

Antioral Cancer Effect of Fucoxanthin on 7,12-Dimethylbenz[a] Anthracene-Induced Experimental Cancer Model Hamster through Changes of Apoptosis and Cell Proliferation

Zhizheng Zhuang[#], Jingxuan Wang^{1#}, Yingshun Yang, Yujiao Hou, Song Li, Yifan Wang, Yan Hu, Fan Wu

Department of Stomatology, Affiliated Hospital of Hebei University, ¹The Second Department of Stomatology, Baoding No.1 Central Hospital, Baoding, Hebei, 071000, China

[#]These authors are contributed equally

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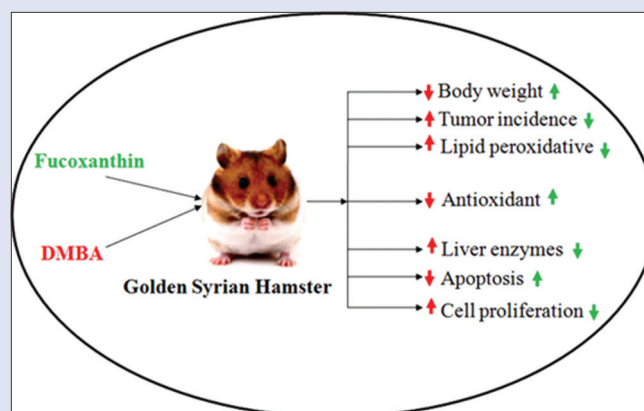
ABSTRACT

Background: In this study, we investigated the chemopreventive efficacy of fucoxanthin (Fx) on 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral cancer in hamsters. **Materials and Methods:** Twenty-four male Syrian golden hamsters were randomly allotted to four groups with six hamsters each. Squamous cell carcinogenesis was initiated by administering 0.5% DMBA in the left oral mucosa of hamsters for 10 weeks. The complete formation of oral tumor (OT) was confirmed via hematoxylin and eosin staining and biochemical analysis, as well as via molecular markers using plasma samples and in buccal and liver tissue samples. **Results:** Significant increase in the level of antioxidant, lipid peroxides (LPOs), and liver marker enzymes was observed in control animals. Increased rate of cell proliferation and decreased expression of apoptotic proteins were observed in buccal tumor in control animals. Treatment of DMBA-induced animals with Fx (50 mg/kg body weight) resulted in mild-to-moderate premalignant lesions, such as hyperplasia and dysplasia, but control animals showed the development of OT. Furthermore, the levels of LPO, antioxidants, and xenobiotic agents were altered due to Fx-administered DMBA-induced hamster showed reduced expression pattern of proliferating cell nuclear antigen and moderate expression pattern of caspases-9 and 3 and p53 were observations. **Conclusion:** In this study, the chemoprotective potential of Fx was due to the antiproliferative, antiapoptotic, and antioxidant effects of Fx, as well as due to anti-LPO effects in the DMBA-induced hamster cheek pouch carcinogenesis.

Key words: 7,12-dimethylbenz[a]anthracene, apoptosis, cell proliferation, fucoxanthin, hamster, oral cancer

SUMMARY

- Oral carcinomas are widespread in Southeast Asian countries such as Sri Lanka, India, and Taiwan
- Fucoxanthin shows potential activity by improving antioxidant status, by increasing the level of xenobiotic enzymes, and by altering the levels of lipid peroxides.



Abbreviations Used: Fx: Fucoxanthin; DMBA: 7,12-dimethylbenz[a]anthracene; SCC: Squamous cell carcinogenesis; LME: Liver marker enzymes; LPO: Lipid peroxide; OT: Oral tumor.

Correspondence:

Dr. Yan Hu,
Department of Stomatology, Affiliated Hospital of
Hebei University, Baoding 071000, Hebei, China.
E-mail: 13733397167m@sina.cn
Dr. Fan Wu,
Department of Stomatology, Affiliated Hospital of
Hebei University, Baoding 071000, Hebei, China.
E-mail: wufanxufang@sina.com
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INTRODUCTION

Oral carcinomas are the most prevalent ones among the Southeast Asian countries such as Sri Lanka, India, and Taiwan.^[1] Approximately 50% of the 5-year survival rate oral cancer, appear most important health burden leads mortality due to ill health, we are in the need of special attention.^[2] The transformation of the cells from premalignant to malignant in the oral mucosa. Alcohol consumption has been shown to increase leukoplakia and epidermoid carcinoma in hamster model. The hamster cheek pouches (HCPs) are extended backward along the oral cavity and lined with keratinizing squamous epithelium similar to the human palate or the gingiva. The hamster buccal pouch (HBP) carcinomas induced by 7,12-dimethylbenz[a]anthracene (DMBA) exhibit extensive similarities to that of human oral squamous cell carcinoma with respect to the morphology, histology, preneoplastic lesions, propensity to invade and metastasize,

expression of biochemical and molecular markers, and genetic and epigenetic alterations.^[3-6]

Even forth hard works for common teaching and broadcast, the percentage of victims in early phase of causes was defaulted during the

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past 40 decades,^[7] with the incidence of second primary malignancy ranging 10%–35% of the cases. Consequently, preliminary discovery and prevention are believable to potent to developing viable by diminishing the frequency and severity of OT.

