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RESEARCH ARTICLE

CHEMOPREVENTIVE AND ANTIOXIDANT EFFICACY OF FISETIN IN EXPERIMENTAL ORAL CARCINOGENESIS

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ABSTRACT

The present study aimed to investigate the chemopreventive and antioxidant efficacy of fisetin on 7,12-dimethylbenz(a)anthracene(DMBA) induced hamster buccal pouch carcinogenesis in golden Syrian hamsters. Oral squamous cell carcinoma was developed in the buccal pouch of golden Syrian hamsters by painting with 0.5% DMBA in liquid paraffin three times a week for 14 weeks. We observed 100% tumour formation as well as, the status of lipid peroxidation, antioxidants and phase II detoxification enzymes were siginificantly altered during DMBA induced oral carcinogenesis. Oral administration of fisetin at a dose of (10mg/kgb.wt) to DMBA treated animals significantly prevented the tumour formation in the hamster buccal pouch and regained the status of lipid peroxidation, antioxidant. The above results suggest that the fisetin has potent chemopreventive and anti lipidperoxidative effect during DMBA induced oral carcinogenesis.

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INTRODUCTION

Oral cancer is a serious and growing problem in many parts of the world, is the sixth most common cancer in the world, and 3 – 4% of all cancers in western countries (Ferlay *et al.,* 2004). The morbidity and mortality of oral cancer is still higher in developing countries including India and pakistan, the highest incidence rate (40-50%) of all cancers (Jemal *et al.,* 2008). The etiology of oral cancer is multifactorial and most important etiological factors are tobacco, excess consumption of alcohol and betal quid usage, these factors act separately or synergistically(La Vecchia *et al.*,1997). It has recent epidemiological data reporting that chewing of betel quid with tobacco or without tobacco is the major aetiological factor for oral carcinogenesis in India (Johnson, 2001). Alcohol consumption are also the principal risk factor for oral carcinogenesis, and 80% of paitents with oral cancer are habituated to tobacco and alcohol consumption (Blot *et al.,* 1988).

Golden Syrian hamsters have used siginificantly to the understanding of precancerous and cancerous lesions of oral carcinogenesis. 7, 12-dimethylbenz (a) anthracene (DMBA), a potent carcinogen, site and organ specific carcinogen is widely used to induce oral squamous cell carcinoma in hamsters. Since it has many morphological and histological similarities with human oral carcinoma (Miyata *et al*, 2001; Shklar, 1999). DMBA manifests its carcinogenic effect in the target tissue through formation of DNA adducts, reactive oxygen species (ROS) readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Alias *et al*, 2009). Over production of reactive oxygen species in the human body can induce strand break and modify DNA bases, contributing to mutagenesis and carcinogenesis (Ray *et al*, 2002).

Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) , oxidative stress resulting from ROS and RNS is associated with an imbalance of oxidant /antioxidant status of the cell, which leads to a cascade of reaction in cell and affects the structural and functional integrity at the cell membrane level (Finkle and Holbrook, 2000). The human body has primary and secondary defenses against free radicals. The primary defence consists of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The secondary defense system consists of vitamins A, C, E, ß- carotenes and many others, which can act as antioxidants (Halliwell and Gutteridge, 2007).

Lipid peroxidation, a free radical mediated chain reaction, causes oxidative deterioration of lipids, particularly polyunsaturated fatty acids, play an important role in the control of cell divison (Janero, 1990). It has been suggested that DMBA, on metabolic activation, induces cancer through an oxidative mediated genotoxicity by incorporating diolepoxide and other ROS into DNA (Dipple *et al* ,1983). Reduced the levels of (TBARS) thio barbituric acid reactive substances have been well documented in tumours of the oral cavity (Kolanjiappan et *al* 2003). Over production of ROS in the activities of antioxidants diminished are implicated in the pathogenesis of several disorders including cancer (Valko *et al*, 2007).

