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Protective effect of sugar cane extract against dextran sulfate sodium-induced colonic inflammation in mice

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ABSTRACT

Sugar cane extract (SCE) exhibits various biological effects and has been reported to enhance animal growth performance. However, the effect of SCE on inflammation in animals is still obscure. To study the effects and underlying mechanism of SCE on dextran sulfate sodium (DSS)-induced colonic inflammation, forty female ICR mice (26.63 ± 0.19 g, 6-week-old) were assigned into four groups: a control group (Cont), a DSS-challenged group (DSS), a SCE-supplemented group (SCE), and a DSS+SCE group (DSS+SCE). Mice in Cont group and DSS group were fed basic diet and other mice received 1% SCE supplemented in basic diet from 6-week to 8-week-old. Mice in DSS and DSS+SCE groups were also given a 4% DSS solution from 7-week to 8-week-old via drinking water to induce colonic inflammation. After 2 weeks, mice were sacrificed and samples were collected. The results showed that dietary SCE alleviated DSS induced growth suppression, splenic damage, colonic histological changes, colonic inflammation, oxidative stress, and colonic dysfunction of tight junctions. Meanwhile, the DSS exposure activated nuclear transcription factor kappa B p65 and inhibited nuclear factor E2-related factor 2 (Nrf2), while SCE markedly attenuated the DSS-promoted effect on the p65 nuclear accumulation and the DSS-inhibited effect on the Nrf2 nuclear accumulation. In conclusion, SCE conferred a protective role in the DSS-induced inflammation and the mechanism might be associated with the activated signals of the nuclear factor kappa B p65 and Nrf2.

1. Introduction

Sugar cane extract (SCE) is natural byproducts in sugar cane industry after removing glucose, fructose, and sucrose, and contains large abundances of phenolics and flavonoids. Previous reports suggest that dietary SCE improves growth performance in pigs and chickens (Hikosaka et al., 2007; Lo et al., 2006). Recent evidence suggested that SCE exhibited various biological effects, such as immunostimulation and antioxidative functions (Amer et al., 2004; Bazer et al., 2015; Chung et al., 2011). In pseudorabies virus infection, dietary supplementation with SCE improved the immune function and enhanced the activity of nature kill cells in pigs (Lo et al., 2005). Chen et al. also reported that dietary SCE enhanced the biological function of neutrophils and the anti-inflammatory function that might play a beneficial role in bacterial infections in mice. In addition, SCE might play an antioxidative role in many physiological conditions via scavenging free radical species (Chung et al., 2011; Valli et al., 2012). However, it is not clear about the mechanism underlying these positive effects on animals fed with SCE.

Recently, some studies reported an anti-inflammatory effect of SCE in different animal models. In the zymosan-induced arthritis, sugar cane alleviated the inflammatory response through its inhibitory effects on the arachidonic acid metabolism in mice (Ledon et al., 2007). In the lipopolysaccharide (LPS)-challenged mice, dietary SCE markedly inhibited the expression and amount of inflammatory cytokines (Hikosaka et al., 2006). Colonic inflammation is a chronic intestinal inflammatory response and has been considered as one of the most intractable gastrointestinal diseases, which may further develop into colorectal cancer and influence life quality of patients (Utrilla et al., 2015; Wurth et al., 2015;

Abbreviations: SCE, sugar cane extract; DSS, dextran sulfate sodium; Nrf2, nuclear factor E2-related factor 2; CAT, catalase; SOD1, superoxide dismutase 1; Gpx1, glutathione peroxidase 1; Gpx4, glutathione peroxidase 4; TNF-α, tumor necrosis factor-alpha; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidant capability; ZO-1, zona occludens-1; IBD, inflammatory bowel disease.

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Xiong et al., 2015). Currently, aminosalicylic, immunosuppressor and steroid hormone are main drugs for treating colonic inflammation. Previous studies were carried to find new drugs from extracts and activate compounds of herbs to prevent or treat the colonic inflammation. However, there is little reference about the effects of SCE on colonic inflammation. Thus, we hypothesized that dietary SCE alleviates inflammatory response in animals.

In the present study, the anti-inflammatory effect and the antioxidative activity were investigated to verify the protective function of dietary supplementation with SCE on the dextran sulfate sodium (DSS)-induced colonic inflammatory response in mice, since this model induced production of multiple inflammatory and pro-inflammatory mediators. We hypothesized that SCE would protect mice against the DSS-induced colonic inflammation via enhancing the anti-inflammatory and antioxidative functions.

2. Material and methods

2.1. SCE composition

SCE was extracted from sugar cane juice (*Saccharum officinarum* L.) by chromatographic separation on an ion exchange column and kindly provided by Mitsui Sugar Co., Japan. The composition of SCE powder consists of 10.47% crude protein, 1.33% crude fiber, 3.02% crude fat, 67.83% nitrogen-free extracts and 9.08% ash. The relative content of sugarcane polyphenols is 8.54 mg/g. The amino acid contents (mg/g) of SCE determined via an isotope dilution liquid chromatography-mass spectrometry were as following: Asp+Asn 9.876, Thr 2.727, Ser 4.091, Glu+Gln 30.675, Gly 3.079, Ala 3.209, Cys 0.746, Val 3.826, Met 0.207, Ile 2.882, Leu 5.717, Tyr 1.088, Phe 4.653, Lys 1.529, His 1.707, Arg 2.259, and Pro 4.187. SCE was mixed with bread flour (1:4) and then used for dietary supplementation in this study.

