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Protective effects of yacon (*Smallanthus sonchifolius*) intake on experimental colon carcinogenesis

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ABSTRACT

Yacon (*Smallanthus sonchifolius*), a tuberous root native to the Andean region of South America, contains high concentration of fructans with potential for colon cancer prevention. This study investigated the potential beneficial of yacon intake on colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) in male Wistar rats. After 4 weeks of DMH-initiation, groups were fed basal diet (G1 and G6) or basal diet containing dried extract of yacon root at 0.5% (G2), 1.0% (G3 and G5) or a synbiotic formulation (G4) (1.0% yacon plus *Lactobacillus casei* at 2.5×10^{10} CFU per g diet) for 13 weeks. At week 20, a significant reduction in number and multiplicity of aberrant crypt foci (ACF) and in number of invasive adenocarcinomas was observed in the groups orally treated with 1.0% yacon (G3) or the synbiotic formulation (G4) ($0.05 < p < 0.001$). Tumor multiplicity (noninvasive plus invasive) was significantly lower in the group fed synbiotic formulation ($p < 0.02$). A significant reduction in cell proliferation in colonic crypts and tumors and short chain fatty acids (SCFA) caecal contents was observed in the groups orally treated with 1.0% yacon (G3) or the synbiotic formulation (G4). Therefore, the findings this study indicate that yacon and yacon plus *L. casei* intake may reduce the development of chemically-induced colon cancer.

1. Introduction

Colon cancer is a significant cause of mortality from cancer among men and woman in industrialized Western societies (Jemal et al., 2011). Although its etiology is multifactorial and complex, it is generally accepted that the hereditary genetic component associated with dietary habits such as low intake of fibers, proteins, fruits and vegetables as well as diets with high red and processed meat and fat levels are among the greatest risk factors (Khan et al., 2010; van Engeland et al., 2011; Bastide et al., 2011; Watson and Collins, 2011). Compelling laboratory animal and epidemiologic evidences indicate that lifestyle and dietary habits have been associated with up- or down-regulation of the risk for colon cancer onset and progression (Erdelyi et al., 2009; van Engeland et al., 2011). Recently, there are great scientific and public interest in studies of colon cancer chemoprevention for identification of naturally occurring substances present in food (Tarapore et al., 2012; Poulsen et al., 2011). Chemically-induced models of colon carcinogenesis in rodents have been suitable for the study of risk factors, prevention and tumor development (Raju, 2008; Namasivayam, 2011).

Colon cancer has been postulated as a complex and multistage process well established in both humans and experimental models (Takahashi and Wakabayashi, 2004; Tanaka, 2009). The stages of colon carcinogenesis are accompanied by several biochemical, morphological, genetic, and epigenetic alterations in the colonic mucosa (Takahashi and Wakabayashi, 2004). Development of rodent and human colon cancer includes a series of pathological alterations ranging from discrete microscopic mucosal lesions like aberrant crypt foci (ACF) to malignant tumors (Gupta and Schoen, 2009; Tanaka, 2009). ACF are considered putative preneoplastic lesions since they are found in the colonic mucosa of rodents treated with chemical carcinogens (Bird and Good, 2000; Cheng and Lai, 2003) and in patients at high risk for development of colon cancer (Khare et al., 2009). ACF can be easily induced in rodent colon after single or multiple administrations of 1,2-dimethylhydrazine (DMH) or its metabolite azoxymethane (Tanaka, 2009). ACF detection and quantification have been proposed for identification of potential chemopreventive agents in rodent short- and medium-term bioassays of colon carcinogenesis (Bird and Good, 2000; Raju, 2008).

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Aberrant crypts with altered β-catenin expression have been designated as β-catenin-accumulated crypts (BCAC) or surrogate premalignant lesions, and seem to progress more likely colon cancer as compared to classical ACF (Yoshimi et al., 2004; Mori et al., 2005; Suehiro and Hinoda, 2008). β-catenin is a structural protein encoded by CTNNB1 gene, that acts as a component of the cadherin-mediated cell-cell adhesion complex and as a transcriptional activator mediating Wnt signal transduction of immediate gene targets (Barker and Clevers, 2007). Free β-catenin in the cytoplasm binds to the glycogen synthase kinase-3 (GSK-3β)-axin-adenomatous polyposis coli (APC) protein complex and it is phosphorylated by GSK-3β occurring their proteosome-mediated degradation (26S proteosome) (Barker and Clevers, 2007). Nuclear β-catenin activated transcription factors such as T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) where it serves as a transcription regulator for several genes that regulate tumor progression and invasion. β-catenin aberrant expression is frequently detected in chemically-induced colon tumors in rats and mice (Ochiai et al., 2003; Hata et al., 2004; Takahashi and Wakabayashi, 2004) and in human colon cancer (Hao et al., 2001) suggesting that alterations of β-catenin may be early events in rodent and human colorectal carcinogenesis.

Yacon (*Smallanthus sonchifolius* Poepp. and Endl., Asteraceae) is a perennial plant originally native of Andean region of South America (Zardini, 1991; Valentová et al., 2006). There are reports on yacon cultivation in other countries, including EUA, Europe, New Zealand and Brazil (Valentová et al., 2006). Yacon has been cultivated in south-eastern Brazil as a crop since 1991 yielding up to 100 t/ha (Vilhena et al., 2000). In folk medicine, yacon tuberous roots and infusions from dried leaves are consumed by people suffering from diabetes or from various digestive or renal disorders (Valentová et al., 2006). Its tuberous roots are consumed fresh or cooked and it has been considered a functional food because of the large amounts of fructans (i.e., inulin and fructooligosaccharides) (Valentová et al., 2006). Fructans are carbohydrates reserve which contains up to 70 fructose units linked or not to a terminal sucrose molecule, may have linear or branched structure held together by frutosil-fructose bonds (Roberfroid and Delzenne, 1998; Pedreschi et al., 2003). Studies have shown that the best period to harvest yacon in tropical regions is between the 31st and 35th week after cultivation, regarding the concentration of fructans and their proportion in relation to mono- and disaccharides (Oliveira and Nishimoto, 2004). The yacon plants present a high hydrolytic activity at maturation phase of the tuberous roots, contributing to the predominance of a low degree of polymerization such as fructooligosaccharides (FOS, DP < 10) rather than fermentable long-term fraction fructans (DP > 10) (Itaya et al., 2002; Lobo et al., 2007).

