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Ellagic Acid Modulates Antioxidant Status, Ornithine Decarboxylase Expression, and Aberrant Crypt Foci Progression in 1,2-Dimethylhydrazine-Instigated Colon Preneoplastic Lesions in Rats

Kasinathan Nirmal Kumar, Subramaniya Bharathi Raja, Natarajan Vidhya, and Sivachithamparam Niranjali Devaraj*

Department of Biochemistry, School of Life Sciences, Maraimalai (Guindy) campus, University of Madras, Chennai 600025, Tamilnadu, India

ABSTRACT: Chemoprevention offers a novel approach to control the incidence of colorectal cancer (CRC), which is a fatal cause of malignancies in both Western and Asia countries. Ornithine decarboxylase (ODC) functions as a cell transition factor by regulating the biosynthesis of polyamines, which, allied with aberrant crypt foci (ACF) proliferation, cause early lesions of CRC. This study exemplifies the chemopreventive efficacy of ellagic acid (EA) in 1,2-dimethylhydrazine (DMH) initiated CRC in rats. Subcutaneous injection of DMH (40 mg/kg body weight twice a week for 2 weeks) to the rats resulted in elevated expression of ODC, a genetic marker for CRC, and its transcription factor myelocytomatosis oncogene (c-myc). Furthermore, increased levels of lipid peroxidation and hydroperoxides with diminished levels of antioxidants including superoxide dismutase, catalase, and reduced glutathione were also observed in the tissues of DMH-intoxicated rats. Oral supplementation of EA significantly influences maintenance of antioxidant status and transcriptional inactivation of ODC expression, reducing ACF proliferation and/or progression, thus signifying the chemopreventive efficacy of EA against CRC.

KEYWORDS: *aberrant crypt foci, colon cancer, DMH, ellagic acid, preneoplastic colon cancer*

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies in the Western world, whereas in Asia it is the third most fatal cause of all malignant diseases in both genders.[1] CRC development is a multistep process, and the most relevant etiologies are Western diet, sedentary lifestyle,[2] and genetic abnormalities,[3] characterized by a series of pathological alterations arising from discrete microscopic mucosal lesions such as aberrant crypt foci (ACF).[4] In 90% of cases, early-stage disease can be cured by surgery alone. However, without postsurgical treatment, 45% of patients succumb to regional disease within 5 years of diagnosis.[5]

ACF are presumed precursors (preneoplastic lesions) of colon cancer. They are clusters of abnormal tube-like glands in the lining of the colon and rectum consisting of enlarged crypts with thickened layers of epithelial cells, increased pericryptal spaces, and irregular or slit-shaped lumina, which are visible by methylene blue staining and microscopic examination without sectioning or histological preparation.[4,6] Thus the detection of ACF as an endpoint structure is a best tool to study the pathophysiology of preneoplastic lesions of colon before phenotypically characterized cancer,[7] our investigations were directed toward primary prevention of these ACFs that progress to colon cancer.

The search for an effective mode of prevention of CRC is still in progress. Despite much progress in recent years, this situation is due to limited chances for cure by chemotherapy. Modern scientific concern is projected toward the chemopreventive potential of naturally occurring components of the diet, which can prevent the formation and proliferation of ACF.[8] Plant phytochemicals are receiving considerable attention for their potential role in reducing cancer risk. Most established drugs endure inadequate specificity toward tumor cells by developing drug resistance and menacing side effects. In the 1980s and 2002, 69% of approved anticancer drugs are either natural products or their derivatives.[9]

Ellagic acid (EA, $C_{14}H_6O_8$), a naturally occurring dietary polyphenolic compound present in several plants and fruits (strawberries, raspberries, muscadine grapes, and nuts), has been shown to reduce the incidence of a variety of carcinogen-induced tumors[10,11] through diverse mechanisms. EA is also a primary constituent of several tannin-bearing plants that produce the category of tannins known as ellagitannins (ETs).[12] EA is a dimeric derivative of gallic acid, which occurs in fruits and nuts either in the free form, as EA-glycosides, or as bound ETs.[13] EA has antioxidant, antifibrotic, and anticarcinogenic properties.[14,15] The anticarcinogenic effect of EA was shown in several types of cancers including skin and esophageal, and there are many mechanisms associated with the colon cancer chemopreventive effects of EA.[16−18]

The present study was executed to reveal experimentally that EA modulates ACF proliferation, which is considered to be the earliest hallmark of colon carcinogenesis and also acts as an antioxidant enhancer to regress the 1,2-dimethylhydrazine (DMH) initiated carcinogenesis (preneoplastic lesion) in rats.

