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EVALUATION OF CHEMOPREVENTIVE EFFICACY OF *CASSIA FISTULA* IN 7, 12–DIMETHYL BENZ(a) ANTHRACENE (DMBA) INDUCED ORAL CARCINOGENESIS

K. VASUDEVAN, S. MANOHARAN*, LINSA MARY ALIAS, S. BALAKRISHNAN, L. VELLAICHAMY and M. GITANJALI

Department of Biochemistry and Biotechnology, Annamalai University, ANNAMALAINAGAR – 608002 (T. N.) INDIA

ABSTRACT

Cassia fistula is used in Indian traditional medicine as a remedy for various diseases including tumours. The present study has investigated the chemopreventive efficacy of *Cassia fistula* bark extracts in 7, 12–dimethyl benz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. The mechanistic pathway for its chemopreventive potential was assessed by measuring the status of lipid peroxidation by-products, antioxidants and detoxification enzyme activities in the circulation and tissues of tumour bearing animals. Oral squamous cell carcinoma was developed in buccal pouches of hamsters by painting with 0.5% DMBA, three times a week for 14 weeks. We observed 100% tumour formation in DMBA alone painted animals. Oral administration of *Cassia fistula* bark extract to DMBA painted animals completely prevented the formation of oral squamous cell carcinoma. The bark extract also restored the status of lipid peroxidation by-products, antioxidants and detoxification enzymes in DMBA painted animals. These results suggest that *Cassia fistula* bark extract has prominent chemopreventive effect during DMBA induced oral carcinogenesis, which is probably due to the presence of one or more potent anticarcinogenic principles and their synergistic effect. The chemopreventive potential of *Cassia fistula* may also be due to its antilipid peroxidative, antioxidative and modulation of detoxification agents during DMBA induced oral carcinogenesis.

Key words : DMBA, Oral cancer, Lipid peroxidation, Anti-oxidants, Detoxification agents.

INTRODUCTION

Cancer usually forms as a tumor and can travel to other parts of the body, where they begin to grow and replace normal tissue. Oral cancer is the cancer that starts in the

* Author for correspondence; Phone : +91-4144-238343 (Extn. 230) (Off); + 91= 41442 = 232788 (Res); Fax : +91- 4144 - 238080; E-Mail : manshisak@yahoo.com

mouth, also called oral cavity. Oral cavity includes the lips, cheeks (buccal mucosa), the gums, the front two-thirds of the tongue, the floor of the mouth below the tongue, the bony roof of the mouth (hard palate) and the area behind the wisdom teeth[1]. Oral squamous cell carcinoma (OSCC) constitutes a major public health problem globally, with almost half a million oral and pharyngeal cancers are diagnosed every year, with three-quarters of these from the developing world[2]. The American Cancer Society reported about 34, 360 new cases of oral cavity and oropharyngeal cancer in the United States in 2007[3]. In India, oral cancer is the leading cancer, comprising about 40-50% of all malignancies. The higher incidence of oral cancer in India is primarily associated with the habits of tobacco and areca nut chewing, tobacco smoking and alcohol consumption[4]. 7, 12-Dimethylbenz(a) anthracene (DMBA) induced hamster buccal pouch carcinogenesis is widely employed to study the chemopreventive potential of medicinal plants and their bioactive constituents.

DMBA on metabolic activation produces diol-epoxide (ultimate carcinogen), which mediates carcinogenic process by inducing chronic inflammation, over production of reactive oxygen species and oxidative DNA damage. It is a potent organ specific carcinogen and the pre-cancerous and cancerous lesions induced by DMBA are morphologically and histologically similar to that of human oral carcinoma[5].

Free radicals are chemical species with unpaired electrons that are formed in the body in response to stress, poor diet and infection and in illness. They act as mediators of the phenotypic and genotypic alterations, contributing to mutagenesis and carcinogenesis. The important reactive oxygen species in biological systems include superoxide radical (O_2^{-}), hydrogen peroxides (H_2O_2), hydroxy radical ($^{\bullet}OH$), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^{-}$). Although physiological levels of free radicals are essential to maintain various cell function such as aiding in the destruction of microorganisms, over production of free radicals results in oxidative stress that cause damage to structure and function of cells[6]. Free radicals induced oxidative stress results in the etiopathogenesis of several cancers including oral cancer[7]. Free radicals on attacking biomembranes can lead to oxidative destruction of the polyunsaturated fatty acids (PUFA) by a process called lipid peroxidation, a multistep process[8].