The HBP system for OT is a well-recognized system for studying various chemicals and their effects on carcinogenesis. The experimental model of hamster for buccal pouch cancer analysis is an easy technique.^[4] The HBP carcinogenesis (HBPC) is an extremely reliable model in which case leukoplakia begins at 6–8 weeks. After 10 weeks, carcinomas develop, and after 12 weeks, it can be easily distinguished. There are multiple and extensive gross lesions that appear after 14 weeks and large invasive lesions after 18 weeks.^[6] A previous study has described similarities in molecular and biochemical aspects as well. Increased synthesis of reactive oxygen species (ROS) induces the chronic inflammatory response which causes DNA damage, which is the main mechanism of action of DMBA in HBPC.^[8] In tumor progression, uncontrolled proliferation of cells was observed during the multistep activity of oncogenesis characterized by the imperfect arrangement of genes related to apoptosis, inflammation, and cell proliferation on the experimental, cellular, and genetic level.^[9]

Fucoxanthin (Fx), a brown pigment which exists in abundant quantities in brown algae, has an unusual allenic bond, 5,6-monoepoxide, 9-linked dual bonds, and a few oxygenic functional groups, as well as epoxy, hydroxyl, carbonyl, and carboxyl groups, which are mainly involved in free radical scavenging mechanism. Therefore, it exerts broad therapeutic categories anti-inflammatory, antiproliferative, antimicrobial, antioxidant, antidiabetic, and neuroprotective effects.^[10] Furthermore, Fx inhibits obesity through the induction of fatty acid oxidation in adipose tissue. Phytochemicals derived from various plants showed signs of chemoprevention such as antioxidation, anti-inflammation, cell cycle arrest, antiproliferation, developed programmed cell death, and antiangiogenesis. Numerous studies have demonstrated the anticancer activity of Fx.^[11,12] To the best of our knowledge, there are no studies that demonstrate the chemopreventive potential of Fx. Therefore, in this study, we aimed to explore the chemopreventive capability of Fx on DMBA-induced HBPC.

MATERIALS AND METHODS

Reagents and chemicals

Fx (≥95% purity, CASNO: 3351-86-8) and DMBA (≥95% purity, CASNO: 57-97-6) were procured from Sigma-Aldrich Pvt. Ltd. Antibodies against proliferating cell nuclear antigen (PCNA) and p53 were procured from Santa Cruz Biotechnology (CA, USA). Caspases-3 and 9 colorimetric test kits were purchased from Biovision Research Products (Mountain View, CA, USA). All other chemicals used were of analytical grade and procured from Hi-Media Laboratories Pvt. Ltd.

Animals

Golden male hamsters were maintained and the study protocol was approved by the ethical committee (2019–2134). The animals were housed in four propylene cages, with six hamsters in each cage. The cages were maintained separately, and the animals received supplemented pellet diet and water *ad libitum*. All animals were maintained in well-organized environment with warmth (27°C ± 2°C) and moisture (55% ± 5%) for 12 h of light and dark sequence.

Experimental plan

The effective dose was planned as follows: A total of 24 hamsters in the age group of 8–10 weeks (weighing 80–120 g) were randomly separated to four groups with six hamsters in each. Group I consisted of control hamsters, which did not receive any treatment. Groups II–III were given

a 0.5% DMBA with liquid paraffin vehicle thrice a week up to 10 weeks on their left hamster oral pouch (HOP) using a number four brushes. In addition, Group III hamsters received effective dosage of Fx dissolved in dimethyl sulfoxide as the vehicle (50 mg/kg body weight [bw]) through oral administration once in a day beginning 1 week earlier to DMBA exposure; this continued till the completion of the experiment. Group IV hamsters were administered with Fx only for the 16 weeks. In this study, it was found that Fx (50 mg/kg bw) orally administered hamster showed the inhibition of tumor development.

After 24 h of final treatment, the animals were fasted overnight and anesthetized. The experimental hamsters body weight (HBW) was calculated by subtracting the initial bw. Blood was collected using to heparinized tubes from each animal and plasma was retained for biochemical studies. The hepatic tumor tissues and oral tumor tissues (OTTs) were removed and homogenized using appropriate homogenizing buffer. The homogenized material was centrifuged at 4000 ×g, and there sultant supernatant was further analyzed for biochemical parameters. OTT samples were preserved in 10% formalin, and approximately 2–5 μm thick sections were prepared and stained with hematoxylin and eosin (H and E) staining for observation under microscope.

Measurement of oral tumor parameters

Oral tumors (OTs) were measured by using Vernier caliper marked as percentage. The total number of tumors for every hamster was analyzed through with a Vernier caliper. The volume of the OT and the burden of the OT were determined by applying the following formula: $V = 4/3 \pi (D1/2) (D2/2) (D3/2)$, where D1, D2, and D3 are diameters (mm³) of tumors, respectively.

Biochemical analysis

Sample collection

Excised hepatic tumor tissues and OTTs were cleaned with ice-cold buffered saline. Briefly, 100 mg of excised and cleaned tissue was homogenized with chilled Tris–HCl solution (0.1 M) with a mechanical homogenizer and was centrifuged at 4000 ×g for 14 min at 4°C. Then, the supernatant was collected for further biochemical assays. The proteins from hepatic and buccal cells were separated and quantified by applying the procedure of Lowry *et al.*^[13]

Lipid peroxides (LPO), as confirmation with the improvement of lipid peroxidation (LOOH) and conjugated dienes (CD) plasma and OM, were investigated based on the methods described by Ohkawa *et al.*,^[14] Jiang *et al.*,^[15] and Rao and Recknagel,^[16] respectively. The antioxidant enzymes, namely catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), were analyzed in the plasma and HOP tissues based on the method described by Kakkar *et al.*,^[17] Rotruck *et al.*,^[18] and Sinha,^[19] respectively. The non-enzymatic antioxidant activities, GSH levels, and the level of Vitamin-E in the plasma and OM tissues were assessed based on the method described by Beutler and Kelly,^[20] Desai,^[21] and Palan *et al.*,^[22] respectively.

The status of liver marker enzymes (LMEs) such ascut-p450 and cut-b5, GST, DTD, GR, GSH, and GSSG in the hepatic and HCP tissues was estimated based on the methods described by Omura and Sato,^[23] Lind *et al.*,^[24] Habig *et al.*,^[25] Carlberg and Mannervik,^[26] Anderson,^[27] Tietze,^[28] and Ernster,^[29] respectively.