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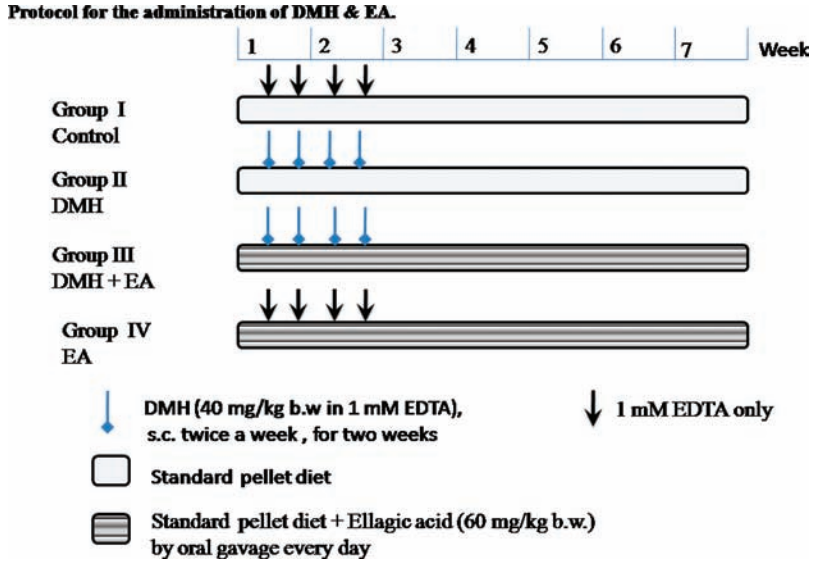

Figure 1. Protocol for the administration of DMH and EA.

Table 1. Primer Sequences for Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

accession no.	gene name	gene symbol	primer	product size (bp)
NM_012615	ornithine decarboxylase	ODC	left: 5′- AAAGTTGGTTTTGCGGATTG-3′ right: 5′- AGGGTCAGTACACCCACTGC-3′	169
NM_012603	myelocytomatosis oncogene	c-myc	left: 5′-CGAGCTGAAGCGTAGCTTTT-3′ right: 5′-CTCGCCGTTTCCTCAGTAAG-3′	170
NM_017008	glyceraldehyde-3-phosphate dehydrogenase	GAPDH	left: 5′-ACAGTCAGCCGCATCTTCTT-3′ right: 5′-TTGATTTTGGAGGGATCTCG-3′	312

MATERIALS AND METHODS

Chemicals. 1,2-Dimethylhydrazine and ellagic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Animals. Male Wistar rats aged 6 weeks and weighing 160 g were obtained from the Laboratory Animal Maintenance Unit, Tamilnadu Animal Science and Veterinary University, Chennai, India. The animals were acclimatized to the laboratory conditions for a period of 2 weeks. Thereafter, the rats were maintained under controlled conditions of temperature (24 ± 2 °C) and humidity (50 ± 10%). They were allowed access to standard rat feed (Hindustan Lever Ltd., Bangalore, India) and water, ad libitum. Animal experiments were carried out in strict accordance with the guidelines set by the Institutional Animal Ethical Committee9 (No. 01/036/09) for the use of small animals in biomedical research at the University of Madras, Chennai, India.

Experimental Procedure. The animals were randomly divided into four groups (n = 6 per group). Group I rats (control) received subcutaneous (sc) injections of 1 mM EDTA throughout the course of the experiment. Group III and IV rats were given 60 mg/kg body weight of EA in distilled water orally by gavage throughout the experimental period. Group II and III rats received four sc injections of DMH dissolved in 1 mM EDTA (adjusted to pH 6.5 with 1 mM NaOH) for 2 weeks (40 mg/kg body weight twice a week).

The time schedule and administration of DMH/EA are given in Figure 1. At the end the of experimental period (7 weeks), animals were sacrificed by cervical dislocation after an overnight fast. The colon was opened longitudinally and immediately fixed for analyzing ACF.

Identification of ACF. The method of Bird[19] was used to assess ACF. The colon was removed, flushed with saline, and opened from the anus to cecum. For counting the number of ACF, the opened colon was fixed flat on a filter paper in 10% buffered formalin for 24 h. Fixed colons were stained with 0.5% methylene blue in saline. ACF were identified by their increased size, irregular and dilated luminal opening, thickened epithelial lining, and pericryptal zone. The number of ACF/colon and the number of aberrant crypts in each focus were determined microscopically at 40× magnification.

