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Açaí Berries Inhibit Colon Tumorigenesis in Azoxymethane/Dextran Sulfate Sodium-Treated Mice

Yoon Jin Choi[1], Yoon Jeong Choi[1], Nayoung Kim[1,2], Ryoung Hee Nam[1], Seonmin Lee[1], Hye Seung Lee[3], Ha-Na Lee[4], Young-Joon Surh[4], and Dong Ho Lee[1,2]

[1]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, [2]Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, [3]Department of Pathology, Seoul National University Bundang Hospital, Seongnam, and [4]Tumor Microenvironment Global Core Research Center, Seoul National University College of Pharmacy, Seoul, Korea

Background/Aims: The aim of this study was to investigate the protective effect of açaí against azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced colorectal cancer development. **Methods:** The effect of açaí on tumorigenesis was assessed by evaluating tumor incidence, multiplicity and invasiveness in the mouse colon. The levels of myeloperoxidase (MPO) and proinflammatory cytokines (tumor necrosis factor α [TNF-α], interleukin [IL]-1β, and IL-6) were measured via enzyme-linked immunosorbent assay. Protein levels of cyclooxygenase 2 (COX-2), proliferating cell nuclear antigen (PCNA), B-cell lymphoma 2 (Bcl-2), Bcl-2-associated death promoter (Bad) and cleaved-caspase-3 were assessed by immunoblotting. **Results:** Administration of pellets containing 5% açaí powder reduced the incidences of both colonic adenoma and cancer (adenoma, 23.1% vs 76.9%, respectively, p=0.006; cancer, 15.4% vs 76.9%, respectively, p=0.002). In the açaí-treated mice, the MPO, TNF-α, IL-1β and IL-6 levels in the colon were significantly down-regulated. Açaí inhibited PCNA and Bcl-2 expression and increased Bad and cleaved-caspase-3 expression. *In vitro* studies demonstrated that açaí treatment reduced lipopolysaccharide-induced expression of TNF-α, IL-1β, IL-6 and COX-2 in murine macrophage RAW 264.7 cells. **Conclusions:** Açaí demonstrated protective effects against AOM/DSS-induced colon carcinogenesis, which suggests that the intake of açaí may be beneficial for the prevention of human colon cancer. **(Gut Liver 2017;11:243-252)**

Key Words: Açaí berry; Colorectal neoplasms; Anti-inflammatory; Proapoptotic

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and the fourth most frequent cause of cancer-related death.[1] Chronic inflammation is a risk factor for tumorigenesis, and epidemiological data suggest that up to 15% of human cancers are associated with inflammation.[2,3] Colitis-associated cancer (CAC) is a type of colon cancer preceded by clinically detectable inflammatory bowel disease, such as Crohn's disease or ulcerative colitis (UC).[4] Indeed, UC has been reported to increase the cumulative risk of CAC by up to 18% to 20%.[5]

Accumulating evidence suggests that chronic inflammatory conditions predispose cells to malignant transformation, thereby promoting tumorigenesis. In the inflammatory site, activated inflammatory cells produce reactive oxygen species (ROS) and reactive nitrogen intermediates, the persistent formation of which can induce DNA damage and mutation in surrounding cells.[6] Moreover, ROS produced by immune cells can stimulate epithelial cells to induce sustained production of intracellular ROS, leading to severe mutations or epigenetic silencing of tumor suppressor genes.[7] As well as oxidative stress, chronic exposure to proinflammatory cytokines, such as interleukin (IL)-6, IL-1β, and tumor necrosis factor α (TNF-α), causes tumorigenesis.[8-11]

As chronic inflammation is a risk factor for colon tumorigenesis, nonsteroidal anti-inflammatory drugs have been considered to be effective chemopreventive agents for CRC. However, their long-term administration results in gastrointestinal side effects; therefore, alternative therapeutic approaches are needed to manage or prevent inflammation-associated CRC.[12] In this regard, fruits and vegetables containing various compounds with antioxidant and anti-inflammatory properties have been con-

Correspondence to: Nayoung Kim
Department of Internal Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea
Tel: +82-31-787-7008, Fax: +82-31-787-4051, E-mail: nayoungkim49@empas.com
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Yoon Jin Choi and Yoon Jeong Choi contributed equally to this work as first authors.

sidered promising sources of chemopreventive agents for CRC due to their low toxicity.[13] The açaí berry has attracted much attention in this regard. The açaí species *Euterpe oleracea Mart*. is an exotic fruit that contains high levels of polyphenols, especially anthocyanin and proanthocyanidin (mainly cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside).[14] Dietary administration of açaí attenuated atherosclerosis in apolipoprotein-E-deficient mice, and cigarette smoke-induced lung inflammation through its anti-inflammatory and antioxidant activities.[15,16] Açaí also exerts anticancer effects by promoting apoptosis of cancer cells (e.g., human SW-480 colon cancer cells and human leukemic-60 cells).[17,18] Furthermore, açaí inhibits dimethylhydrazine-induced colon carcinogenesis and N-nitrosomethylbenzylamine-induced esophageal cancer development in rats.[19,20]

Although anticancer properties of açaí have been suggested, the protective effect of açaí on inflammation-associated CRC has not been investigated to date. This prompted us to investigate the chemopreventive effect of dried açaí powder on azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced colorectal adenoma and cancer in mice, with a focus on its anti-inflammatory, proapoptotic and antioxidant properties.

