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Antioxidant and Anti-inflammatory Effects of Yam (*Dioscorea batatas* Decne.) on Azoxymethane-induced Colonic Aberrant Crypt Foci in F344 Rats

In Suk Son, Jeong Soon Lee, Ju Yeon Lee, and Chong Suk Kwon

Department of Food Science and Nutrition, Andong National University, Gyeongbuk 760-749, Korea

ABSTRACT: Yam (*Dioscorea batatas* Decne.) has long been used as a health food and oriental folk medicine because of its nutritional fortification, tonic, anti-diarrheal, anti-inflammatory, antitussive, and expectorant effects. Reactive oxygen species (ROS), which are known to be implicated in a range of diseases, may be important progenitors of carcinogenesis. The aim of this study was to investigate the modulatory effect of yam on antioxidant status and inflammatory conditions during azoxymethane (AOM)-induced colon carcinogenesis in male F344 rats. We measured the formation of aberrant crypt foci (ACF), hemolysate antioxidant enzyme activities, colonic mucosal antioxidant enzyme gene expression, and colonic mucosal inflammatory mediator gene expression. The feeding of yam prior to carcinogenesis significantly inhibited AOM-induced colonic ACF formation. In yam-administered rats, erythrocyte levels of glutathione, glutathione peroxidase (GPx), and catalase were increased and colonic mucosal gene expression of Cu/Zn-superoxide dismutase (SOD), Mn-SOD, and GPx were up-regulated compared to the AOM group. Colonic mucosal gene expression of inflammatory mediators (i.e., nuclear factor kappaB, inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor alpha, and interleukin-1beta) was suppressed by the yam-supplemented diet. These results suggest that yam could be very useful for the prevention of colon cancer, as they enhance the antioxidant defense system and modulate inflammatory mediators.

Keywords: yam (*Dioscorea batatas* Decne.), azoxymethane, antioxidant enzymes, inflammatory mediators

INTRODUCTION

Colorectal cancer (CRC) is a leading cause of mortality and morbidity in the western world (1). In Korea, the morbidity of CRC has increased dramatically in the last decade. According to the 2011 cancer statistics of the National Cancer Information Center, between 1999 and 2011, the age-standardized morbidity per 100,000 inhabitants increased from 27.0 to 52.7% for men and from 17.1 to 27.6% for women. The overall morbidity of CRC increased annually by 6.1% in men and 4.5% in women during the same period (2). Epidemiological studies have shown that a diet high in calories and animal fats and low in fruits, vegetables, and fiber; a lack of physical activity; obesity; and alcohol consumption are associated with an increased risk of CRC (3). Consumption of fruits and vegetables that are rich in antioxidant nutrients may help to prevent colon cancer by minimizing the genotoxic effects of free radicals and lipid peroxidation by-products (4). It has been estimated that 70~90% of colon cancer deaths can be linked to diet (5).

Reactive oxygen species (ROS), which are known to be implicated in a range of diseases, may be important progenitors of carcinogenesis (6). Under conditions of oxidative stress, where levels of ROS are not in balanced with antioxidants, ROS can be detrimental to cells, leading to uncontrolled proliferation, inflammation, or apoptosis (7). These deleterious actions of ROS can be countered by antioxidant defense systems, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). These enzymes are important for the inactivation of many environmental mutagens and play a central role in protecting cells from oxidative injury (8). ROS are also critical to several signal transduction pathways, as they act as second messengers involved in the activation of NF-κB (9). Activated NF-κB further induces the transcription of pro-inflammatory mediators, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α), and interleukin-1 (IL-1) (10,11). NF-κB is

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Correspondence to Chong Suk Kwon, Tel: +82-54-820-5484, E-mail: cskwon@andong.ac.kr

a promoter of tumorigenesis that regulates inflammation through the overexpression of iNOS, COX-2, and inflammatory cytokines, such as TNF-α and IL-1β (12,13).

