Chemopreventive efficacy of curcumin and piperine during 7,12-dimethylbenz [a]anthracene-induced hamster buccal pouch carcinogenesis

Manoharan S, Balakrishnan S, Menon V P, Alias L M, Reena A R

ABSTRACT

Introduction: Oral carcinoma accounts for 40–50 percent of all cancers in India. Tobacco chewing, smoking and alcohol consumption are the major risk factors associated with the high incidence of oral cancer in India. Our aim was to investigate the chemopreventive potential of curcumin and piperine during 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis.

Methods: Oral squamous cell carcinoma was developed in the buccal pouch of Syrian golden hamsters, by painting them with 0.5 percent DMBA in liquid paraffin, three times a week for 14 weeks. The tumour incidence, tumour volume and burden were determined in the buccal pouches. The status of phase II detoxification agents, lipid peroxidation and antioxidants were estimated by specific colorimetric methods.

Results: We observed 100 percent tumour formation in DMBA-alone painted hamsters. Disturbances in the status of lipid peroxidation, antioxidants and phase II detoxification agents were noticed in DMBA-alone painted hamsters. Oral administration of curcumin (80 mg/kg body weight) and piperine (50 mg/kg body weight) to DMBA-painted hamsters on alternate days to DMBA painting for 14 weeks completely prevented the formation of oral carcinoma. Also, curcumin and piperine restored the status of lipid peroxidation, antioxidants and detoxifying agents in DMBA-painted hamsters.

Conclusion: The chemopreventive efficacy of curcumin and piperine is probably due to their antilipidperoxidative and antioxidant potential as well as their modulating effect on the carcinogen detoxification process.

Keywords: 7,12-dimethylbenz[a]anthracene, antioxidants, buccal pouch carcinogenesis, curcumin, lipid peroxidation, oral carcinoma, piperine

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INTRODUCTION

Oral carcinoma is defined as cancer of the mouth and pharynx including cancer of the lips, tongue, floor of the mouth, palate, gingiva, alveolar mucosa, buccal mucosa, oropharynx, tonsils, uvula and salivary glands. India has recorded the highest incidence of oral cancer, where it accounts for 40%–50% of all cancers. The major risk factors for this cancer include the use of tobacco products (tobacco smoking and chewing) and alcohol, which accounts for 75% of all oral cancers. Oral cancer most often occurs in people over the age of 40 years, and about half of the patients afflicted will die within five years of diagnosis.[1,2] The 7,12-dimethylbenz[a]anthracene (DMBA), an organ-specific carcinogen, on metabolic activation produces the ultimate carcinogen, dihydrodiol epoxide, which can mediate neoplastic transformation by inducing DNA damage, generating excess reactive oxygen species (ROS) and mediating the chronic inflammatory process. Oral carcinogenesis induced by DMBA closely resembles a human oral tumour, both histologically and morphologically. DMBA-induced experimental oral carcinogenesis can thus be used as an ideal model for studying chemoprevention of oral cancer.[3,4] Measurement of the activities of detoxification agents such as glutathione-S-transferase (GST) and glutathione reductase (GR) in the liver may help to assess the chemopreventive potential of the test compound. GST detoxifies carcinogens either by destroying their active reactive centres or by facilitating their excretion through a conjugation process. GR, an important enzyme required for reduced glutathione (GSH) maintenance, catalyses reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of glutathione disulphide to GSH.[5]

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608002, Tamil Nadu, India

Manoharan S, MSc, MPhil, PhD
Reader

Balakrishnan S, MSc, MPhil, PhD
Research Scholar

Menon VP, MSc, PhD
Professor and Head

Alias LM, MSc, MPhil
Research Scholar

Reena AR, MSc, MPhil
Research Scholar

Correspondence to:
Dr Shanmugam Manoharan
Tel: (91) 4144 238 343
Fax: (91) 4144 238 080
Email: sakshiman@rediffmail.com

Table I. Incidence of oral squamous cell carcinoma in DMBA alone, DMBA + piperine and DMBA + curcumin treated hamsters.

Groups	Tumour incidence (oral squamous cell carcinoma)	Total no. of tumours/total no. of animals tested	Tumour volume (mm^3)/animals	Tumour burden (mm^3)/animals
DMBA alone	100% tumour formation	37/10	378.3 ± 32.1	1,399.7 ± 98.6
DMBA + piperine	No tumour formation	0/10	–	–
DMBA + curcumin	No tumour formation	0/10	–	–

Tumour volume was measured using the formula, $v = \frac{4}{3}\partial\left[\frac{D_1}{2}\right]\left[\frac{D_2}{2}\right]\left[\frac{D_3}{2}\right]$, where D_1, D_2 and D_3 are the three diameters (mm) of the tumour. Tumour burden was calculated by multiplying tumour volume and the number of tumours/animal.

