 9D). Thus, FX-BAE treatments were more effective than treatment with curcumin, in which 75 mg curcumin/kg bodyweight every 2 days increased the survival rate in CACC mice from 40%, to only 50%.

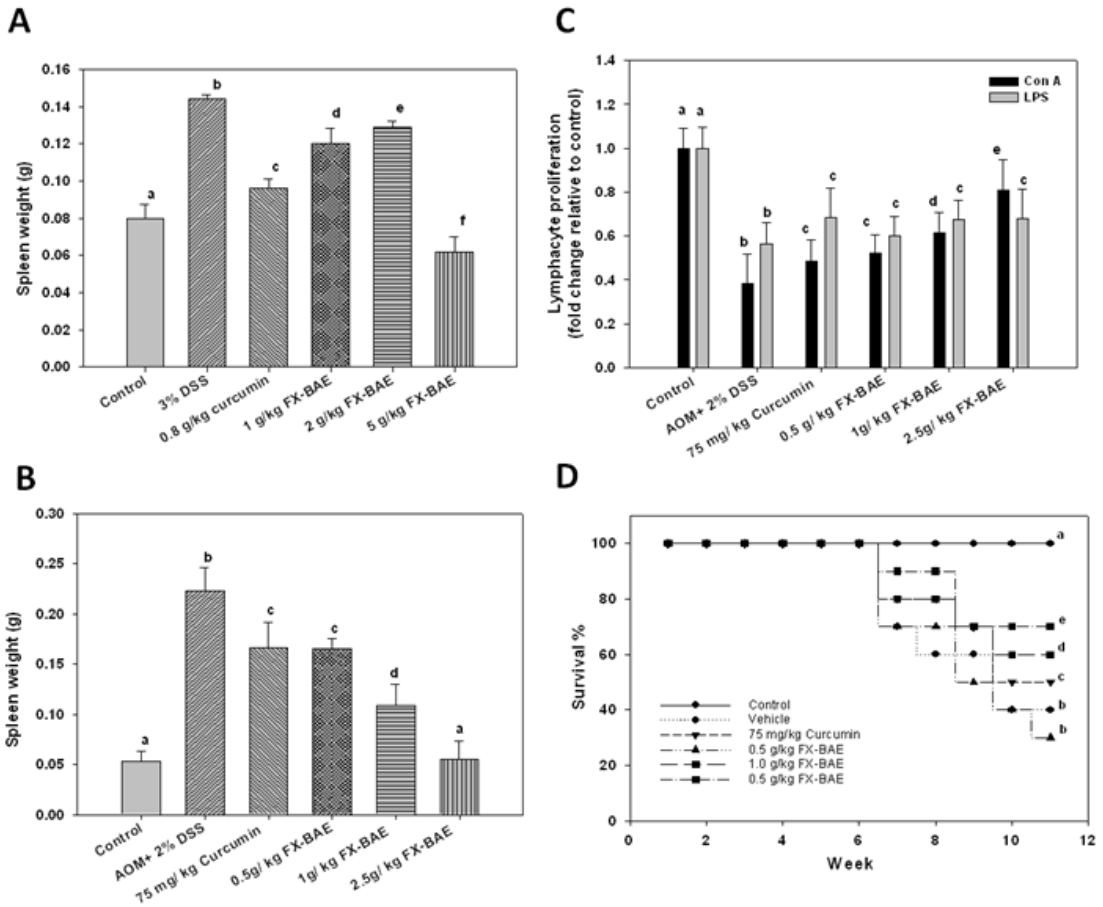

Figure 9. Fucoxanthin-rich brown algae extract induces lymphocyte **proliferation and increases survival rates in the CACC mouse model.** Spleen weight was obtained from DSS-induced colitis and CACC mice ($p < 0.05$; Figure 8A-B). Splenocytes were obtained from CACC mice and separated into 96-well culture plates containing RPMI medium with Con A or LPS. After culturing under 5% CO_2 for 48 h at 37°C, T cell and B cell proliferations were determined by MTT assay. (C) FX-BAE induced proliferation of lymphocyte T cells and B cells in CACC mice, the optimal treatments for increased immunity were determined as dosages of 1 or 2.5 g/kg every 2 days. (D) groups treated 1 and 2.5 g/kg of FX-BAE every 2 days exhibited improvements in survival rates from 40% to 60% and from 40% to 70%, respectively, compared with the CACC only group. Curcumin (75 mg/kg every 2 days) was used as a positive control. Data are reported as means ±SD (n = 10 mice/group). Different letters indicate significant differences, with at $p < 0.05$