Yam is a perennial trailing rhizome plant of the *Dioscorea* genus (Dioscoreaceae family), that has long been used as health food and oriental folk medicine because of its nutritional fortification, tonic, anti-diarrheal, anti-inflammatory, antitussive, and expectorant effects (14). Recent studies have shown that yam had *in vivo* and *in vitro* antioxidant and anti-inflammatory properties and numerous biological and pharmacological activities (15-19). Therefore, this study aimed to investigate the efficacy of yam at preventing azoxymethane (AOM)-induced colon tumors by modulating the antioxidant and anti-inflammatory systems.

MATERIALS AND METHODS

Materials

The root of *Dioscorea batatas* Decne was obtained from Andong Bukhu Nonghyup (Gyeongbuk, Korea), freeze-dried and powdered. AOM was purchased from Sigma (St. Louis, MO, USA). The assay kits for glutathione (GSH), SOD, GPx, and CAT were obtained from Cayman Chemical Co. (Ann Arbor, MI, USA). The AIN-76A diet was obtained from Teklad Lab Animal Diets; Harlan (Madison, WI, USA). All other chemicals were of reagent grade.

Animals and experimental diets

Male Fisher 344 rats (5 week-old) were obtained from Central Lab. Animal Inc. (Seoul, Korea), housed under a 12-h light/dark cycle in a temperature (23±2°C) and humidity (50±5%) controlled room. Rats were acclimatized to the above conditions for one week with free access to standard laboratory rodent chow and drinking water until the initiation of the experiment. The rats were randomly assigned to receive one of three treatment diets (n=12 rats per group) modified from the AIN-76A diet (Table 1): control diet, control diet with 2% yam, and control diet with 5% yam. After one week of experimental diet consumption, all rats were injected with AOM subcutaneously once a week for 2 weeks at a dose of 15 mg/kg body weight. All animals were euthanized by CO_2 inhalation 8 weeks after the initial AOM injection. Throughout the experimental period, weekly body weight and food consumption were recorded. This animal study was approved by the university committee for the care and use of laboratory animals.

Table 1. Composition of the experimental diets (unit: g/kg diet)

Ingredients	Group		
	AOM	AOM+2%Y	AOM+5%Y
Starch	400	388.5	358.7
Sugar	250	250	250
Corn oil	50	49.3	48.2
Casein	200	198.6	196.6
Cellulose	50	48.9	47.3
Mineral[1)]	35	35	35
Vitamin[2)]	10	10	10
Choline	2	2	2
Methionine	3	3	3
Yam powder	-	20	50

[1)]Mineral mixture: $CaCO_3$ (292.9 g/kg), $CaHPO_4 \cdot 2H_2O$ (4.3 g/kg), KH_2PO_4 (43.1 g/kg), NaCl (250.6 g/kg), $MgSO_4 \cdot 7H_2O$ (99.8 g/kg), $Fe(C_6H_5O_7) \cdot 6H_2O$ (6.23 g/kg), $CuSO_4 \cdot 5H_2O$ (1.56 g/kg), $MnSO_4 \cdot H_2O$ (1.21 g/kg), $(NH_2)_6Mo_7O_{24} \cdot 4H_2O$ (0.025 g/kg), $Na_2SeO_3 \cdot 5H_2O$ (0.015 g/kg), $ZnCl_2$ (0.005 g/kg).

[2)]Vitamin mixture: vitamin D_3 (58.2 mg/kg), α-tocopherol-acetate (1,200.0 mg/kg), retinol-acetate (93.2 mg/kg), vitamin K_3 (6.0 mg/kg), thiamin-HCl (59.0 mg/kg), vitamin B_{12} (0.2 mg/kg), vitamin C (588.0 mg/kg), pyridoxine-HCl (29.0 mg/kg), D-biotin (1.0 mg/kg), folic acid (2.0 mg/kg), inositol (1,176.0 mg/kg), Ca-pantothenate (235.0 mg/kg), riboflavin (59.0 mg/kg), nicotinic acid (294.0 mg/kg), sucrose (96,257.017 mg/kg).

Sample preparations

After euthanasia, blood samples were collected from the abdominal aorta and centrifuged at 3,000 rpm for 20 min to separate the plasma from the blood cells. Erythrocytes were lysed in four volumes of ice-cold water and centrifuged at 10,000 *g* for 15 min at 4°C. The supernatant (hemolysate) was collected and stored at −80°C. The entire colon was excised, flushed with ice-cold saline, and longitudinally opened. The colonic mucosa was collected by scraping with a rubber policeman, immediately treated with RNAlater, and stored at −80°C until subsequent molecular analysis.