A prebiotic, as defined by Gibson and Roberfroid (1995), is "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon with the potential to improve host health. A number of poorly digested carbohydrates fall into the category of prebiotics, including certain fibers and resistant starches, but the most widely described prebiotics are non-digestible oligosaccharides. Combinations of probiotics and prebiotics can result in additive or synergistic effects on gastrointestinal function (Pool-Zobel and Sauer, 2007; Fotiadis et al., 2008). FOS are prospective prebiotics as they are fermented by beneficial species of gut bacteria (Gibson and Roberfroid, 1995; Havenaar, 2011; DuPont and DuPont, 2011). They are also used as a source of natural sweeteners and syrups suitable for persons suffering from digestive problems (Charalampopoulos and Rastall, 2012). Oral treatment with yacon syrup markedly accelerated colonic transit time in healthy individuals (Geyer et al., 2008) and increased defecation frequency and satiety sensation in obese and slightly dyslipidemic pre-menopausal women (Genta et al., 2009).

Considering the large amounts of fructooligosaccharides detected in yacon tuberous roots and its increased popularity and consumption, the present study aimed to investigate the potential chemopreventive action of 0.5% and 1.0% dried extract of yacon root intake on the ACF and tumor development in the rat colon carcinogenesis induced by DMH. In addition, the beneficial effects of yacon root in association with *Lactobacillus casei* (i.e., a synbiotic formulation) were also evaluated.

2. Material and methods

2.1. Animals and treatment

The animals used in this study were handled in accordance with the Ethical Principles for Animals Research adopted by the Brazilian College of Animal Experimentation (COBEA). The experimental protocol used herein was approved by the local University Ethics Committee for Animal Research.

Four-week-old male Wistar rats were obtained from Centro de Tecnologia do Paraná (TECPAR, Araucária PR, Brazil). The animals were housed in polypropylene cages covered with metallic grids in a room maintained at 22 ± 2 °C, 55 ± 10% humidity under a 12-h light–dark cycle. After a 2-week acclimation period to the housing environment, the animals were randomly distributed into six groups (5 or 12 rats each): Groups 1 to 4 were treated with four subcutaneous injections of 40 mg/kg body weight 1,2-dimethylhydrazine hydrochloride (DMH, Sigma–Aldrich, Co, USA) (Dias et al., 2006, 2010), twice a week for 2 weeks. Groups 5 and 6 received similar injections of EDTA solution at pH 6.0 (DMH vehicle). After 4 weeks of DMH-initiation,G1 and G6 groups were fed basal diet (Nuvilab-CR-1, Curitiba-PR, Brazil) and G2, G3, G4 and G5 groups received basal diet containing dried extract of yacon root at 0.5%, 1.0%, a synbiotic formulation (dried extract of yacon root at 1.0% plus *Lactobacillus casei* at 2.5×10^{10} de CFU per g diet) or dried extract of yacon root at 1.0%, respectively, for 13 weeks (week 7–20). At the end of the week 20, the animals were killed by exsanguination under sodium pentobarbital anesthesia (45 mg/kg body weight). Individual body weight and food consumption were recorded twice a week during the experimental period.

2.2. Preparation of dried extract of yacon root, synbiotic formulation and determination of fructans

The probiotic *Lactobacillus casei* (lyophilized power containing 2×10^{10} live cells/g, Future Ceuticals, USA, Lot. 90715LC10N4710) was incorporated into basal diet at 2.5×10^{10} de CFU per g diet. The yacon roots were washed, cut into 1.5 cm thick cubes and heated for 10 min at 90 °C in water under slowly stirring. Then, cubes were grounded and its pulp was sieved (150 mesh) and heated for 10 min at 90 °C under slowly stirring. Samples of dried extract of yacon root were obtained by spray dryer process using maltodextrin as a carrier agent. The spray drying process was performed in a laboratory scale spray dryer with a 0.2 mm diameter nozzle (R.A.B Ranazzi and Co. Ltd., Brazil). The feed flow rate used was 25 ml/min and the inlet and outlet air temperatures were 230–250 °C and 100–110 °C, respectively. A synbiotic formulation was produced by dietary association between yacon root at 1.0% plus *Lactobacillus casei*.

The fructooligosaccharides (FOS) was analyzed by high performing liquid chromatography (HPLC) using ProStar Varian model (Hubbardston, MA, USA). The sugars separation was achieved on a Biorad Aminex HPX-87P model column and RI350 detector. Mobile phase consisted of deionized water and detection of maltodextrin, FOS, sucrose, glucose and fructose (12.120, 13.307, and 19.608, 24.425 and 37.926 retention time) was performed at flow rate of 0.8 mL/min. The concentrations of maltodextrin, FOS, sucrose, glucose and fructose were calculated from standard curves prepared with purified standards (Sigma–Aldrich, Co, USA). The chemical analysis revealed that dried extract of yacon root used in this study contained 20.4% FOS 4.8% sucrose, 2.8% glucose, 6.8% fructose and 16.7% maltodextrin. Equivalent quantities from sucrose, glucose, fructose and maltodextrin were added into basal diet from G1 and G6 groups. Thus, control diet and diet containing dried extract of yacon root at 1.0% were formulated in order to provide similar caloric energy.

2.3. Colon processing and histopathological analysis

At necropsy, caecal content was collected on ice into a vial, and frozen at −80 °C for short chain fatty acids (SCFA) analyses The colon was removed, opened longitudinally and gently rinsed with saline to remove residual bowel contents and, then, fixed flat in 10% buffered formalin for 24 h at room temperature. The localization and volume of each tumor were registered. Then middle and distal colon were stained with 1.0% methylene blue dissolved in phosphate-buffered salt solution (PBS) for analysis and quantification of classical ACF (Dias et al., 2006, 2010). ACF were identified according to Bird criteria (Bird and Good, 2000). The number of ACF/colon, number of aberrant crypts in each ACF (multiplicity)/colon and the number of ACF with 1–3, 4–9 or >9 aberrant crypts (AC)/colon were determined under

light microscopy at 40× magnification. After ACF evaluations, the colons were Swiss-rolled (4–6, 2-mm width segments) and embedded in paraffin. Histological evaluation was carried out on hematoxylin-eosin (HE)-stained colon sections and ACF were classified as conventional or dysplastic lesions, according to Yoshimi et al. (2004). Colon tumors were classified as adenomas or adenocarcinomas, according to Hamilton and Aaltonen (2000). In addition, the colonic adenocarcinomas were classified into invasive and non-invasive type (i.e., based on invasion into submucosa and muscularis) and their incidence (% rats with tumor), and multiplicity (number of tumor/rat) were also calculated.