Table II. Histopathological changes in the buccal pouch of hamsters in control and experimental animals in each group.

Groups	Hyperkeratosis	Hyperplasia	Dysplasia	Squamous cell carcinoma
Control	Not observed	Not observed	Not observed	Not observed
DMBA	Severe	Severe	Severe	Well differentiated
DMBA + piperine	Moderate	Moderate	Mild	–
DMBA + curcumin	Moderate	Moderate	Mild	–
Piperine alone	Not observed	Not observed	Not observed	Not observed
Curcumin alone	Not observed	Not observed	Not observed	Not observed

A large number of chemical carcinogens, including DMBA, mediate carcinogenesis through free radical-mediated oxidative damage to cells and tissues. Enormous production of ROS due to oxidative stress in the system can induce strand breaks and can modify DNA bases contributing to mutagenesis and carcinogenesis.[6] The major target of ROS *in vivo* or *in vitro* is membrane lipid peroxidation that leads to abnormalities in the structural integrity and function of the cell membrane.[7] Lipid peroxidation, a free radical-mediated chain reaction, has been implicated in the pathogenesis of several disorders including oral carcinoma.[8] Increased levels of lipid peroxidation byproducts play a role in the early phases of tumour growth. An inverse association between lipid peroxidation and the rate of cell proliferation in tumour tissues has been reported.[9] Mammalian cells possess an array of enzymatic (superoxide dismutase [SOD], catalase [CAT] and glutathione peroxidase [GPx]) and non-enzymatic (Vitamins C and E, and GSH) defence mechanisms to protect the cells and tissues from the deleterious effects of free radical-mediated oxidative damage. The administration of antioxidants has been shown to have beneficial effects on the regression of tumours in animals. Anticarcinogenic activity has been demonstrated with vitamin E, vitamin C and glutathione.[10-12]

Chemoprevention is a promising and novel strategy for the prevention, inhibition, suppression and reversal of carcinogenesis through the use of natural plant products and synthetic agents. It has been suggested that compounds that possess antimutagenic, anticarcinogenic, inhibitory effects on cell proliferation and antioxidant function are considered to be good chemopreventive agents. A large number of active principles from traditional medicinal plants have been reported to have chemopreventive properties.[13,14] Circulatory lipid peroxidation and antioxidant status could be used as biomarkers of chemoprevention in experimental carcinogenesis.[15]

Curcumin, the active principle in *Curcuma longa* (turmeric), has been shown to have potent anti-inflammatory, antioxidant and anticarcinogenic properties.[16] Several studies have pointed out that curcumin has the ability to inhibit carcinogen-DNA adduct formation and the inhibition of tumours in experimental carcinogenesis.[17] It has been demonstrated that the topical application of curcumin inhibits DMBA initiation and 12-O-tetradeconoylphorbol 13-acetate promotion of skin tumours.[18] Curcumin is a potent scavenger of superoxide anion, hydroxyl radical, singlet oxygen, nitric oxide and peroxinitrite.[16] *In vitro*, curcumin has been shown to induce the apoptosis of a wide variety of cancer cells.[19] Piperine, a pungent alkaloid constituent of black and long peppers, possesses diverse pharmacological actions, such as anti-inflammatory, antimicrobial, hepatoprotective and anticancer effects.[20] It has been reported that piperine inhibits xenobiotic (drug) metabolising enzymes and enhances cellular antioxidant status.[21] Piperine has been shown to reduce liver lipid peroxidation and protect against oxidative damages induced by a number of chemical carcinogens.[22] To the best of our knowledge, we have found no scientific studies on the chemopreventive efficacy of piperine in DMBA-

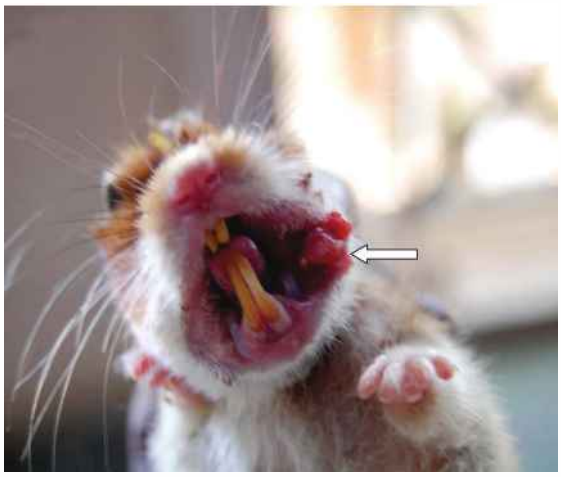

Fig. 1 Photograph shows the gross appearance of oral squamous cell carcinoma (⇦) noticed in DMBA-alone painted animals.

