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Anti-clastogenic potential of carnosic acid against 7,12-dimethylbenz(a) anthracene (DMBA)-induced clastogenesis

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Abstract:
Carnosic acid, a primary phenolic compound found in the leaves of rosemary (*Rosmarinus officinalis*), has diverse pharmacological and biological activities. The aim of the present study was to investigate the anti-clastogenic effect of carnosic acid in DMBA-induced clastogenesis. The frequency of bone marrow micronucleated polychromatic erythrocytes (MnPCEs), chromosomal aberrations (cytogenetic end points), the status of Phase I and II detoxification enzymes, lipid peroxidation by-products and antioxidants (biochemical endpoints) were analyzed to assess the anti-clastogenic effect of carnosic acid in DMBA-induced clastogenesis. Oral pretreatment of carnosic acid for five days to DMBA-treated hamsters significantly protected DMBA-induced clastogenesis as well as biochemical abnormalities. Although the exact mechanism of anti-clastogenic effects of carnosic acid is unclear, the antioxidant potential and effect on modulation of Phase I and II detoxification enzymes could play a possible role.

Key words:
carnosic acid, clastogenesis, antioxidant, detoxification enzymes

Abbreviations: CAT – catalase, DMBA – 7,12-dimethylbenz(a)-anthracene, GPx – glutathione peroxidase, GR – glutathione reductase, GSH – reduced glutathione, GST – glutathione-S-transferase, MnPCEs – micronucleated polychromatic erythrocytes, NCEs – normochromatic erythrocytes, PCEs – polychromatic erythrocytes, SOD – superoxide dismutase, TBARS – thiobarbituric acid reactive substances

Introduction

Human cancer cells exhibit specific alterations in the chromosomal structure. Abnormalities in chromosome number and structure are responsible for changes in the morphology and biochemical behavior of the cell, which lead to uncontrolled and rapid proliferation. Chemical carcinogen-induced an euploidy causes alterations in the expression of oncogenes and tumor suppressor genes [13, 33]. Micronuclei mainly originate from chromosome fragments or whole chromosomes that fail to engage with the mitotic spindle during cell division. Micronuclei frequency and chromosomal aberrations in bone marrow cells are widely used as cytogenetic end points to assess the genotoxic or anti-genotoxic potential of test compounds under *in vitro* and *in vivo* conditions.

The alkaline comet assay (single-cell gel electrophoresis) is also used as a promising and sensitive

tool for early genotoxicity testing of new pharmaceutical drugs, chemicals and environmental contaminants. This assay has been widely used to detect DNA damage (DNA single strand breaks, alkali labile sites and cross links) due to the fact that the test is simple to perform and only requires minute amounts of test substance [10]. The comet assay measures DNA damage in terms of the size and shape of the comet, DNA tail length and various measures of tail movement using commercially available software programs.

7,12-Dimethylbenz(a)anthracene (DMBA), a potent organ specific and site-specific carcinogen, is widely employed to induce clastogenic effects in the bone marrow of experimental animal models. Several studies demonstrated DMBA-induced DNA damage and mutations in experimental animal models [4, 15]. Guerin et al. [5] showed polyploidy and sister chromatid exchanges in DMBA-induced genotoxicity. Reports also suggested that H-ras mutation is the earliest event in DMBA-induced leukemogenesis [12]. Phase I (cytochromes P_{450} and b_5) and II detoxification enzymes [glutathione-S-transferase (GST) and glutathione reductase (GR)] play a crucial role in the metabolic activation and detoxification of several carcinogenic agents in the liver. Assays of phase I and II detoxification enzymes in liver could therefore be indirectly helpful to assess the clastogenic or anti-clastogenic effect of test compounds [28].