Immunohistochemistry

Tissue portions that are embedded in paraffin were subjected to the removal and were rehydrated by merging it in ethanolic solution. Then, HBP samples were kept in the universal protein-blocking solution for 15 min at 37°C to block the binding sites. Then, the samples were treated with

relevant tumor suppressor protein (p53), and cell proliferation (PCNA) antibodies were utilized in immunohistochemistry (IHC) coloring of proteins. Then, it was treated with secondary antibody conjugated with horse radish peroxidase and 3,3'-diaminobenzidine substrate for observing the bound antibodies. After obtaining an intense color, the slides were cleaned and colored with hematoxylin and covered with mounting medium (dibutylphthalate polystyrene xylene). Each sample on the glass slide was investigated through the microscope, and the percentage of positive cells was determined by applying the procedure of Lyzogubov *et al.* (2005).^[30]

Estimation of caspases-3 and 9 activities

The activities of caspases-3 and 9 were assayed in HBP tissues using enzyme-linked immunosorbent assay (ELISA) kits (Cayman's calorimetric assay kits) by following the manufacturer's instructions. Briefly, HOP was homogenized with phosphate-buffered saline and centrifuged at 4000 rpm. The supernatant was placed in a microtiter plate that is precoated with biotin-conjugated antibodies. Caspases-3 and 9 were analyzed based on spectrophotometric discovery of chromophore *p*-nitroanilide following the split through labeled substrate DEVD-pNA and LEHD-pNA at 405 nm on an ELISA plate reader (Bio-Rad).

Statistical study

The data are presented as mean $\pm$ standard deviation (SD). Statistical evaluations for biochemical parameters were executed with one-way analysis of variance followed by Duncan's multiple range test. Results were regarded as statistically significant if $P < 0.05$.

RESULTS

Effects of fucoxanthin on body weight

Figure 1 depicts the effects of Fx on DMBA-induced HBW. Body weight (BW) was notably ($P < 0.05$) decreased in hamsters with HBP tumor. Moreover, Fx treatment decreased the BW on DMBA-induced hamsters. There was no difference in the BW of hamsters that were treated with Fx alone when compared with control hamsters.

Effect of fucoxanthin on tumor incidence, number, and volume

Figure 2 and Table 1 demonstrate the rate of tumor incidence (100%), augmented tumor burden, and tumor volume on DMBA-alone-administered hamsters OT. Oral supplementation of Fx to DMBA-treated hamsters considerably lowered the volume, incidence, and burden of tumor. Fx alone treated hamsters buccal tissues no tumor growth observed, its similar appearance of normal tissues.

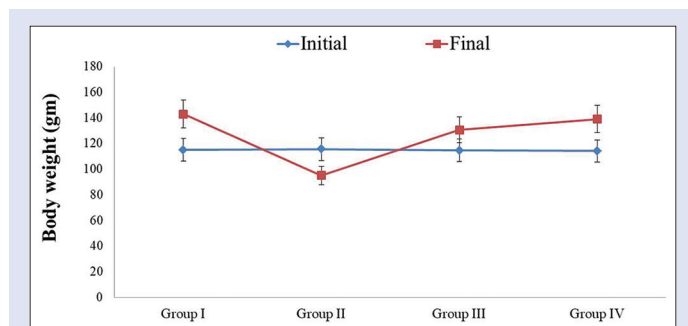


Figure 1: Initial and final body weight of 7,12-dimethylbenz[a]anthracene-induced oral cancer in hamsters

Histopathological evaluation of hamster buccal pouch tissue

Figure 3 and Table 2 show the histopathological changes in cheek pouch of the control and treated hamsters. We found 100% tumor proliferation and severe hyperkeratosis, hyperplasia, dysplasia, and well-documented OT in the buccal mucosal epithelium of hamsters. Although well-differentiated oral carcinogenesis was not seen in the buccal pouch epithelium of DMBA-induced hamsters with Fx supplementation, they showed moderate-to-mild keratosis and hyperplastic epithelium. Oral administration of Fx alone and control demonstrated well-differentiated and intact epithelial layers.

Levels of lipid peroxides in plasma and oral mucosa

Figure 4 shows the activities of the CD, thiobarbituric acid reactive substances (TBARS), and LOOH in the plasma and cheek pouch of control and treated hamsters. CD, TBARS, and LOOH status was considerably ($P < 0.05$) increased in the plasma and decreased in the buccal mucosal tissues. Oral supplementation of Fx to DMBA-induced hamsters notably ($P < 0.05$) reverted the significance LPO in the blood cells and OM then control hamsters. Control and Fx-only-supplemented hamsters did not show any differences.

Effect of fucoxanthin on enzymatic status

Figure 5 shows the enzyme levels of CAT, GPx, and SOD in the plasma and OM of control and treated hamsters. The activity of CAT and SOD was notably ($P < 0.05$) decreased, whereas that of GPx was increased ($P < 0.05$) in the DMBA-induced hamsters when compared with control animals. Oral presupplementation of Fx potentially ($P < 0.05$) reverted to near-normal level of antioxidant enzyme status in DMBA-induced hamsters when compared with control animals. No significant variation was demonstrated in the Fx-alone-supplemented and control hamsters.

Non-enzymatic antioxidant levels in plasma and cheek pouch

Figure 6 shows the non-enzymatic levels of GSH and Vitamin E in the plasma and HBP of control and treated hamsters. The status of

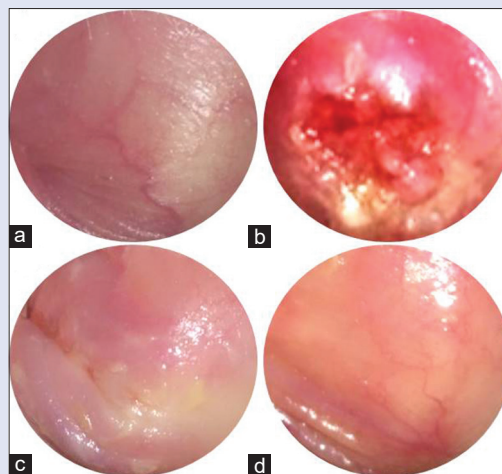


Figure 2: Photomicrograph showing the gross appearance of squamous cell carcinogenesis of experimental hamsters. (a) Exophytic and well-defined tumor mass in the oral mucosa painted with 7,12-dimethylbenz[a]anthracene alone. Untreated control (b), 7,12-dimethylbenz[a]anthracene + fucoxanthin (c), and fucoxanthin alone (d) treated hamsters revealed that the normal appearance of the oral tissue

Table 1: Tumor incidence, tumor number, tumor volume, and tumor burden of experimental hamsters