Liver plays prominent and profound role in the metabolism and detoxification of mutagenic and carcinogenic substances. Glutathione – S- transferase (GST), glutathione reductasse (GR) and reduced glutathione act together to detoxify

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carcinogen either by destroying their reactive centres or excretion by conjugation process. Measurement of phase II detoxification enzymes in liver could help to assess the chemopreventive efficacy of the test compound (Kleiner *et al*, 2008). Cancer chemoprevention, a novel approach and recent trends in experimental oncology, deals with anti-tumour initiation and promoting potential of natural and synthetic agents. chemopreventive agents possibly exert their role by inhibiting mutagenesis, cell proliferation or by inducing apoptosis and modulating the activies of detoxification agents (Breimer ,1990).

Fisetin (3, 3, 4, 7- tetrahydroxy flavones), a major plant flavonoid, naturally occurring polyphenolic phytoconstituents. They are present in fruits, vegetables, tea, wine and grains. It also exerts wide properties such as anti cancer, anti allergic as well as inhibition of angiogenesis, antithyroid effects (Hertog *et al*, 1993; Rice-Evans *et al*, 1990; Arai *et al*, 2000). Many invitro studies have been performed to study the effect of fisetin. Very few reports were available on animal models (Sengupta, B. and Swenson.J, 2005). To the best of our knowledge, there were no scientific studies on chemopreventive efficacy of fisetin in DMBA induced hamster buccal pouch carcinogenesis. The chemopreventive efficacy of fisetin was assessed by analyzing the tumour incidence, tumour burden and volume and by measuring the status of lipid peroxidation, antioxidants and detoxification agents in DMBA treated hamsters.

MATERIALS AND METHODS

Chemicals

The carcinogen, DMBA and Fisetin were obtained from Sigma- Aldrich Chemical Pvt.Ltd., Bangalore, India. All other chemicals used were of analytical grade, purchased from Hi-media Laboratories, Mumbai, India.

Animals

Male golden Syrian hamsters 8-10 weeks old weighing 80-120g were purchased from National Institute of Nutrition, Hyderabad, India and were maintained in Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai Univeriesty. The animals were housed five in polypropylene cages 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 (Registar number 160/1999/CPCSFA), Annamalai University, Annamalai Nagar, 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.

Experimental Protocol

A total of 40 hamsters were randomised into four groups of 10 animals each. Group 1 animals were served as control and were painted with liquid paraffin alone. Group 2 and 3 animals were painted with 0.5% DMBA in liquid paraffin three times a week for 14 weeks on the left buccal pouches(NO: 4 Brush). Group 2 animals received no other treatment. Group 3 animals were orally administrated with Fisetin [10mg/kg b.wt](dissolve in 0.5%DMSO), starting Ist week before the exposure to the carcinogen and continued on days alternate to DMBA painting, until the sacrification of the animals. Group 4 animals orally anministred with fisetin alone throughtout the experimental period. The experiment was first terminated at the end of 16th week 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, 2-3µm sections were cut in a rotary microtome and stained with haematoxylin and eosin.

Macroscopic Observation

The numbers of tumours in the buccal pouches were counted and the diameter of each tumour was measured with a vernier caliper. Tumour volume was measured using the formula $v=(4/3)\pi\ [(D_1/2)(D_2/2)(D_3/2)]$ 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.

Samples (plasma and tissue preparation)

Blood samples were collected into heparinzed tubes. Plasma was separated by centrifugation at 1000 x g for 15 min. Tissue samples from the animals were washed with ice cold saline and homogenized using an appropriate buffer [(GST)-0.3 M phosphate buffer , pH6.5; glutathione reductase (GR)-0.1M phosphate buffer, pH7.4; (GSH and GPx)-0.4 M phosphate buffer, pH7.0; TBARS:0.025 M Tris-Hcl buffer, pH7.5; SOD:0.025 M sodium pyrophosphate buffer, pH8.3; CAT;0.01M phosphate buffer, pH7.0) in all-glass homogenizer with a teflon pestle and used for biochemical estimations.