Exposure of cells to carcinogenic agents results in the excessive generation of reactive oxygen species (ROS), whose cellular targets are DNA, lipids and proteins. Overproduction of ROS can cause single and double strand breaks as well as formation of purine and pyrimidine lesions in genomic DNA [14, 32]. ROS-mediated lipid peroxidation has been implicated in the pathogenesis of several diseases, including cancer [32]. The human body possesses an array of sophisticated enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] and non-enzymatic [reduced glutathione (GSH)] antioxidant defense mechanisms to protect the cells and tissues from the deleterious effects of ROS-mediated oxidative stress. Profound chemical carcinogens, including DMBA, mediate genotoxicity and carcinogenesis through ROS-mediated oxidative stress [11].

Recent reports suggest that agents possessing antioxidant and anti-lipid peroxidative potential as well as inhibitory effects on carcinogen-induced DNA damage are considered potent anti-genotoxic agents [24]. Carnosic acid (4a-(2H)-phenanthrene carboxylic acid), an important bioactive phenolic diterpene of *Rosmarinus officinalis*, has received considerable attention in recent years because of its diverse biological and pharmacologic properties. Approximately 90% of the antioxidant activities of *Rosmarinus officinalis* are attributed to its carnosic acid content. Maurin et al. [16] showed radio-protective and anti-mutagenic effects of carnosic acid against gamma ray-induced chromosomal damage in human lymphocytes. Offord et al. [18] reported photo-protective potential of carnosic acid in UVA-irradiated human strain fibroblast. Carnosic acid significantly lowered DNA damage induced by ferric acid hydroperoxide [17]. Offord et al. [19] also suggested that carnosic acid has the potential to decrease metabolic activation and increase detoxification of an important carcinogen, benz(a)pyrene, identifying it as a promising candidate for chemopreventive programs. Indeed, carnosic acid showed potent cytotoxic activity against human cancer cell lines [30]. Wijeratne and Cuppett [34] have reported that carnosic acid inhibited lipid peroxidation by 88–100% under oxidative stress condition.

To the best of our knowledge, the anti-clastogenic potential of carnosic acid has not been reported so far. The present study thus validated the anti-clastogenic effect of carnosic acid by measuring the frequency of MnPCEs, chromosomal aberrations and DNA damage as well as by analyzing the status of lipid peroxidation by-products (TBARS), antioxidants and detoxification enzymes in DMBA-induced clastogenesis.

Materials and Methods

Animals

Twenty-four male golden Syrian hamsters, 8 weeks old, weighing 80–120 g, were obtained from National Institute of Nutrition, Hyderabad, India, and maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. Five animals per polypropylene cage were housed and were maintained under controlled conditions of temperature and humidity with a 12-h light/dark cycle.

Chemicals

Carnosic acid, DMBA (95% purity, soluble in mineral oil), colchicine (95% purity, soluble in water), Giemsa and May-Grunwald's stains were purchased from Sigma-Aldrich Chemical Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade.

Experimental design

The experimental design was approved by the Institutional Animal Ethics Committee, (Reg.no:160/1999/CPCSEA) Annamalai University, Annamalainagar. A total number of 24 animals were divided into 4 groups of 6 animals. Group 1 (95.6 ± 6.4 g) animals served as the negative control. Group 2 (96.4 ± 6.1 g) animals were pretreated with carnosic acid (10 mg/kg, b.w. *po*) alone for 5 days and did not receive DMBA. Group 3 (96.1 ± 6.6 g) animals were given an intraperitoneal injection of DMBA (30 mg/kg, b.w.) on the 5th day. Group 4 (96.9 ± 7.1 g) animals were pretreated with carnosic acid (10 mg/kg, b.w. *po*) for five days and were intraperitoneally injected with DMBA (30 mg/kg, b.w.) on the 5th day after a 2 h administration of carnosic acid. All animals were provided a standard pellet diet and water *ad libitum*. All animals were sacrificed after 24 h of DMBA injection by cervical dislocation for the assessment of micronucleus frequency and chromosomal aberrations.