Biochemical Assay. Colon tissue was immediately excised, weighed, and then homogenized in 0.1 M Tris-HCl buffer (pH 7.4). Homogenates were taken for the analyses described below. The levels of lipid peroxides were estimated in colon tissue according to the method of Ohkawa et al.[20] Hydroxy radical production was measured in tissue homogenate according to the method of Cederbaum and Cohen.[21] Superoxide dismutase (SOD) was assayed following the method of Misra and Fridovich.[22] Catalase (CAT) was assayed according to the method of Takahara et al.[23] Glutathione reductase (GR) was assayed by using the method of Staal et al.[24] The activity of glutathione peroxidase (GPx) was assayed by using the method of Rotruck et al.[25] Reduced glutathione (GSH) was assayed according to the method of Moron et al.[26] The protein content was determined by using the Lowry et al.[27] method. The sera of the control and experimental groups were isolated, and the level of cathepsin-D (CD) was assayed according to the method of Sapolsky et al.[28] Alkaline phosphatase (ALP) was determined according to the method of King[29] and lactate dehydrogenase (LDH) also according to the method of King.[30]

DNA Fragmentation Analysis. DNA was isolated according to the method of Higuchi and Linn.[31] Colon homogenate was extracted with phenol followed by extraction with phenol/chloroform (1:1) mixture. The DNA in the aqueous phase was precipitated and washed with 70% ethanol and resuspended in TE buffer. Analysis of DNA fragments was performed in a 1.2% agarose gel in 1× Tris−borate−EDTA buffer.

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Histological Analysis. A portion of the colonic tissue was fixed in 10% formaldehyde solution and embedded in paraffin. Sections were cut at 4 mm thickness, stained with hematoxylin and eosin, and viewed under light microscope for histological changes.

RNA Isolation and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from colon tissues of normal and cancer-bearing animals. The method of RNA extraction was similar to the TRIzol RNA extraction protocol. The concentration of RNA extracted was determined at 260 nm using a U-2000 spectrophotometer. Primer sequences were designed using the Primer 3 software for optimal primer design and were synthesized commercially. The primer sequences for ODC and c-myc are given in Table 1. Ten microliters of each amplification reaction was analyzed by electrophoresis using 1.2% agarose gel in the presence of 5 ng/mL ethidium bromide. DNA was detected under UV light. Results were expressed as a ratio of the DNA signal relative to the corresponding GAPDH signal from each sample.

Statistical Analysis. All of the grouped data were significantly evaluated with SPSS/10 software. Hypothesis testing methods included one-way analysis of variance followed by a least significant difference test. Significant differences were subjected to Duncan's test to compare the means of two specific groups. p values of <0.05 were considered to indicate statistical significance. All of these results were expressed as the mean ± standard deviation (SD) for six animals in each.

RESULTS

Effect of DMH and EA on Body Weight and Food Intake of the Control and Experimental Animals. Table 2 represents the body weight change and food intake of control and experimental groups. DMH-induced animals (group II) showed significantly ($p < 0.05$) reduced body weight when compared to control animals. Body weight loss was reversed and returned to normal upon EA treatment (group III). No significant difference was found between the control (group I) rats and EA alone treated rats (group IV).

Table 2. Effect of DMH and EA on Body Weight and Food Intake of the Control and Experimental Animals[a]

group/ parameter	initial body wt (g)	final body wt (g)	food consumption (g/rat/day)
control	165.5 ± 1.5 a	186.1 ± 8.01 a	19.5
DMH	162.6 ± 4.04 b	174.6 ± 7.9 b	17.5
DMH + EA	166.6 ± 3.7 c	183.5 ± 3.3 c	18.2
EA	164.5 ± 3.9 ns	187.83 ± 6.5 ns	20.1

[a]Values are expressed as the mean ± SD. Comparisons: b, control vs DMH; c, DMH vs DMH + EA; ns, nonsignificant, $P < 0.05$.

Effect of EA on Antioxidant Status. Table 3 represents the levels of antioxidants SOD, CAT, GPx, GR, and GSH in the colon tissue homogenate of rat in control and experimental groups. Enzymatic antioxidants SOD, CAT, GPx, GR, and GSH were observed to be significantly ($p < 0.05$) reduced in DMH induction (group II) rats, whereas EA supplementation significantly ($p < 0.05$) increased the antioxidant enzymes in group III rats when compared to group II. No significant difference was found between the control rats (group I) and EA alone treated rats (group IV).