2.4. Immunohistochemical procedures

Proliferating cell nuclear antigen (PCNA), cleaved caspase-3 and β-catenin expression in colon sections were immunohistochemically detected using a polymer system (MACH 4 Universal HRP polymer Detection, Biocare, CA, USA). Briefly, deparaffinated 5-μm colon sections on silanized slides were treated sequentially with 0.01 M citrate buffer (pH 6.0) at 120 °C for 5 min in a Pascal Pressure Chamber (Dako Cytomation Denmark A/S), 3% H_2O_2 in phosphate-buffered saline (PBS) for 10 min, nonfat milk for 60 min, mouse anti-PCNA (clone PC10, 1:200 dilution, DakoCytomation Denmark A/S, Glostrup, Denmark), rabbit polyclonal cleaved anti-caspase-3 (clone Asp 175, 1:100 dilution, Cell Signalizing Technology, Inc., Danvers, MA, USA) or anti-β-catenin (clone ab16051, Abcam, MA, USA) antibodies overnight at 4 °C, mouse probe and HRP polymer for 30 min each at room temperature. Chromogen color development was accomplished with 3,3′-diaminobenzidine tetrahydrochroride (Sigma–Aldrich, Co, USA). The slides were counterstained with Harris hematoxylin.

2.5. PCNA, cleaved caspase-3 and β-catenin analysis

At least 20 perpendicular well-oriented normal-appearing crypts from the middle and distal colon were examined in each animal (groups G1–G6) under light microscopy The index of colonic crypt cells expressing proliferating cell nuclear antigen (PCNA) or cleaved caspase-3 was evaluated as the percentage among the total number of cells analyzed in a perpendicular colonic crypt. Also, PCNA and cleaved caspase-3 indexes (~500 cells) and β-catenin expression were assessed in colon tumors. The abnormal expression of β-catenin (i.e., cytoplasm and nucleus) was evaluated in each colon tumor according to Yoshimi et al. (2004) and Femia et al. (2004).

2.6. Determination of caecal short chain fatty acids (SCFA)

The amount of SCFA in caecal contents was analyzed by gas chromatograph detection according to the methodology described by Zhao et al. (2006). Quantification of acetic, propionic, butyric, acid was carried out by comparing peak areas to those of pure standards of known concentration. Acetic acid, 100%, propionic acid, 99%, butyric acid, 99.5% were used by external calibration standard curves.

2.7. Statistical analysis

The statistical analysis was performed using the Jandel Sigma Stat software (Jandel Corporation, San Rafael, CA, USA). Data from body weight gain, food consumption, ACF and tumor number and multiplicity/colon, cell proliferation and apoptosis indexes and SCFA were compared among the groups. Besides, incidence of conventional and dysplastic ACF, colon tumor and altered β-catenin expression were compared by chi-square or the Fischer exact tests. The differences among groups were considered significant when $p < 0.05$.

3. Results

3.1. General findings

The mean body weights of respective DMH- and vehicle-treated groups fed yacon or the synbiotic formulation did not differ from the groups receiving basal diet (data not showed). Among DMH-initiated groups (G1–G4), the survival rate was higher in the group orally receiving synbiotic formulation (G4) when compared to the group fed basal diet (G1) (Fig. 1A), although without significant difference. Oral treatment throughout 13 weeks (week 7–20) with yacon or the synbiotic formulation did not change body-weight gain or food consumption (Table 1). At sacrifice (week 20), serum glucose, triglycerides and total cholesterol levels were similar among the different groups, but the relative liver weight was significantly lower ($p = 0.04$) in the group receiving synbiotic formulation (G4) than in the DMH-treated group fed basal diet (G1) (Table 1). The mean daily intake estimated of FOS was approximately 73.90, 150.74, 147.65 and 123.26 mg/kg of body weight for G2, G3, G4 and G5 groups, respectively.

Specific SCFA concentrations (μmol/g) of caecal content in DMH-initiated groups are shown in Fig. 2. Acetic and butyric acid content were significantly higher ($0.01 < p < 0.001$) in the groups fed 1.0% yacon (G3) or the synbiotic formulation (G4) than in the group fed basal diet (G1). Caecal propionate concentrations were not significantly modified by the different treatments.

3.2. Classical ACF analyses

Classical ACF were evaluated in methylene blue-stained whole mount colon at the end of week 20 (Fig. 3A and B). Data from the number and multiplicity of stereoscopically-analyzed ACF in the different groups are summarized in Table 2. All DMH-initiated animals developed classical ACF in the colon (G1–G4) while no preneoplastic lesion was detected in the colon from vehicle-treated groups (G5 and G6).

The total number of aberrant crypts AC and ACF and multiplicity (AC/ACF) were significantly lower ($p = 0.013$, $p < 0.05$ and $p = 0.003$, respectively) in the groups orally treated with 1.0% yacon (G3) or the synbiotic formulation (G4) when compared to the group fed basal diet (G1). The number of ACF containing 4–9 crypts (Fig. 3A) was significantly lower ($p = 0.018$) in the group receiving 1.0% dried root yacon (G3) and the synbiotic formulation (G4) when compared to the respective control group (G1). Also, the number of ACF containing more than 9 crypts (Fig. 3B) was significantly lower ($p = 0.001$) in groups fed 0.5% and 1.0% yacon (G2 and