Groups/treatment	Control	DMBA	DMBA+Fx (50 mg/kg body weight)	Fx-Alone (50 mg/kg body weight)
Tumor incidence	0	100%	0	0
Total number of tumor/hamsters	0	9±0.71	0	0
Total volume (mm ³)/hamsters	0	176.86±13.47	0	0
Tumor burden (mm ³)/hamsters	0	1591.47±121.88	0	-

Values are expressed as mean±SD for six hamsters in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$. SD: Standard deviation

Table 2: Histopathological changes in the buccal mucosa of experimental hamsters

Groups/treatment	Control	DMBA	DMBA+Fx (50 mg/kg body weight)	Fx-Alone (50 mg/kg body weight)
Keratosis	0	+++	++	0
Hyperplasia	0	+++	+	0
Dysplasia	0	+++	0	0
OSCC	0	+++	0	0

No change (0); Mild (+); Moderate (++); Severe (+++). Fx: Fucoxanthin; DMBA: 7,12-dimethylbenz[a]anthracene; OSCC: Oral squamous cell carcinoma

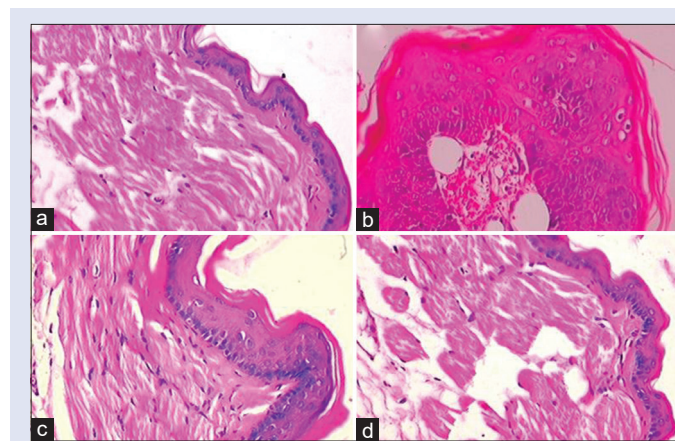


Figure 3: The histopathological changes in the oral tissue of experimental hamsters (H and E; ×40). (a and d) hamsters oral mucosal epithelium showed a normal cellular architecture with no signs of cell proliferation. (b) Hamsters showed well-differentiated squamous cell carcinogenesis. (c) Hamsters showed normal cellular architecture with mild-to-moderate hyperkeratosis and hyperplasia

Vitamin E and GSH was found to be statistically ($P < 0.05$) declined in the plasma, whereas they were elevated in OM of OT. The oral presupplementation of Fx was notably ($P < 0.05$) shown the status increased expression of GSH and Vitamin E in DMBA-treated hamsters. However, there was no significant variation in the Fx-alone and control hamsters.

Effect of fucoxanthin on the level of hepatic enzymes in liver tissues

Figure 7 shows the xenobiotic levels of phase II (GST, DTD, GSH, and GR) and phase I (cyt-b5 and cyt-p450) enzymes of liver tissues of control and treated hamsters. The levels of cyt-b5 and cyt-p450 were notably ($P < 0.05$) decreased, whereas the GST, DTD, GSH, and GR enzymes were drastically increased ($P < 0.05$) augmented in tumor cell development hamsters. The presupplementation of Fx to DMBA-induced hamsters ($P < 0.05$) helped to revert back the levels of xenobiotic enzymes to near-normal levels as compared with control hamsters. Moreover, hamster treatment with Fx-only and control hamsters revealed no significant variation in LME levels.

Table 3: Immunohistochemistry scoring of cell proliferation and apoptotic marker expressions examined in experimental hamsters

Groups/treatment	PCNA				p53			
	0	1+	2+	3+	0	1+	2+	3+
Control	0	6	0	0	10	0	0	0
DMBA	0	0	2	4	0	0	1	5
DMBA + Fx (50 mg/kg bw)	0	3	2	1	1	3	2	0
Fx-Alone (50 mg/kg bw)	0	6	0	0	3	3	0	0

Results are given as number of hamsters ($n=6$). The percentage of positive cells was scored as: 3+: Strong staining, >50% of cells were stained; 2+: Moderate staining, between 20% and 50% of cells were stained, 1+: Weak staining between 1% and 20% of cells were stained, 0: Negative, <1% of cell staining. Fx: Fucoxanthin; DMBA: 7,12-dimethylbenz[a]anthracene; PCNA: Proliferating cell nuclear antigen

Effect of fucoxanthin on levels of liver marker enzymes in oral tissues

Figure 8 shows the status of phase I (cyt-b5 and cyt-p450) and phase II (GST, GR, GSH, GSSG, and GSH/GSSG) enzymes in OM of control and treated hamsters. The levels of cyt-b5 and cyt-p450 were drastically increased ($P < 0.05$), and the levels of GST, GSH, and GR (the GSH/GSSG ratio was improved; GSSG was inhibited) were altered in tumor-bearing hamsters. The oral presupplementation of Fx in DMBA-induced hamsters caused ($P < 0.05$) the reversal of LMEs to near-normal levels as compared with control hamsters. No significant change was evaluated in the Fx-only and control hamsters.

Proliferating cell nuclear antigen and p53 protein expression

Figures 9 and 10 and Table 3 show the IHC staining of p53 and PCNA expression in control and treated hamsters. Upregulation of p53 and PCNA protein appearance were observed expression in DMBA-induced hamsters groups. In addition, the oral supplementation of Fx (50 mg/kg bw) in DMBA-induced hamsters exhibited drastically nearby normal expression of p53 and PCNA proteins. Furthermore, treatment with Fx only and control hamsters revealed no significant variations.

Fucoxanthin treatment modulates caspases-3 and 9 activity

caspases-3 and 9 activity was analyzed colorimetrically after 16 weeks of oral supplementation of Fx to DMBA-induced hamsters [Figure 11].