Quantification of aberrant crypt foci (ACF)

ACF analysis was done according to the method described by Bird (20). Briefly, colons were longitudinally opened, rinsed with saline and fixed flat between two pieces of filter paper in 10% buffered formalin for a minimum of 24 h. The colons were then cut into 3 segments (proximal, middle, and distal), stained with 0.2% methylene blue in phosphate buffered saline for 5 to 10 min, placed on a microscope slide with the mucosal side up, and visualized through a light microscope at 40× magnification. ACF were distinguished from surrounding normal crypts by their increased size, a significantly increased distance from the lamina to the basal surface of the cells, and an easily discernible pericryptal zone. The total number of ACF and the number of aberrant crypts in each focus were counted. ACF were categorized by crypt multiplicity (1, 2, 3, or ≥4 crypts). ACF containing four or more aberrant crypts/focus were categorized as multicrypts.

Measurement of glutathione and antioxidant enzyme activity

GSH and antioxidant enzymes (i.e., SOD, GPx, CAT)

were measured in hemolysates using commercially available detection kits (Cayman Chemical Co.) according to the manufacturer's instructions.

Analyses for gene expression in colonic mucosa

Total RNA was extracted from the scraped colonic mucosae using an RNA preparation kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Real-time reverse transcriptase (RT)-PCR was carried out using a One Step SYBR PrimeScript RT-PCR kit (Takara Bio Inc., Shiga, Japan). mRNA levels were analyzed with Exicycler3 (Bioneer Co., Daejeon, Korea). Primers were designed with Primer3 software, and purchased from Bioneer Co. The PCR primer pair sequences are listed in Table 2. A quantitative analysis of gene expression was conducted using the comparative cycle threshold ($2^{-\Delta\Delta CT}$) method with β-actin as an internal control (21).

Statistical analyses

Data are expressed as the mean±SD. One-way ANOVA and subsequent Duncan's multiple range tests were used for multiple comparisons. Significance was set at α=0.05.

Table 2. Primer sequences

Target gene	Primer sequence (5'→3')
β-Actin	F: CAC CAG TTC GCC ATG GAT
	R: ATC ACA CCC TGG TGC CTA
Cu/Zn-SOD	F: GTC GTC TCC TTG CTT TTT GC
	R: TCT GCT CGA AGT GAA TGA CG
Mn-SOD	F: CGT CAC CGA GGA GAA GTA CCA CGA
	R: CAG CCT GAA CCT TGG ACT CCC ACA
GPx	F: GAA GGT AAA GAG CGG GTG AG
	R: AGG AGA ATG GCA AGA ATG AAG
CAT	F: AAG CTG GTT AAT GCG AAT GG
	R: CAA GTT TTT GAT GCC CTG GT
NF-κB (p65)	F: ACG ATC TGT TTC CCC TCA TC
	R: CAC TTG TAA CGG AAA CGC AT
COX-2	F: ACT ACG CCG AGA TTC CTG AC
	R: CAA TGT TCC AGA CTC CCT TG
iNOS	F: GTG TTC CAC CAG GAG ATG TT
	R: ACC GCT TTC ACC AAG ACT G
IL-1β	F: GCC TCA AGG GGA AGA ATC TAT
	R: CAA ACC GCT TTT CCA TCT TCT
TNF-α	F: GAG CTC AAG CCC TGG TAT G
	R: CGG ACT CCG TGA TGT CTA AG

SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; NF-κB, necrosis factor-kappaB; COX, cyclooxygenase; iNOS, inducible nitric oxide synthase; IL, interleukin; TNF-α, tumor necrosis factor-alpha.

RESULTS AND DISCUSSION

General observations

There were no considerable differences in body weight gain and food intake among control and yam-fed groups until the end of the study. In addition, liver weight and colon length were not significantly different among groups (Table 3).