4. Discussion

The prevalence of colonic cancer is currently increasing. Aside from hereditary factors, life-style and abnormal inflammation of the intestine are thought to be important factors in the genesis of colonic cancer. Our aim was to apply a novel approach for the prevention and treatment of colonic cancer by identifying and collecting compounds from common foods. The World Health Organization (WHO) report that fucoxanthin (FX), a component in seaweed, is a functional ingredient [11]. During past decades, FX was researched for its antioxidant and anti-inflammation properties [21,34,40]. However, the details of FXs between antioxidant and anti-inflammatory role in the treatment of colitis and CACC remain unclear. Heo et al. provided that FX inhibits the iNOS/NO pathway in LPS-induced macrophage cells, which are associated with mediation in TNF-α and IL-6 production [19], and Heo's report is in agreement with our previous findings (data not show). Accordingly, in this study, we investigated the effect of FX-BAE on colitis in the CACC mouse model, and then compared our results with those seen for the curcumin positive control group. Curcumin is a natural polyphenolic compound from the turmeric (*Curcuma longa)*; it is a powerful antioxidant that can reduce inflammation [1]. C. longa has inhibits the activation of NF-κB and AP-1 transcription factors, and exhibits free-radical scavenging (antioxidant) properties [36]. Historically, curcumin has been researched for its activities in several chronic disease and cancer prevention applications [24]. According to our findings, FX-BAE offers similar benefits to those of curcumin, but with greater efficacy. In addition to its beneficial role in treating intestinal injury, FX-BAE has a lower DAI score than curcumin has, suggesting fewer side-effects such as diarrhea and intestinal bleeding. Treatment with FX-BAE resulted in increased body weight (data not show) and greater colonic weight/length ratio in DSS-induced colitis mice. We have shown that DSS causes significant inflammation and tissue injury to the mouse colon, which is characterized by infiltration of inflammatory cells, epithelial disruption, edema, and hemorrhage. These symptoms of DSS-induced colitis were substantially reduced in mice treated with FX-BAE, suggesting that FX-BAE may inhibit the inflammatory response to DSS-induced colitis and intestinal injury [13]. Other inflammation related factors for intestinal injury include oxidative stress that results from an imbalance between production of free radicals (FR) and the body's ability to neutralize them. FX is a carotenoid, and has been implicated as an important dietary nutrient with

antioxidant properties. Carotenoids can quench singlet oxygen by a physical process whereby the excess energy of the singlet oxygen transfers to the long central chain of conjugated double bonds in the carotenoid molecule [3,43]. FX can donate an electron as a part of its free-radical quenching function [11]. We have shown that the anti-oxidative and anti-inflammatory effects of FX-BAE involve downregulation of NO, MDA, TNF-α, and IL-6 in the colonic tissue and plasma in experimental animals. We observed FX-BAEs ability to alleviate DSS-induced colitis. Furthermore, treatment with FX-BAE results in increased SOD production. Taken together, FX-BAE offers an effective treatment for inflammation by decreasing the levels of pro-inflammatory mediators [38].

Similar effects were observed in the CACC mouse model. In this study, we used a well-established mouse model of intestinal tumorigenesis to examine the effects of the anti-inflammatory dietary component, FX-BAE, on intestinal TNF-α and IL-6, and on associated tumorigenesis in the form of polyps. Our findings revealed that a dose of 1 g FX-BAE/kg body weight every 2 days diminished the incidence of colonic neoplasm by at least 40%. CACC mice treated with 2.5 g FX-BAE/kg showed a 50% reduction in the incidence of colonic neoplasm, and significantly decreased the DAI score for CACC mice. Decreases in pro-inflammatory mediator levels might mitigate cell damage resulting from oxidative stress, and help to maintain normal physical bioactivity in CACC and DSS-induced colitis animals [17]. Carcinoma is a malignant tumor of epithelial cell origin. The immune-mediated spontaneous regression of carcinoma has been described by many clinical research reports [30,45]. Accumulating evidence indicates that anti-carcinoma immunity activates with the rise and progression of carcinoma, and is characterized by the appearance of TICs T, B, and NK cells [14], thus, an investigation into the effects of FX-BAE on the CACC mouse model immunity may provide an important marker. Our results show that FX-BAE not only decreases the oxidative damage but also induces an increase in splenocyte proliferation, which in turn increases the immunity of CACC mice to carcinoma. Moreover, treatment with FX-BAE (2.5 g/kg every 2 days) resulted in increases in CACC mouse survival rates from 30% to 70%. We suggest that treating CACC by using FX-BAE will provide significant benefits for the prevention and treatment of disease. In conclusion, FX-BAE significantly attenuated the development of DSS-induced colitis and CACC in a mouse model. Being a nontoxic and natural dietary product, FX-BAE could be useful in the treatment of human IBD and CACC. We are currently conducting research to identify FX-BAEs precise target and detailed mechanism in the prevention of carcinogenesis, and expect to provide more evidence of FX-BAE chemopreventive activities in the future.

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