MATERIALS AND METHODS

1. Chemicals

Açaí berries were collected in Belem, Brazil, and spray-dried using an industrial spray-dryer system with maltodextrin DE10 as a carrier agent.[21] Açaí pulp powder was produced by Centroflora Group Brazil (Botucatu, Brazil) with the following characteristics: moisture 6%, volumetric density 350 to 650 g/L, and total polyphenol content 0.5%.[21] Freeze-dried açaí pulp powder was purchased through Boto Superfood Co., Ltd. (Seoul, Korea) which imported the end product. Freeze-dried açaí powder was stored at –20°C until analyzed. A cereal-based commercial diet for mice containing 2.5% and 5% açaí powder was specially formulated by the Orient Bio Group (Seongnam, Korea) by a natural drying method according to the National Research Council's recommendation to meet rodent nutritional needs.[21]

For açaí treatment *in vitro*, açaí powder (0.5 g) was freshly dissolved in 5 mL of phosphate-buffered saline, pH 7.4. This mixture was vortexed repeatedly and allowed to sit at room temperature for 2 hours. Prior to use, insoluble particles were removed by centrifugation and subsequent filtration using a 0.22 μm cellulose-acetate syringe filter.

2. Animal experiments

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital (BA1310-139/091-01) on 21 October 2013. The procedure was in accordance with the ARRIVE (Animals in Research: Reporting *In Vivo* Experiments) statement. Male ICR mice (4 weeks of age) were purchased from Orient Co., Ltd. (Seoul, Korea) and housed in a cage maintained at 23°C, with a 12/12 hour light/dark cycle under specific pathogen-free conditions. Experimental groups included group 1 (untreated control, n=8); group 2 (n=13, treated with AOM and DSS); group 3 to 4 (n=13 per group, were treated AOM/DSS and açaí [2.5% for group 3 and 5% for group 4]); group 5 (n=8) was treated with only açaí (5%) (Fig. 1A). Mice in groups 2 to 4 were given a single intraperitoneal injection of 10 mg/kg AOM (Sig-

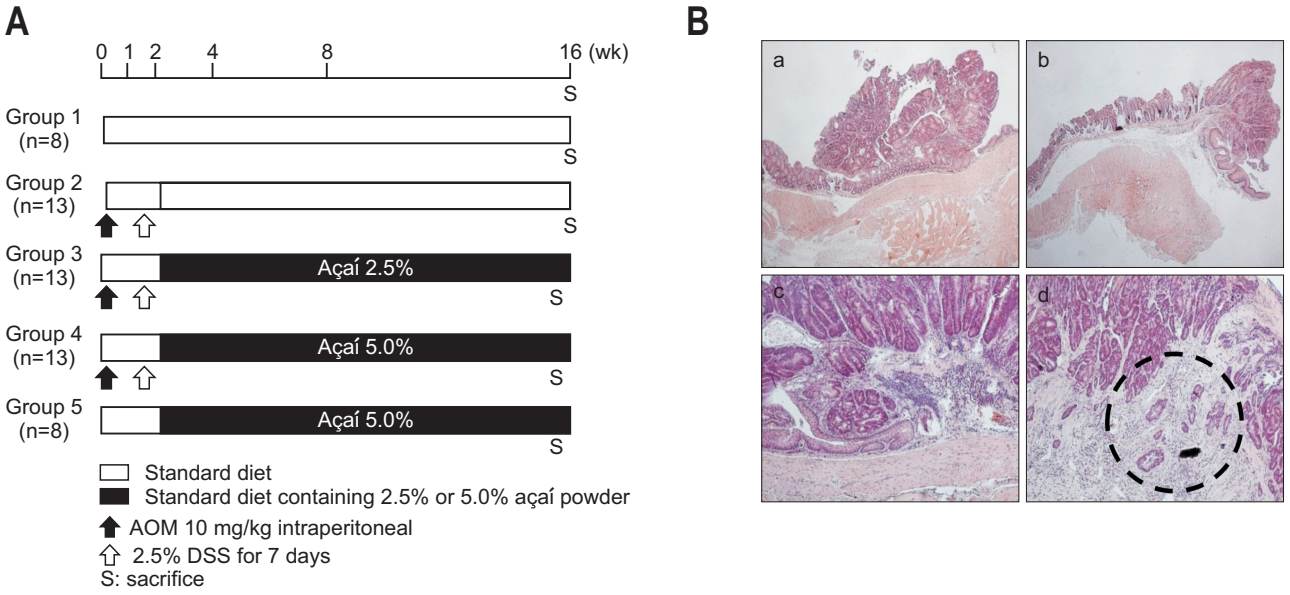

Fig. 1. Azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced mouse colon carcinogenesis model. (A) Total experimental protocols. (B) Representative histological images of (a) adenoma, (b) cancer, (c) mucosal cancer, and (d) submucosal invasion of cancer (H&E stain; a, b, ×40; c, d, ×100). Cancer cells were identified in the submucosa (circle).

ma-Aldrich, St. Louis, MO, USA). For induction of colitis, DSS (MP Biomedicals, Aurora, OH, USA) was prepared in drinking water at a concentration of 2.5% (w/v).[22] Starting 1 week after AOM injection, mice received 2.5% DSS in drinking water for 7 days. Subsequently, groups 3 to 4 received 2.5% and 5% açaí-containing diets for 14 weeks, respectively. All animals were euthanized at 16 weeks.

3. Gross and histopathological evaluation of colonic mucosa

Complete autopsies were performed and the colons from the cecum to rectum were immediately removed, flushed with phosphate buffered saline, and opened longitudinally. Polypoid lesions were counted in the whole colon by three gastroenterologists in a blinded manner and tumor multiplicity was defined as the number of gross polyps approved by all of the three gastroenterologists.

The rectum (up to 3 cm from the anal verge) and other segments including any grossly proven polyps larger than 2 mm in diameter were fixed in phosphate-buffered formalin and stained with hematoxylin and eosin for histopathological examination. Another portion was flash-frozen in lipid nitrogen and kept at −70°C for enzyme-linked immunosorbent assay (ELISA), Western blot and polymerase chain reaction (PCR) analyses. The tumors were classified as adenomas or adenocarcinomas according to Hamilton and Aaltonen[23] (Fig. 1B). In addition, the depth of invasion by colonic adenocarcinomas was described as mucosa and "into the submucosa and muscularis" (Fig. 1B) and their incidence (percentage of rats with tumor) was assessed.