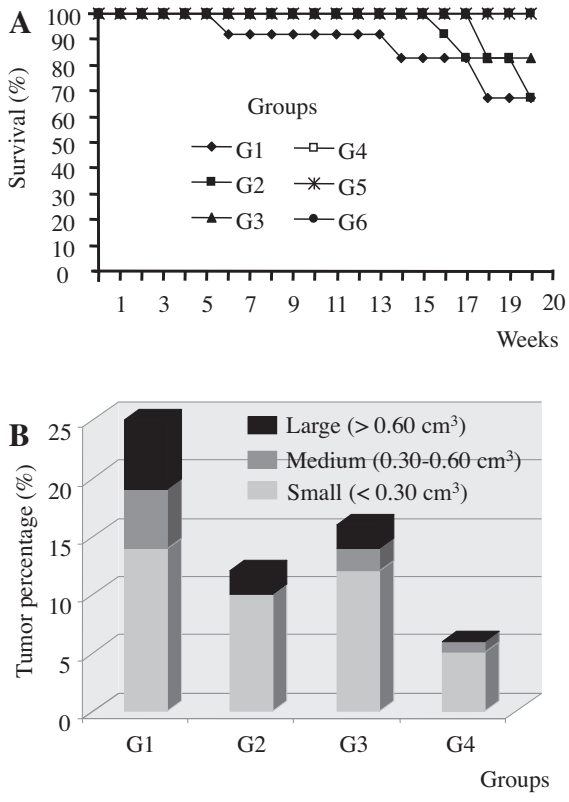

Fig. 1. (A) Kaplan–Meier analysis of animal survival in the different groups during experiment. (B) Data from tumor size (cm^3) in the colon of DMH-treated animals. G1 = DMH-initiated and fed basal diet, G2 = DMH-initiated and fed basal diet containing 0.5% yacon, G3 = DMH-initiated and fed basal diet containing 1.0% yacon; G4 = DMH-initiated and fed basal diet containing synbiotic formulation (*L. casei* at 2.5×10^{10} CFU/kg basal diet *plus* 1.0% yacon), G5 = Vehicle-treated and fed basal diet containing 1.0% yacon, G6 = Untreated. DMH = 1,2 dimethylhydrazine dihydrochloride (4 × 40 mg/kg b.wt., s.c.).

Table 1
Effects of dietary yacon on general data, relative weight organs and biochemical parameters in different groups[a].

Parameters	Group/treatment[b]					
	DMH-initiated				Non-initiated	
	(G1) Untreated 12/08[c]	(G2) Yacon 0.5% 12/08	(G3) Yacon 1.0% 12/10	(G4) Syn 12/12	(G5) 1.0% yacon 05/05	(G6) Untreated 05/05
General data						
Final body weight (g)	388.0 ± 18.27	401.37 ± 10.55	401.70 ± 11.02	422.0 ± 12.70	479.0 ± 13.25*	452.6 ± 13.84
Body-weight gain (g)	78.0 ± 17.04	99.29 ± 10.70	95.22 ± 16.50	91.92 ± 7.72	104.80 ± 6.35	79.40 ± 2.31
Food consumption (g/rat/day)[4]	25.53 ± 0.61	23.62 ± 0.83	23.89 ± 0.84	24.37 ± 0.71	23.22 ± 0.76	24.52 ± 1.14
Yacon consumption (mg/rat/day)	0	125.16 ± 4.06	252.97 ± 8.09	254.86 ± 7.44	243.05 ± 8.97	0
Relative weight organs (%)						
Liver	2.86 ± 0.17	2.42 ± 0.10	2.56 ± 0.11	2.37 ± 0.11*	2.42 ± 0.12	2.59 ± 0.12
Right kidney	0.29 ± 0.01	0.27 ± 0.01	0.27 ± 0.02	0.27 ± 0.02	0.25 ± 0.01	0.27 ± 0.01
Left kidney	0.29 ± 0.02	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
Biochemical data						
Glucose (mg/dL)	111.85 ± 7.84	104.06 ± 11.35	106.72 ± 4.91	102.01 ± 7.55	112.94 ± 7.04	114.60 ± 17.21
Triglycerides (mg/dL)	68.94 ± 3.32	60.10 ± 5.18	66.54 ± 6.19	66.10 ± 4.77	69.80 ± 6.07	62.50 ± 6.49
Cholesterol (mg/dL)	68.00 ± 2.86	64.33 ± 2.98	64.28 ± 3.27	61.87 ± 1.94	74.79 ± 2.73	78.18 ± 5.28

[a] Values are mean ± SE.
[b] DMH = 1,2 dimethylhydrazine dihydrochloride (4 × 40 mg/kg b.wt., s.c.); yacon = 0.5% or 1.0% dried extract of yacon root in the basal diet for 13 weeks; Syn = synbiotic (*L. casei* at 2.5 × 10^{10} CFU/kg basal diet *plus* 1.0% yacon).
[c] Number of animals: initial/final.

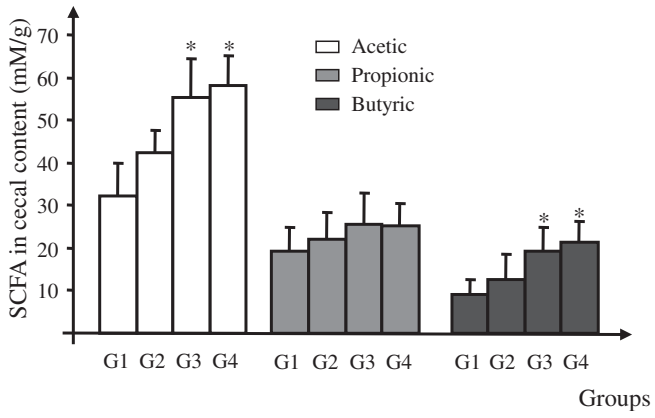

Fig. 2. SCFA concentration in caecal contents in DMH-initiated groups ($n = 8$). G1 = Fed basal diet, G2 = Fed basal diet containing 0.5% yacon, G3 = Fed basal diet containing 1.0% yacon; G4 = Fed basal diet containing synbiotic formulation (*L. casei* at 2.5 × 10^{10} CFU/kg basal diet *plus* 1.0% yacon). G1 = DMH-initiated and fed basal diet, G2 = DMH-initiated and fed basal diet containing 0.5% yacon, G3 = DMH-initiated and fed basal diet containing 1.0% yacon; G4 = DMH-initiated and fed basal diet containing synbiotic formulation (*L. casei* at 2.5 × 10^{10} CFU/kg basal diet *plus* 1.0% yacon). DMH = 1,2 dimethylhydrazine dihydrochloride (4 × 40 mg/kg b.wt., s.c.). *Different from G1 group, $0.01 < p < 0.001$.

G3) or the synbiotic formulation (G4) when compared to the respective control group fed basal diet (G1).