Biochemical Analysis

The activity of glutathione-S-transferase (GST) in liver and buccal pouch mucosa tissue homogenate was assayed using the method of Habig *et al* (Habig *et al.,* 1974). Glutathione reductase activity in liver tissue homogenate was assayed by the method of carlberg and mannervik (Carlberg and Mannervik , 1985).

The erythrocyte membrane was prepared by the method of Dodge *et al* (Dodge *et a.,* 1968). Modified by Quist (Quist, 1980). Thiobarbituric acid reactive substances were assayed in plasma, erythrocytes, and buccal mucosa according to the methods of Yagi (Yagi, 1987), Donnan (Donnan ,1950) and Ohkawa *et al* (Ohkawa *et al*.,1979) , respectively Vitamin C and E were measured according to the methods of Omaye *et al* (Omaye *et al*.,1979) and Desai (Desai, 1984), respectively. The activities of enzymatic antioxidants , SOD , CAT and GPx were estimated by the methods of Kakkar *et al* (Kakkar *et al.,* 1984). Sinha (Sinha, 1972) and Rotruck et *al* (Rotruck *et al.,* 1973) respectively.

Statistical Analysis

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

RESULTS

Table 1: Shows the tumour incidence, tumour volume and tumour burden of control and experimental animals in each group. 100% of tumour formation with mean tumour volume (375.5 ±34.4 mm3) and tumour burden (1242.7 ±102.9 mm3) in DMBA alone painted animals (Group 2) were obsreved. Oral administration of Fisetin at a dose of 10 mg/kg b.wt siginificantly prevented the tumour incidence, tumour volume and tumour burden in DMBA painted hamsters (Group 3). No tumours were observed in control and Fisetin alone administered animals (Group 1 and 4).

Oral administration of Fisetin alone (Group 4) showed no significant differences were observed as compared to control animals.

Status of plasma and erythrocyte TBARS and antioxidants

The status of thiobarbituric acid reactive substances and antioxidants (vitamin E and C), in plasma and erythrocyte of control and DMBA treated animals in each group are shown in Table 4 and 5. The levels of TBARS were increased, whereas the status of non-enzymatic antioxdants [GSH,Vitamin C and E] and the activities of enzymatic antioxidants [SOD,CAT and GPx] were significantly decreased in tumour-bearing animals (Group 2) as compared to control animals. Oral administration of Fisetin to DMBA treated animals (Group 3) significantly brought back the levels of TBARS and antioxidants to near normal levels.

Table 1 Incidence of oral neoplasm in control and experimental animals in each group (n=10).

Groups	Treatment	Tumour incidence (Oral squamous cell carcinoma)	Total number of tumours/animals	Tumour volume	Tumour burden (mm³)
1	Control	–	–	–	–
2	DMBA	100%	33(10)	375.5±34.4^{b}	124.7±102.9^{b}
3	DMBA+Fisetin (10mg/kg b.wt)	50%	5(2)	88.5±16.2^{c}	166.2±17.4^{c}
4	Fisetin – alone (10mg/kg b.wt)	–	–	–	–

Tumor volume was measured using the formula, $V= (4/3)\pi [D_1/2][D_2/2][D_3/2]$ where D1, D2 and D3 are the three diameters (mm) of the tumor. Tumor burden was calculated by multiplying tumour volume and the number of tumours/animals.Tumour multiplicity = average number of tumours per animal. Tumour frequency = number of tumours per group.