4. Cytokine measurement

An ELISA was performed to measure cytokine levels using the appropriate kits from R&D systems (Minneapolis, MN, USA). All assays were performed in triplicate, and data are shown as means±standard error (SE).

5. Western blot analysis

Protein extracts were isolated using RIPA buffer (Cell Signaling Technology, Beverly, MA, USA). Protein samples were mixed with an equal volume of 5× SDS sample buffer, boiled for 5 minutes, and then separated in 8% to 12% SDS-PAGE gels. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline with Tween-20 buffer (TBS-T) for 1 hour at room temperature. Membranes were incubated overnight at 4°C with specific antibodies. Primary antibodies were removed by washing the membranes three times in TBS-T, and incubated for 2 hours with horseradish peroxidase-conjugated antirabbit or antimouse immunoglobulin (Santa Cruz Biotechnology, Dallas, TX, USA). Following three washes with TBS-T, antigen-antibody complexes were detected using the SuperSignal West Pico Chemiluminescence System (Thermo Fisher Scientific, Rockford, IL, USA). The incubation conditions were as follows: anti-cyclooxygenase2 (COX-2) antibody (1:1,000; Cayman Chemical, Ann Arbor, MI, USA), anti-proliferating cell nuclear antigen (PCNA) antibody (1:1,000; Santa Cruz Biotechnology), anti-B-cell lymphoma 2 (Bcl-2) antibody (1:1,000; Santa Cruz Biotechnology), anti-Bcl-2-associated death promoter (Bad) antibody (1:1,000; Santa Cruz Biotechnology), anti-cleaved caspase 3 antibody (1:1,000; Cell Signaling Technology), anti-heme oxygenase 1 (HO-1) antibody (1:1,000; Abcam Inc., Cambridge, UK) or anti-NQO 1 [NAD(P)H:quinone oxidoreductase 1] antibody (1:1,000; Abcam Inc.).

6. Quantitative real-time PCR analysis

Total RNA from was isolated using RNeasy Plus Mini Kits (Qiagen, Valencia, CA, USA). Reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time qPCR for mRNA expression was performed using SYBR Green probes and an ABI 7500 instrument. The mRNA expression of all genes was normalized to that of GAPDH. The primer sequences were as follows: *TNF-α*, 5′-TCT CAT GCA CCA CCA TCA AGG ACT-3′and 5′-ACC ACT CTC CCT TTG CAG AAC TCA-3′; *IL-1β*, 5′-ACT CAT TGT GGC TGT GGA GA-3′ and 5′-TTG TTC ATC TCG GAG CCT GT-3′; *COX-2*, 5′-TGC CTG GTC TGA TGA TGT ATG CCA-3′ and 5′-AGT AGT CGC ACA CTC TGT TGT GCT-3′; *GAPDH*, 5′-TGA AGC AGG CAT CTG AGG G-3′ and 5′-CGA AGG TGG AAG AGT GGG AG-3′ (forward and reverse, respectively).

7. Cell culture

Mouse RAW 264.7 macrophages were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco BRL, San Francisco, CA, USA) at 37°C in a humidified 5% CO_2 atmosphere. RAW 264.7 cells were treated with lipopolysaccharide (LPS) (200 ng/mL; Sigma Aldrich) in the presence or absence of açaí (20, 40, 80, or 100 μg/mL). Human colonic epithelial cells (CCD841CoN) were kindly provided by Y.J.S. (Seoul National University, Korea) and were maintained in DMEM containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin.[22]

8. Statistical analysis

Data are expressed as means±SE. Statistical analyses were conducted using the GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) and SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) software. Statistical significance was determined using the Mann-Whitney U test and $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

1. Açaí attenuates AOM/DSS-induced colon carcinogenesis

To investigate whether açaí has preventive effects on inflammation-induced carcinogenesis, we used the AOM-initiated and DSS-promoted mouse CRC model. After 2.5% DSS administration, mice were fed either a normal or açaí-containing diet for 14 weeks (Fig. 1). At week 16, nodular colonic adenomas were macroscopically found in the middle and distal colon of mice treated with AOM/DSS (Fig. 2A). Administration of 5% açaí reduced the incidence of both colonic adenoma and cancer (adenoma, 23.1% vs 76.9%, p=0.006; cancer, 15.4% vs 76.9%,