Human body, however, contains an array of antioxidant defense mechanisms including non-enzymatic (Vitamin E, C and reduced glutathione (GSH) and enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) to fight against reactive oxygen species. Vitamin E, vitamin C and reduced glutathione (GSH) are primary defense antioxidants that provide protection against oxidative stress mediated dysfunctions and act as inhibitors of carcinogenesis[9]. Reduced

glutathione (GSH), an important intracellular antioxidant plays a crucial role in normal cell function as well as in toxicology, carcinogenesis, radiotherapy, chemotherapy and other diverse areas[10]. Superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx) are important antioxidant enzymes which can protect cell and tissues causes by from free radicals mediated oxidative damage[11].

Cancer chemoprevention is a novel approach in oncology, to arrest or reverse the process of cancer development by the use of natural or synthetic agents. India is rich in medicinal plants biodiversity where most of the prescribed modern medicine contains the active principles of medicinal plants or their derivatives. Several medicinal plants and their constituents have been reported to prevent multistage carcinogenesis[12,13]. Although a large number of medicinal plants and their bioactive constituents have been reported to have potent chemopreventive potential, several other medicinal plants and their active principles remain to be investigated.

Cassia fistula Linn (Caesalpinaceae) tree is one of the most widespread in the forests of India, usually occurring in deciduous forests. It is native to India, the Amazon and Sri Lanka and is now widely cultivated worldwide as an ornamental flowering tree. *Cassia fistula* is an important source of naturally occurring bioactive compounds such as polyphenolic compounds. In Ayurvedic medicine, *Cassia fistula* is widely employed and used as a remedy for the tumours of liver, stomach and throat[14]. The whole plant possesses medicinal properties, which are useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice[15]. To the best of our knowledge we have found no scientific literature for the chemopreventive efficacy of *Cassia fistula* in DMBA induced experimental oral carcinogenesis. The present study was, thus, designed to provide scientific validity for the chemopreventive effect of ethanolic *Cassia fistula* bark extract during DMBA induced hamster buccal pouch carcinogenesis.

EXPERIMENTAL

Materials and methods

Chemicals

The carcinogen, 7, 12-dimethylbenz(a)anthracene (DMBA) and other biochemicals such as reduced glutathione, reduced nicotinamide adenine dinucleotide, 1, 1', 3, 3'-tetramethoxypropane, bovine serum albumin, were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. Heparin, thiobarbituric acid (TBA), trichloroacetic acid, 2, 4-dinitrophenylhydrazine (DNPH), 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), 1-

chloro-2, 4-dinitrobenzene (CDNB), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), cysteine hydrochloride and sodium metaarsenate were purchased from Hi-Media Laboratories, Mumbai, India. All other chemicals and solvents used were of analar grade.

Animals

Male golden Syrian hamsters 8-10 weeks old weighing 80-120 g were purchased from National Institute of Nutrition, Hyderabad, India and were maintained in Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed five in a polypropylene cage and provided standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12 h light/dark cycle. The local institutional animal ethics committee (Register number 160/1999/CPCSEA), Annamalai University, Annamalainagar, India, approved the experimental design (Proposal No. 421 : dated. 21-03-2007); 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.

Plant materials

Cassia fistula barks were obtained from in and around Chidambaram, Tamil Nadu, India. The identity of the plants were verified by the Botanist, Dr. S. Sivakumar, Reader, Department of Botany, Annamalai University and a voucher specimen (Cassia fistula AU06167) was also deposited in the Department of Botany, Annamalai University.

Preparation of ethanolic extract of *Cassia fistula* bark

The ethanolic extract of *Cassia fistula* barks was prepared according to the method of Hossain et al.[16]. 500 g of *Cassia fistula* barks were dried, powdered and then soaked separately in 1500 mL of 95% ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 h and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapour at 40-50°C under reduced pressure. A 16% semisolid dark brown material obtained was stored at 0-4°C until used.

DMBA induced oral carcinogenesis

7, 12-dimethylbenz (a) anthracene (DMBA), a potent organ specific carcinogen, is widely employed to induce oral carcinoma in hamsters buccal pouches. It has been

suggested that DMBA on metabolic activation induce cancer through an oxidative mediated genotoxicity by incorporating diol epoxide and other reactive oxygen species in to DNA. DMBA induced hamster buccal pouch carcinogenesis is a well suited model for studying precancerous and cancerous lesions of human oral squamous cell carcinoma, Since the carcinoma induced by DMBA is morphologically and histologically similar to human tumours, as well as express many biochemical and molecular markers that are expressed in human oral carcinoma.