3.3. Tumor size and multiplicity and histopathology analyses

At sacrifice, all macroscopic tumors were measured and classified into three categories: small (<0.30 cm^3), medium (0.30–0.60 cm^3) and large (>0.60 cm^3) (Fig. 1B). A reduction in the mean tumor size (cm^3) was observed in groups orally receiving 0,5% and 1.0% yacon (G2 and G3) or the synbiotic formulation (G4) when compared to the group fed basal diet (G1) (Table 3), although without a significant difference.

Nondysplastic ACF (Fig. 3C) were observed in all DMH-treated groups (G1–G4), but a few number of animals presented dysplastic ACF (Fig. 3D). A significant reduction ($p < 0.05$) in incidence of dysplastic ACF was observed in groups fed 1.0% yacon (G3) or the synbiotic formulation (G4) when compared to the respective control group fed basal diet (G1) (Table 3). In general, dyplastic ACF showed altered β-catenin-accumulated crypts (Fig. 3E). The histopathological analysis by HE-staining revealed that more than 95% of colon tumors were invasive adenocarcinomas and less than 5% the remaining were *in situ* adenocarcinomas.

The mean number of invasive adenocarcinomas were significantly lower ($p = 0.02$) in the groups fed 1.0% yacon (G3) or the synbiotic formulation (G4). A significant reduction ($p = 0.02$) in tumor multiplicity (noninvasive and invasive tumor) was observed in the group fed synbiotic formulation (G4) (Table 3). No tumors were identified macroscopically or by histology in the non-initiated groups fed basal diet (G6) or fed 1.0% yacon (G5).

3.4. Cell proliferation, apoptosis and β-catenin analyses

Cell proliferation (PCNA-positive epithelial cells, Fig. 4) and apoptosis (cleaved caspase-3-positive epithelial cells, Fig. 5) indexes were analyzed in the "normal-appearing" crypts and in colon tumors. The proportion of PCNA and cleaved caspase-3 immunoreactivity was markedly and significantly higher ($p < 0.001$) in colon tumors than in "normal-appearing" colonic crypts (Figs. 4C and 5C). Also, a significant reduction ($p < 0.001$, $p = 0.039$, respectively) in the cell proliferation indexes was observed in the groups fed 1.0% yacon (G3 and G5) or the synbiotic formulation (G4) when compared to the respective control groups (G1 and G6) for both "normal-appearing" colonic crypts or colon tumors analyses (Fig. 4A1–A6, B1–B4 and C). The apoptosis index was not modified by dietary yacon (G2 and G3) or the synbiotic formulation (G4) when compared to the respective control groups fed basal diet (G1 and G6) (Fig. 5C).

Altered β-catenin expression was analyzed in colon tumors and compared to the "normal-appearing" crypts showing only membrane β-catenin expression. All adenocarcinomas expressed altered β-catenin in the cytosol and some malignant tumors also presented nuclear positivity for β-catenin (Fig. 3F). A significant reduction (40% and 50%, $p < 0.001$) in the incidence of colon tumors showing lower levels of altered β-catenin expression was observed in the groups fed 1.0% yacon (G3) or the synbiotic formulation (G4), respectively (Fig. 3G and H) when compared to the respective control group fed basal diet (G1). (Fig. 3F).

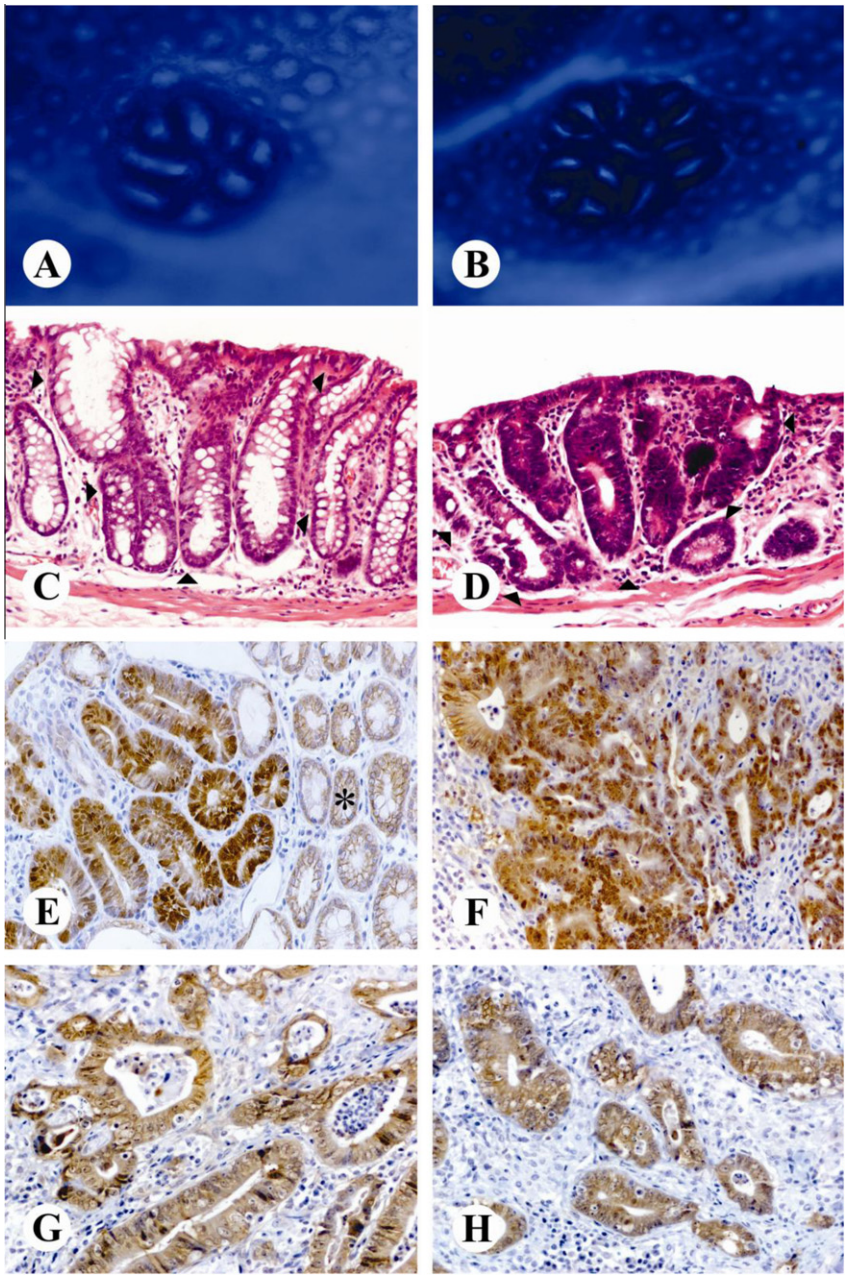

Fig. 3. (A–B) Representative topographic view of classical ACF in a methylene blue-stained colon whole mount containing eight and fourteen aberrant crypts, respectively (20× objective). (C–D) Representative nondysplastic and dysplastic ACF detected in HE-stained Swiss-rolling sections, respectively (arrows 20× objective). (E) Dysplastic ACF showing altered β-catenin expression in the cytoplasm and nucleus (40× objective). (F–H) Colon tumors showing altered β-catenin expression in both cytoplasm and nucleus (strong expression), and mainly in cytoplasm (weak expression) from the groups G1, G3 and G4, respectively (40× objective). *Membrane β-catenin expression in a "normal-appearing" crypts.