Table 2 Histopathological changes in the buccal pouches of control and experimental animals in each group.(n=10)

Groups	Treatment	Hyperkeratosis	Hyperplasia	Dysplasia	Squamous Cell Carcinoma
1	Control	No change	No change	No change	No change
2	DMBA	Severe	Severe	Severe	Well differentiated
3	DMBA+Fisetin (10mg/kg b.wt)	Moderate	Moderate	Moderate	Mild differentiated
4	Fisetin alone (10mg/kg b.wt)	No change	No change	No change	No change

Histopathological Studies

The histopathological features observed in buccal mucosal tissues of control and experimental animals in each group are shown in Table 2 and Figure 1. We observed severe hyperplasia, dysplasia and keratosis and squamous cell carcinoma in the buccal mucosa tissues of hamsters in DMBA alone painted animals (Fig1.2). A mild to moderate hyperplasia, keratosis and dysplasia were observed in (Fig1.3) DMBA painted animals administered with fisetin (Group 3). Control animals as well as animals treated with Fisetin alone (Fig1.1 and 4) showed well defined oral epithelial tissues (Groups 1 and 4).

Status of phase II detoxification enzymes

The status of phase II detoxification agents in the liver of experimental and control animals in each group are shown in Table 3. The status of phase II detoxification agents (GST, GSH and GR) were significantly decreased in the liver of DMBA painted animals (Group 2) as compared to control animals (Group 1). Oral administration of Fisetin to DMBA painted animals (Group 3) reverted the status of phase II detoxification agents to near normal range.

Status of buccal mucosa TBARS and antioxidants

Fisetin alone treated hamsters (Group 4) showed no significant difference in TBARS and antioxidants status as compared to control hamsters (Group 1).

The status of TBARS and antioxidants in the buccal mucosa of control and DMBA treated animals in each group are shown in Table 6. Decreased the levels of TBARS and disturbances in antioxidant status (Vitamin E, GSH and GPx were increased; SOD and CAT were decreased) were noticed in tumour bearing animals (Group 2) as compared to control animals. Oral administration of fisetin to DMBA treated animals (Group 3) brought back the levels of TBARS and antioxidants to near normal range. Hamsters treated with Fisetin alone (Group 4) showed no significant difference in TBARS and antioxidants status as compared to control hamsters (Group 1).

