Fucoxanthin Modulates the Development of 7, 12-dimethyl benz (a) anthracene-induced Skin Carcinogenesis in Swiss Albino Mice *in vivo*

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ABSTRACT

Background: Fucoxanthin (Fx), an distinctive carotenoid occurs on brown seaweed, contains several benefits including anti-cancer effects. To investigate the chemopreventive effectual of Fx on 7,12-dimethylbenz[a] anthracene (DMBA)-alone skin tumor development in Swiss albino mice. Materials and Methods: The skin sarcoma being provoked on the hairless flipside of the mice's skin, twice weekly for 8 weeks through challenging through DMBA (25 µg on 0.1 ml acetone/mice). Thereafter, the mice were oral supplementation with 50 mg/kg body weight (BW), Fx for 25th week a frequency of three times/week. Tumor size, change in BW, the cumulative quality of papillomas and some oxidative stress-related parameters were measured. Results: Orally administered Fx were notably increased BW, delayed tumor incidence with no abnormal pathology in DMBA-induced skin tumor mice. Fx effectively modulates the level of xenobiotic enzymes, brought back to the statuses of lipid peroxidation (LPO) enzymes and increased antioxidant enzymes status in squamous cell carcinomas (SCC). A considerable decrease in the protein expression of proinflammatory markers that is interleukin (IL) 1-6, tumor necrosis factor-alpha (TNF- α), and IL- β and restored the expression status of inflammatory regulators such as nuclear factor-kappa B (NF- κ B), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) on serum. These findings were supported through histopathological examinations. Hence, it was clear that Fx possessed a good anticancer activity against skin cancer. Conclusion: The chemopreventive prospective of Fx was eventually owing to its altering capacity on the levels of proinflammatory (IL 1-6, TNF- α , and IL- β) cytokines, inflammatory (NF-KB, COX-2, and iNOS) markers, LPO, antioxidants, and toxin-eliminating mediators in DMBA-provoked skin SCC. Thus, it can be concluded that the FX indicates the antitumor potential on DMBA challenged skin tumor proliferation model on mice.

Key words: Chemoprevention, fucoxanthin, oxidative stress, papilloma, skin sarcoma

SUMMARY

- Skin cancer (SC) is a foremost reason of morbidity and mortality in global. Acute skin-related illness was caused by immunosuppression and finally SC, due to skin overexposure to ultraviolet irradiations, chemicals, and different viruses.
- Fucoxanthin (Fx) diminished inducible nitric oxide synthase, overexpression in SC in the 7,12-dimethylbenz[a] anthracene (DMBA)-induced mice and considerably augmented interleukin-6 (IL-6), serum tumor necrosis factor-alpha and IL-1b, representative that Fx abridged DMBA-treated oxidative damage in skin tumor growth and also bring apoptosis in SC cells thereby toward skin carcinoma.



Abbreviations used: FX: Fucoxanthin; DMBA: 7,12-dimethylbenz[a] anthracene; SCC: Squamous cell carcinoma; BW: Bodyweight; LPO: Lipid peroxidation; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; COX-2: Cyclooxygenase-2.

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INTRODUCTION

Skin cancer (SC) is well thought out as the major widespread infection in the human people.^[1] The report, consideration illustrates that SC represents about 40% of every one of new cancer cases identified globally. Among all the human cancers, in India, survey conducted mostly accounts about 1%–2% of skin neoplasms in 2014.^[2] An augment in the SC burden was programmed due to daily life alteration and ecological risks. The keratin stratum with resident mechanism of the human skin offers an outstanding defense anti-harmful contacts.^[3] 7,12-dimethylbenz[a] anthracene (DMBA), an environmental pollutant, procarcinogen, which is commonly located in cigarette smoke, that mostly interferes through xenobiotics metabolism with causes DNA breakage. In addition, it disturbs linkage between proteins and nucleic acid, generate makes genomic alteration and exaggeration, those mechanisms.^[4,5] A crucial molecular system of an inflammation provokes ailments in organisms, include skin illness. According to earlier investigation, a kind of proinflammatory markers, for instance, tumor necrosis factor-alpha (TNF- α) and interleukins (ILs) and the anti-inflammatory factor, transforming growth factor-beta, are enhanced expression in the