4. Discussion

Prebiotics, probiotics or their combination (synbiotics) have also been associated to the reduction of experimental colon carcinogenesis (Pool-Zobel, 2005; Fotiadis et al., 2008). Fructans present in foods such as garlic, onion, artichoke, asparagus and yacon have been demonstrated to increase the levels of probiotics and short chain fatty acids (SCFA) concentrations in intestinal contents (Valentová et al., 2006; Pool-Zobel, 2005; Pool-Zobel and Sauer, 2007; Fotiadis et al., 2008). Thus, the present study evaluated the potential protective effects of the ingestion of yacon root or a synbiotic formulation. Thus, the present study evaluated the potential protective effects of yacon root or a synbiotic formulation (i.e., potential addictive effect) against chemically-induced colon carcinogenesis in male Wistar rats.

A significant reduction in development of the classical and dysplastic ACF and in the number of invasive colon tumors was observed in the groups fed 1.0% yacon root. These protective effects of dietary yacon containing high FOS concentrations were associated to a significant increase of SCFA concentrations and a reduction in the cell proliferation and incidence of colon tumors expressing altered β-catenin. These findings are in agreement with previous observations indicating that prebiotics may reduce the incidence and multiplicity of chemically-induced colon tumors (Pool-Zobel, 2005; Pool-Zobel and Sauer, 2007; Fotiadis et al., 2008).

Table 2
Effects of dietary yacon on development of colonic aberrant crypt foci (ACF) [a].

Group/treatment[b]	Number of rats[c]	Number of ACF			Total number		
		1–3 crypt	4–9 crypts	>9 crypts	AC[d]	ACF	AC/ACF
DMH-initiated							
(G1) Untreated	12/8	30.78 ± 4.79	15.33 ± 4.47	1.89 ± 0.48	304.26 ± 51.27	91.63 ± 11.58	3.25 ± 0.31
(G2) 0.5% yacon	12/8	23.62 ± 5.60	6.37 ± 1.53	0.25 ± 0.25*	153.82 ± 32.44	62.73 ± 11.85	2.40 ± 0.33
(G3) 1.0% yacon	12/10	22.60 ± 5.90	4.10 ± 1.00*	0.30 ± 0.15*	110.04 ± 19.41*	52.82 ± 12.10*	2.19 ± 0.24*
(G4) Syn	12/12	16.08 ± 2.90	3.17 ± 0.90*	0.33 ± 0.26*	103.04 ± 22.08*	51.11 ± 10.03*	1.79 ± 0.20*,**
Non-initiated							
(G5) 1.0% yacon	5/5	0	0	0	0	0	0
(G6) Untreated	5/5	0	0	0	0	0	0

[a] Values are mean ± SE;
[b] DMH = 1,2 dimethylhydrazine dihydrochloride (4 × 40 mg/kg b.wt., s.c.); Yacon = 0.5% and 1.0% dried extract of yacon root in the basal diet for 14 weeks; Syn = synbiotic (*L. casei* at 2.5 × 10^{10} CFU/kg basal diet *plus* 1% yacon);
[c] Number of animals: Initial/Final.
[d] AC = aberrant crypt.
*,** Different from G1 group and G1 and G2 groups, respectively, $0.05 < p < 0.001$.

Table 3
Effects of dietary yacon on development of dysplastic ACF (incidence) and colon tumors (incidence and multiplicity).

Group/treatment[a]	Number of rats[b]	Displastic ACF[c] (%)	Tumor (%)	Multiplicity[d]			Tumor volume[d] (cm^3)
				Invasive	Non-invasive	Total	
DMH-initiated							
(G1) Untreated	12/8	8 (100)	7 (70)	4.1 ± 1.2	1.5 ± 0.5	5.0 ± 1.4 (38)[e]	0.61 ± 0.14
(G2) 0.5% yacon	12/8	5 (63)	8 (100)	2.2 ± 0.6	1.5 ± 0.3	3.4 ± 0.4 (27)	0.27 ± 0.06
(G3) 1.0% yacon	12/11	5 (45)*	7 (64)	1.3 ± 0.7*	0.8 ± 0.6	2.9 ± 1.3 (20)	0.32 ± 0.12
(G4) Syn	12/12	3 (25)*	9 (75)	1.2 ± 0.2*	0.7 ± 0.3	1.4 ± 0.2*(16)	0.22 ± 0.10
Non-initiated							
(G5) 1.0% yacon	5/5	0	0	0	0	0	0
(G6) Untreated	5/5	0	0	0	0	0	0

[a] DMH = 1,2 dimethylhydrazine dihydrochloride (4 × 40 mg/kg b.wt., s.c.); yacon = 0.5% and 1.0% dried extract of root yacon in the basal diet for 14 weeks; Syn = synbiotic (*L. casei* at 2.5 × 10^{10} CFU/kg basal diet plus 1% yacon).
[b] Number of animals: initial/final.
[c] ACF = aberrant crypt foci.
[d] Values are mean ± SE.
[e] Total number of colon tumors.
*,** Different from G1 group and G1 and G2 groups, respectively, $0.02 < p < 0.001$.