Effect of EAcid on Colonic Lipid Peroxidation (LPO) and Hydroxyl Radical (•OH) Levels in Control and Experimental Groups of Animals. Figure 2 shows the levels of LPO and •OH in colonic tissue in control and experimental animals. Significant ($p < 0.05$) increases in LPO and •OH levels were seen in DMH-administered (group II) animals when compared to control animals. EA treatment resulted in significantly ($p < 0.05$) decreased LPO and OH levels in group III animals. No significant difference was found between control rats and EA alone treated rats (group IV).

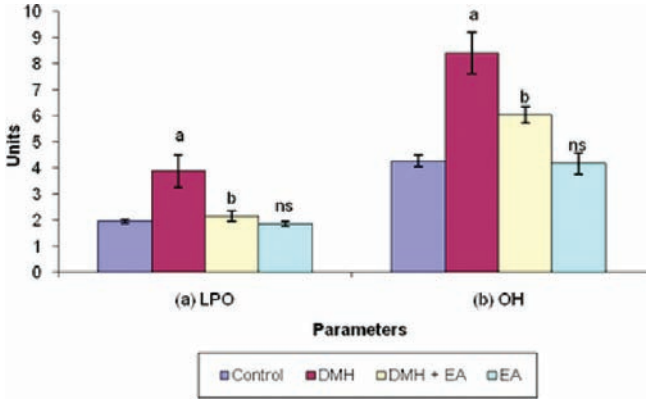

Figure 2. Effect of ellagic acid on colonic lipid peroxidation (LPO) and hydroxyl radical (•OH) levels in control and experimental animals: (a) levels of lipid peroxidation in colon of control and experimental animals (LPO, mol of MDA released/mg of protein); (b) levels of hydroxyl radical in the colon of control and experimental groups (•OH, ng/mg of protein). Values are expressed as the mean ± SD. Comparisons: a, control vs DMH and EA; b, DMH vs DMH + EA, $P < 0.05$; ns, nonsignificant.

Effect of EA on Pathophysiological Marker Enzyme Levels in Control and Experimental Animals. Figure 3 shows the pathophysiological marker enzyme levels, namely, cathepsin D (CD), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), in the serum of the control and experimental groups. In contrast with the control group of animals, significant ($p < 0.05$) increases in CD, ALP, and LDH were seen in DMH-administered animals (group II). Upon treatment with EA, significant ($p < 0.05$) decreases of these

Table 3. Effect of DMH and EA on Antioxidant Status in Control and Experimental Animals[a]

parameter	control	DMH	DMH + EA	EA
SOD	7.64 ± 0.23	4.19 ± 0.49 a	5.14 ± 0.55 b	7.71 ± 0.44 ns
CAT	62.9 ± 2.64	37.4 ± 4.37 a	45.6 ± 4.4 b	63.2 ± 3.27 ns
GPx	104.7 ± 7.83	70.5 ± 7.12 a	83.1 ± 4.65 b	105.3 ± 4.43 ns
GR	153.2 ± 3.64	106.4 ± 11.08 a	133.3 ± 11.23 b	153.9 ± 6.41 ns
GSH	44.2 ± 1.85	18.38 ± 1.76 a	25.28 ± 1.78 b	45.4 ± 1.84 ns

[a]Values are expressed as the mean ± SD for six rats in each group. Comparison: a, control vs DMH; b, DMH vs DMH + EA; ns, nonsignificant, $P < 0.05$. SOD activity is expressed as units/mg protein; 1 unit is equal to the amount of enzyme that inhibits the autoxidation reaction by 50% of epinephrine/min/mg of protein. CAT, μmol of H_2O_2 consumed/min/mg of protein. GPx, μmol of glutathione consumed/min/mg of protein. GR, μmol of NADPH oxidized/min/mg of protein. GSH, mmol/mg protein.