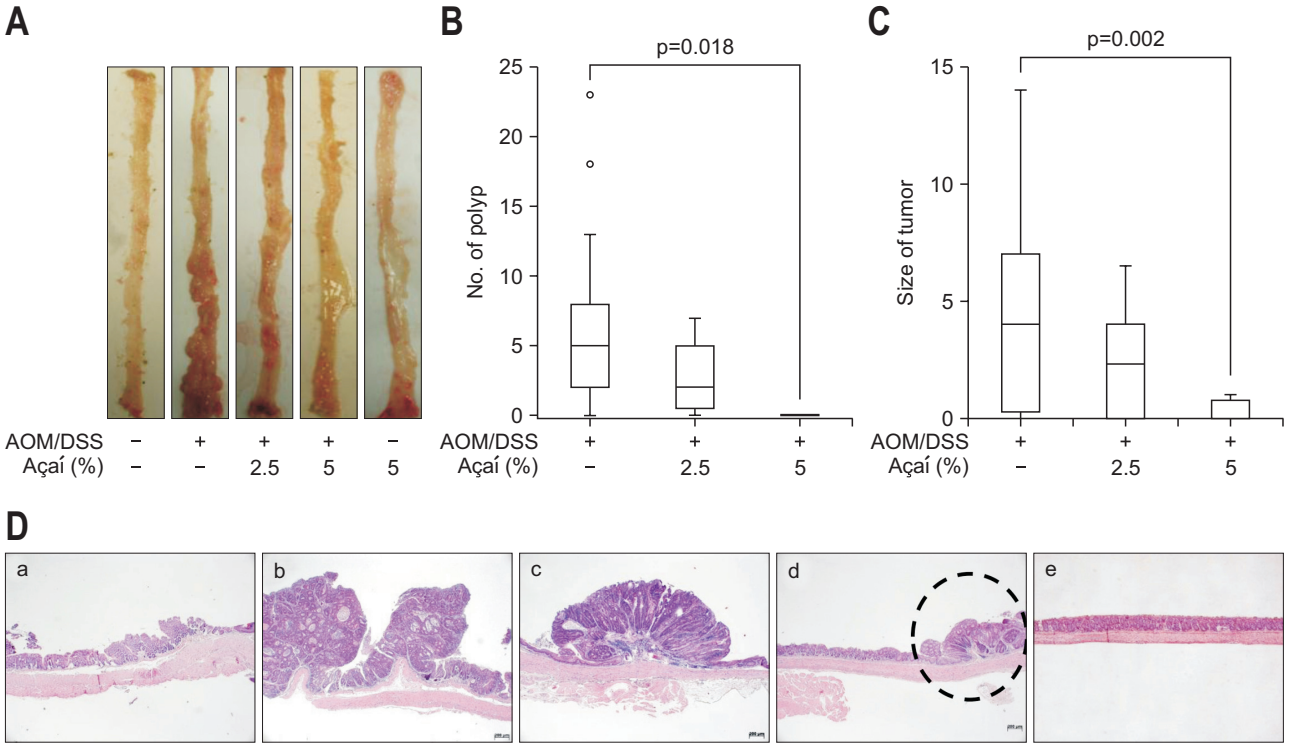

Fig. 2. Açaí inhibits azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced mouse colon carcinogenesis. (A) Representative macroscopic view of the mouse colon in each group. (B) More colonic tumors were identified in the distal colon and rectum of the AOM/DSS-only-treated group than in those of the AOM/DSS+açaí 5% group (p=0.018). (C) Larger colonic tumors were identified in the distal colon and rectum of the AOM/DSS-only-treated group than in those of the AOM/DSS+açaí 5% group (p=0.002). (D) Representative histological images (H&E stain, ×40, and scale bar, 200 μm) in (a) control, (b) AOM/DSS, (c) AOM/DSS+açaí 2.5%, (d) AOM/DSS+açaí 5% with the tumor portion indicated in a circle, and (e) açaí 5% alone. Animal treatments and other experimental conditions are described in the MATERIALS AND METHODS section.

Table 1. Incidence and Multiplicity of Colon Adenoma and Cancer

Treatment group	No.	Adenoma incidence	Cancer incidence	Adenoma/cancer incidence	Adenoma/cancer multiplicity	Size of adenoma/tumor	Depth of invasion (n)
Control	8	0	0	0	0.00	0.00	
AOM/DSS	13	76.9 (10/13)	76.9 (10/13)	76.9 (10/13)	6.62±2.01	4.21±0.89	Mucosa (9), submucosa (1)
AOM/DSS+açaí 2.5%	13	38.5 (5/13)	53.8 (7/13)	53.8 (7/13)	2.75±0.94	2.29±0.69	Mucosa (6), submucosa (1)
AOM/DSS+açaí 5%	13	23.1 (3/13)	15.4 (2/13)	23.1 (3/13)	0.85±0.53	0.83±0.38	Mucosa (1), submucosa (1)
Açaí 5%	8	0	0	0	0.00	0.00	
p trend		0.007*	0.002*	0.007*			
p-value†		0.018*	0.007*	0.022*	0.016*,§	0.002*	
p-value‡		0.006*	0.002*	0.006*	0.018*,§	0.002*,§	
p-value‖		0.047	0.216	0.411	0.222	0.361	

Data are presented as percent (number/total number) or mean±standard error.
AOM, azoxymethane; DSS, dextran sulfate sodium.
*Represents statistical significance; †Among three AOM/DSS-treated groups; ‡Between AOM/DSS and AOM/DSS+açaí 5% groups; §Using one-way analysis of variance followed by a Scheffe test; ‖Between AOM/DSS and AOM/DSS+açaí 2.5% groups.

p=0.002) (Table 1). Multiplicity of colonic adenoma or cancer was decreased in the 5% açaí-fed mouse group compared to the AOM/DSS-only treated mouse group (0.85±0.53 vs 6.62±2.01, p=0.018) (Table 1, Fig. 2B). Administration of 5% açaí reduced the tumor size (0.83±0.38 mm vs 4.21±0.89 mm) (Table 1, Fig. 2C). Histological examination also showed that AOM/DSS-induced CRCs or adenomas were alleviated by açaí administration in a dose-dependent manner (Fig. 2D). The groups fed 2.5% açaí did not show a significant reduction in the incidence or multiplicity of adenomas (Table 1).

Although all of the three AOM/DSS-treated groups had adenomas (Fig. 2D, b-d) and cancer with mucosal or submucosal invasion, even the group fed 5% açaí (Fig. 2D, d), the incidence and multiplicity of cancer were significantly decreased in the AOM/DSS-treated group with 5% açaí.

2. Açaí suppresses proinflammatory cytokine production, inhibits cell proliferation, and induces apoptosis

Increased inflammatory cell influx and proinflammatory cytokine production is a hallmark of colorectal tumors. Therefore, we tested the effects of açaí on myeloperoxidase (MPO) expression and proinflammatory cytokine production in the AOM/DSS-induced CRC model. The expression levels of MPO and proinflammatory cytokines such as TNF-α, IL-1β and IL-6 were significantly increased in the AOM/DSS group, and açaí administration reduced the proinflammatory cytokine levels (Fig. 3). Açaí administration also inhibited AOM/DSS-induced COX-2 expression in the mouse colon (Fig. 4A), the major proinflammatory enzymes whose expression is regulated by nuclear factor-κB (NF-κB).[24] These results indicate that açaí administration exerts an anti-inflammatory effect in the AOM/DSS-induced CRC model.