Yacon root or syrup intake is apparently well-tolerated without evident toxic effects in experimental and human studies (Genta et al., 2009; Valentová et al., 2006; Geyer et al., 2008). In an intervention study in obese and dyslipidemic pre-menopausal women, the recommended and safety daily consumption of yacon syrup was 0.14 g fructooligosaccharides/kg (Genta et al., 2009). In other human intervention study, the intake of yacon tubers capsules at daily dose of 2.4 g was safety and well tolerated in people suffering from or at risk of metabolic syndrome (Valentová et al., 2006). However, yacon syrup intake at daily dose of 20 g (equal to 6.4 g fructooligosaccharides) induced a marked acceleration of colonic transit time in healthy volunteers included in a placebo-controlled crossover study (Geyer et al., 2008). The results of present study indicate that daily dose of FOS in the 1.0% yacon root intake was ~150 mg/kg b.w. (0.15 g/kg). This dose did not cause deleterious effects on the gastrointestinal system of male Wistar rats and it was a safe dose similar to the recommended for humans (Genta et al., 2009).

Various studies indicate that gut flora fermentation products of FOS beneficially modulate tumor progression markers in human and rat colon tumor cells (Pool-Zobel, 2005; Pool-Zobel and Sauer, 2007; Fotiadis et al., 2008). In the present study, acetate and butyrate contents in the caecum were significantly increased in DMH-initiated groups fed 1.0% yacon. In special, butyrate is a protective agent against colon cancer by promoting cell differentiation, cell-cycle arrest and apoptosis of transformed colonic epithelial cells; inhibiting the enzyme histone deacetylase and decreasing the transformation of primary to secondary bile acids as a result of colonic acidification (feces) (Wong et al., 2006; Pool-Zobel and Sauer, 2007). Although yacon fructooligosaccharides contain low degree of polymerization (Ohyama et al., 1990), a significant increase in SCFA production was observed as well as to inulin and Synergy® (high degree of polymerization) that are more slowly fermented in the large bowel (Roberfroid, 2007). Thus, the protective effects against colon carcinogenesis by dietary yacon could involve an increase in SCFA production associated to the reduction of development of ACF and colon tumor.

The present study evaluated the modifying effects of yacon intake on cell proliferation and apoptosis in both "normal appearing" crypts and colonic tumors. Cell proliferation may lead to an increased risk of developing cancer whereas apoptosis is a protective innate mechanism taking to elimination of the epithelial cells with DNA damage or genomic instability (Hanahan and Weinberg, 2011). Increase in both cell proliferation and apoptosis indexes has been documented during development of colon tumors (Mełeń-Mucha and Niewiadomska, 2002), as also observed in this present study by differences in levels of cell proliferation/apoptosis between "normal appearing" crypts and tumor epithelial cells. In fact, some studies have described an increase in apoptosis levels in the colon by prebiotics or synbiotic interventions shortly after carcinogen treatment (Hughes and Rowland, 2001; Le Leu et al., 2005) rather than in late stages of carcinogenesis (Femia et al.,

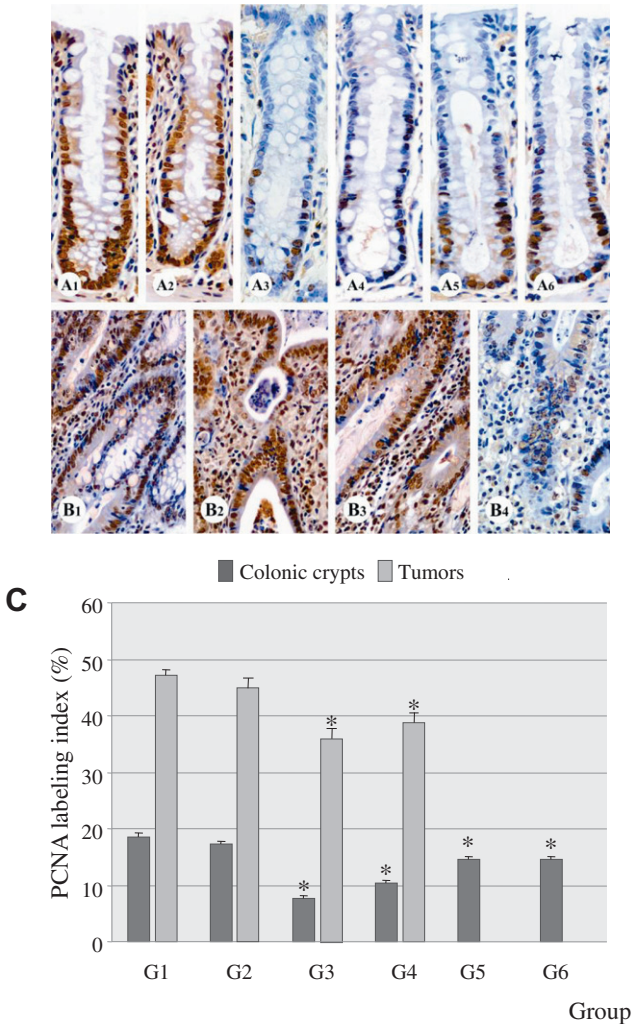

Fig. 4. (A1–A6) Representative "normal-appearing" crypts immunoreactivity for PCNA (dark nuclei) from the groups G1, G2, G3, G4, G5 and G6, respectively (40× objective). (B1–B4) Representative tumor immunoreactivity for PCNA (dark nuclei) from the groups G1, G2, G3 and G4, respectively (40× objective). (C) PCNA labeling indexes for "normal-appearing" crypts and colon tumors in the different groups. G1 = DMH-initiated and fed basal diet, G2 = DMH-initiated and fed basal diet containing 0.5% yacon, G3 = DMH-initiated and fed basal diet containing 1.0% yacon; G4 = DMH-initiated and fed basal diet containing synbiotic formulation (*L. casei* at 2.5 × 10^{10} CFU/kg basal diet *plus* 1.0% yacon), G5 = Vehicle-treated and fed basal diet containing 1.0% yacon, G6 = Vehicle-treated and fed basal diet. DMH = 1,2 dimethylhydrazine dihydrochloride (4 × 40 mg/kg b.wt., s.c.). *Different from G1 group, $p < 0.001$.