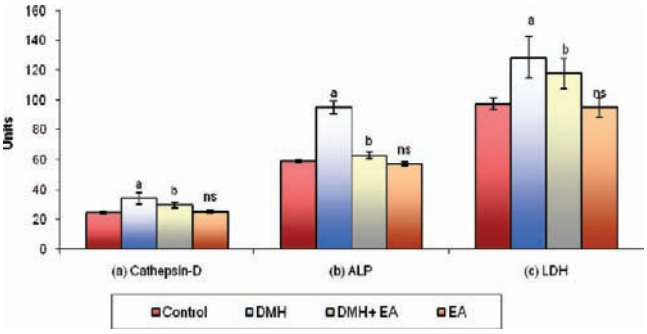

Figure 3. Effect of ellagic acid on pathophysiological markers enzymes level in control and experimental animals: (a) cathepsin D (CD), nmol of tyrosine liberated/min/mg protein/h; (b) alkaline phosphatase (ALP), μmol of phenol liberated/min/mg protein; (c) lactate dehydrogenase (LDH), μmol of pyruvate liberated/min/mg protein. Values are expressed as the mean ± SD for six rats in each group. Values are statistically significant at $p < 0.05$; ns, not significant; a, control vs DMH; b, DMH vs DMH + EA.

enzyme levels were seen in group III animals. No significant difference was found between control rats and EA alone treated rats (group IV).

Effect of EA on the Cellular DNA Damage and DNA Fragmentation in Control and Experimental Groups of Animals. Figure 4 illustrates DNA fragmentation analysis.

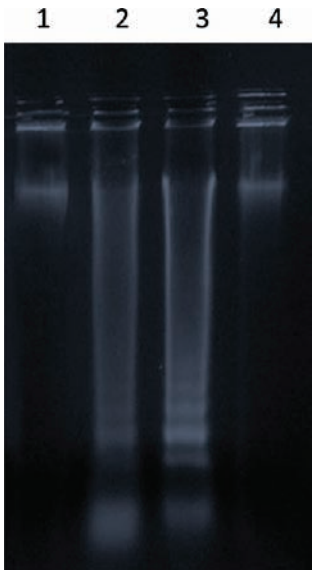

Figure 4. Effect of ellagic acid on the cellular DNA damage and DNA fragmentation in control and experimental animals. Lanes: 1, control; 2, DMH; lane 3, DMH + EA; 4, EA.

Because DNA fragmentation is a unique marker of cells undergoing apoptosis, we analyzed the DNA fragmentation in control and experimental groups. Rats treated with EA showed significantly inhibited DMH-induced DNA damage.

Influence of EA on the Incidence of ACF. The incidence of ACF and AC multiplicity are shown in Table 4. ACFs developed in rats treated with DMH, with or without EA treatment (groups III and II). ACF was not observed in control (group I) and EA (group IV) groups at the end of the experimental period. The frequency of ACF and multiplicity of AC per colon in the DMH + EA group (III) were significantly ($p < 0.05$) lower as compared to DMH group (II).

Histological Analysis. Figure 5 shows the histological view of ACF in the control and experimental animals.

The tissue sections of control and treated rat colon were stained with hematoxylin−eosin and viewed under an optical microscope for identification of crypts and ACF. Photomicrographs of control rat colon (Figure 5A) showed normal crypt, DMH-induced rats (Figure 5Bi) with ACF with one crypt, ACF (Figure 5Bii) with two crypts, ACF (Figure 5Biii) with three crypts, and ACF (Figure 5Biv) with four crypts. Administration of EA to DMH-treated rats (Figure 5C) showed reduced DMH-induced abnormalities.

Effect of EA on Myelocytomatosis Oncogene (c-myc) and Ornithine Decarboxylase (ODC) Expression. Figure 6 shows the mRNA expression levels of ODC and c-myc during DMH-induced carcinogenesis. RT-PCR showed that the expressed ODC and c-myc were significantly increased in DMH alone treated group when compared to the control group. DMH-induced group III animals when administered EA showed significantly decreased expression of ODC and c-myc compared to DMH-induced group II.

DISCUSSION

Various aspects of carcinogenesis can be mimicked in experimental animal models, providing valuable information on the process of disease progression and treatment. The most frequently used experimental model of colon carcinogenesis in rodents is by administering the genotoxic chemical DMH,[32] which resembles the characteristics of human carcinogenesis.