PCNA was evaluated as an important marker of cell proliferation in the colonic mucosa.[25] As shown in Fig. 4A, the level of PCNA was significantly elevated in the colon of AOM/DSS-treated mice, compared to control. However, in a dose-dependent manner, açaí administration suppressed AOM/DSS-induced PCNA expression in the mouse colon, indicating that açaí inhibits cell proliferation.

Dysregulation of apoptosis plays a pivotal role in tumor progression and therapy resistance.[18] We investigated the effect of açaí on the apoptosis pathway in AOM/DSS-induced colon cancer progression. The protein levels of Bcl-2 were significantly inhibited by administration of 2.5% and 5% açaí, while 5% açaí induced expression of Bad and cleaved-caspase-3 in the mouse colon (Fig. 4B). This suggested that açaí triggers cell apoptosis by targeting the mitochondrial intrinsic proapoptotic pathway, which results in caspase-3 cleavage.

3. Açaí inhibits LPS-induced proinflammatory gene expression in RAW 264.7 cells

Following evaluation of the anti-inflammatory activity of açaí in the AOM/DSS-induced mouse colon cancer model, we examined its effect on cytokine expression in a mouse macrophage cell line (RAW 264.7) upon stimulation with LPS, which is one of the most potent proinflammatory stimuli for monocytes and macrophages. RAW 264.7 cells were pretreated with açaí extract at concentrations ranging from 20 to 100 μg/mL for 1 hour and then stimulated with 200 ng/mL LPS for a 6 hours incubation period. As shown in Fig. 5, mRNA expression levels of TNF-α, IL-1β and COX-2 were significantly increased by LPS stimulation (Fig. 5A-C). mRNA expressions of TNF-α and COX-2 in RAW 264.7 cells treated with LPS were significantly reduced by 40 and 80 μg/mL of açaí extract compared with only LPS-treated macrophages, while that of IL-1β significantly decreased at 40, 80 and 100 μg/mL. However, 20 μg/mL açaí

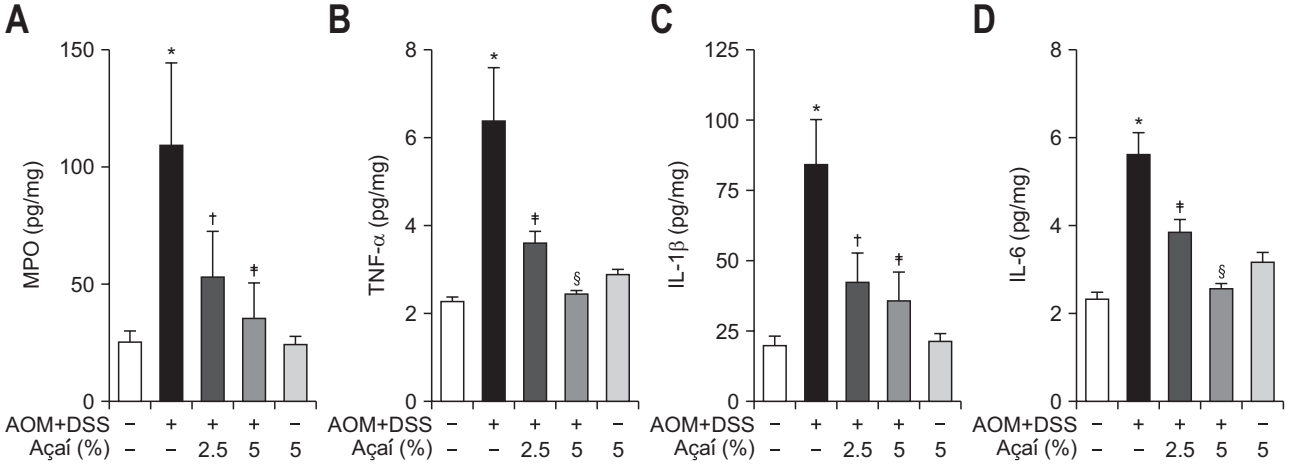

Fig. 3. Açaí downregulates the myeloperoxidase (MPO) and proinflammatory cytokine levels in the mouse colon. (A) MPO, (B) tumor necrosis factor α (TNF-α), (C) interleukin (IL)-1β, and (D) IL-6 levels in the supernatants of colon strips of control mice (n=5), azoxymethane (AOM)/dextran sulfate sodium (DSS)-treated mice (n=10), AOM/DSS plus açaí-treated mice (n=10) and açaí-only-treated mice (n=5). The data are expressed as the mean±standard errors. *p<0.05 compared with the control; †p<0.05, ‡p<0.01, §p<0.001 compared with the AOM/DSS-only-treated group.