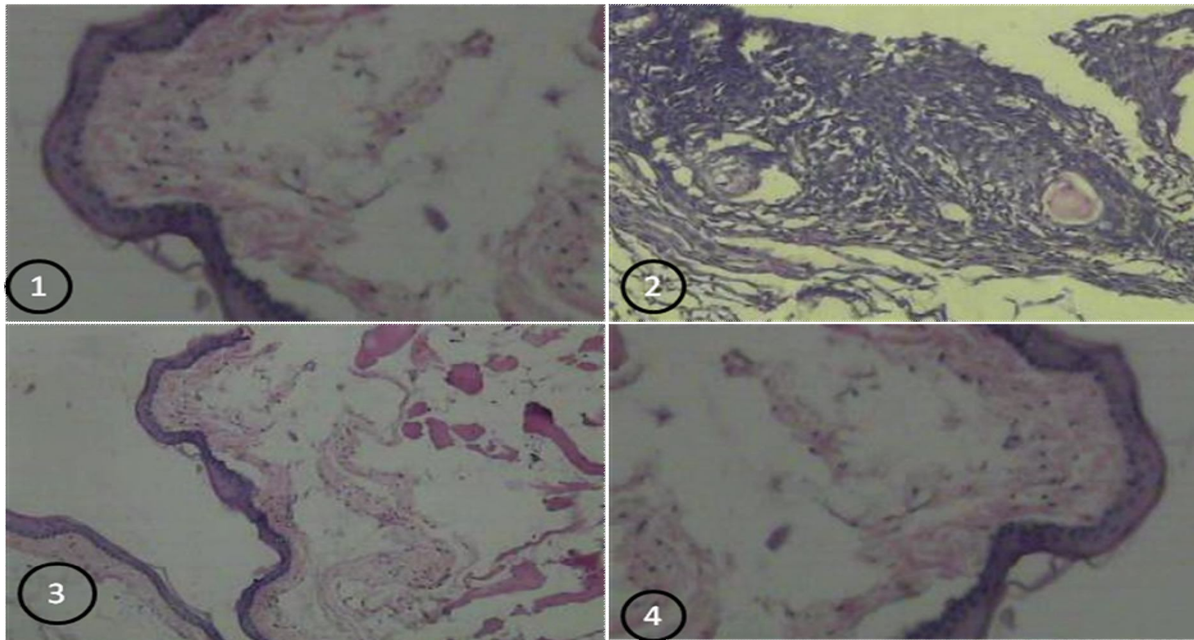

Figure 1 Shows histological features observed in the buccal mucosa of control and experimental animals in each group (1and 4). Photomicrographs showing well- defined buccal pouch epithelium from control and Fisetin alone treated hamsters respectively (H & E,40 X) .
(2) Photomicrograph showing well – defferentiated squamous cell carcinoma with keratin pearls in hamster treated with DMBA alone (H & E ,40X).
(3) Photomicrograph showing moderate dyplastic epithelium in hamster treated with DMBA + Fisetin alone (H & E,40X).

Table 3 Status of phase II detoxification agents in the liver of control and experimental animals in each group

Groups	Treatment	GSH (nmol/mg protein)	GST (nmol ofCDNB-GSH conjugate formed/min/mg protein)	GR (nmol of NADPH oxidised/min/mg protein)
1	Control	2.54±0.21[a]	24.67±1.83[a]	18.55±1.11[a]
2	DMBA	1.35± 0.15[b]	12.1±1.06[b]	8.90± 0.89[b]
3	DMBA+Fisetin (10mg/kg b.wt)	2.15±0.17[c]	18.73±1.53[c]	15.40±1.10[c]
4	Fisetin alone (10mg/kg b.wt)	2.57±0.16[a]	22.65±1.43[a]	17.93±1.08[a]

Values are expressed as mean ± SD for 10 hamsters in each group. Values that do not share a common superscript in the same column differ significantly at p <0.005(DMRT).

Table 4 Status of plasma TBARS and antioxidants of control and experimental animals in each group

Groups	Treatment	TBARS (nmol/ml)	GSH (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)	SOD (U*/ml)	CAT (U**/ml)	GPx (U***/ml)
1	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]
2	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]
3	DMBA + Fisetin (10mg/kg b.wt)	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]
4	Fisetin alone (10mg/kg b.wt)	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]	108.0±8.3[a]

Values are expressed as mean ± SD for 10 hamsters in each group. Values not sharing a common supercript letter differ significantly at P<0.005(DMRT)
*Amount of enzyme required to inhibit 50% NBT reduction
**µmole of H_2O_2 utilized /sec.
*** µmole of glutathione utilized /sec.

Table 5 TBARS and antioxidant status in erythrocytes of control and experimental animals in each group

Groups	Treatment	TBARS (µmoles/ mgHb)	GSH (mg/dl)	VitaminE (mg/dl)	SOD (U*/ml)	CAT (U**/ml)	GPx (U***/ml)
1	Control	1.48±0.12[a]	50.3±4.1[a]	2.56±0.16[a]	2.50±0.17[a]	1.36±0.08[a]	15.62±1.30[a]
2	DMBA	2.85±0.33[b]	31.4±2.6[b]	1.71±0.19[b]	1.52±0.19[b]	0.64±0.09[b]	8.21±0.93[b]
3	DMBA + Fisetin (10mg/kg b.wt)	1.68±0.13[c]	44.6±3.9[c]	2.23±0.23[c]	2.19±0.21[c]	1.24±0.07[c]	13.3±1.28[c]
4	Fisetin alone (10mg/kg b.wt)	1.49±0.15 a	51.1±4.6[a]	2.25±0.21[a]	2.51±0.15[a]	1.37±0.08[a]	15.61±1.40[a]

Values are expressed as mean± SD for 10 hamsters in each group. Values not sharing a common supercript letter differ significantly at P<0.005(DMRT)
*Amount of enzyme required to inhibit 50% NBT reduction
**µmole of H_2O_2 utilized /sec.
*** µmole of glutathione utilized /sec.