Experimental design

A total number of 24 hamsters were divided into 4 groups of 6 animals each. Group 1 animals were served as untreated control. Animals in groups 2 and 3 were induced oral carcinogenesis by painting with 7, 12-dimethybenz(a)anthracene (DMBA) in liquid paraffin three times per week for 14 weeks. Group 2 received no other treatment. Groups 3 animals were orally administered ethanolic *Cassia fistula* bark extract, starting 1 week before the exposure to the carcinogen and continuing till the sacrification of the animals. Groups 4 animals were orally administered ethanolic *Cassia fistula* bark extract alone throughout the experimental period. Tumour tissues and normal buccal mucosa tissues were fixed in 10 % formalin and were routinely processed and paraffin embedded, 2-3 μm sections were cut in a rotary microtome and were stained with hematoxylin and eosin.

Biochemical analysis

The activity of glutathione –S-transferase (GST) was assayed by the method of Habig et al[17]. Glutathione reductase (GR) activity was assayed by the method of Carlberg and Mannervik[18]. Thiobarbituric acid reactive substances in plasma were assayed by the method of Yagi[19] and in erythrocyte membrane by the method of Donnan[20]. Reduced glutathione was assayed by the method of Beutler and Kelley[21]. Vitamin C and E were measured according to the methods of Omaye et al.[22] and Desai[23], respectively. The activities of SOD, CAT and GPx were estimated according to the methods of Kakkar et al.[24] Sinha[25] and Rotruck et al.[26] respectively.

Statistical analysis

The values are expressed as mean ± SD. The statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT), using SPSS version 12.0 for windows (SPSS Inc. Chicago; http : //www. spss. com). The values are considered statistically significant if the p value was less than 0.05.

RESULTS AND DISCUSSION

Table 1 depicts the effect of *Cassia fistula* ethanolic bark extract on tumour incidence, tumour volume and tumour burden in DMBA induced hamster buccal pouch carcinogenesis. We have observed 100% tumour formation with mean tumor volume 208.1 mm^3 and tumour burden (628.5 mm^3) in DMBA alone painted hamsters (Group 2). Oral administration of *Cassia fistula* ethanolic bark extract at a dose of 300 mg/kg bw completely prevented the tumour incidence, tumour volume and tumour burden in DMBA painted hamsters in the pre-initiation phase (Group 3). No tumours were observed in *Cassia fistula* ethanolic bark extract alone administered animals (Group 4) and in control animals (Group 1).

Table 1. Effect of *Cassia fistula* on formation of squamous cell carcinoma in 0.5% DMBA painted golden syrian hamsters

Parameters	Group			
	Control	**DMBA**	**DMBA + *Cassia fistula***	***Cassia fistulla* alone**
Tumor incidence coral squamous cell carcinoma	0	100 %	0 %	0
Total number of tumors / animals	0	20 (6)	0 (6)	0
Tumor volume	0	208.1 ± 17.5	0	0
Tumor burden	0	628.5 ± 70.4	0	0

Values are expressed as ± SD for 6 animals in each group. Tumor volume was measured using the formula $V = \frac{4}{3}\pi \left(\frac{D_1}{2}\right)\left(\frac{D_2}{2}\right)\left(\frac{D_3}{2}\right)$ where D_1, D_2 and D_3 are the three diameters (mm) of the tumor. The tumor burden was calculated by multiplying tumor volume and the number of tumors / animal. ( ) - indicates total number of animals bearing tumors.

Table 2 shows the status of plasma, erythrocytes and erythrocyte membrane

TBARS in control and experimental animals in each group. The concentration of TBARS was increased in plasma, erythrocytes and erythrocyte membranes whereas decreased in DMBA painted hamsters (Group 2) as compared to control animals. Oral administration of ethanolic extract of *Cassia fistula* barks at a dose of 300 mg/kg bw, respectively, significantly revert back the concentration of TBARS to near normal range in the pre-initiation phase (Group 3). Hamsters treated with ethanolic extract of *Cassia fistula* barks alone (Group 4) respectively showed no significant difference in TBARS levels as compared to control animals.