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skin under different conditions.^[6] IL-1 β is produced by macrophages is also known as catabolism, which is activated as proteins in macrophages. IL-1 β , crucial regulator of the inflammatory response and various cellular functions, including cell differentiation and apoptosis.^[7] TNF- α , establish in tumor extracts.^[8] TNF- α , as well identified as cachectin, is a main function in anti-tumor effect, inflammatory response, immunomodulating activities macrophage-mediated cytokine through several activities.^[9] Hence that we have now investigated the preventive action of fucoxanthin (Fx) on cancerous progression in tumor-induced mice. The serum statuses of TNF- α and IL-6 were also deliberated to learn the own immune method.^[10]

The herbal plants provide enormous natural bioactive compounds with the purpose of protection alongside stress and pathogenic injury. Extensive-term use of sure medicinal plants overcomes carcinomas in all animal and human parts of the body.^[11] Consequently, it is the significant role to recognize natural plant effect that possibly will repress or change the progression of carcinoma.^[12] Fx is an active phytochemical and it belongs in carotenoids which enormously occurs in brown seaweed among a different forms with having of an allenic bond, epoxide group, and linked carbonyl grouping in a polyene chain.^[13]

Several considerations have been confirmed the different pharmacological actions of Fx, together with anti-mutagenic,^[14] anti-obesity,^[15] anti-diabetic,^[16] anti-inflammatory,^[17] neuroprotective activity,^[18] and anti-tumor activity.^[19] Liu *et al.*^[20] have been demonstrated that Fx have several anti-cancer found in glioblastoma, colon, melanoma, prostate, liver, leukemia, lung cancer, breast, bladder, osteosarcoma, cervical, and gastric. Fx represses tumor delay through an enhancing gap junctional intercellular communication, a variety of machinery, arresting the cell cycle at G1/G0 and inducing cell death.^[19,21]

The current study was explored to the possible anti-cancer efficacy of Fx in Swiss albino mice (SAM)-treated DMBA alone skin carcinogens. We examined the tumor incidence (TI), histological evaluation, and levels of liver xenobiotic agents, antioxidant, and lipid peroxidation (LPO) in the control and treated mice. In addition, appearance of numerous protein markers nuclear factor-kappa B (NF- κ B), inducible nitric oxide synthase (iNOS), IL1- β , IL-6, TNF- α , and cyclooxygenase-2 (COX-2) was analyzed using enzymes-linked immunosorbent test (ELISA) and Western blotting.

MATERIALS AND METHODS

Chemicals

Fx and DMBA was obtained from Sigma-Aldrich Chemical Pvt. Ltd (USA). IL-6, IL1- β , and TNF- α calorimetric evaluate kits were obtained from BioVision research products, USA. Antibodies adjacent to NF- κ B, COX-2, and iNOS were procured from Santa Cruz, CA, USA.

Animals and diet

For this study, male SAM (68 weeks age), weighing 15–20 g was purchased and kept in acclimatized and under the controlled conditions as per the all experimental guidelines were followed the ethical committee for the careful handling of test animals. Standard pellet diet was consists of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorous, 3.4% glucose, 2% vitamin, and 55% nitrogen-free extract (carbohydrates).

Tumor induction and assessment

SAM was separated into four groups of six mice in each. All mice (totally 24 mice) hair removed were posterior side of our study. For skin carcinomas initiation, the DMBA was administered topically to mice and keep under observation for 8 weeks. In the last part of the

25th week, all mice are admitted to slay through cervical displacement. Cell proliferation in the skin tumor was removed from everyone mice skin tissues and total number of tumor and diameters were determined microscopically, with a Vernier caliper. Skin tumor burden (TB) was measured through multiplying the tumor volume (TV) and number of tumors in the mice. The TV was determined via the formula, V = (4/3) π (D1/2) (D2/2) (D3/2) where D1, D2, and D3 are the three diameters (mm³) of the SC.

Experimental protocol

All mice were separated into four groups of 6 mice (n = 6 every). Group I mice was provided as control, whereas Group II mice was induced by DMBA only 25 µg in 100 µL acetone. Group III mice received DMBA and Fx at 50 mg/kg body weight (BW), in 1% DMSO correspondingly. Group IV mice received an Fx (50 mg/kg BW) alone. Oral supplementation of Fx (50 mg/kg BW) thrice a week, starting from a week beginning DMBA treated mice, of the 25 weeks. Toward the end of 25 weeks, experiment was concluded. Biochemical and molecular-level analysis were euthanized and immediately skin tissue was dissected out control and experimental mice.