Dose selection

Different doses of carnosic acid (5, 10 and 15 mg/kg, b.w.) were assessed to determine the anti-clastogenic effect of carnosine acid in DMBA-induced clastogenesis. A dose of 10 mg/kg, b.w. carnosic acid has shown potent anti-clastogenic potential in DMBA treated hamsters as compared to the rest of the doses. Due to this reason, 10 mg/kg, b.w. was chosen for this study.

Assessment of chromosomal aberrations

Assessment of chromosomal aberrations in bone marrow was carried out according to the procedure of Kilian et al. [9]. The femur bones were removed from negative control and experimental animals injected with 0.1% colchicine (1 ml/100 g b.w. *ip*), 90 min before sacrificing the animals. The bone marrow contents were flushed into 5 ml of physiological saline and centrifuged at 500 × g for five min. The sediments obtained were resuspended in 6 ml of hypotonic KCl (0.075 M) and incubated at 37°C for 25 min. The pellets were then fixed using methanol : acetic acid (3:1, v/v) fixative and stained with Giemsa stain. One hundred well-spread metaphase cells were scored for each animal and structural chromosomal aberrations were observed and recorded.

Bone marrow micronucleus test

Bone marrow micronucleus tests were carried out according to the method of Schmid [27]. The femur bones removed from golden Syrian hamsters were cleaned and the contents were flushed into tubes containing 1 ml of calf serum and centrifuged at 500 × g for 10 min. The obtained pellet was suspended with a few drops of fresh serum, and slides were prepared and air-dried for 18 h. After drying, the slides were stained with May-Grunwald stain followed by Giemsa stain. The frequency of MnPCEs in each group was calculated by scoring 2500 polychromatic erythrocytes (PCEs) per animal.

Single cell gel (comet) assay

The single cell gel (comet) assay is a rapid, simple, and reliable technique used to assess the DNA damage in bone marrow cells [31].

The femur bone marrow cells were flushed with Hank's balanced salt solution (HBSS) and then filtered through a 50-µm nylon filter. The cells were counted and diluted to a final suspension of 50,000 – 100,000 cells/ml. A mixture of 10 µl bone marrow cells and 200 µl of 0.5% low melting point agarose was layered onto precoated slides, which contained 1% normal melting point agarose, and then was covered with a cover slip. The slides were placed in a chilled lysing solution containing 2.5 M NaCl, 100 mM EDTA 2Na, 100 mM Tris-HCl, pH 10 and 5% DMSO, 1% Triton X 100 and 1% sodium sarcosinate for 1 h at 4°C and followed by alkaline buffer (pH > 13) for 20 min. The electrophoresis was carried out for 20 min at 25 V and 300 mA. The slides were stained with 50 µl of ethidium bromide (EtBr, 20 µg/ml) and analyzed under a fluorescence microscope. The images (25 cells/slide) were viewed with a high-performance Nikon camera. For each sample, 50 randomly selected cells (i.e., two coded slides/sample) were analyzed.

Biochemical estimations

Blood samples were collected into heparinized tubes. Plasma was separated by centrifugation at 1000 × g for 15 min. The buffy coat was removed, and the packed cells were washed three times with physiological saline. Liver tissue from animals were washed with ice-cold saline and homogenized using an appropriate buffer in an all-glass homogenizer with teflon pestle and used for biochemical estimations.