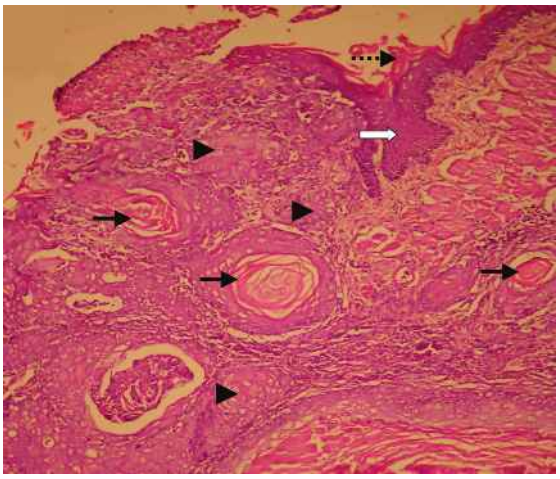

Fig. 2 Photomicrograph shows hyperkeratosis (▪▪▪▶), hyperplasia (⇨), dysplasia (▶) and well-differentiated squamous cell carcinoma with keratin pearls (➡) observed in the buccal pouch of DMBA-alone painted hamsters (Haematoxylin & eosin, × 10).

induced hamster buccal pouch carcinogenesis. Although the cancer chemopreventive potential of curcumin and piperine has been repeatedly reported in various experimental carcinogenesis, the biochemical mechanistic pathway for their chemopreventive effects has not been reported in DMBA-induced oral carcinogenesis. Thus, the present study was undertaken to find out the biochemical mechanistic pathway for the chemopreventive efficacy of curcumin and piperine during DMBA-induced oral carcinogenesis.

METHODS

DMBA, curcumin and piperine were obtained from Sigma-Aldrich Chemical Pvt Ltd, Bangalore, India. All other chemicals used were of analytical grade, purchased from Himedia Laboratories, Mumbai, India. Male golden Syrian hamsters, aged 8–10 weeks, weighing 80–120g, were purchased from the National Institute of Nutrition, Hyderabad, India and were maintained in the Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed five in a polypropylene cage and provided with a standard pellet diet (Agro Corporation Pvt Ltd, Bangalore, India) and water *ad libitum*. The standard pellet diet is composed of 21% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamin and 55% nitrogen-free extract (carbohydrates). The animals were maintained under controlled conditions of temperature (27 ± 2°C) and humidity (55 ± 5%) with a 12 h light/dark cycle. Tumours were induced in each hamster's buccal pouch through the topical application of 0.5% DMBA in liquid paraffin three times a week for 14 weeks.[4,10] The total number of tumours in the hamster's buccal pouch was determined macroscopically at the time of sacrifice. The total number of tumours was counted macroscopically by an oral pathologist (CRR).

The institutional animal ethics committee (Reg. no.160/1999/CPCSEA), Annamalai University, Annamalainagar, India, approved the experimental design. The animals were maintained as per the principles and guidelines of the ethical committee for animal care of Annamalai University in accordance with Indian National Law on animal care and use. A total of 60 hamsters were randomised into six groups with ten animals in each group. Group I served as the control group, where the animals were painted with liquid paraffin (vehicle) alone three times a week for 14 weeks on their left buccal pouches. Groups II–IV animals were painted with 0.5% DMBA in liquid paraffin three times a week for 14 weeks on their left buccal pouches. Group II animals received no other treatment. Groups III and IV animals were orally administered with piperine (50 mg/kg body weight) and curcumin (80 mg/kg body weight) respectively, starting one week before exposure to the carcinogen and continued on alternate days to DMBA painting until the animals were sacrificed. Groups V and VI animals received oral administration of piperine (50 mg/kg body weight) and curcumin (80 mg/kg body weight) alone, respectively, throughout the experimental period. The experiment was terminated at the end of 14 weeks and all animals were sacrificed by cervical dislocation. Biochemical studies were conducted on the blood and buccal mucosa of control and experimental animals in each group. For histopathological examination, buccal mucosa tissues were fixed in 10% formalin and routinely processed and embedded with paraffin, 2–3 µm sections were cut in a rotary microtome and stained with haematoxylin and eosin.