Table 6 Status of buccal mucosa TBARS and antioxidants of control and experimental animals in each group

Groups	Treatment	TBARS (nmol/mg protein)	GSH (mg/100 mg Tissues)	VitaminE (mg/100 mg Tissues)	SOD (U*/ mg Protein)	CAT (U**/ mg Protein)	GPx (U***/ g protein)
1	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]
2	DMBA	39.8±4.1 [b]	12.3±1.30 [b]	2.91±0.31 [b]	2.98±0.31 [b]	20.1±2.6 [b]	12.7±1.20 [b]
3	DMBA +Fisetin (10mg/kg b.wt)	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]
4	Fisetin alone (10mg/kg b.wt)	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]

Values are expressed as mean± SD for 10 hamsters in each group. Values not sharing a common supercript letter differ significantly at P<0.005(DMRT)
*Amount of enzyme required to inhibit 50% NBT reduction
**µmole of H2O2 utilized /sec.

DISCUSSION

Oral cancer, one of the leading causes of cancer death in western countries, has become a common malignancy in Asian countries, following recent changes in diet and life style (Moore *et al.,* 2000). DMBA induced hamster buccal pouch carcinogenesis is widely used to examine the chemopreventive and antioxidant enhancing effect of phytochemicals and their synthetic derivatives (Balasenthil *et al.,* 1993). We observed 100% tumour formation in the buccal pouches of hamsters painted with DMBA alone. The tumour was histopathologically, morphologically and biochemically confirmed as moderately differentiated squamous cell carcinoma. Oral administration of fisetin at a dose of 10mg/kg b.wt to DMBA painted animals significantly reduced and delayed the tumour formation. Our results concluded that fisetin has significantly chemopreventive and antitumour potential during DMBA painted hamster buccal pouch carcinogenesis. The antitumour principle of fisetin is probably due to its suppressing effect of abnormal cell proliferation.

DMBA, a member of polycyclic aromatic hydrocarbon that are present in the environment and complex hydrocarbons , requires metabolic activation by cytochrome P^{450} to form dihydro diol epoxide and generates excessive ROS during oral carcinogenesis. An abnormal Reactive oxygen species reacts with membrane essential fatty acids like poly unsaturated fatty acids cause serious damage to cell membrane by inducing abnormal membrane lipid peroxidation (Machlin *et al.,* 1987).

Liver, multifaced chemical industry, perform an important role in the elimination of toxic substances and carcinogen and there by measuring the detoxification enzymes help to determine the antioxidant effects on the fisetin. Thus, in the present study the liver phase II detoxifying enzymes were decreased in DMBA painted animals when compared to control animals. Recent data , reported that decreased the activation of phase II detoxification enzymes in malignant condition especially oral cancer caused by toxic metabolite DMBA disturbance the hepatic cells (Wilkinson and Clapper,1997). Our results corroborate these observations. Oral administration of fisetin at a dose of 10mg/kg b.wt to DMBA painted animals increased the activity of phase II detoxification enzymes. The finding of the present study suggest that fisetin have potent detoxifying effects on DMBA mediated process an evidenced by increasing the levels of phase II detoxifying enzymes (McLellan and Wolf, 1999). Oxidative mechanisms like generation of O_2 play a major role in carcinogenesis has been reported (Dix and Marnett, 1983). High concentration of TBARS in plasma due to extend of tissue damage and increased levels of plasma TBARS could therefore be due to over production and diffusion from damaged red blood cells and other host tissues with subsequent leakage into plasma. Changes in the levels of lipidperoxidation by products have been described during buccal pouch carcinogenesis (Gutteridge, 1995). Diminished PUFA content in oral tumour tissuses is responsible for decreased levels of lipid peroxidation in oral carcinoma. Oral administration of fisetin significantly decreased the levels of plasma and erythrocyte TBARS in DMBA painted hamsters, which suggest that it has been potent antioxidant potential during oral carcinogenesis.