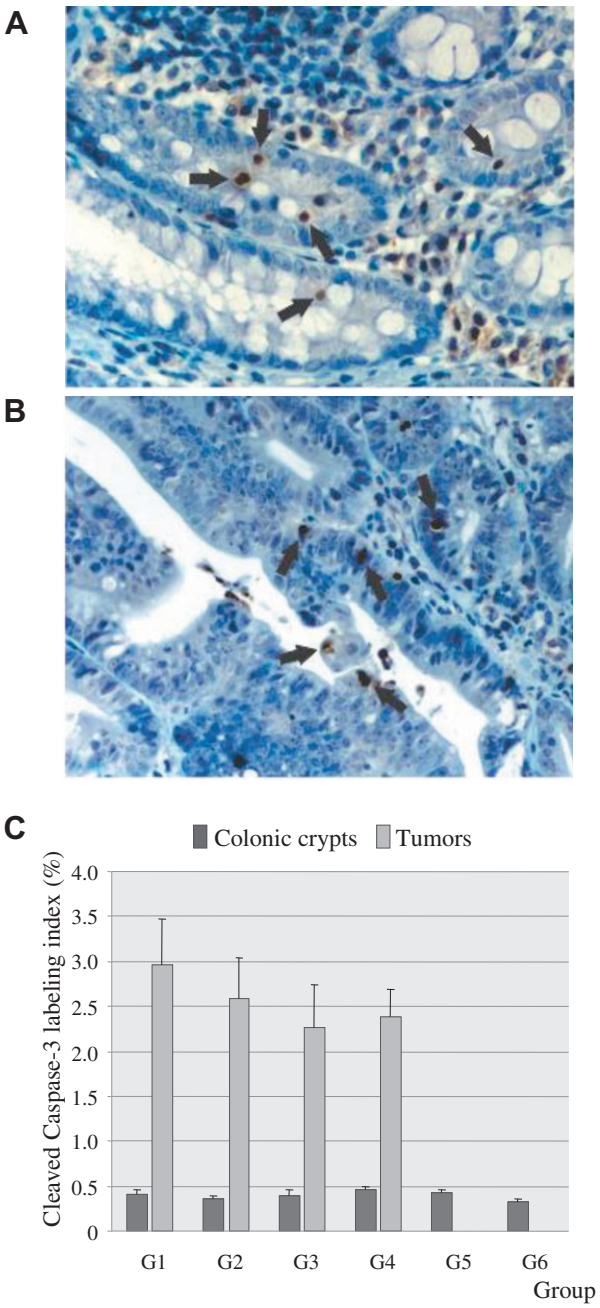

Fig. 5. (A–B) Representative "normal-appearing" crypts and tumor immunoreactivity for cleaved caspase-3 (arrows, 40× objective), respectively. (C) Cleaved caspase-3 labeling indexes for "normal-appearing" crypts and colon tumors in the different groups. G1 = DMH-initiated and fed basal diet, G2 = DMH-initiated and fed basal diet containing 0.5% yacon, G3 = DMH-initiated and fed basal diet containing 1.0% yacon; G4 = DMH-initiated and fed basal diet containing synbiotic formulation (*L. casei* at 2.5 × 10^{10} CFU/kg basal diet *plus* 1.0% yacon), G5 = Vehicle-treated and fed basal diet containing 1.0% yacon, G6 = Untreated. DMH = 1,2 dimethylhydrazine dihydrochloride (4 × 40 mg/kg b.wt., s.c.).

2002; Le Leu et al., 2010). In addition, similar findings for protective effects of prebiotics on colonic cell proliferation have been observed in rats fed fermentable substrates as inulin, FOS and resistant starch (Femia et al., 2002; Le Leu et al., 2010). Thus, increased SCFA concentrations resulting from fermentation of great content of FOS contained in the yacon root could contribute to the beneficial effects on cell proliferation rather than apoptosis.

Several rodent studies have shown that prebiotics (i.e., fructooligossacharides or frutans) probiotics and their combination synbiotics can provide protective effects against early biomarkers and tumor development in the colon of carcinogen-treated rats (Reddy et al., 1997; Reddy, 1998; Pool-Zobel et al., 2002; Pool-Zobel and Sauer, 2007; Sivieri et al., 2008). Importantly, prebiotics and probiotics can survive and transit through the human and rodent gastrointestinal tract (Femia et al., 2002; Roberfroid et al., 2010; Yan and Polk, 2010.) Yacon root saccharides, particularly β-(2 → 1) fructooligosaccharides appear to be a good candidate as a prebiotic supplement (Valentová et al., 2006). In addition, it has been shown that *Lactobacillus acidophilus* NRRL-1910, *Lactobacillus plantarum* NRRL B-4496, and *Bifidobacterium bifidum* ATCC 15696 (Pedreschi et al., 2003) and *Lactobacillus casei* Shirota were able to ferment yacon FOS under anaerobic conditions (Buriti and Saad, 2007). In general, synbiotic treatment is more beneficial for reducing colon carcinogenesis than treatment with probiotics or prebiotics (Gallaher and Khil, 1999; Femia et al., 2002; Klinder et al., 2004). In the present study, the greatest decrease in the number of and multiplicity

of aberrant crypt foci (ACF) and in the number of invasive and non-invasive adenocarcinomas (multiplicity) was observed in the group fed synbiotic formulation. This mild additive effect against colon carcinogenesis could be attributed to the probiotic (*Lactobacillus casei*) effect since various studies have suggested several health-promoting mechanisms by which probiotics exert beneficial effects in human and laboratory animals (Fotiadis et al., 2008; Yan and Polk, 2010; Wallace and Mackay, 2011).

A significant reduction in the incidence of colon tumors showing lowest altered β-catenin expression (i.e., loss or reduced β-catenin protein at the cell-to-cell borders but an over-expression in the cytoplasm and nucleus) was observed in the groups fed 1.0% yacon or the synbiotic formulation. Since β-catenin is an important Wnt signaling pathway activator, its level in the non-altered tissue is adequately regulated by GSK-3β complex that targets its rapid degradation in the cytoplasm to prevent translocation of β-catenin into the nucleus and transcription of target genes, including genes related to cell proliferation (Karim et al., 2004). In fact, c-myc and cyclin D1 genes have been identified as targets of the β-catenin/APC signaling (Karim et al., 2004). Thus, a potential role of dietary yacon on the reduction of aberrant β-catenin expression could be associated to a lower cell proliferation in colon tumors, indicating that yacon could have a beneficial effect on tumor progression involving β-catenin/Wnt signaling pathway (Tarapore et al., 2012).

In conclusion, the findings this present investigation suggest that yacon root intake may have potential as a chemopreventive agent against colon carcinogenesis. The underlying mechanisms of chemoprevention of colon carcinogenesis by yacon root still must be investigated.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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