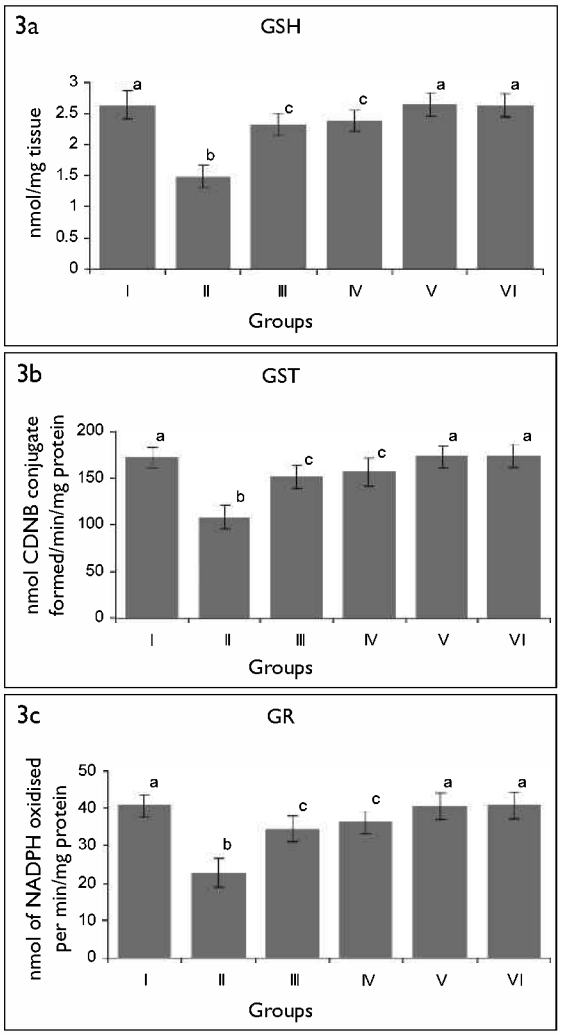

Fig. 3 Bar charts show the activities of detoxification agents, (a) GSH; (b) GST; and (c) GR, in liver homogenate of control and experimental animals in each group (n = 10).
Groups I: Control; II: DMBA; III: DMBA + piperine; IV: DMBA + curcumin; V: Piperine alone; VI: Curcumin alone. Values are expressed as mean ± SD for ten hamsters in each group. Values that do not share a common superscript in the same graph differ significantly at $p < 0.05$ (DMRT).

Blood samples were collected into heparinised tubes. Plasma was separated by centrifugation at 1,000 g for 15 min. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte membrane was prepared using the method employed by Dodge et al[23] and modified by Quist.[24] The erythrocytes remaining after the removal of plasma were washed three times with 310 mM isotonic Tris-HCl buffer (pH 7.4). Haemolysis was carried out by pipetting out the washed erythrocyte suspension into polypropylene centrifuge tubes, which contained 20 mM hypotonic Tris-HCl buffer (pH 7.2). The erythrocyte membranes were sedimented in a high-speed cooling centrifuge at 20,000 g for 40 min. The supernatant was decanted and the erythrocyte membrane pellet was made up to a known volume using 0.2 M isotonic Tris-HCl buffer (pH 7.4). Aliquots from these preparations were used for the estimation of thiobarbituric acid reactive substances (TBARS) and vitamin E.

Tissue samples from the animals were washed with ice cold saline and homogenised using an appropriate buffer (GST: 0.3 M phosphate buffer, pH 6.5; GR: 0.1 M phosphate buffer, pH 7.4; TBARS: 0.025 M Tris-HCl buffer, pH 7.5; GSH and GPx: 0.4 M phosphate buffer, pH 7.0; SOD: 0.025 M sodium pyrophosphate buffer, pH 8.3; CAT: 0.01 M phosphate buffer, pH 7.0) in an all-glass homogeniser with a teflon pestle and used for biochemical estimations. The activity of GST in liver tissue homogenate was assayed using the method employed by Habig et al.[25] GST activity was measured by incubating the tissue homogenate with the substrate 1-chloro-2,4-dinitrobenzene. The absorbance was followed for 5 min at 540 nm after the reaction was started by the addition of GSH. GR activity in liver tissue homogenate was assayed using the method employed by Carlberg and Mannervik.[26] The enzyme activity was assayed by measuring the formation of GSH when the oxidised glutathione is reduced by NADPH.