Lipid peroxidation was estimated by the formation of thiobarbituric acid reactive substances (TBARS). TBARS in plasma were assayed by the method previously described by Yagi [35]. Plasma was deproteinized with phosphotungstic acid and treated with thiobarbituric acid at 90°C for 1 h. The pink color that formed gives a measure of TBARS, which was read at 530 nm. Tissue lipid peroxidation was conducted by the method of Ohkawa et al. [20]. The color formed by the reaction of thiobarbituric acid with breakdown products of lipid peroxidation was measured colorimetrically at 532 nm. Superoxide dismutase activity in plasma and liver was assayed by the method of Kakkar et al. [8], based on the 50% inhibition of formation of NADH-phenazine methosulfate nitroblue tetrazolium (NBT). The color developed was read at 520 nm. One unit of the enzyme was taken as the amount of enzyme required to give 50% inhibition of NBT reduction. The activity of GPx in plasma and liver was determined using the method of Rotruck et al. [26], based on the utilization of GSH by the enzyme. One unit of the enzyme was expressed as μM of GSH utilized per minute. The activity of catalase in plasma and liver was assayed by the method of Sinha [29], based on the utilization of H_2O_2 by the enzyme. The color developed was read at 620 nm. One unit of the enzyme was expressed as μM of H_2O_2 utilized per min. The reduced glutathione level in plasma and liver was determined by the method of Beutler and Kelley [2]. The technique involves protein precipitation by meta-phosphoric acid and spectrophotometric assay at 412 nm of the yellow derivative obtained by the reaction of the supernatant with 5,5'-dithiobis-2-nitrobenzoic acid.

The levels of cytochrome P_{450} and b_5 in liver were determined according to the method of Omura and Sato [21]. Cytochrome P_{450} was measured by the formation of pigment on reaction between reduced cytochrome P_{450} and carbon monoxide. The pigment was read with an absorbance maximum at 450 nm. The difference spectrum between reduced and oxidized cytochrome b_5 was used as an index to measure the level of cytochrome b_5.

The activity of GST in liver tissue homogenate was assayed by the method of Habig et al. [6]. GST activity was measured by incubating the tissue homogenate with the substrate – 1-chloro-2,4-dinitrobenzene (CDNB). The absorbance was followed for 5 min at 540 nm after the reaction was initiated by the addition of reduced glutathione. GR activity in liver tissue homogenate was assayed by the method of Carlberg and Mannervik [3]. The enzyme activity was assayed by measuring the formation of reduced glutathione when the oxidized glutathione (GSSG) was reduced by reduced nicotinamide adenine dinucleotide phosphate (NADPH). All of the above mentioned biochemical assays were carried out in duplicate in plasma or liver tissue homogenates.

Statistical analysis

The data are expressed as the mean ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the p values were 0.05 or less.

DNA damage

DNA damage, as reflected by % DNA in tail (tail intensity), tail length, tail moment (product of tail DNA/total DNA by the center of gravity) and olive tail moment (the product of the distance between the barycenters of the head and tail and the proportion of DNA in the tail) of the stored images, was investigated in 25 cells per treatment using CASP software.

Results

Tables 1 and 2 show the frequency of MnPCEs and chromosomal aberrations, respectively, in negative control and experimental hamsters in each group. Hamsters treated with DMBA alone (Group 3) showed the highest frequency of MnPCEs and chromosomal aberrations (chromosomal gap, chromatid break, chromosomal break, fragment and minute) as compared to negative control hamsters (Group 1).

Tab. 1. Frequency of MnPCEs in negative control and experimental animals in each group

Group	Parameters	MnPCEs/2500 PCEs	PCEs/NCEs	PCE (%)[#]
1	Negative control	4.68 ± 0.36[a]	1.02 ± 0.05[a]	50.4
2	Carnosic acid alone	4.59 ± 0.51[a]	1.01 ± 0.06[a]	50.2
3	DMBA alone	40.81 ± 5.1[b]	0.69 ± 0.07[b]	40.8
4	DMBA + carnosic acid	12.7 ± 1.5[c]	0.88 ± 0.09[c]	46.8

Values are expressed as the mean ± SD for six animals in each group (2500 PCEs were scored per animal). Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT). [#] Percentage of polychromatic erythrocytes was calculated as follows: PCEs / (PCEs + NCEs) 100

Tab. 2. Mitotic index and chromosomal abnormalities in negative control and experimental animals in each group