Effect of yam on colonic ACF formation

To determine the effect of yam on the induction of ACF by AOM, rats were fed the control diet (AOM) or the control diet containing 2% (AOM+2%Y) or 5% yam (AOM+5%Y) for 10 weeks. Colons were removed, and ACF number and multiplicity were determined. The number and distribution of ACF in the colon are shown in Table 4. The mean number of ACF/colon in the rats administered AOM alone was 171.5±55.1 ACF/colon. However, compared to the AOM group, dietary feeding of yam at a dose of 2% (118.6±36.8 ACF/colon) or 5% (110.8±23.8 ACF/colon) reduced AOM-induced ACF formation in a dose-dependent manner. More importantly, the number of ACF containing 1, 2, 3, and 4 or more crypts was decreased by yam-feeding compared to the AOM only treated group. Compared to the AOM group, rats in the AOM+5%Y group had 35% fewer one-crypt foci, 37% fewer two-crypt foci, and 42% fewer three-crypt foci (P<0.05). In addition, rats in the AOM+2%Y group had 45% fewer ACF with four or more crypts (P<0.05) compared to the AOM group. The number of ACF in the proximal region of the colon was particularly reduced in yam-supplemented groups. On average, there were 47.5±16.2 ACF in the proximal region of the colons of rats in the AOM+2%Y group and 40.8±30.4 ACF in the proximal region of the colons of rats in AOM+5%Y group, while there were 81.8±28.4 ACF in the proximal region of the colons of rats in the AOM group.

ACF are microscopic lesions that are postulated to precede the development of adenomas and are consid-

Table 3. Effect of yam on body weight, food intake, liver weight, and colon length in rats

Group	Body weight gain (g/rat)	Food intake (g/day/rat)	FER (%)	Liver (g)	Colon length (cm)
AOM	228.0±33.9^{ns}	11.5±1.6^{ns}	21.8±2.6^{ns}	7.10±0.54^{ns}	4.22±0.38^{ns}
AOM+2%Y	223.7±13.3	12.1±1.6	21.0±1.2	6.97±0.47	4.33±0.54
AOM+5%Y	224.4±19.0	11.7±1.0	21.0±1.5	7.15±0.59	4.38±0.72

Values are mean±SD (n=12).
FER: Food efficiency ratio (%)=body weight gain (g)/food intake (g)x100.
ns: not significant.

Table 4. Effect of yam on AOM-induced colonic ACF formation in rats

	AOM	AOM+2%Y	AOM+5%Y
Total no. of ACF/colon	171.5±55.1[a]	118.6±36.8[ab]	110.8±23.8[b]
Crypt multiplicity of ACF			
1 crypt	39.1±14.4[ns]	27.5±14.0	25.4±6.9
2 crypts	45.0±16.7[ns]	37.9±17.0	28.5±10.4
3 crypts	34.4±11.8[a]	24.9±7.9[ab]	19.8±6.3[b]
≥4 crypts	53.0±16.3[a]	29.2±11.9[b]	35.8±17.1[ab]
Distribution of ACF			
Proximal colon	81.8±28.4[a]	47.5±16.2[b]	40.8±30.4[b]
Middle colon	64.0±25.5[ns]	49.0±18.0	41.2±27.1
Distal colon	25.4±12.3[ns]	22.4±11.5	27.4±5.6

Values are mean±SD (n=6). Values are the number of ACF.
Different superscripts (a,b) within a row indicate significant differences among groups (P<0.05).
ns: not significant.

Table 5. Effect of yam on erythrocyte antioxidant enzyme activities in rats

Antioxidant enzymes	AOM	AOM+2%Y	AOM+5%Y
GSH (nmol/mg Hb)	3.74±2.32[a]	5.33±3.25[a]	15.29±8.90[b]
SOD (U/mg Hb)	215.3±36.2[a]	193.3±26.7[a]	159.1±14.0[b]
GPx (μmole/min/mg Hb)	1.15±0.19[a]	1.40±0.22[b]	1.50±0.19[b]
CAT (μmole/min/mg Hb)	3.13±0.53[a]	4.26±0.61[b]	4.02±0.54[b]

Values are mean±SD (n=12).
Different superscripts (a,b) within a row indicate significant differences among groups (P<0.05).

ered to be the earliest premalignant lesions of colon carcinogenesis (20). ACF multiplicity increases with time and appears to be a predictor of tumor outcome (22). These observations justify the use of the colonic ACF assay as a tool for the screening of potential chemopreventive agents in colon tumors prior to the evaluation of such agents in clinical studies.