The effective approach of prevention of CRC is still in progress. One important issue is reducing mortality, which involves the use of oral agents that can prevent colorectal cancer. Such pharmacological intervention is called chemoprevention. Studies involving plant constituents and their derivatives showed significant effects on decreasing the incidence of ACF in vivo and have the ability to control tumor cells in vitro.[33]

For early lesion CRC the short-term ACF assay was used to identify agents that modulate the development of colonic neoplasms.[34] Our aim was to investigate the effect of EA on the

Table 4. Influence of DMH and EA on the Incidence of ACF[a]

			no. of foci containing			
group	incidence	total no. of ACF/colon	1 crypt	2 crypts	3 crypts	4 crypts
control	0/6	0	0	0	0	0
DMH	6/6	87.3 ± 23.55 a	39.8 ± 15.7 a	24.8 ± 17.4 a	11.8 ± 5.7 a	4.6 ± 1.8 a
DMH + EA	6/6	43.5 ± 7.06 b	22.8 ± 7.6 b	10.6 ± 4.3 b	6.6 ± 2.5 b	3.3 ± 1.75 b
EA	0/6	0	0	0	0	0

[a]Values are given as the mean ± SD for six rats in each group. Values not sharing a common letter differ significantly at $p < 0.05$. a, significantly different from control, $p < 0.05$; b, significantly different from DMH, $p < 0.05$.

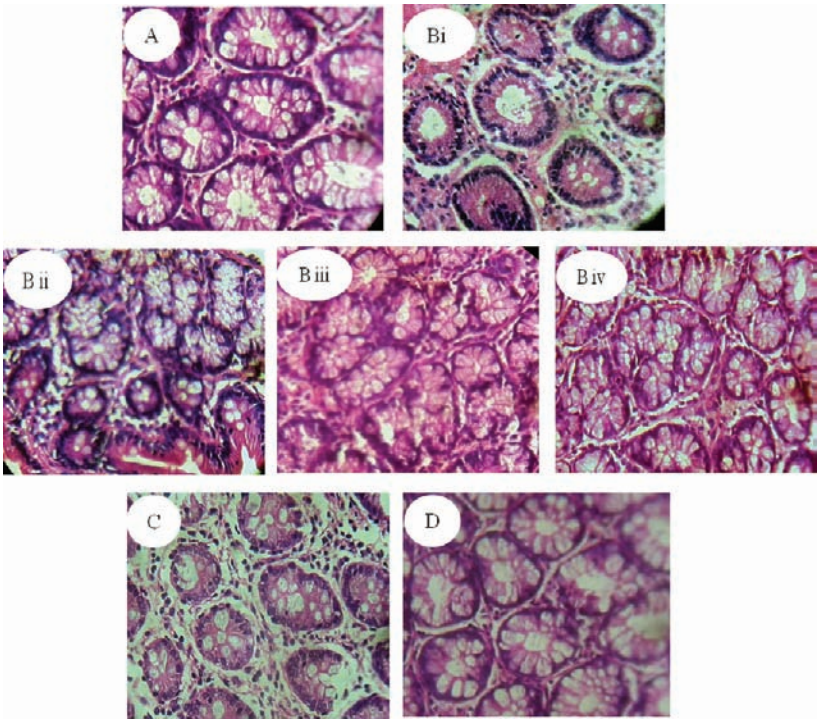

Figure 5. Photomicrography of colon tissue of control and experimental animals; histopathological analysis of control and experimental rat colon: (A) (control) normal histological appearance of colon tissue; (B) (DMH group) i, aberrant crypt focus (ACF) with 1 crypt; ii, ACF with 2 crypts; iii, ACF with 3 crypts; iv, ACF with 4 crypts; (C) (DMH + EA group) sustained normal crypt architecture; (D) (EA group) histological appearance was similar to that of control.