Group	Parameter	Mitotic index (%)[A]	Chromosomal aberrations[B] hamsters $^{-1}$					Total aberration hamsters^{-1}	Abnormal metaphase hamsters^{-1}
			G[*]	B'	B''	F	M		
1	Negative control	4.26 ± 0.51[a]	0.41 ± 0.04[a]	1.02 ± 0.11[a]	0[a]	1.14 ± 0.09[a]	0.30 ± 0.02[a]	2.46 ± 0.29[a]	1.56 ± 0.19[a]
2	Carnosic acid alone	4.29 ± 0.52[a]	0.43 ± 0.05[a]	1.06 ± 0.14[a]	0[a]	1.17 ± 0.13[a]	0.34 ± 0.03[a]	2.57 ± 0.30[a]	1.51 ± 0.18[a]
3	DMBA alone	2.01± 0.27[b]	10.7 ± 1.30[b]	5.46 ± 0.66[b]	2.44 ± 0.26[b]	5.27 ± 0.64[b]	2.46 ± 0.29[b]	15.63 ± 1.90[b]	13.41 ± 1.80[b]
4	DMBA + carnosic acid	3.49 ± 0.36[c]	4.2 ± 0.52[c]	2.39 ± 0.28[c]	1.06 ± 0.10[c]	2.76 ± 0.34[c]	0.94 ± 0.08[c]	7.15 ± 0.90[c]	4.38 ± 0.56[c]

Values are expressed as the mean ± SD for six animals in each group. Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT). G – Gap, B' – chromatid break, B'' – isochromatid break, F – fragment, M – minute. A – Mitotic index has been calculated by analyzing 1000 cells/animal (for a total of 6000 cells/treatment) and percentage of the mitotic cells calculated for each treatment group. B - Frequency per 100 cells. Each chromosomal aberration has been counted by analyzing 100 cells/animal (6 animals/group, for a total of 600 cells/treatment) and the mean ± SD were calculated per treatment group. * Gaps were not included in total chromosomal aberrations

Tab. 3. Changes in the levels of DNA damage (% DNA in tail, tail length, tail moment and olive tail moment) in the hamsters' bone marrow cells

Group	Parameters	% DNA in tail	Tail length	Tail moment	Olive tail moment
1	Negative control	0.18 ± 0.02[a]	3.20 ± 0.21[a]	0.02 ± 0.004[a]	0.06 ± 0. 01[a]
2	Carnosic acid alone	0.22 ± 0.02[a]	3.6 ± 0.24[a]	0.03 ± 0.004[a]	0.05 ± 0.01[a]
3	DMBA alone	12.6 ± 1.40[b]	39.6 ± 2.5[b]	6.04 ± 2.20[a]	5.87 ± 0.62[c]
4	DMBA + carnosic acid	2.15 ± 0.36[c]	20.5 ± 0.21[c]	3.18± 0.29[c]	2.51 ± 0.40[c]

Values are expressed as the mean ± SD for six animals in each group. Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT)

Oral pretreatment of carnosic acid to DMBA treated hamsters (Group 4) significantly decreased the frequency of MnPCEs and protected chromosomal abnormalities in their structure. Oral pretreatment of carnosic acid alone to hamsters (Group 2) displayed no significant differences in MnPCEs frequency and chromosomal aberrations as compared to negative control hamsters.

The extent of DNA damage (% DNA in tail, tail length, tail moment and olive tail moment) in bone

Tab. 4. TBARS and antioxidants status in plasma and liver of negative control and experimental animals in each group