Supplementation with yam not only inhibited the growth of ACF by decreasing the total number of ACF, and the number of ACF with multicrypts, but also affected the distribution ACF within the colon, especially in the proximal colon. Typically, ACF develops as early as 2～4 weeks after carcinogen administration and appears predominantly in the middle-colon during early time points. With time, ACF begin to appear in the distal and proximal colon (23). Our results clearly demonstrate that the dietary feeding of yam prior to carcinogenesis significantly inhibits AOM-induced colonic ACF formation in rats. The suppressive effect of yam on total colonic ACF formation was also associated with a reduction in crypt multiplicity. ACF with crypt multiplicity are more likely to persist, increase in size through multiplication and develop into malignant tumors (24). Also, yam may intervene with the development of ACF at later time points by suppressing of the number of ACF that from in the proximal colon.

Effect of yam on antioxidant enzyme activities

The effect of yam on erythrocyte antioxidant enzyme activities (Table 5) was evaluated in this study. The concentration of GSH was 4-fold higher in the 5% yam group and the activities of GPx and CAT were 25% and 36% higher, respectively, in the 2% yam group, and 30% and 28% higher, respectively, in the 5% yam group. However, SOD activity was significantly decreased by yam supplementation.

Red blood cell (RBC) changes have been detected in a number of human pathologic conditions and after exposure to xenobiotics that induce oxidative stress. Erythrocytes are in constant contact with potentially damaging levels of oxygen, but under normal conditions their metabolic and antioxidant defense systems are capable of reversing any damage incurred (25). The antioxidant defense system includes SOD, CAT, GPx, glutathione-*S*-transferase, and glutathione reductase, and GSH. Oxidation of erythrocytes is associated with membrane injury, methemoglobin formation, osmotic fragility, and cell destruction. Oxidative stress in RBCs can be an indicator of RBC-related disorders or systemic oxidative stress. For example, decreased GPx and CAT activities and increased Cu/Zn-SOD activity have been reported in the erythrocytes of patients with gastric cancer. Several studies have reported that mucilage and phenolic compounds extracted from yam exhibit strong hydroxyl and superoxide anion radical scavenging activities (26-28). In this study, the erythrocyte levels of GSH, GPx, and CAT in yam-administered rats were significantly increased, whereas SOD activity was similar to that of the

AOM group. Our study shows that yam could act as a free radical scavenger and enhance the function of oxidative stress protective systems (e.g., GSH, GPx, and CAT). We hypothesize that the superoxide scavenging activity of yam decreased erythrocyte levels of the substrate for SOD, thus preventing yam-induced changes in erythrocyte SOD activity.

Effect of yam on antioxidant enzyme gene expression

We evaluated the mRNA expression of antioxidant enzymes in the colonic mucosa (Table 6). Compared to the AOM group, Cu/Zn-SOD expression was 2.45-fold greater in the 2% yam-supplemented group and 2.12-fold greater in the 5% yam-supplemented. Mn-SOD and GPx expressions were 1.51-fold and 1.83-fold greater, respectively, in the 5% yam-supplemented group. CAT expression was also increased in the yam-treated groups.

The cytotoxicity of AOM, a potent colon cancer inducing agent, is considered to be mediator of oxidative stress (29). Two characteristics of tumor cells are increased ROS generation and decreased capacity to eliminate ROS (30). Cancer cells use ROS to stimulate proliferation, invasion, migration and angiogenesis. Importantly, cancer cells have also developed mechanisms to use ROS to evade apoptosis (31). Antioxidant enzymes are a major mechanism for protecting cells against endogenous and exogenous toxic compounds such as ROS and chemical carcinogens (32). CAT, GPx, and SOD are considered to be "first line of defense" antioxidants and are extremely important in the prevention of oxidative damage. Treatment with carcinogens or tumor promoters usually decreases levels of antioxidant enzymes in the colon. A decline in these enzymes may enhance subsequent oxidative stresses, leading to increased oxidation of DNA bases and subsequent mutagenesis and carcinogenesis (33,34).