Table 2. The levels of thiobarbituric acid reactive substances (TBARS) in plasma, erythrocytes and erythrocyte membrane of control and experimental animals in each group.

Group	TBARS		
	Plasma (nmol/mL)	**Erythrocytes (pmol/mg Hb)**	**Erythrocyte membrane (nmol/mg protein)**
Control	2.07 ± 0.12a	1.69 ± 0.11a	0.27 ± 0.10a
DMBA	4.16 ± 0.52b	2.27 ± 0.24b	0.96 ± 0.08b
DMBA + *Cassia fistula* ethanolic bark extract	2.32 ± 0.22c	1.97 ± 0.15c	0.44 ± 0.05c
Cassia fistula ethanolic bark extract alone	2.11 ± 0.14a	1.68 ± 0.11a	0.36 ± 0.05a

Values are expressed as mean ± SD; n = 6.Values not sharing a common superscript significantly differ at $p < 0.05$.(DMRT)

Tables 3 and 4 show the levels of plasma, erythrocytes and erythrocyte membrane non-enzymatic antioxidants, respectively in control and experimental animals in each group. The concentration of non - enzymatic antioxidants (GSH, vitamin C and vitamin E) were significantly decreased in plasma erythrocytes and erythrocyte membrane whereas disturbances in antioxidant status (vitamin E and GSH were increased) were noticed in buccal mucosa of cancer animals as compared to normal animals. Oral administration of ethanolic extract of *Cassia fistula* barks at a dose of and 300 mg/kg bw revert back the status to normal range in the pre-initiation phase (group 3). Hamsters treated with ethanolic extract of *Cassia fistula* barks alone showed no significant difference in antioxidants status as compared to control animals.

Table 4. The levels of non-enzymatic antioxidants in erythrocytes, erythrocyte membrane of control and experimental animals in each group

Group	Erythrocyte membrane vitamin E (µg/mg protein)	Erythrocyte GSH (mg/dL)
Control	2.19 ± 0.10^{a}	51.8 ± 3.2^{a}
DMBA	1.59 ± 0.16^{b}	35.8 ± 3.8^{b}
DMBA + *Cassia fistula* ethanolic bark extract	1.92 ± 0.30^{c}	45.8 ± 9.01^{c}
Cassia fistula ethanolic bark extract alone	2.05 ± 0.11^{a}	54.6 ± 8.50^{a}

Values are expressed as mean ± SD; n = 6 Values not sharing a common superscript significantly differ at $p < 0.05$.(DMRT)

Tables 5 and 6 show the activities of plasma and erythrocytes enzymatic antioxidants in control and experimental animals in each group.

Table 5. The activities of enzymatic antioxidants in plasma of control and experimental animals in each group

Group	Plasma		
	SOD (UA/mL)	CAT (UB/mL)	GPx (UC/l)
Control	2.46 ± 0.16^{a}	0.38 ± 0.02^{a}	109.5 ± 4.7^{a}
DMBA	1.58 ± 0.22^{b}	0.28 ± 0.03^{b}	84.3 ± 6.1^{b}
DMBA + *Cassia fistula* ethanolic bark extract	2.00 ± 0.35^{c}	0.33 ± 0.06^{c}	100.4 ± 9.1^{c}
Cassia fistula ethanolic bark extract alone	2.56 ± 0.43^{a}	0.35 ± 0.07^{a}	117.2 ± 13.3^{a}

Values are expressed as mean ± SD; n = 6.Values not sharing a common superscript significantly differ at $p < 0.05$.(DMRT)

A - Amount of enzyme required to inhibit 50% nitroblue tetrazolium reduction/min.

B - µ moles of H_2O_2 utilized /min.

C - µ moles of GSH utilized / min.

Table 6. The activities of enzymatic antioxidants in erythrocytes of control and experimental animals in each group

Group	Erythrocyte lysate		
	SOD (UA/mg Hb)	**CAT (UB/mg Hb)**	**GPx (UC/g Hb)**
Control	2.07 ± 0.11^{a}	1.16 ± 0.09^{a}	13.2 ± 1.1^{a}
DMBA	1.52 ± 0.20^{b}	0.93 ± 0.09^{b}	8.2 ± 0.72^{b}
DMBA + *Cassia fistula* ethanolic bark extract	1.81 ± 0.25^{c}	1.11 ± 0.15^{c}	10.0 ± 1.93^{c}
Cassia fistula ethanolic bark extract alone	2.14 ± 0.31^{a}	1.37 ± 0.22^{a}	12.8 ± 2.90^{a}

Values are expressed as mean ± SD; n = 6.Values not sharing a common superscript significantly differ at $p < 0.05$.(DMRT)

A - Amount of enzyme required to inhibit 50% nitroblue tetrazolium reduction/min.