Parameters	Group 1 Negative control	Group 2 Carnosic acid alone	Group 3 DMBA alone	Group 4 DMBA + carnosic acid
Plasma				
TBARS (nmol/ml)	1.41 ± 0.10[a]	1.38 ± 0.11[a]	2.36 ± 0.27[b]	1.89 ± 0.14[c]
GPx (U^A/L)	92.1 ± 7.6[a]	95.8 ± 7.1[a]	126.4 ± 13.1[b]	107.3 ± 9.6[c]
SOD (U^B/ml)	2.31 ± 0.16[a]	2.26 ± 0.19[a]	4.27 ± 0.39[b]	2.96 ± 0.24[c]
CAT (U^C/ml)	1.24 ± 0.09[a]	1.20 ± 0.10[a]	2.69 ± 0.22[b]	1.67 ± 0.13[c]
Liver				
TBARS (nmol/100 mg protein)	54.1 ± 3.9[a]	52.9 ± 4.8[a]	87.6 ± 9.2[b]	65.8 ± 7.4[c]
GPx(U^A/g protein)	4.59 ± 0.32[a]	4.50 ± 0.38[a]	10.71 ± 1.2[b]	6.23 ± 0.72[c]
SOD (U^B/ mg protein)	3.86 ± 0.27[a]	3.79 ± 0.29[a]	6.14 ± 0.52[b]	4.73 ± 0.51[c]
CAT (U^C/ mg protein)	23.4 ± 2.1[a]	22.6 ± 2.4[a]	40.6 ± 5.1[b]	30.1 ± 3.4[c]

Values are expressed as the mean ± SD for six animals in each group. Values that do not share a common superscript letter in the same row differ significantly at $p < 0.05$ (DMRT). A – μM of glutathione utilized/min; B – the amount of enzymes required to inhibit 50% nitroblue-tetrazolium (NBT) reduction; C – μM of H_2O_2 utilized/second

marrow cells of negative control and experimental animals in each group is shown in Table 3. Extensive DNA damage, as reflected by an increase in DNA tail length, tail moment, % DNA in tail, and olive tail moment, was noticed in hamsters treated with DMBA alone. Oral pretreatment of carnosic acid significantly protected DNA damage in DMBA-treated hamsters. Oral pretreatment of carnosic acid alone in hamsters showed a similar comet pattern as observed in negative control hamsters.

The status of TBARS and enzymatic antioxidants in plasma and liver of negative control and experimental hamsters in each group is shown in Table 4. TBARS levels and enzymatic antioxidant activities were increased in hamsters treated with DMBA alone (Group 3) as compared to negative control hamsters (Group 1). Oral pretreatment of carnosic acid significantly decreased the levels of TBARS and antioxidant activities in hamsters treated with DMBA (Group 4). No significant difference in the status of TBARS and antioxidants was observed between negative control hamsters (Group 1) and carnosic-acid-treated hamsters (Group 2).

The activities of phase I (cytochrome P_{450} and b_5), phase II (GST and GR) detoxification enzymes and reduced glutathione (GSH) content in liver of negative control and experimental hamsters in each group are shown in Table 5. The activities of detoxification enzymes and GSH content were significantly increased in hamsters treated with DMBA alone (Group 3) as compared to negative control hamsters (Group 1). Oral pretreatment of carnosic acid significantly de-

Tab. 5. Activities of phase I and II detoxification enzymes and glutathione content in the liver of negative control and experimental animals in each group

Group	Parameters	GSH (nM/mg rotein)	GST (nM of CDNB-GSH conjugate formed/min/mg protein)	GR (nM of NADPH oxidized/min/mg protein	Cyt P_{450} (U^X/mg protein)	Cyt b_5 (U^Y/mg protein)
1	Negative control	3.22 ± 0.21[a]	126.4 ± 11.5[a]	30.8 ± 2.6[a]	0.49 ± 0.03[a]	0.99 ± 0.10[a]
2	Carnosic acid alone	3.17 ± 0.26[a]	125.7 ± 12.1[a]	28.9 ± 3.2[a]	0.48 ± 0.05[a]	1.04 ± 0.12[a]
3	DMBA alone	5.09 ± 0.62[b]	173.7 ± 20.1[b]	54.8 ± 5.9[b]	0.86 ± 0.09[b]	1.78 ± 0.14[b]
4	DMBA + carnosic acid	4.01 ± 0.38[c]	148.9 ± 12.9[c]	39.9 ± 4.1[c]	0.64 ± 0.08[c]	1.27 ± 0.17[c]

Values are expressed as the mean ± SD for six animals in each group. Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$ (DMRT). X – μM of cytochrome P_{450} formed; Y – μM of cytochrome b_5 formed

creased the activities of detoxification enzymes and glutathione content in hamsters treated with DMBA (Group 4). No significant difference was observed in the status of detoxification enzymes and GSH content between negative control hamsters (Group 1) and carnosic-acid-treated hamsters (Group 2).