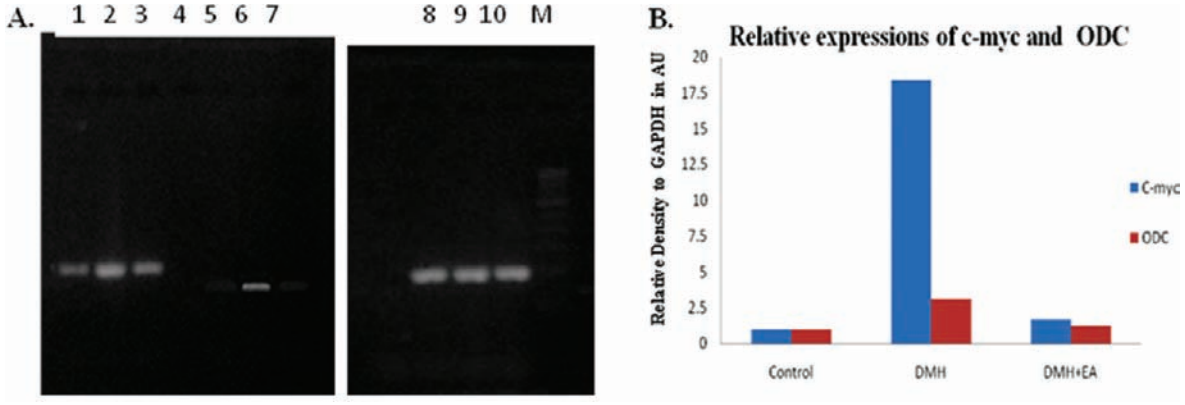

Figure 6. Effect of ellagic acid on c-myc and ornithine decarboxylase (ODC) expression asssy by RT-PCR: (A) c-myc and ODC mRNA expression analysis by RT-PCR (lanes: 1−3, c-myc; 5−7, ODC; 8−10 GAPDH; 1, 5, and 8, control (group I); 2, 6, and 9, DMH (group II); 3, 9, and 10, DMH induced + EA (group III); M, marker); (B) densitometry analysis.

severity of crypt foci development and progression by enhancing the antioxidant status and inhibiting the expression of ODC in the initiation phase of DMH -induced intestinal preneoplasm.

The initiation and progression of carcinogenic transformation are mainly due to the contribution of reactive oxygen species (ROS) and organic free radical intermediates formed from many carcinogens.[35] The generated ROS can directly produce DNA damage, causing carcinogenic promotion either by arrest or induction of transcription and genomic instability.[36] A significant increase in the level of hydroxyl radical was observed in DMH-administered group, which is due to oxidative metabolism of DMH to electrophilic diazonium ions by gut microbes.[37] EA had attenuated the DMH-induced hydroxyl radical accumulation by enhancing the antioxidant status.[18]

LPO, a free radical mediated process that is a well-established mechanism of cellular injury, is used as an indicator of oxidative stress. The hallmarks of LPO are formation of lipid radicals, rearrangement of the unsaturated lipids that leads to the formation of a variety of degraded products such as alkanes, malondialdehyde (MDA), conjugated dienes, and lipid hydroperoxides, and, eventually, damage to cells.[38] The mutagenic and genotoxic agent MDA may contribute to the development of human cancer.[39] The most commonly used method for monitoring LPO is by determination of MDA in the form of TBARS.[40] In the present study, increased LPO in group II (Figure 2) cancer-bearing animals may be due to the excessive free radicals produced by administration of DMH, whereas

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significantly decreased levels of lipid peroxides were seen in EA-treated group III induced with DMH. This contributed to the decreased level of hydroxyl radical in EA treatment of DMH-induced rats.

Disturbance of dynamic balance between pro-oxidants and antioxidants leads to oxidative stress. The primary defenses in maintaining this balance are provided by the antioxidant enzymes such as SOD, CAT, GPx, and GR, which scavenge intermediates of oxygen reduction. Activities of antioxidant enzymes such as SOD, CAT, GPx, and GR along with the level of GSH were depleted in DMH-administered rat colon tissue (Table 3). The decreased activities of SOD and CAT are due to the aberrant increase in the levels of ROS and, thus, enhanced oxidative stress coupled with proliferation of colonocytes in colorectal malignant carcinoma.[41] On EA treatment, the activities of these enzymes were markedly elevated in group III animals when compared to group II (Table 3). This might be due to the direct reaction of EA with superoxide, hydroxyl, and alkoxyl radicals.

The decreased level of GPx in DMH-induced animals is due to the increased neutralization of H_2O_2 and singlet oxygen.[10] A large flux of H_2O_2 and/or $^{\bullet}OH$ causes disturbances in the GSH/GSSG ratio. Thus, EA restored its value in the case of DMH-treated animals (group III), and GSH was protected from depletion. Therefore, direct scavenging of free radicals is an important mechanism by which the EA may exert its cytoprotective effect, not only by sparing GSH utilization by free radicals but also by preventing the free radical mediated tissue damage.[18]

The progression of several human tumors, including colorectal cancer, involves overexpression of CD.[42] Normal colon functions were determined by ALP and LDH levels, which serve as prognostic tools, because frequent elevation of these markers was seen in CRC patients.[43] In our study, elevated activities of these serum markers were found in DMH-induced rats (group II) (Figure 3). EA treatment almost renormalized these levels, which is probably due to the oxidative stress modulating effect of EA.[44] Thus, supplementation of EA enhances the antioxidant status, which, in turn, reduces free radical formation and oxidative damage during initiation of CRC.