B - μ moles of H_2O_2 utilized /min.

C - μ moles of GSH utilized / min.

The activities of enzymatic antioxidants (SOD, CAT, GPx) were significantly decreased in plasma and erythrocytes of cancer animals as compared to normal animals. Oral administration of ethanolic extract of *Cassia fistula* bark at a dose of 300 mg/kg bw revert back the status to normal range in the pre-initiation phase in the circulation. Hamsters treated with ethanolic extract of *Cassia fistula* bark alone showed no significant difference in enzymatic antioxidants status as compared to control animals.

Table 7 shows the activities of liver detoxication agents in control and experimental animals in each group. The activities of glutathione–S–transferase, glutathione reductase and reduced glutathione content were significantly decreased in liver of cancer animals as compared to normal animals. Oral administration of ethanolic extract of *Cassia fistula* bark at a dose of 300 mg/kg bw revert back the status to normal range in liver. Hamsters treated with ethanolic extract of *Cassia fistula* bark alone showed no significant difference in detoxication agents as compared to control animals.

Table 7. Activities of enzymatic antioxidants in liver of control and experimental animals in each group

Group	GST (U^A/mg protein)	GR (U^B/mg protein)	GSH (mg/g tissue)
Control	22.62 ± 2.2[a]	16.74 ± 1.82[a]	2.87±0.19[a]
DMBA	17.81 ± 1.56[b]	13.16 ± 0.94[b]	1.57±0.20[b]
DMBA + *Cassia fistula* ethanolic bark extract	19.76 ± 1.85[c]	15.17 ± 1.47[c]	2.63±0.16[c]
Cassia fistula ethanolic bark extract	22.68 ± 2.08[a]	17.81 ± 1.86[a]	2.80±0.14[a]

Values are expressed as mean ± SD; n = 6 values not sharing a common superscript significantly differ at $p < 0.05$.(DMRT)

A - μ moles of CDNB-GSH – conjugate formed /min.

B - μ moles of NADPH oxidized per hour.

In the present study, we have investigated the chemopreventive effect of *Cassia fistula* bark extract in DMBA induced hamster buccal pouch carcinogenesis. The mechanistic pathway for chemopreventive effect of *Cassia fistula* was assessed by analyzing the percentage of tumour bearing animals, tumour volume and burden as well as by estimating the status of detoxification enzymes, lipid peroxidation and antioxidants in DMBA painted animals. In the present study, we have observed hundred percentage tumour formations in DMBA alone painted animals. Oral administration of ethanolic bark extract of *Cassia fistula* to DMBA painted animals completely prevented the formation of oral squamous cell carcinoma. We have observed well-defined and intact epithelial layer in untreated hamsters whereas severe hyperkeratosis, hyperplasia, dysplasia and well-differentiated squamous cell carcinoma were noticed in DMBA alone painted hamsters. The tumour tissues from buccal pouches of golden Syrian hamsters showed pleomorphic hyperchromatic nucleic with epithelial pearl formation. Oral administration of ethanolic *Cassia fistula* bark to DMBA painted animals revealed mild hyperkeratosis and hyperplasia.

The chemopreventive potential ethanolic bark extract is probably due its suppressing effect on cell proliferation. The chemopreventive effect is also due to the presence of one or more bioactive chemopreventive principles and their synergestic effects in *Cassia fistula* barks.

Lipid peroxidation and antioxidants play a vital role in the genesis of various cancers. Over production of reactive oxygen species and disturbed antioxidant defense mechanism have been documented well in several cancers[27]. Free radical mediated lipid peroxidation causes abnormalities in structure and functions of cell membranes and can cause damages to cellular DNA[28]. In the present study, altered lipid peroxidation disturbed enzymatic and non enzymatic antioxidants were noticed in DMBA alone painted animals as compared to untreated control animals. Oral administration of *Cassia fistula* bark extracts restored the status of lipid pcroxidation and antioxidants in DMBA painted animals.