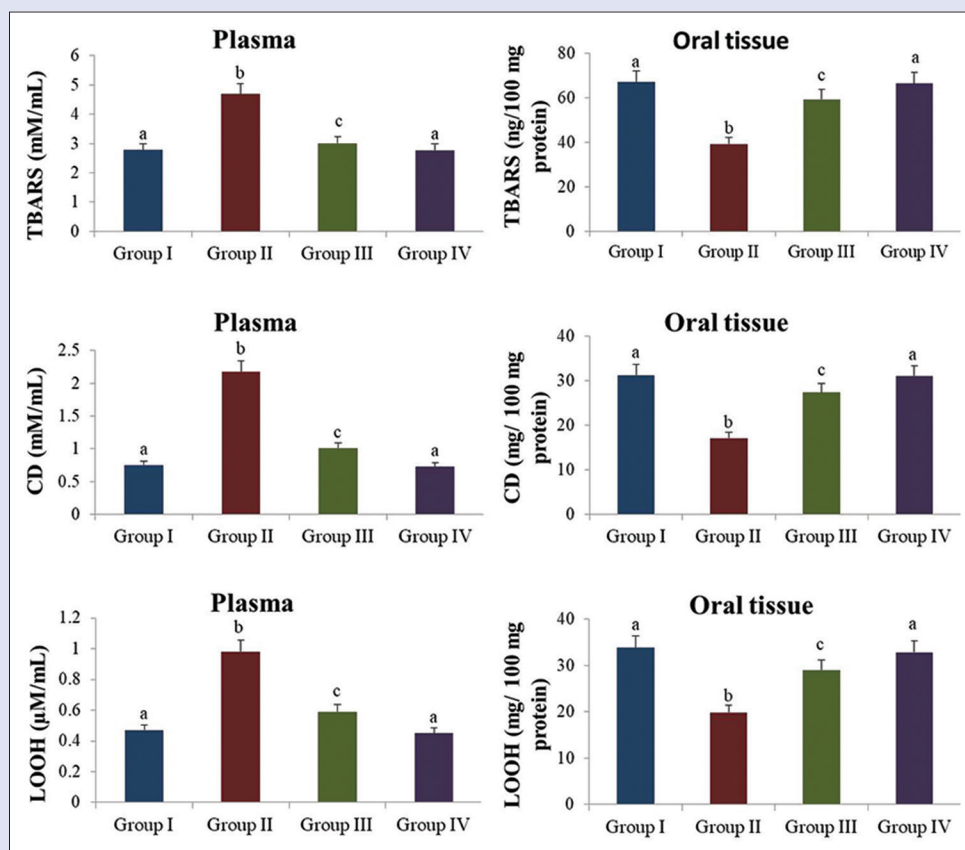


Figure 4: The status of lipid peroxides by-products in the plasma and oral tissues of experimental hamsters. Results are expressed as mean $\pm$ standard deviation for six hamsters in each group. Data not sharing a common superscript ^{a-c}Differ significantly at $P < 0.05$ (Duncan's multiple range test)

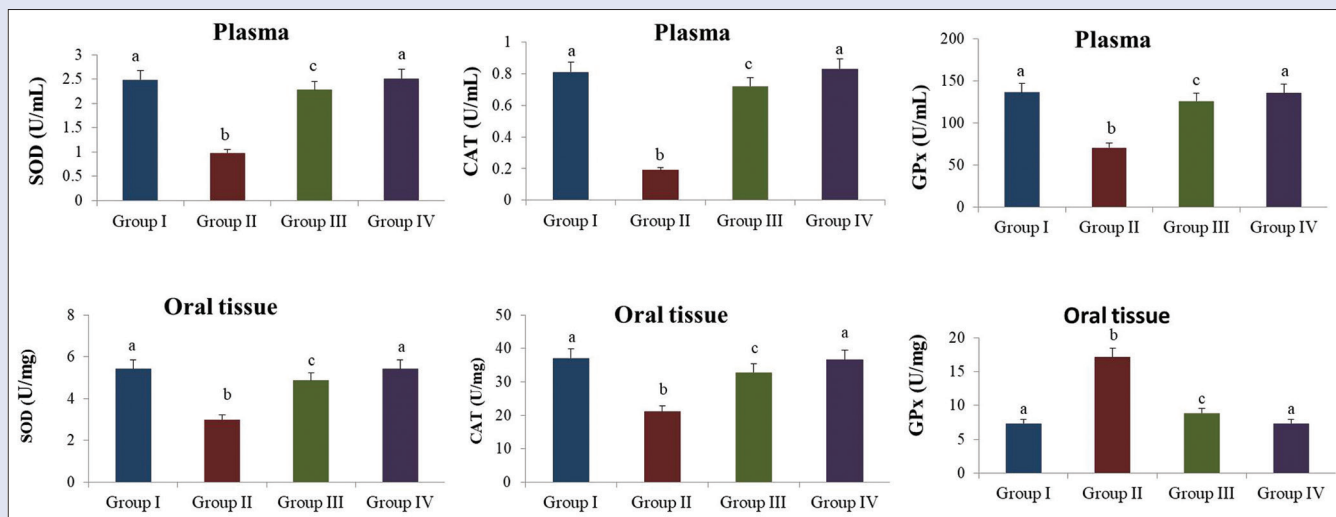


Figure 5: The status of enzymatic antioxidants in the plasma of experimental hamsters. Results are expressed as mean $\pm$ standard deviation for six hamsters in each group. Data not sharing a common superscript ^{a-c}Differ significantly at $P < 0.05$ (Duncan's multiple range test)

Levels of caspases-3 and 9 were notably decreased in OTP hamsters. The activity of caspases-3 and 9 was increased in the hamsters that received Fx when compared to tumor hamsters. Taken together, our results revealed that Fx decreases DMBA-induced increase in the levels of caspases 3 and 9 in hamster OM.