In this study, the expression of GPx, Cu/Zn-SOD and Mn-SOD in the colon was significantly up-regulated. The expression of CAT was also up-regulated, but not significantly. SOD catalyzes the dismutation of superoxide anions into hydrogen peroxide, while GPx and CAT catalyzes the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and oxygen (34). Many studies have demonstrated that yam exhibits a wide range of biological activities including antioxidant and anti-inflammatory effects (15-19). Many biologically active compounds in yam, including polyphenols, flavonoids, steroid saponins, and mucilage proteins, have direct ROS scavenging ability and stimulate the upregulation of antioxidant enzyme expression, thus contributing to the antioxidant activity of yam (14). Our results suggest that yam has an antioxidant capability in animals experiencing AOM-induced oxidative stress and that this effect occurs through the modulation of the activities and gene expression of antioxidant enzymes.

Effect of yam on the gene expression of inflammatory mediators

Table 7 shows the colonic mucosal gene expression of inflammatory mediators, NF-κB, iNOS, COX-2, TNF-α, and IL-1β. Compared to the AOM group, the relative mRNA expression of NF-κB/p65 was significantly down-regulated to 0.53 and 0.57 in the 2% and 5% yam-supplemented groups, respectively. The expression of inducible inflammatory enzymes, iNOS (0.16 in AOM+2%Y, 0.14 in AOM+5%Y) and COX-2 (0.03 in AOM+

Table 6. Effect of yam on gene expression of antioxidant enzymes in the colonic mucosa of rats

Antioxidant enzyme	AOM	AOM+2%Y	AOM+5%Y
Cu/Zn-SOD			
ΔC_T	1.04±1.07[a]	−0.25±0.69[b]	−0.04±0.34[b]
$2^{-\Delta\Delta C_T}$	1.00	2.45	2.12
Mn-SOD			
ΔC_T	3.96±0.70[ab]	4.22±1.06[a]	3.36±0.57[b]
$2^{-\Delta\Delta C_T}$	1.00	0.83	1.51
GPx			
ΔC_T	1.73±0.86[a]	1.36±0.23[ab]	0.86±0.19[b]
$2^{-\Delta\Delta C_T}$	1.00	1.30	1.83
CAT			
ΔC_T	2.22±0.43[ns]	2.28±0.89	1.78±0.35
$2^{-\Delta\Delta C_T}$	1.00	0.96	1.36

Values are mean±SD (n=6).
Different superscripts (a,b) within a row indicate significant differences among groups ($P<0.05$).
ΔC_T, mean of C_T value for the target gene − mean of C_T value for β-actin; $2^{-\Delta\Delta C_T}$, differential gene expression in the target sample compared to the normal sample counterpart.
ns: not significant.

Table 7. Effect of yam on gene expression of NF-κB, inflammatory enzymes and cytokines in the colonic mucosa of rats

	AOM	AOM+2%Y	AOM+5%Y
NF-κB/p65			
ΔC_T	3.36±0.35[a]	4.28±0.44[b]	4.16±0.60[b]
$2^{-\Delta\Delta C_T}$	1.00	0.53	0.57
iNOS			
ΔC_T	8.62±0.61[a]	11.2±0.39[b]	11.5±0.97[b]
$2^{-\Delta\Delta C_T}$	1.00	0.16	0.14
COX-2			
ΔC_T	5.70±1.27[a]	10.8±1.91[b]	9.42±0.50[b]
$2^{-\Delta\Delta C_T}$	1.00	0.03	0.08
TNF-α			
ΔC_T	3.29±1.24[a]	3.07±0.50[a]	5.42±0.52[b]
$2^{-\Delta\Delta C_T}$	1.00	1.16	0.35
IL-1β			
ΔC_T	3.98±0.48[a]	4.09±0.45[a]	5.20±0.90[b]
$2^{-\Delta\Delta C_T}$	1.00	0.93	0.43

Values are mean±SD (n=6).
Different superscripts (a,b) within a row indicate significant differences among groups ($P<0.05$).
ΔC_T, mean of C_T value for the target gene − mean of C_T value for β-actin; $2^{-\Delta\Delta C_T}$, differential gene expression in the target sample compared to the normal sample counterpart.