Increase in lipid peroxidation and poor antioxidant systems have been reported in both human and experimental carcinogenesis[29]. Susceptibility of erythrocytes to lipid peroxidation has been well documented in cancerous conditions[30]. Measurement of plasma TBARS help to assess the extent of tissue damage in the host. Lipid peroxides formed at primary sites can traverse through the circulation to the other sites in order to propagate the process of lipid pcroxidation[31]. The observed increase in plasma TBARS in DMBA painted animals is probably due to over production of reactive oxygen species during DMBA exposure with subsequent leakage into plasma. Elevated TBARS in plasma is also due to over production and diffusion from erythrocytes with subsequent leakage into plasma. Several studies have documented that tumour tissues sequester nutrients and antioxidants from circulation for their nutrient demand and growth[32, 33]. A positive association between lipid peroxidation and antioxidants has been reported[34]. Lowered levels of non-enzymatic antioxidatants such as vitamin E, vitamin C and reduced glutathione were reported in human and experimental oral cancers[35, 36]. Elevated lipid peroxidation in plasma of tumour bearing animals can therefore be correlated to insufficient antioxidant mechanism. Lowered non-enzymatic antioxidants in plasma are probably due to utilization by tumour tissues or to combat the deleterious effects of excessively generated lipid peroxides in the system. Decease in the activities of enzymatic antioxidants, due to exhaustion of these enzymes in combating the deleterious effects of ROS, has been reported in several type of cancers[37, 38]. Our results lend credibility to these observations. Oral administration of *Cassia fistula* bark extract to DMBA painted animals normalized the status of enzymatic and non-enzymatic antioxidants in the circulation. Our results reveal that *Cassia fistula* bark extract has potent antioxidant activity during DMBA induced hamster buccal pouch

carcinogenesis.

Estimation of liver detoxification enzyme activities can be used as reliable biochemical markers to assess the chemopreventive efficacy of the test compound. Several studies on chemoprevention suggested that many medicinal plants that induce the activity of glutathione-S-transferase have potent chemopreventive activity[39, 40]. Many chemopreventive agents convert DNA damaging entities into excretable metabolites through the induction of GST[41]. Glutathione reductase catalyses NADPH dependent reduction of glutathione disulfide to reduced glutathione. This enzyme therefore has a crucial role in the maintenance of reduced glutathione in the system[42]. Decreased activities of GST and GR were reported in tumour bearing animals as compared to control animals. Oral administration of *Cassia fistula* significantly restored the activities of these enzymes during pre-initiation phase, which indicates its crucial role in the detoxification process of the chemical carcinogens.

Oral administration of *Cassia fistula* bark extract not only prevented the tumour formation but also improved the status of lipid peroxidation, antioxidants and detoxification enzyme activities in DMBA painted animals, which clearly indicates its potent chemopreventive, antilipid peroxitative and detoxification potential during DMBA induced hamster buccal pouch carcinogenesis. The present investigation, thus, warrants further studies to isolate and characterize bioactive chemopreventive principles from the ethanolic extract of *Cassia fistula* bark.

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REFERENCES

1. P. N. Notani, Curr. Sci., 81, 465-474 (2001).
2. J. Ferlay, F. Bray, P. Pisani and D. M. Parkin, International Agency for Research On Cancer, Iarc Cancer Base, 5 (2001).
3. US Cancer Statistics Working Group. United States Cancer Statistics, (2001) Incidence and Mortality. Atlanta, Ga, Centers for Disease Control and Prevention and National Cancer Institute (2004).