DISCUSSION

Head and neck cancer is a widespread malignant neoplasm with poor prognosis and multiple modification and major oncological problem in the world.^[31] The chemopreventive effect of the Fx in the hamster

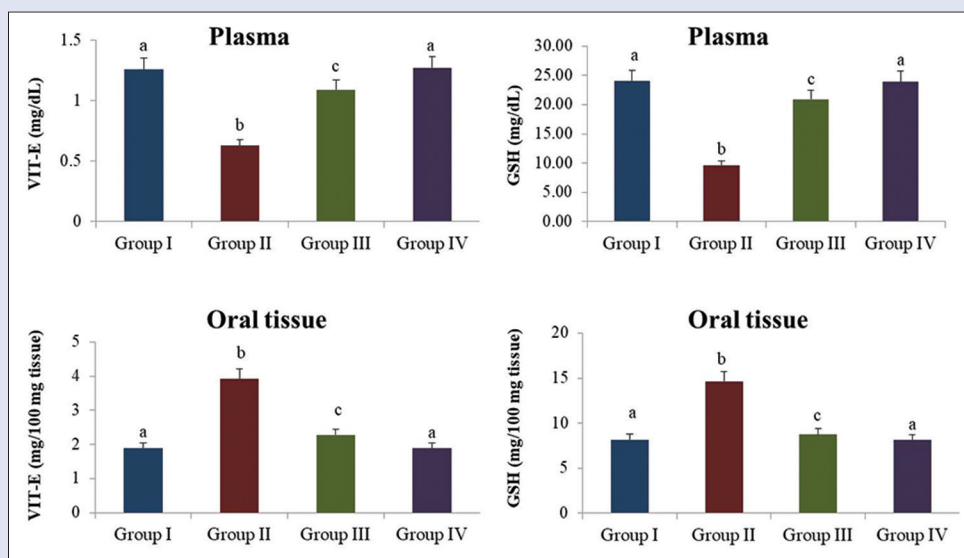


Figure 6: The status of non-enzymatic antioxidant in the plasma of experimental hamsters. Results are expressed as mean $\pm$ standard deviation for six hamsters in each group. Data not sharing a common superscript ^{a-c}Differ significantly at $P < 0.05$ (Duncan's multiple range test)

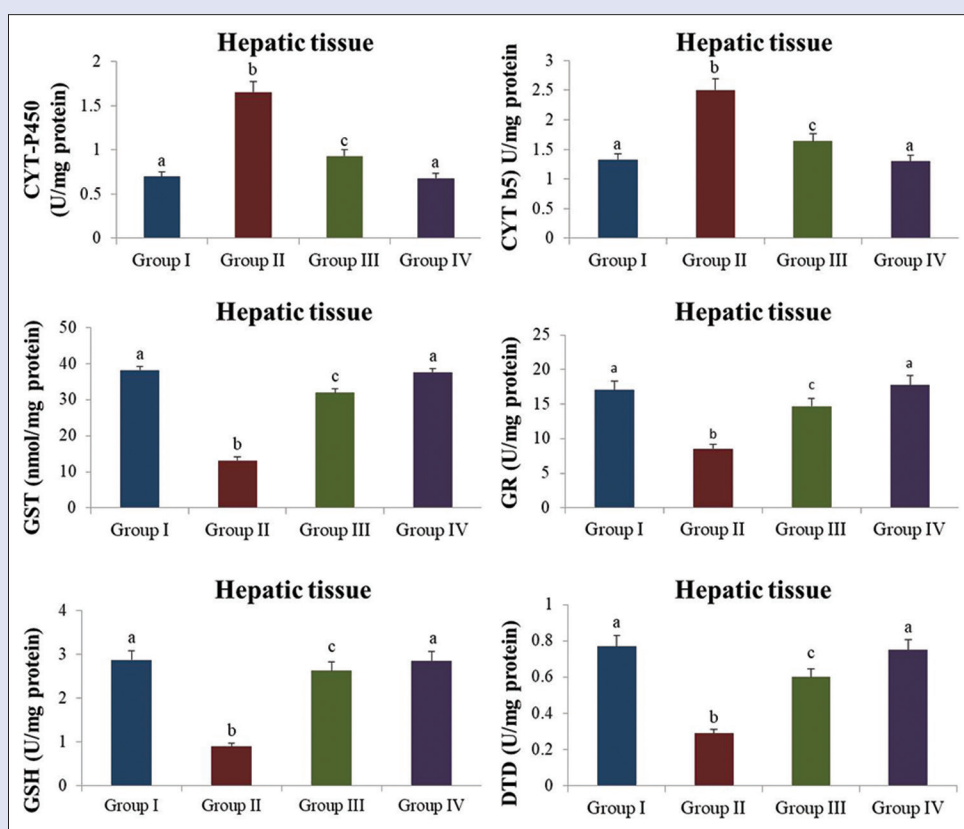


Figure 7: The levels of xenobiotic enzymes in the hepatic tissue of experimental hamsters. Results are expressed as mean $\pm$ standard deviation for six hamsters in each group. Data not sharing a common superscript ^{a-c}Differ significantly at $P < 0.05$ (Duncan's multiple range test)

model system was given the information about comparison of the DMBA-induced carcinogenesis progression compared with normal and FX + DMBA-induced hamster. Otherwise, promising effect of DMBA via the conversion of diepoxide from DMBA during metabolism, which reduces the binding of DMBA to DNA, thereby diminishing the carcinogenicity. DMBA-induced HBPC has proven to be good animal

model to investigate the anticancer ability of dietary compounds such as Fx. Despite current development in the analysis and helpful modalities for OC. In this study, we examined the chemoprotective capacity of Fx in DMBA-induced oral carcinoma. Therefore, our results showed a 100% OTP and loss of HBW, and histopathological investigation distinguished squamous cell carcinogenesis (SCC) in hamsters induced with DMBA