Free radicals formed in the cells, including oxygen free radicals, can oxidize a number of cellular constituents such as lipids, protein, and DNA. DMH itself does not attack DNA, however, during reduction; DMH metabolites can subsequently attack macromolecules and lead to DNA damage. There are numerous types of DNA damage such as strand breaks (single and double), which are mainly ascribed to ROS.[45] DNA fragmentation that occurs due to free radical formation is a hallmark of apoptosis. Hence, apoptosis was analyzed by visualizing the DNA fragmentation on ethidium bromide stained agarose gels. DNA shear was noted in DMH-induced animals (group II) when compared to control animals (group I), which might be attributed to the increase in DNA strand breaks mainly contributed by ROS (Figure 4). On the other hand, there was a ladder-like pattern in experimental animals treated with DMH and supplemented with EA (group III), indicating induction of apoptosis in cells with abnormal DNA content.

The microscopic observation of colon tissues reveals that crypts were normal without any ACFs in the control group of rats (Figure 5A). Due to increased free radical toxicity and lipid peroxidation, DMH-treated rats exhibited hyperplasic crypts with two to many aberrant foci (Figure 5Bi–iv). Dietary supplementation of EA during the initiation phase significantly inhibited DMH-mediated colon carcinogenesis as revealed by inhibition of ACFs and by maintaining the normal crypt architecture of the colon, suggesting the antiproliferative activity of EA (Table 4; Figure 5C). The antiproliferative role of EA might be due to its ability to increase cellular resistance to LPO and to restore the activities of enzymic antioxidants SOD and GPx, which help to scavenge both $O_2^{\bullet}$ and H_2O_2 effectively.[26]

ACF has been suggested to be the earliest precursor to lesion formation in chemically induced colon cancer.[4,6] Formation of ACF was associated with elevated levels of ODC, which plays a key regulatory role in the biosynthesis of polyamines (spermine, spermidine, putrescine).[46] Colonic luminal polyamines are also produced by enteric bacteria; both diet and enteric bacteria provide sources of potential tumor-promoting polyamines for colonic epithelial cells. Colonic bacteria provide sources of other luminal risk factors for colon cancer. These bacteria metabolize primary bile acids to secondary bile acids, which have been associated with colon cancers in humans and are capable of promoting colon carcinogenesis in rodent models.[47] Bile acid and chenodexycholic acid are well-known for their tumorogenic activities. They promote CRC through induction of cell proliferation and DNA damage by increasing ODC activity.[48] Garewal et al.[49] showed the association of bile acids with observed resistance to apoptosis in colon cancer/adenoma patients. Induction of ODC occurs in cellular transformation induced by chemical carcinogens, viruses, and oncogenes.[50] ODC plays a significant role in cellular proliferation and carcinogenic processes.[51] Luk et al.[52] demonstrated a biphasic induction of ODC during azoxymethane-induced colon carcinogenesis in rats. Expression of ODC is dependent on c-myc, which acts as a transcription factor for ODC.[53] As expected, there was increased expression of ODC in the DMH-induced group, correlating to increased incidence of ACF formation. Dietary administration of EA considerably decreased the expression of ODC by inhibiting the expression of c-myc (Figure 6).

The result in this study exemplifies the abrogative effect of EA in DMH-mediated colon carcinogenesis initiation by lowering lipid peroxidation, DNA damage by enhancing the cellular antioxidant status, and inhibition of ACF incidence by altering expression of c-myc-dependent ODC, providing chemoprotection against DMH-induced cancer initiation. Hence, EA might be developed as a successful chemopreventive agent to control the incidence of colon cancer.

AUTHOR INFORMATION

Corresponding Author

*Phone: 91-44-22202730. Fax: 91- 44 - 22352494. E-mail: niranjali@yahoo.com or kasinathan.nirmalkumar@gmail.com.

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Notes

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ABBREVIATIONS USED

CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; EA, ellagic acid; ODC, ornithine decarboxylase; c-myc, myelocytomatosis oncogene; ACF, aberrant crypt foci; LPO, lipid peroxidation; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; $^{\bullet}OH$, hydroperoxides; EDTA, ethylenediaminetetraacetic acid; MDA, malonyldialdehyde; ROS, reactive oxygen species; CD, cathepsin-D; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

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