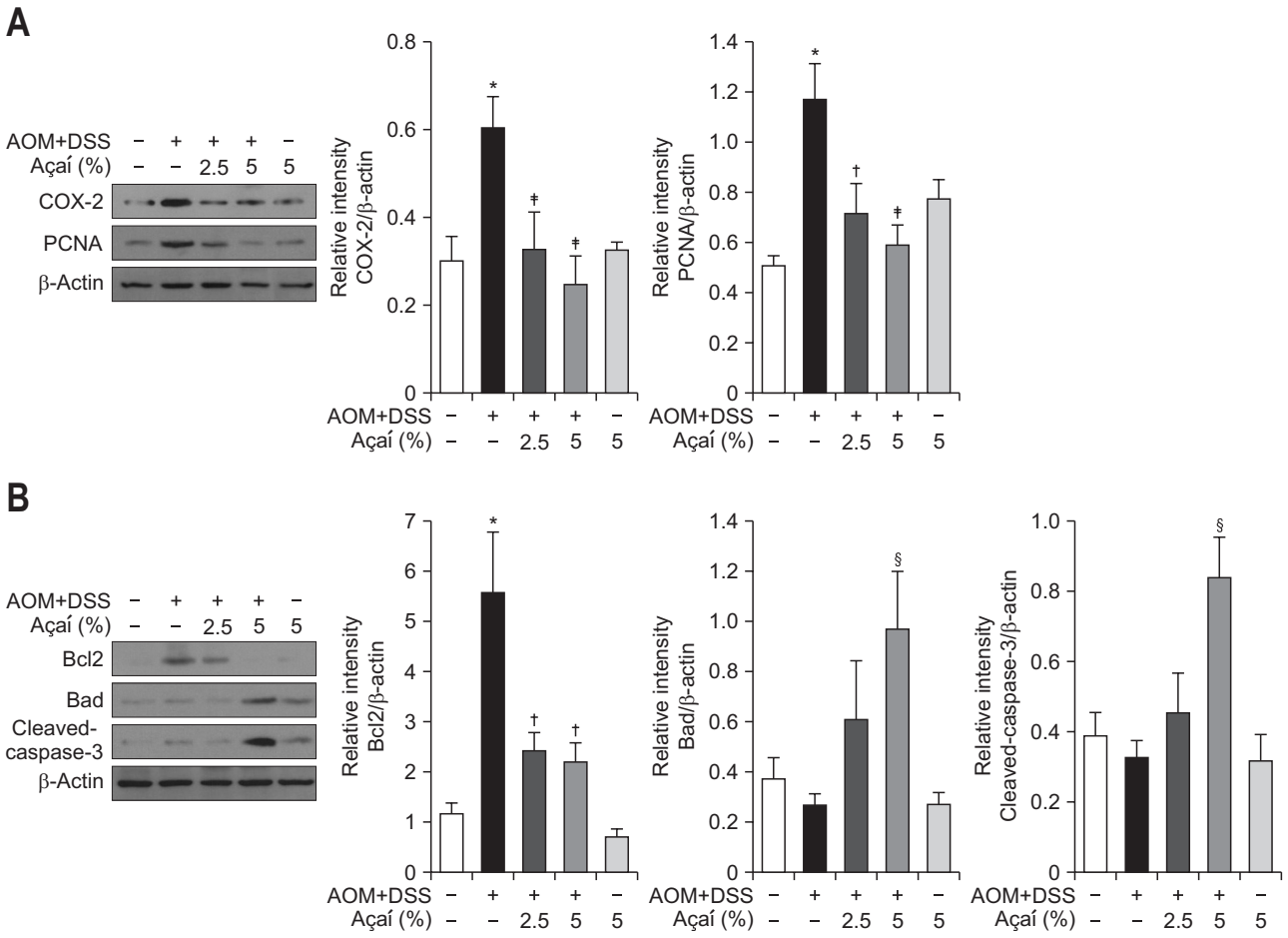

Fig. 4. Açaí inhibits cyclooxygenase 2 (COX-2) and proliferating cell nuclear antigen (PCNA) expression and promotes apoptosis. (A) COX-2 and PCNA and (B) B-cell lymphoma 2 (Bcl-2), Bcl-2-associated death promoter (Bad) and cleaved caspase-3 levels in the supernatants of colon strips of control mice (n=5), azoxymethane (AOM)/dextran sulfate sodium (DSS)-treated mice (n=10), AOM/DSS plus açai-treated mice (n=10) and acai-only-treated mice (n=5). The data are expressed as the mean±standard errors. *p<0.05 compared with the control; †p<0.05, ‡p<0.01, §p<0.001 compared with the AOM/DSS-only-treated group.

extract did not show any significant inhibitory effects.

Regarding cytokine production, RAW 264.7 cells were pretreated with açaí extract for 1 hour, stimulated by LPS for 24 hours and TNF-α, IL-1β and IL-6 levels were measured by ELISA. In line with the mRNA findings, those levels were significantly increased upon LPS stimulation (Fig. 5D-F). The TNF-α level was reduced significantly by 20 to 100 μg/mL of açaí treatment, while IL-1β was decreased significantly by only 40 and 100 μg/mL, and IL-6 was reduced significantly by 20 and 40 μg/mL of açaí extract. Taken together, these data suggest that açaí may regulate the function of macrophages in terms of proinflammatory cytokine production, thereby ameliorating the development of AOM/DSS-induced CRC.

4. Açaí upregulates the expression of antioxidant enzymes in CCD841CoN cells

Açaí has a high antioxidant capacity.[26] Since inflammation often accompanies oxidative stress, we investigated whether açaí could potentiate the antioxidant capacity of intestinal epithelial cells in the context of induction of antioxidant enzymes. CCD841CoN human colonic epithelial cells were treated with açaí extract (from 40 to 100 μg/mL) for 24 hours and then the protein expression of antioxidant enzymes, including HO-1 and NQO-1, were estimated by Western blotting.

Açaí treatment at 40 to 100 μg/mL had no effects on the viability of either RAW 264.7 or CCD841CoN cells (Supplementary Fig. 1). As shown in Fig. 6, açaí treatment (from 40 to 100 μg/mL) increased the HO-1 protein level in CCD841CoN cells (Fig. 6). The protein level of NQO-1 was also elevated by açaí treatment (20 to 100 μg/mL) (Fig. 6).

