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Dietary intervention with green dwarf banana flour (*Musa* sp AAA) prevents intestinal inflammation in a trinitrobenzenesulfonic acid model of rat colitis

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Abstract

Dietary products are among the therapeutic approaches used to modify intestinal microflora and to promote protective effects during the intestinal inflammatory process. Because the banana plant is rich in resistant starch, which is used by colonic microbiota for the anaerobic production of the short-chain fatty acids that serve as a major fuel source for colonocytes: first, green dwarf banana flour produces protective effects on the intestinal inflammation acting as a prebiotic and, second, combination of this dietary supplementation with prednisolone presents synergistic effects. For this, we used the trinitrobenzenesulphonic acid (TNBS) model of rat colitis. Our results revealed that the protective effect produced by a combination of 10% green dwarf banana flour with prednisolone was more pronounced than those promoted by a single administration of prednisolone or a diet containing 10% or 20% banana flour. This beneficial effect was associated with an improvement in the colonic oxidative status because the banana flour diet prevented the glutathione depletion and inhibited myeloperoxidase activity and lipid peroxidation. In addition, the intestinal anti-inflammatory activity was associated with an inhibition of alkaline phosphatase activity, a reduction in macroscopic and microscopic scores, and an extension of the lesions. In conclusion, the dietary use of the green dwarf banana flour constitutes an important dietary supplement and complementary medicine product to prevention and treatment of human inflammatory bowel disease.

Keywords: Banana; Dietary products; Functional foods; Inflammatory bowel disease; *Musa* sp AAA; Ulcerative colitis; Trinitrobenzenesulfonic acid (TNBS); Rat

Abbreviations: ANOVA, analysis of variance; AP, alkaline phosphatase; GSH, glutathione; IBD, inflammatory bowel disease; IFN-γ, interferon-γ; MPO, myeloperoxidase; PPAR, peroxisome proliferator–activated receptors; RS, resistant starch; SCFA, short-chain fatty acids; TNBS, trinitrobenzenesulfonic acid.

1. Introduction

Inflammatory bowel disease (IBD) refers essentially to 2 different but closely related chronic intestinal disorders: Crohn disease and ulcerative colitis. Although much progress has been made in understanding the pathogenesis of human IBD, its etiology has not yet been defined. However, accumulating evidence suggests that this disease results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host [1]. Furthermore, intestinal microbiota is linked to IBD pathogenesis because of its role in modulating

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intestinal homeostasis and immunologic functions [2]. In fact, increasing experimental evidence supports the role of luminal bacteria in the initiation and development of the intestinal inflammatory process [3,4]. On the basis of these findings, 2 approaches have been used to modify intestinal microflora, the administration of probiotics or prebiotics, which are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of limited bacteria in the colon [5].

Dietary fiber, defined as plant substances that resist hydrolysis by small bowel digestive enzymes, has been proven to be beneficial in maintaining remission in human ulcerative colitis, and this protective effect has been related to an increase in the luminal production of short-chain fatty acids (SCFAs), which are considered to be an important factor in the maintenance of healthy function in colorectal mucosa [6]. In fact, several studies have reported that some prebiotics including dietary fiber, germinated barley foodstuff, inulin, lactulose, and polydextrose exert beneficial effects in both human and experimental colitis models [7,8].

Banana is the fourth most important crop in developing countries, with a worldwide production of about 100 metric tons [9]. Fruits of the green dwarf banana (*Musa* sp AAA) are rich in starch granules containing 73.6% to 79.4% starch, and of the total amount of starch (14%), 47.3% to 54.2% is considered to be resistant starch [10-12]. Resistant starch is a nondigestible polysaccharide used as a dietary fiber that is resistant to digestion in the small intestine and used by colonic microbiota for the anaerobic fermentation production of SCFA [10-14].