Lipid peroxidation was estimated as evidenced by the formation of TBARS. TBARS in plasma were assayed by the method described by Yagi.[27] Plasma was deproteinised with phosphotungstic acid and the precipitate was treated with thiobarbituric acid at 90°C for an hour. The pink colour formed gave a measure of the TBARS, which was read at 530 nm. TBARS in erythrocytes and erythrocyte membrane was estimated using the method employed by Donnan.[28] The pink chromogen formed by the reaction of thiobarbituric acid with breakdown products of lipid peroxides was read at 535 nm. Tissue lipid peroxidation was done using the method employed by Ohkawa et al.[29] The colour formed by the reaction of thiobarbituric acid with breakdown products of lipid peroxidation was measured colorimetrically at 532 nm. The GSH levels in the plasma, erythrocytes, liver and buccal mucosa were determined by the method described by Beutler and Kelley.[30] The technique involved protein precipitation by metaphosphoric 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 level of plasma vitamin C was determined by the method described by Omaye et al.[31] The dehydro-ascorbic acid formed from the oxidation of vitamin C by copper, formed a coloured product on treatment with 2,4-dinitrophenylhydrazine, whose absorbance was measured at 520 nm.

The vitamin E level in the plasma and erythrocyte

Table III. Status of plasma TBARS and antioxidants in control and experimental animals in each group.

Groups	TBARS (nmoles/ml)	GSH (mg/dL)	Vitamin C (mg/dL)	Vitamin E (mg/dL)	SOD (U^A/ml)	CAT (U^B/ml)	GPx (U^C/L)
Control	2.02 ± 0.18[a]	27.3 ± 1.8[a]	1.34 ± 0.08[a]	1.38 ± 0.12[a]	2.61 ± 0.23[a]	0.93 ± 0.07[a]	109.7 ± 9.1[a]
DMBA	4.78 ± 0.42[b]	17.2 ± 2.3[b]	0.72 ± 0.09[b]	0.74 ± 0.13[b]	1.69 ± 0.19[b]	0.51 ± 0.09[b]	76.6 ± 9.3[b]
DMBA + piperine	2.32 ± 0.23[c]	24.3 ± 2.2[c]	1.18 ± 0.11[c]	1.20 ± 0.09[c]	2.32 ± 0.20[c]	0.80 ± 0.08[c]	94.2 ± 8.5[c]
DMBA + curcumin	2.27 ± 0.19[c]	25.1 ± 1.7[c]	1.22 ± 0.09[c]	1.24 ± 0.10[c]	2.37 ± 0.18[c]	0.84 ± 0.07[c]	98.6 ± 8.7[c]
Piperine alone	2.02 ± 0.17[a]	27.5 ± 1.6[a]	1.35 ± 0.12[a]	1.37 ± 0.09[a]	2.62 ± 0.17[a]	0.93 ± 0.05[a]	108.9 ± 8.3[a]
Curcumin alone	2.01 ± 0.14[a]	27.6 ± 1.7[a]	1.36 ± 0.07[a]	1.38 ± 0.07[a]	2.62 ± 0.21[a]	0.94 ± 0.07[a]	109.5 ± 8.9[a]

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

[A] The amount of enzyme required to inhibit 50% NBT reduction.

[B] Micromoles of hydrogen peroxide utilised/sec.

[C] Micromoles of glutathione utilised/min.

membranes were determined colorimetrically using the method described by Desai.[32] Vitamin E present in the lipid residue forms a pink-coloured complex with bathophenanthroline-phosphoric acid reagent, which was measured at 536 nm. Tissue vitamin E was measured using the fluorimetric method described by Palan et al.[33] The lipid extracts were dried under nitrogen and the residues were suspended in 66% ethanol, followed by the addition of 4 ml of hexane and 0.6 ml of 60% H_2SO_4. The fluorescence intensity of vitamin E extracted to the hexane layer was measured at an excitation of 295 nm and emission of 320 nm. SOD activity was assayed using the method employed by Kakkar et al,[34] based on the 50% inhibition of NADH-phenazine methosulphate nitroblue tetrazolium (NBT) formation. The colour developed was read at 520 nm. One unit of enzyme is taken as the amount of enzyme required to give 50% inhibition of NBT reduction. The activity of CAT was assayed using the method described by Sinha,[35] based on the utilisation of hydrogen peroxide by the enzyme. The colour developed was read at 620 nm. One unit of the enzyme is expressed as µmoles of hydrogen peroxide utilised per minute. The activity of GPx was determined using the method employed by Rotruck et al,[36] based on the utilisation of GSH by the enzyme. One unit of the enzyme is expressed as µmoles of GSH utilised per minute. The data is expressed as mean ± standard deviation (SD). Statistical comparisons were performed by one-way analysis of variance followed by Duncan's multiple range test (DMRT). The results were considered statistically significant if the p-values were less than 0.05.