DISCUSSION

In the present study, we investigated the anticarcinogenic effects of açaí using experimental models of inflammation-associated colon carcinogenesis. Açaí reduced the incidence of adenoma and cancer and multiplicity of tumors in the AOM/DSS mouse model. Açaí alleviated the expression of proinflam-

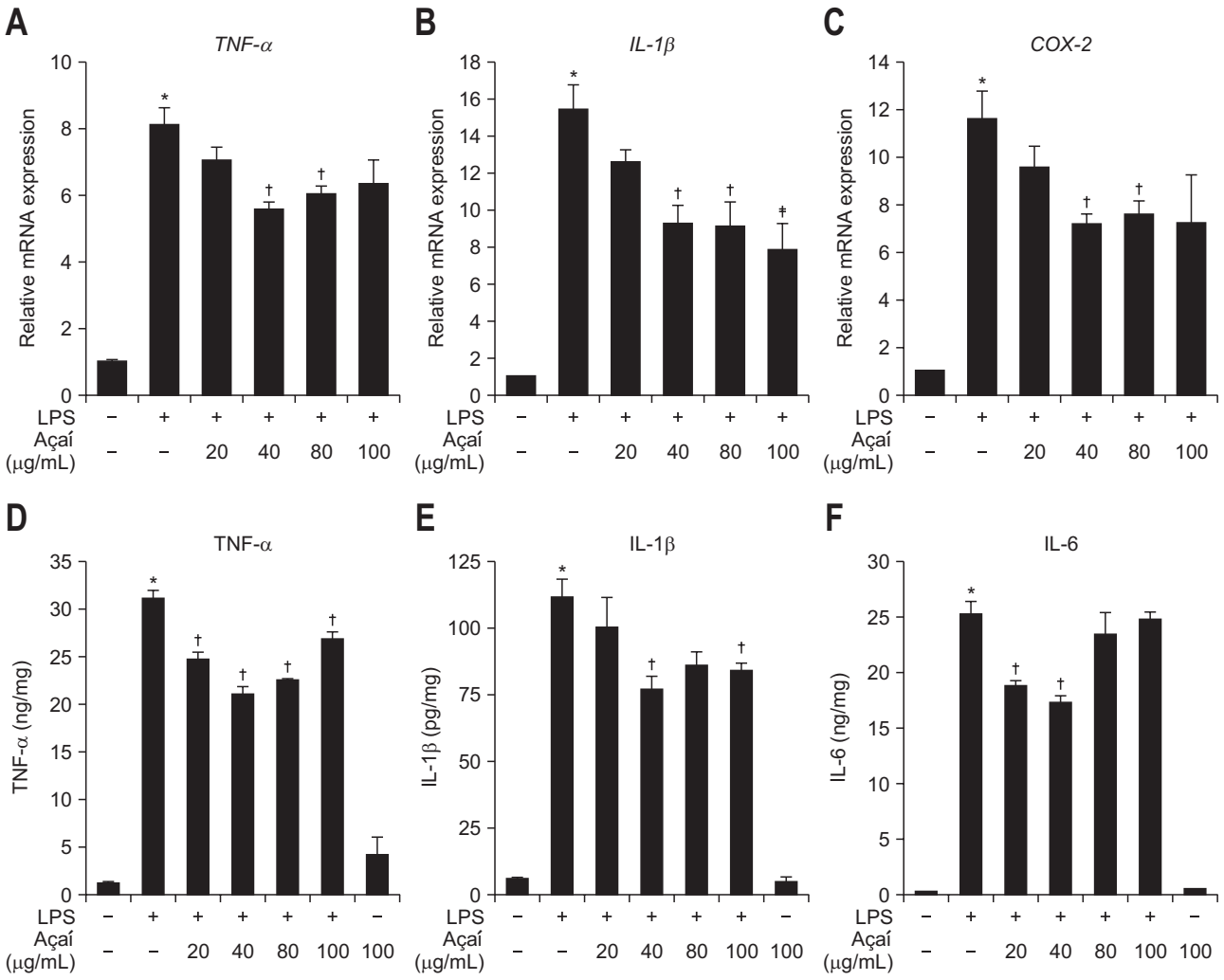

Fig. 5. Açaí reduces lipopolysaccharide (LPS)-induced proinflammatory gene expression in RAW 264.7 cells. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of LPS-induced mRNA expression of tumor necrosis factor α (*TNF-α*) (A), interleukin (*IL*)-*1β* (B) and cyclooxygenase 2 (*COX-2*) (C) and protein levels of TNF-α (D), IL-1β (E) and IL-6 (F) in RAW 264.7 cells. The data represent three independent experiments (n=3). The results are expressed as the mean±standard errors. *p<0.05 compared with the control; †p<0.05 and ‡p<0.01 compared with the LPS-only-treated group.

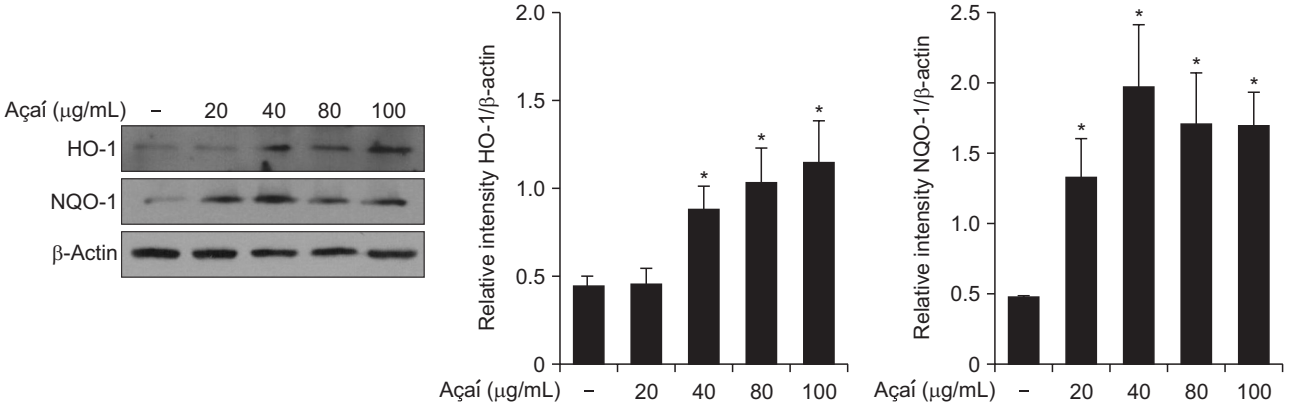

Fig. 6. Açaí induces the expression of antioxidant enzymes in CCD841CoN cells. CCD841CoN cells were treated with the indicated concentrations of açaí for 24 hours. Total cellular lysates were collected for the detection of heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO-1) protein expression by Western blot analysis. The data represent three independent experiments (n=3). The results are expressed as the mean±standard errors. *p<0.05 compared with the control.

matory cytokines and induced apoptosis and production of antioxidant enzymes.