2%Y, 0.08 in AOM+5%Y) was also significantly suppressed by the yam-supplemented diet. The expression of pro-inflammatory cytokines, TNF-α and IL-1β, was significantly inhibited to 0.35 and 0.43, respectively, in the 5% yam-supplemented group compared to the AOM group.

NF-κB is an important regulator of cellular responses because it is a "rapid-acting" primary transcription factor (i.e., a transcription factor that is present in cells in an inactive state and do not require new protein synthesis to become activated). This allows NF-κB to be a first responder to harmful cellular stimuli. Known inducers of NF-κB activity are highly variable and include ROS, TNF-α, IL-1β, and bacterial lipopolysaccharides (LPS) (35). Expression of NF-κB has been shown to promote cell proliferation, whereas inhibition of NF-κB activation blocks cell proliferation. Thus, the use of NF-κB as a target for anticancer therapy is currently a subject of active research by pharmaceutical companies (36). In the present study, supplementation with yam suppressed the expression of NF-κB in rats with AOM-induced colon carcinogenesis; this suppressive effect is one of the key mechanisms for the prevention of colorectal tumors by yam supplementation.

TNF-α is one of the most important proinflammatory cytokines. TNF-α is primarily produced by monocytes and macrophages, is secreted during the early phase of acute and chronic inflammatory diseases, and triggers diverse inflammatory cascades such as the secretion of other cytokines, including IL-1, IL-6, and IL-8 (37). Several studies have shown that the expression of TNF-α during inflammation is dependent upon the activation of NF-κB. Cells exposed to TNF-α activate NF-κB, leading to the expression of inflammatory genes, such as COX-2, lipoxygenase-2, cell-adhesion molecules, inflammatory cytokines, chemokines, and iNOS (37,38). In addition, TNF-α has been found to mediate initiation, promotion, and metastasis for most tumor cells (36). In agreement with these observations, mice deficient in TNF-α are resistant to skin carcinogenesis (39). This study revealed that yam has several anti-inflammatory abilities, including its ability to down-regulate the expression of TNF-α and IL-1β in AOM-induced rats and its ability to inhibit NF-κB activation through the suppression of inflammatory cytokines. Therefore, our data suggest that yam may have the potential to prevent colon carcinogenesis by modulation of inflammatory processes.

The enzyme responsible for prostaglandins (PGs) synthesis exists in two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed in many tissues, but the expression of COX-2 is regulated by mitogens, tumor promoters, cytokines, and growth factors. Overexpression of COX-2 and subsequent PGs production have been implicated in colon carcinogenesis (37). Thus, dietary agents that can suppress NF-κB have the potential to inhibit COX-2 expression. Nitric oxide synthase (NOS) is responsible for the release of the gaseous free radical nitric oxide (NO) during the transformation of L-arginine to L-citrulline. Excessive and prolonged iNOS-mediated NO generation has been linked to inflammation and tumorigenesis. iNOS is overexpressed in human colon tumors and in rats treated with a colon carcinogen (40). COX-2 and iNOS expression are regulated by the transcription factor, NF-κB (38). Several recent studies have reported that the anti-inflammatory activity of yam occurs via the inhibition of iNOS and COX-2 expression (15-18). In the present study, yam substantially reduced gene expression of COX-2 and iNOS.

In conclusion, the results of our study strongly suggest that yam plays a protective role against AOM-induced colon tumors. In addition, our findings indicate that yam exerts its protective effect by inhibiting ACF formation (a preneoplastic marker), enhancing the antioxidant defense system, and hampering colonic inflammation by inhibiting the NF-κB pathway. Inhibition of NF-κB subsequently down regulates inflammatory markers such as COX-2, iNOS, TNF-α, and IL-1β. These effects may be attributed to the antioxidant and anti-inflammatory nature of yam. However, further investigation into the altered activity of antioxidant enzyme and the altered content of signal transduction proteins in the colonic mucosa is necessary to further elucidate the potential of yam as a chemopreventive agent against colon tumors.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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