Currently, the pharmacologic treatments for IBD include corticosteroids, aminosalicylates, immunomodulators, and anti–tumor necrosis factor-α antibodies, but these pharmacologic therapies result in serious adverse events, particularly after a long-term use. Because of these adverse effects and the chronic nature of IBD, there is dissatisfaction with current traditional therapies, which has led to an increase in the use of complementary and alternative medicine approaches including prebiotics and probiotics. The use of these compounds is currently estimated to be 49.5% [15,16].

Given that the green dwarf banana (*Musa* spp AAA) is an important source of resistant starch with several physiological effects consistent with those of dietary fibers and prednisolone, a drug that presents serious adverse effects from long-term use, two hypothesis of this study were evaluated. First: dietary supplementation with green dwarf banana flour produces protective effects on the intestinal inflammatory process acting as a prebiotic. Second: combination of dietary supplementation with prednisolone presents synergistic effects. For this purpose, we assayed the effects of the green dwarf banana flour and their combination with prednisolone in preventing the acute inflammatory response induced by trinitrobenzensulphonic acid (TNBS). In this experimental model, macroscopic, microscopic, and biochemical parameters were evaluated.

2. Methods and materials

2.1. Chemicals

All chemicals were supplied by Sigma (St Louis, Mo) and were freshly prepared for each animal administration or biochemical evaluation. The enriched diet with green dwarf banana flour was manufactured in the School of Medicine, São Paulo State University, UNESP, São Paulo, Brazil.

2.2. Plant material and diet preparation

Green dwarf banana fruits (*Musa* spp AAA) were collected in Botucatu City, São Paulo, Brazil, in December 2010. The plant was identified by taxonomists from Irina Felanova Gemtchjnicov Herbarium (Institute of Biosciences, São Paulo State University, UNESP), where a voucher specimen was deposited.

After collection, the green banana fruits were washed, chopped, and dried at 50°C for 72 hours in a hothouse with forced air circulation and renewal. After drying, the dried fruits were powdered to produce flour. For the preparation of the enriched diet, the flour was added at a ratio of 10% and 20% in previously sprayed Labina-Purine food for rodents. After homogenization, water was added to produce a paste. The paste was then placed in a pelletizer to produce diet pellets containing 10% or 20% green dwarf banana flour.

Table 1
Ingredient composition of the diets fed to rats (g/100 g)

Ingredients	Control diet	10% Banana diet	20% Banana diet
Protein mix	23.0	20.7	18.5
Mineral mix [a]	12.0	10.8	9.7
Fiber	5.0	4.5	3.6
Vitamin mix [b]	1.0	0.9	0.8
Fat	10.0	9.0	8.0
Fatty acids	5.5	4.95	4.4
Corn starch	32.0	28.8	25.7
Sugar mix	6.0	5.4	4.9
Soybean meal	2.5	2.25	2.0
Wheat bran	3.0	2.7	2.4
Banana flour [c]	–	10.0	20.0

[a] Mineral mixture provided the following amounts (in milligrams per kilogram): Mg, 1.7; Mn, 110.0; I, 1.0; Co, 2.0; Fe, 180.0; Zn, 110.0; Cu, 30.0, Se, 0.2, Na, 2.8, P, 8.5; and Ca, 13.0.

[b] Vitamin mixture provided the following amounts (in milligrams per kilogram per diet): vitamin A (25 600 UI); vitamin D_3 (4000 UI); vitamin E (82 mg); vitamin K (6.4 mg); vitamin B_{12} (40 μg); vitamin B_6 (11 mg); folic acid (13 mg); choline (2800 mg); biotin (0.16 mg); niacin (220 mg); thiamine (11 mg); and pantothenic acid (90 mg).

[c] Fruits of green dwarf banana (*Musa* sp AAA) containing starch (73.6%-79.4%), amylose (20.9%-23.5%), protein (2.61%-2.99%), soluble fiber (2.29%-2.49%), insoluble fiber (5.35%-5.39%), ash (3.44%-3.56), and traces of lipids [16].