RESULTS

The tumour incidence, tumour volume and tumour burden in DMBA-painted animals, DMBA + piperine treated animals and DMBA + curcumin treated animals are shown in Table I. We observed oral squamous cell carcinoma formation in all the hamsters' buccal pouches painted with DMBA alone for 14 weeks; therefore the tumour incidence was 100%. The total number of oral tumours in the buccal pouches was counted and the diameter of each tumour was measured with a vernier caliper. The tumour volume was calculated by the formula, $v = \frac{4}{3}\partial\left[\frac{D_1}{2}\right]\left[\frac{D_2}{2}\right]\left[\frac{D_3}{2}\right]$, where D_1, D_2 and D_3 are the three diameters (mm) of the tumours. Tumour burden was calculated by multiplying tumour volume and the number of tumours/hamster. The mean tumour volume and burden of tumour-bearing hamsters (Group II) were found to be 378.3 mm^3 and 1,399.7 mm^3, respectively. Oral administration of piperine (50 mg/kg body weight) and curcumin (80 mg/kg body weight) to DMBA-painted hamsters (Groups III and IV, respectively) on alternate days to DMBA painting for 14 weeks completely prevented the formation of oral squamous cell carcinoma. The gross appearance of oral squamous cell carcinoma noticed in DMBA-alone painted hamsters is shown in Fig. 1.

The histopathological features observed in the buccal mucosa of the control and experimental animals in each group are shown in Table II. We observed severe hyperkeratosis, hyperplasia, dysplasia and well-differentiated squamous cell carcinoma in the buccal pouches of DMBA-alone painted hamsters (Fig. 2). Although well-differentiated squamous cell carcinoma was not developed in the buccal pouches of DMBA + piperine (Group III) and DMBA + curcumin (Group IV) treated hamsters, hyperplasia, hyperkeratosis and dysplasia were observed. Control animals treated with piperine and curcumin showed well-defined and intact epithelial layers similar to that of the control animals. The severity of pathological changes was determined by the oral pathologist (CRR), when examining the histopathological slides under the microscope.

Table IV. TBARS and antioxidants status in erythrocytes of control and experimental animals in each group.

Groups	Erythrocyte TBARS (pmoles/ mg Hb)	Erythrocyte membrane TBARS (nmoles/ mg protein)	Vitamin E (μg/mg protein)	Erythrocytes GSH (mg/dL)	Erythrocyte lysate SOD (U^A/mg Hb)	Erythrocyte lysate CAT (U^B/mg Hb)	Erythrocyte lysate GPx (U^C/g Hb)
Control	1.48 ± 0.12[a]	0.32 ± 0.03[a]	2.56 ± 0.16[a]	50.3 ± 4.1[a]	2.50 ± 0.17[a]	1.36 ± 0.08[a]	15.62 ± 1.30[a]
DMBA	2.85 ± 0.33[b]	1.12 ± 0.12[b]	1.71 ± 0.19[b]	31.4 ± 2.9[b]	1.52 ± 0.19[b]	0.64 ± 0.09[b]	8.21 ± 0.93[b]
DMBA + piperine	1.68 ± 0.13[c]	0.40 ± 0.06[c]	2.23 ± 0.23[c]	44.6 ± 3.9[c]	2.19 ± 0.21[c]	1.24 ± 0.07[c]	13.34 ± 1.28[c]
DMBA + curcumin	1.61 ± 0.11[c]	0.38 ± 0.05[c]	2.28 ± 0.26[c]	45.7 ± 4.3[c]	2.24 ± 0.29[c]	1.26 ± 0.08[c]	13.92 ± 1.31[c]
Piperine alone	1.49 ± 0.15[a]	0.32 ± 0.08[a]	2.57 ± 0 21[a]	51.1 ± 4.6[a]	2.51 ± 0.15[a]	1.37 ± 0.08[a]	15.61 ± 1.40[a]
Curcumin alone	1.47 ± 0.12[a]	0.31 ± 0.05[a]	2.58 ± 0.17[a]	51.7 ± 4.1[a]	2.52 ± 0.18[a]	1.37 ± 0.09[a]	15.73 ± 1.35[a]