4. P. H. Chen, T. Y. Shieh, P. S. Ho, C. C. Tsai, Y. H. Yang, Y. C. Lin, M. S. Ko, P. C. Tsai, S. L. Chiang, H. P. Tu and Y. C. Ko, Bmc Cancer., **7**, 101 (2007).
5. K. Suresh, S. Manoharan, K. Panjamurthy and N. Senthil, J. Med Sci., **7**, 100-105 (2007).
6. T. P. Devasagayam, J. C. Tilak, K. K. Bloor, K. S. Sane, S. S. Ghaskadbi and R. D. Lele, J. Assoc Physicians India., **52**, 794-804 (2004).
7. L. J. Marnett, Toxicology., 181, 219-22 (2002).
8. N. Garg , R. Singh , J. Dixit and A. Jain , J. Periodontal Res., **4**, 405-10 (2006).
9. J. M. C. Gutteridge, Clin. Chem., **41**, 1819-28 (1995).
10. J. D. Hayes and L. I. Mclellan, Free Rad Res., **31**, 273-300 (1999).
11. A. Matkowski and M. Piotrowska, Fitoterapia, **77**, 346-53 (2006).
12. S. F-Guyer, F. Afaq and H. Mukhtar, Photodermotol. Photoimmunol. Photomed., **19**, 56–72 (2003).
13. G. J. Kelloff, Adv. Cancer Res., **78**, 199–334 (2000).
14. T. N. Misra, R. S. Singh, H. S Pandev and R. P. Pandev, Fitoterapial XVII, **57**, 173-174 (1996).
15. L. V. Asolkar, K. K. Kakkar and O. J. Chakre, Publication and Information Directorate, CSIR, New Delhi., (1992) p. 177.
16. M. Z. Hossain, B. H. Shibib and R. Rahman, Ind. J. Exp. Biol., **30**, 418-420 (1992).
17. W. M. Habig, M. J. Pabst and W. B. Jakoby, J. Biol. Chem., **249**, 7130-7139 (1994).
18. I. Carlberg and B. Mannervik, Methods in Enzymology. New York. Academic Press. (1985) pp. 484-490.
19. K. Yagi, Chem. Phys. Lipids., **45**, 337-351 (1987).
20. S. K. Donnan, J. Biol. Chem., **182**, 415-419 (1950).
21. E. Beutler and B. M. Kelley, Experientia., **19**, 96-97 (1963).
22. S. T. Omaye, T. D. Turnbull and H. E. Sauberlich, Methods Enzymol., **62**, 3-11 (1979).
23. F. D. Desai. in, S. Feicher and L. Packer (Eds.), Methods Enzymol., **105**, 138-145 (1984).
24. P. Kakkar, B. Das and P. N. Visvanathan, Ind. J. Biophys., **21**, 130-132 (1984).
25. K. A. Sinha, Anal Biochem., **17**, 389-394 (1972).

26. J. T. Rotruck, A. L. Pope, H. T. Ganther, A. B. Swanson, D. G. Hageman and W. G. Hoekstra, Science, **179**, 588-590 (1973).

27. G. Ozdemiler, H. Pabuccuoglu, T. Bulut et al., J. Cancer Res. Clin. Oncol., **128**, 555-9 (1998).

28. G. Ray and S. A. Husain, Ind. J. Exp Biol., **40**, 1213-1232 (2002).

29. G. Ozdemirler, H. Pabuccuoglu, T. Buluf, D. Bugra, M. Uysal and G. J. Toker, Cancer Res Clin Oncol., **128**, 659-9 (1998).

30. K. V. P. Chandra Mohan and S. Nagini, Nutr. Res., **23**, 1403-16 (2003).

31. P. Evans and B. Halliwell, Br. J. Nutr, **85**, 67 (2001).

32. M. W. Lingen, P. J. Polverini and N. P. Bouch, Cancer Res., **58**, 5551-5558 (1998).

33. K. Kolanjiappan and S. Manoharan, Fund. Clin. Pharmacol., **19**; 687-693 (2005).

34. R. Gonzales, C. Auclair and E. Voisin Et Al., Cancer Res., **44**, 4137-9 (1984).

35. C. Michiels, M. Raes, O. Toussaint and J. Remach, Free Radic. Biol. Med., **17**, 235-48 (1994).

36. S. Li, T. Yan, J. Q. Yang, T. D. Oberley and L. W. Oberley, Cancer Res., **60**, 3927-3939 (2000).

37. S. Balasenthil, S. Arivazhagan and S. Nagini, J. Ethano. Pharmacol., **72**, 429-33 (2000).

38. N. Senthil, S. Manoharan, S. Balakrishnan, Cr. Ramachandran and R. Muralinaidu, J. Appl. Sci., **7**, 1036- 2042 (2007).

39. S. Manoharan, K. Kavitha, N. Senthil and G. L. Renju, Singapore Med. J., **47**, 1038-1042 (2006).

40. V. Bhuvaneswari, S. K. Abraham and S. Nagini, Nutrition, **21**, 726-31 (2005).

41. J. Wilkinson and M. L. Clapper, Proc. Soc. Exptl. Biol. Med., **216**, 192-200 (1997).

42. J. L. Schwartz and G. Shaklar, Nutr. Cancer., **26**, 229-236 (1996).

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