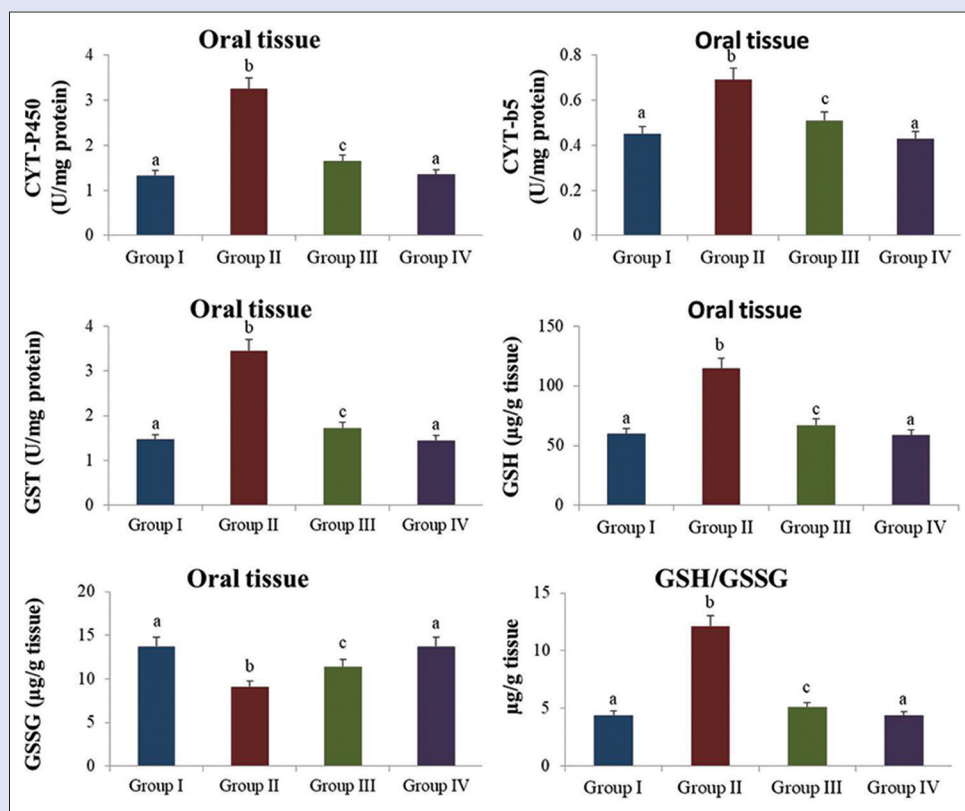


Figure 8: The status of detoxification enzymes in the buccal tissues of experimental hamsters. Results are expressed as mean $\pm$ standard deviation for six hamsters in each group. Data not sharing a common superscript ^{a-c}Differ significantly at $P < 0.05$ (Duncan's multiple range test)

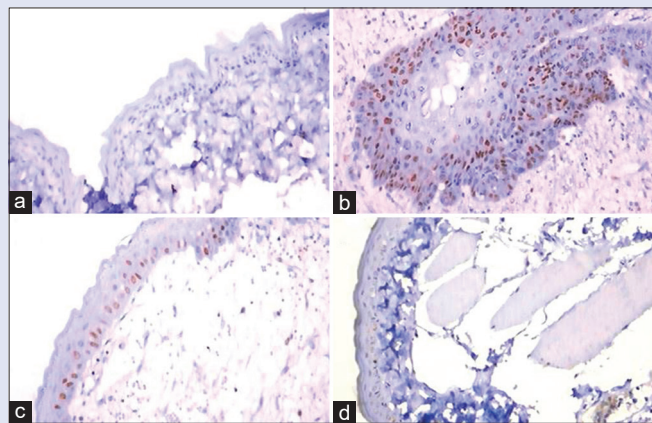


Figure 9: Immunohistochemistry analysis of cell proliferation (proliferating cell nuclear antigen) protein expression observed in the oral tissue of experimental hamsters (x40) (a and d) hamsters showed normal nuclear expression of proliferating cell nuclear antigen; (b) hamster shown higher expression of proliferating cell nuclear antigen was noticed in the tumor bearing hamsters; (c) hamster shown down regulated the expression of proliferating cell nuclear antigen was noticed in mild-to-moderate hyperplastic epithelium of 7,12-dimethylbenz[a]anthracene + fucoxanthin-treated hamster

only. Moreover, oral supplementation of Fx along with DMBA prevented the deleterious effects of Wang *et al.*^[32] has been reported that the Fx take part in a vital role in the prevention of breast cancer stimulated lymph angiogenesis for position the organization to preventing of lymph node metastasis of mammary malignancies. Yamamoto *et al.*^[33] suggested

that Fx inhibited tumor cell growth on stern merged immune-deficient mice immunized through BCBL-1 cells. Chung *et al.*^[34] revealed that Fx declined the number of metastatic nodules in the lungs of mice when treated in B16-F10 cells. Ye *et al.*^[35] found that Fx suppressed the tumor cell proliferation in nude mice implanted with HeLa cells. Nishino^[36] revealed that Fx completely prevented the tumor development in dermal tissues of mouse with second-stage skin carcinogenesis. Similar studies have been observed in following studies. Dong *et al.*^[37] showed that 8-allyl garcinol delayed the dysplastic changes in the DMBA-induced cheek pouch model. Vinoth and Kowsalya^[38] showed that orally administered vanillic acid restored the biochemical variables were when compared to the normal hamsters. Babukumar *et al.* (2017)^[39] showed that hesperetin in topically applied hamster model exhibits the apoptotic and antiproliferative effects.

ROS were derived reactive by-products such as hydroxyl radical and nonhydroxyl-free radical H_2O_2 was synthesized in mitochondria during normal cell metabolism, that particular generation of ROS was most useful for many cellular functions such as phagocytosis and bacterial ingestion.^[40] LPO, an free reactive species-mediated cyclic response in them biological membrane, was accountable to the pathological process of numerous diseases which include carcinoma. Human and animal cells employ both enzymatic and non-enzymatic mechanisms of antioxidant defense which can defend the cell against oxidative injury.^[41] This is supported by very low antioxidant activity in all malignancy cases including oral cavity. Free radical scavenging mechanisms prevent the initiation and progression of cancer and neutralize the immortalization and transformation of cells. The reduced content of polyunsaturated fatty acids in the biomembrane in OCC was caused due to oxidative stress. Various studies have explained the increase in the levels of SOD and H_2O_2 in the ORC tissues. A decrease in the activity of SOD and CAT