Values are expressed as mean ± SD for ten hamsters in each group. Values that do not share a common superscript in the same column differ significantly at $p < 0.05$ (DMRT).
[A] The amount of enzyme required to inhibit 50% NBT reduction.
[B] Micromoles of hydrogen peroxide utilised/sec.
[C] Micromoles of glutathione utilised/min.

The activities of phase II detoxification enzymes (GST and GR) and GSH level in the livers of control and experimental animals in each group are shown in Fig. 3. The activities of liver detoxification agents were significantly decreased in tumour-bearing hamsters (Group II), as compared to control hamsters. Oral administration of piperine (50 mg/kg body weight) and curcumin (80 mg/kg body weight) to DMBA-painted hamsters (Groups III and IV, respectively) significantly brought back the activities of detoxification agents to near normal status. Hamsters treated with piperine and curcumin alone (Groups V and VI, respectively) showed no significant difference in the status of detoxification agents as compared to control hamsters.

The status of TBARS and antioxidants (Vitamins E and C, GSH, SOD, CAT and GPx) in plasma and erythrocytes of control and experimental animals in each group are respectively shown in Tables III and IV. The concentration of TBARS was increased, whereas the status of antioxidants was significantly decreased in tumour-bearing animals (Group II) as compared to control animals. Oral administration of piperine and curcumin to DMBA-painted animals (Groups III and IV, respectively) significantly brought back the concentrations of TBARS and antioxidants to near normal status. Hamsters treated with piperine and curcumin alone (Groups V and VI, respectively) showed no significant difference in TBARS and antioxidants status as compared to control hamsters (Group I).

The status of TBARS and antioxidants in the buccal mucosa of the control and experimental animals in each group is shown in Table V. A decrease in the TBARS level and disturbances in antioxidants status (vitamin E, GSH and GPx were increased; SOD and CAT were decreased) were noticed in tumour-bearing animals (Group II) as compared to control animals. Oral administration of piperine and curcumin to DMBA-painted animals (Groups III and IV, respectively) brought back the concentration of TBARS and antioxidants to near normal range. Hamsters treated with piperine and curcumin alone (Groups V and VI, respectively) showed no significant difference in TBARS and antioxidants status as compared to control hamsters (Group I).

DISCUSSION

In the present study, we have investigated the chemopreventive efficacy of curcumin and piperine in DMBA-induced hamster buccal pouch carcinogenesis. The chemopreventive efficacy was assessed by monitoring the percentage of tumour-bearing animals, the tumour volume and burden as well as by analysing the status of detoxification agents, lipid peroxidation and antioxidants in DMBA-painted animals. Hamsters painted with 0.5% DMBA for 14 weeks in their buccal pouches developed well-differentiated squamous cell carcinoma. Oral administration of piperine and curcumin completely prevented the tumour formation in DMBA-painted hamsters (Groups III and IV). Our results thus suggest that curcumin and piperine may act as either antiproliferators or antipromotors during DMBA-induced oral carcinogenesis.

Chemical carcinogens can bind to DNA and result in mutagenic events that contribute to malignant transformation.[37] A number of studies have reported that chemopreventitive agents convert DNA-damaging entities into excretable metabolites by stimulating the action of detoxification agents such as GST.[5,38] In the present study, the activities of GST, GR and GSH levels were decreased in DMBA-painted animals as compared to control animals. Oral administration of piperine and

Table V. Buccal mucosa TBARS and antioxidants status in control and experimental animals in each group.