The antitumor effect of açaí in the present study is in accordance with the results of Fragoso *et al*.[19] In their study, a 5% açaí-containing diet reduced the number of aberrant crypts and cancers in a dimethylhydrazine-induced rat colon carcinogenesis model. However, the mechanism underlying the preventive effect of açaí on CRC carcinogenesis was not investigated.

We proceeded to evaluate the mechanism underlying the anti-CRC effect of açaí. First, we demonstrated markedly decreased expressions of IL-1β, TNF-α, IL-6 and COX-2 in LPS-stimulated RAW 264.7 cells and in the mouse colon. Reduced TNF-α expression may be associated with the inactivation of extracellular signal-regulated kinase (ERK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and phosphatidylinositide 3-kinase and protein kinase B (PI3K-AKT) pathways in epithelial cells, leading to an inhibition of β-catenin signaling[27,28] in CRC tumorigenesis.

While inflammation can bypass the mutation requirement for tumor initiation, another important role of inflammation is induction of mutations by persistent ROS production.[7] The main substances that link inflammation to cancer via oxidative stress are cytokines.[29] It has been reported that LPS and enhanced proliferation of Gram-negative bacteria in the DSS-treated mouse colon was associated with increased lipid peroxidation.[30] ROS, in turn, affect the expression of genes that regulate cell differentiation and growth, leading to initiation of cancer.[31,32] In the present study, açaí treatment reduced the levels of inflammatory cytokines in colonic mucosa and macrophages and induced production of antioxidant enzymes in CCD841CoN normal colon epithelial cells. This was consistent with the fact that polyphenolics suppress proliferation of colon cancer cells by reducing ROS levels.[33] Overall, ROS production may be down-regulated by açaí, which could be one of the mechanisms underlying involved the reduced incidence of colonic tumors in this study. Further *in vivo* study to evaluate the antioxidant property of açaí is warranted.

Finally, antiapoptotic capacity is crucial for survival of cancer cells. Human CRC is associated with increased inhibition of apoptosis,[34] and mutated colon epithelial cells avoid the normal clearance mechanism, successfully developing to invasive tumors.[35,36] Dias *et al*.[18] demonstrated that açaí inhibited growth of SW-480 cells by inducing cytochrome c, cleaved caspase 3, and reducing the level of the antiapoptotic factor poly [ADP-ribose] polymerase 1 (PARP-1). The down-regulation of Bcl-2 by açaí in the present study is consistent with previous reports.[37,38] Marked decreases in the levels of the proliferation factor PCNA were also noted, suggesting that açaí suppressed CRC by reducing the antiapoptotic capacity of cancer cells or activating the mitochondrial proapoptotic pathway.

Unfortunately, in the present study, the key mechanisms of prevention against CRC were not demonstrated. It is not clear whether açaí is predominantly anti-inflammatory rather than directly suppressing already established cancer cells, since the viability of colon cancer cells was not inhibited by açaí treatment. Moreover, although the incidence of adenoma and adenocarcinoma were decreased in the 5% açaí-feeding group, it did not seem that açaí can inhibit cancer submucosal invasion. Therefore, the anti-inflammatory effect or growth inhibition effect of açaí may reduce colitis and subsequent CRC or adenoma formation at the early stages. Regarding antioxidant property of açaí, HO-1, NQO-1 and Nrf2 *in vivo* as well as *in vitro* should have been measured, but a lack of manpower and funds limited further evaluation. Another limitation of this study is that the açaí berry extract used is not thoroughly characterized. Since we used the soluble part of açaí powder in this *in vitro* study, some effective component in the precipitate might be excluded.

Nonetheless, this is one of few studies to demonstrate an anticolon cancer effect of açaí *in vivo*, together with the anti-inflammatory and antioxidant effects *in vitro*. We evaluated the incidence of macroscopically or microscopically detected tumors, not aberrant crypt foci. Although we did not perform a histological evaluation of the entire colon, three independent and experienced colonoscopists identified adenomas in a blinded manner; and lesions about which there was disagreement underwent histological evaluation. Any lesion suspicious for adenocarcinoma, tumors larger than 0.2 cm in diameter, and the rectum, where tumors occurred most frequently, were also evaluated by an expert pathologist in a blinded manner. Moreover, by dividing tumors into adenoma and adenocarcinoma and assessing the depth of invasion histologically, we aimed to clarify the stage of colon carcinogenesis that was inhibited by açaí.

In conclusion, açaí treatment suppressed AOM plus DSS-induced colonic adenoma and cancer formation in mice with no toxicity by reducing COX-2, TNF-α, IL-1β and IL-6 expression levels in macrophages and the mouse colon, suppressing Bcl-2 and PCNA, and activating the mitochondrial proapoptotic pathway. Furthermore, açaí treatment may protect against ROS production.

Consumption of açaí-containing juice exhibited *in vivo* anti-inflammatory and antioxidant properties in human subjects based on a randomized, double-blind, placebo-controlled crossover study.[39] Further investigations are needed for this formulation to be used against human CRC.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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