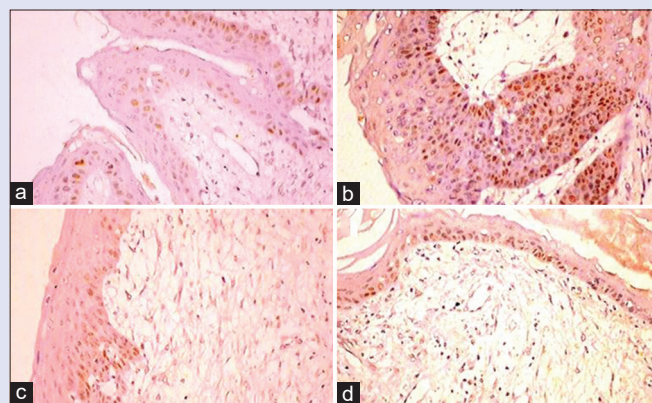


Figure 10: Immunohistochemistry analysis of apoptosis (p53) protein expression observed in the oral tissue of experimental hamsters (x40) (a and d) control and fucoxanthin alone hamsters showed not detectable p53 protein expression were observed; (b) 7,12-dimethylbenz[a] anthracene alone hamster showed higher expression of apoptotic marker (p53); (c) 7,12-dimethylbenz[a]anthracene + fucoxanthin hamsters downregulated the expression of p53 protein

in addition to the increase in the activity of GSH/GPx is common in tumor cells of HBPCs^[42] in this study were similar data were observed also in our study. The presence of GR has been revealed in various solid tumors, including OTP.^[43] Increase in the levels of GPx activity might account for the increased levels of GR in OT tissues. From this result, we suggested that dietary elements such as Vitamins C and E antioxidants like GSH to assemble those nutritional deficiency to down the oxidative stress. In the study, orally administered with Fx level of the LPO were decreased and antioxidant activity was increased, which most probably to control its free radical scavenging property and free radicals in the HCP. Our results agree with those of Neumann *et al.*,^[44] who reported that Fx demonstrated anticell growth and antioxidant activities in RAW 264.7, HepG2, Caco-2, and HeLa cell lines. Our results also agree with those of Jin *et al.*,^[45] who reported that the administration of Fx altered the activities of antioxidants and liver enzymes in diethyl nitrosamine-induced rats.

Liver plays a pivotal role during the removal of xenobiotic substances, including carcinogens. In this study, chemopreventive agents decreased the activity of phase I enzymes and increased the activity of phase II enzymes. However, phases I and II enzymes were toxic which is removed in excretion of carcinogenic metabolites in conjugation with GSH.^[46] Quantification of xenobiotic enzymes in hepatic and OM might help to determine the efficacy of chemopreventive agent. In malignant cancer, including OC, toxic metabolites accumulate in the liver because of the elevated levels of phase I enzymes and diminished levels of phase II enzymes. Thus, in disease condition increased level of DMBA accumulation of carcinogenic metabolites in liver, in addition dihydrodiol epoxide functions were also abnormal expression in liver causes cancer. Everyday exposure of DMBA accounts for the development of hepatic marker enzymes levels development were increased. So that widespread research conducted on DMBA-induced in the need.^[8,47] In this study, the oral supplementation of Fx to hamsters induced with DMBA drastically modulated xenobiotic enzymes. Fx improved the functions of phase II enzymes for the removal of toxic material, i.e., stimulation of malignant metabolites of DMBA in the treatment group with DMBA-induced hamsters.

IHC plays a crucial role in identifying molecular markers in the cancerous cells and is simple and very low cost. PCNA, a nonhistamine nuclear protein of DNA polymerase δ , plays an essential role in the cell

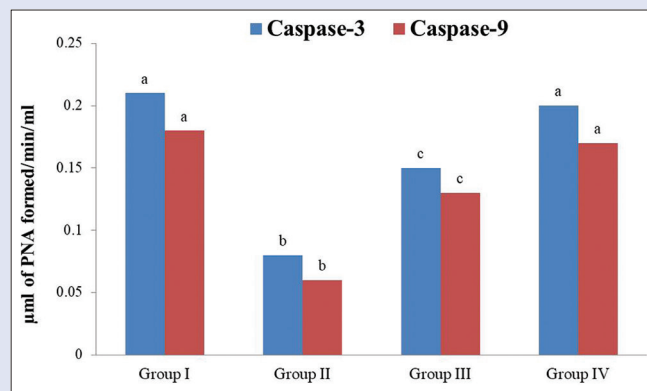


Figure 11: Levels of caspase-3 and 9 activities in the oral tissues of experimental hamsters

development and regulation of cell cycle. Human head and neck cancer was confirmed the overexpression of PCNA through OT proliferation in prepremalignant and malignant sores.^[41,48] In this study, oral supplementation of 50 mg/kg bw, of Fx to hamsters induced with DMBA notably decreased the expression of PCNA. In the recent studies clearly showed that the Fx was noticeably suppressed tumor development via stimulation of pro-apoptotic mediators on Hep-2.^[49] Further, cell growth inhibition and stimulate necrosis resulting on a delayed multiplicity and burden of tumors.

Apoptosis maintains homeostasis by killing the unwanted and poisonous cells.^[50] One such apoptotic marker p53, a gene responsible for the suppression of tumors, performs an essential role in apoptosis, averting the development of cell cycle, and induces DNA repairing mechanism. During OC, early-stage development of p53 proteins is mutated/inactivated.^[51,52] Ding *et al.*^[53] have shown that diminished activity of caspases-3 and 9 is associated with the incursion of vascular metastasis of lymph node and superior tumor in SCC. Current research on the functioning of caspases-3 and 9 has been expressed as protease activity; this contributes to decrease the OT development in DMBA-induced hamsters. Oral supplementation of Fx with DMBA-induced hamsters were induces apoptosis during the oral cancer. Increased expression of p53 and diminished expression of caspases-3 and 9 indicate that Fx induced apoptosis in DMBA-induced HBPC.

CONCLUSION

The results of this study show that Fx prevented DMBA-induced cell proliferation and increased apoptotic induction in HCP cancer. A fascinating examination revealed by this study is the potential of Fx to totally suppress the development of DMBA-induced oral malignancy in cheek pouch of hamsters. Fx potentially improves antioxidant status, modulates the status of xenobiotic enzymes, and alters the levels of LPO. Moreover, other studies have shown the curative effect of Fx as a chemopreventive and therapeutic mediator for treating human head and neck carcinomas.

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Conflicts of interest

There are no conflicts of interest.

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