Groups	TBARS (nmoles/ 100 mg protein)	GSH (mg/ 100 mg tissues)	Vitamin E (mg/ 100 mg tissues)	SOD (U^A/mg protein)	CAT (U^B/mg protein)	GPx (U^C/g protein)
Control	69.3 ± 5.8[a]	6.01 ± 0.48[a]	1.64 ± 0.15[a]	4.91 ± 0.36[a]	36.8 ± 2.9[a]	6.48 ± 0.42[a]
DMBA	39.8 ± 4.1[b]	12.3 ± 1.30[b]	2.91 ± 0.32[b]	2.98 ± 0.31[b]	20.1 ± 2.6[b]	12.7 ± 1.20[b]
DMBA + piperine	59.8 ± 4.9[c]	7.08 ± 0.98[c]	1.98 ± 0.23[c]	4.32 ± 0.35[c]	31.8 ± 3.4[c]	7. 49 ± 0.69[c]
DMBA + curcumin	62.2 ± 4.6[c]	6.84 ± 0.82[c]	1.92 ± 0.23[c]	4.36 ± 0.36[c]	33.1 ± 3.1[c]	7.41 ± 0.72[c]
Piperine alone	69.2 ± 5.9[a]	6.03 ± 0.42[a]	1.65 ± 0.14[a]	4.95 ± 0.38[a]	37.1 ± 2.8[a]	6.52 ± 0.48[a]
Curcumin alone	68.8 ± 5.7[a]	6.04 ± 0.41[a]	1.67 ± 0.13[a]	4.99 ± 0.46[a]	37.6 ± 3.2[a]	6.58 ± 0.51[a]

Values are expressed as mean ± SD for ten hamsters in each group. Values that do not share a common superscript in the same column differ significantly at $p < 0.05$ (DMRT).
[A] The amount of enzyme required to inhibit 50% NBT reduction.
[B] Micromoles of hydrogen peroxide utilised/sec.
[C] Micromoles of glutathione utilised/min.

curcumin to DMBA-painted animals restored the activities of GST, GR, GSH and glutathione, which indicated their role in carcinogen detoxification. The chemopreventive effect of piperine and curcumin is probably due to their inhibitory role on metabolic activation of DMBA by stimulating the activities of phase II detoxification agents.

The measurement of serum or plasma lipid peroxidation byproducts helps in assessing the extent of tissue damage.[8] It has been suggested that excessively generated lipid peroxides at the primary site could be transferred through circulation to other organs and provoke damage by propagating lipid peroxidation.[8] Erythrocytes are highly susceptible to ROS-mediated oxidative damage in pathological conditions.[7] Increased plasma TBARS observed in tumour-bearing hamsters is probably due to the overproduction and diffusion of lipid peroxidation byproducts from the damaged tissues with consequent leakage into plasma. Non-enzymatic and enzymatic antioxidants play a protective role against neoplastic transformation and ROS-mediated oxidative stress.[4,7] Tumour cells sequester essential nutrients from circulation to meet the demands of a growing tumour.[4] Decreased levels of vitamins E and C and GSH content in plasma and erythrocytes are probably due to their utilisation by tumour tissues to meet their nutrient demands for growth. Lowered activities of SOD, catalase and GPx in plasma and erythrocytes are probably due to the exhaustion of these enzymes to scavenge excessively-generated ROS in the system.

Tumours have shown less susceptibility to lipid peroxidation as opposed to normal cells. An inverse association between lipid peroxidation and the rate of cell division in tumours has been suggested.[9,39] The poor availability of peroxidative substrates such as polyunsaturated fatty acids (PUFA) has been demonstrated in tumour tissues.[40] Thus, the decreased susceptibility of tumour tissues to lipid peroxidation can be related to increased cell proliferation occurring in oral carcinogenesis or the poor availability of PUFA in tumour tissues. Glutathione and GPx have regulatory effects on cellular proliferation and are over-expressed in various malignant tumours.[7,41] An increased level of vitamin E has been reported in tumour tissues of several cancers, including oral carcinoma.[4,10] Lowered levels of tumour tissue TBARS also indicate that tumour tissues have sequestered these antioxidants from circulation to enhance their antioxidant capacity as well as to meet their nutrient requirements. Tumour tissues produce substantial amounts of superoxides and hydrogen peroxides.[39] Lowered activities of SOD and CAT in tumour tissues are probably due to the exhaustion of these enzymes to scavenge overproduced superoxides and hydrogen peroxides in tumour cells. Oral administration of piperine and curcumin restored the status of lipid peroxidation and antioxidants in circulation and the buccal mucosa tissues of DMBA-painted animals. Our results thus suggest that piperine and curcumin displayed a potent antioxidant function during oral carcinogenesis.

The present study thus demonstrated the chemopreventive efficacy of curcumin and piperine in DMBA-induced hamster buccal pouch carcinogenesis. The chemopreventive potential of curcumin and piperine are probably due to their antilipidperoxidative and antioxidant potential or modulating effect on the carcinogen detoxification process. Further studies are, however, required in order to better understand the underlying mechanisms of the chemopreventive actions of curcumin and piperine.

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