2.2. Animal model and groups

Forty female ICR mice (26.63 ± 0.19 g, 6-week-old) were randomly divided into four groups: one water control group (n = 10), one DSS challenged group (n = 10), one SCE supplemented group (n = 10), and one DSS+SCE group (n = 10). Mice in control group and DSS group were fed basic diet (Table 1) and the other mice received 1% SCE supplemented in basic diet from 6-week to 8-week-old for 14 days, according to our previous results (unpublished data). At 7-week-old, mice in the DSS and the DSS+SCE groups were also given a 4% DSS solution (KAYON Bio. Technology Co. Ltd) for 7 days (day 8 to day 14 of the experiment) via drinking water to induce the colonic inflammation (Sann et al., 2013).

Table 1
Composition of the basal diet.

Ingredients	Amount, g/kg diet	Nutrient level	Amount
Corn meal	520	Total energy, MJ/kg diet	15.2
Soybean meal	200	Crude protein, %	21.9
Wheat bran	110	Crude fat, %	4.2
Wheat flour	90	Starch, %	50.1
Fish meal	30	Crude fiber, %	4.4
NaCl	2	Crude ash, %	6.3
$CaHPO_4$	10	Calcium, %	1.07
$CaCO_3$	12	Phosphorus, %	0.69
Lard	20		
L-Lysine	2.5		
L-Methionine	2.7		
Mineral mix[a]	0.6		
Vitamin mix[b]	0.2		

[a] Provided the following amount (mg/kg diet): 1) cobalt (as cyanocobalamin), 0.6; 2) copper (as $CuSO_4$-$5H_2O$), 5.0; 3) iodine (as CaI_2), 0.48; 4) iron (as $FeSO_4$), 75.0; 5) manganese (as MnO), 20.0; 6) selenium (as Na_2SeO_3), 0.40; and 7) zinc (as ZnO), 10.0.

[b] Provided the following amount (mg/kg diet): 1) all-rac-a-tocopheryl acetate, 64.0; 2) D-biotin, 0.2; 3) calcium d-pantothenate, 24.0; 4) cholecalciferol, 5.5; 5) folic acid, 6.0; 6) menadione sodium bisulfate, 2.2; 7) nicotinic acid, 30.3; 8) pyridoxine-HCL, 12.0; 9) retinyl acetate, 1.9; 10) riboflavin, 5.5; 11) thiamin-HCL, 13.0; 12) vitamin B-12, 0.022.

After the experimental period (14 days), all mice were sacrificed, and the colonic length and the weights of the liver, spleen, and kidney were recorded (n = 10). Before slaughter, eight blood samples in each group were collected by orbital blood collection. One piece of colonic sample (1 g) was collected in liquid nitrogen and stored at −70 °C for PCR analysis. This study was approved by the animal welfare committee of the Jiangsu Food & Pharmaceutical Science College.

2.3. Histomorphometry determination

The morphological determination was measured using haematoxylin and eosin (HE) staining. Briefly, one piece of each colonic samples (0.5 cm) was stored in 10% formalin, which was further mounted in the paraffin blocks. Six-micrometer-thick sections were cut from the paraffin blocks and then stained with HE. All HE staining specimens were determined under a light microscope (Nikon, Japan). Colonic villus height and crypt depth were investigated by an image-analysis system (Yin et al., 2015).

2.4. Serum oxidative indexes

Blood samples were collected with orbital blood collection and stored at 4 °C for 4 h and then serum from each mice were separated after blood centrifugation at 3500 × *g* and 4 °C for 15 min. Serum malondialdehyde (MDA), glutathione peroxidase (GSH-PX), and total antioxidant capability (T-AOC) were measured (Nanjing Jiancheng, China) (Yin et al., 2015).

2.5. Quantification mRNA

Total RNA from colonic samples were isolated with TRIZOL regent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA) to extract RNA. Synthesis of the first strand (cDNA) was conducted using oligo (dT) 20 and Superscript II reverse transcriptase (Invitrogen, USA).

Primers used in this study were designed by Primer 5.0 to produce an amplification product and the detailed sequences were shown in Table 2. The protocol of RT-PCR was according to previous report (Yin et al., 2014). The relative mRNA expression of target genes were normalized and expressed as a ratio compared with the control group.

2.6. Western bolt

Proteins from colonic tissues were extracted with protein extraction reagents (Thermo Fisher Scientific Inc., USA). Proteins (30–50 μg) were run in a SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to apolyvinylidene difluoride (PVDF) membrane (BioRad, Hercules, CA, USA). Membranes were further blocked and then incubated with the following primary antibodies: ZO-1 (ab59720), Claudin1 (ab115225), Occludin (ab31721), nuclear transcription factor kappa B p65 (p65) (ab16502), and nuclear factor E2-related factor 2 (Nrf2) (ab62352) (Abcam, Inc., USA). Mouse β-actin primary antibody (Sigma) was used as the loading control for western blotting analysis. Membranes were washed five times and incubated with second antibodies, which were further washed five times and quantified using the image J program (NIH) (Yin et al., 2015).