Antioxidants can prevent tissues injury caused by ROS, which is direct scavenging of free radicals. Antioxidants are oxidised by radicals, resulting in more stable and less reactive radicals. Several studies have been demonstrated the antioxidant shown to inhibit both initiation and promotion stages in carcinogenesis and counteract cell immobilization and transformation (Cohen and Ellwein, 1990). Enzymatic and non-enzymatic antioxidants form the first and second line of defence mechanism respectively, against the deleterious effects generated by ROS, which is oxidative mediated cell damage(Hristozov *et al.,* 2001). Antioxidants enzymes that scavenge intermediates of oxygen reduction provide the primary defence against cytotoxic oxygen radical. It is well known that SOD, CAT and GPx play an important role as protective enzymes against lipid peroxidation in tissues. They are involved in the direct elimination of reactive oxygen metabolites, which is probably one of most effective defences of the living body against diseases (Das, 2002). The decreased activities of SOD and CAT may be due to the dangerous increase in the levels of reactive oxygen species and thus, enhanced oxidative stress and proliferation of malignant carcinoma (Cao *et al.,* 1997).

CAT and GPx are important antioxidant enzymes in the inactivation of many environmental mutagens. CAT was found to be inactivated by high OH^- production (Ray and Husian.,1972). GPx, neutralizes hydroxyl radicals and singlet oxygen. When present in high concentration in the cells, it protects cells from malignant tumour as well as carcinogen induced experimental carcinogenesis (Arthur,2000). Lowered levels of vitamin C and E, GSH antioxidants in plasma of tumour bearing hamsters to meet insufficient nutrient of growing tumour the adverse effect of ROS in circulation. Decreased levels of antioxidants cause overproduction of free radicals and lipid peroxides, which in turn induce damage to cell membrane and cellular bio molecules and there by leading

to an active rate of cell proliferation. Lowered activities of SOD and CAT as well as increased activity of GPx, GSH and vitamin E content were reported in oral cancer. Our results tend credence to these observations (Martins *et al.,* 1991).

Flavonoids are low molecular weight compounds rich in seeds, citrus fruits, red wine, tea and olive oil. Flavonoids have diverse biological effects including anti-oxidant, anti-platelet, anti-thrombotic, anti-allergic, anti-viral, anti-carcinogenic activities and anti-inflammatory activities (Park *et al.,* 2007). Fisetin, a naturally occurring flavonoid commonly found in the smoke tree (*Cotinus coggygria*), is also found in fruits and vegetables such as strawberry, apple, persimmon, grape, onion and cucumber (Arai *et al.,* 2000). It exerts a wide variety of activities, including neurotrophic, anti-oxidant, anti-inflammatory and anti-angiogenic effects. It has been reported to inhibit the proliferation of a wide variety of tumor cells, including prostate cancer (Haddad *et al.,* 2006), liver cancer (Chen *et al.,* 2002), colon cancer (Lu *et al.,* 2005), and leukemia cells (Lee *et al.,* 2002). Fisetin modulated cell-cycle arrest, apoptosis and antiproliferation may provide a novel basis for the development of chemotheraphy for cancers. In the present study, oral administration of fisetin at a dose of (10mg/kg b.wt) restored the status of lipid peroxidation and antioxidants in the plasma and buccal mucosa of DMBA treated hamsters. The results of the present study thus demonstrated that fisetin might have maintained the balance of oxidant and antioxidants status during DMBA induced hamster buccal pouch carcinogenesis.

The present study concludes that fisetin have suppressed cell proliferation by inhibiting the metabolic activation of DMBA during DMBA-induced oral carcinogenesis. Also, its antilipidperoxidative (antioxidant) efficacy and modulating the effect of detoxification agents (phase II enzymes, TBARS and antioxidants) in the tumour tissues a rise as a consequence of the process of carcinogenesis. The effect of fisetin could be achieved with time course follow up of these biomarkers by carrying out interim sacrifices of animals during DMBA induced oral carcinogenesis. The effect of fisetin on molecular markers related to cell cycle analysis and apoptotic pathway during DMBA-induced oral carcinogenesis is also under investigation.

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