Discussion

Genomic instability due to loss or gain of chromosomes, point mutation and chromosomal translocation initiates the cancer cell phenotype. It has also been reported that abnormal nuclear morphology, which exhibits micronuclei, is indicative of genomic instability within a cell [7]. Elevated MnPCEs frequency and chromosomal aberrations are associated with an enhanced risk of carcinogenesis. Previous reports from our and other laboratories demonstrated an increased frequency of MnPCEs and chromosomal aberrations in the bone marrow cells of DMBA treated hamsters and rats [4, 22, 25]. Oral pretreatment of carnosic acid to hamsters treated with DMBA significantly reduced the frequency of MnPCEs and chromosomal aberrations. The present study thus suggests that carnosic acid has significant anti-clastogenic potential during DMBA-induced clastogenesis.

The comet assay, frequently used to evaluate the genotoxicity of test substances, is based on the appearance of a comet tail (damage DNA) upon electrophoresis. Direct DNA damage, single strand breaks in the DNA and DNA inter-strand cross-linking can be detected in the comet assay. A significant increase in the DNA migration indicates the presence of increased levels of strand breaks, alkali labile sites or incomplete excision repair sites. Oral pretreatment of carnosic acid in hamsters treated with DMBA significantly reduced the amount of DNA liberated from the head of the comet, which indicates that carnosic acid protected DNA damage caused by DMBA. The present study thus suggests that carnosic acid also shows potent anti-genotoxic effect in DMBA-treated hamsters.

Oxidative stress, an imbalance in oxidant and antioxidant status, plays a crucial role in the pathogenesis of carcinogenesis. Elevated levels of TBARS accompanied by enhanced activities of enzymatic antioxidants in plasma and liver confirm oxidative stress in hamsters treated with DMBA alone. Oral pretreatment of carnosic acid significantly reduced the levels of TBARS and activities of antioxidants in DMBA-treated hamsters. The free radical scavenging activity of carnosic acid is due to the presence of two *o*-phenolic hydroxyl groups found at C-11 and C-12 of the molecule. Pérez-Fons et al. [23] have shown a membrane-rigidifying effect of carnosic acid, which may contribute to carnosic acid antioxidant capacity through the hindrance of diffusion of free radicals. It has also been suggested that the antioxidant activity of carnosic acid could be partly due to their ability to increase or maintain GPx and SOD activities [1]. The present study thus suggests that carnosic acid attenuated DMBA-induced oxidative stress and exhibited a significant antioxidant role against DMBA-induced clastogenesis.

The metabolic activation and detoxification of DMBA mainly takes place in the liver. Increases in the activities of phase I and II detoxification enzymes and GSH content in hamsters treated with DMBA indicates that both Phase I and II detoxification cascades are stimulated to detoxify the carcinogenic agent, DMBA. The present study also noticed that oral pretreatment of carnosic acid to DMBA-treated hamsters attenuated the metabolic activation of DMBA, as evidenced by downregulation of phase I and II detoxification enzymes and GSH content.

The present study thus demonstrated the anti-clastogenic effect of carnosic acid in DMBA-induced clastogenesis. Although the exact mechanism for the anti-clastogenic effect of carnosic acid is unclear, the antioxidant potential and modulation of phase I and II detoxification cascade could account for the anti-clastogenic effect in DMBA-induced clastogenesis.

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