Histopathological examinations

The skin tumor of the experimental mice was quickly removed and cleaned by using the chilled saline solution. Moreover, the normal and tumor skin cells were preserved on 10% formalin and entrenched in paraffin wax. Afterward, tissue portion of $3-5-\mu$ m thickness was cut with a rotary microtome and staining with H and E stain before determine by using an under light microscope. The mice eye blood was collected for proinflammatory cytokine determination.

Biochemical analysis

The skin tumor tissues and hepatic tissues of experimental mice was cleaned with a chilled saline solution and homogenized with the suitable buffer. Whole protein substance was examined by the method of Lowry *et al.*,^[22] The SC tissues were used to analyze LPO, enzymatic and non-enzymatic antioxidant activity.

LPO was measured as conformed by the production of thiobarbituric acid reactive substances (TBARS) were demonstrated through Ohkawa et al. technique.^[23] Reduced glutathione (GSH) was investigated through Beutler and Kelly way^[24] in accordance with the increased yellow color when 5,5'-dithiobis-(2-nitrobenzoic acid) was amalgamated to compounds that having sulfhydryl groups. Glutathione peroxidase (GPx) level was analyzed through the adopting the hydrogen peroxide that the same as depicted in Rotruck et al. method.[25] SOD was determined via Kakkar et al. technique^[26] through 50% inhibition of the development of NADH-phenazinemethosulfate NBT formazan at 520 nm. Levels of catalase (CAT) were analyzed through Sinha, method^[27] in accordance with the utilization of H₂O₂ from the enzyme. Color development was examined at 620 nm. Cytochrome p450 (Cyt-p450) and cytochrome b5 (Cyt-b5) contents were determined via the technique of Omura and Sato,^[28]·Cyt-p450 was examined via the CO various spectra. Reduced Cyt-p450 unites with carbon monoxide to generate a pigment was read at 450 nm. Cyt-b5 was observed through the various spectrums between decreased and oxidized Cyt-b5. The level of Glutathione S-transferases (GST) was investigated as illustrated by Habig et al.^[29] via following the augment on absorbance at 340 nm utilizing 1-chloro-2,4-dinitrobenzene as a substrate. Glutathione reductase (GR) levels were examined from the way of Carlberg and Mannervik, (1985)^[30] the reduction of glutathione disulfide to GR through monitoring the oxidation of NADPH as visualized through diminished absorbance at 340 nm.

Enzymes-linked immunosorbent test

The eye blood was subjected to centrifuge at $12,000 \times g$ for 10 min to carefully collect the upper aqueous phase. Then, IL-6, IL1- β , and TNF- α status in serum was determined using the mice Quantikine ELISA kit (R and D Systems, Inc., Minneapolis, MN, USA) in accordance to the manufacturer's guidelines.

Immunoblot analysis

Immunoblot was employed as follows protein was denatured and equal quantity of protein was loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to PVDF membranes (Bio-Rad, Hercules, CA). Membranes were then blocked with skimmed milk for 2 h to block non-specific binding regions and subsequently kept with primary antibodies specific to anti-NF-KB, COX-2 and iNOS. The membranes were cleaned by using a TBST (TBS-1%; Tween-20) washing buffer three times for 10 min and kept with particular secondary antibody (1:500 dilutions) for 2 h at 37°C. The membrane was cleaned through TBST buffer again for three times. The immune reactive protein band finding was performed with ECL reagents (Bio-Rad, Hercules, CA). Western blots were consequently stripped, re-probed, and practiced for viewing β-actin. The band strength was regularized to the β -actin. Densitometry was employed in IISP-flatbed scanner and quantities with Total lab-1.11 software (SPSS Inc., Chicago, IL, USA).

Statistical study

Values were portrayed as the mean \pm standard deviation in each experiment. Statistical consequence was observed using one-way analysis of variance which was carried out using the SPSS version 17.0 software. The tests were regarded statistically notable values set at *P* < 0.05.

RESULTS

Fucoxanthin increased mice bodyweight in skin carcinogens

As shown in Table 1, at the end of the 25 weeks, mice BW and liver weight (LW) changes were measured in the experimental mice. No mice BW (36.26 ± 2.76 and 37.50 ± 2.87 gm) and LW (2.30 ± 0.18 and 2.33 ± 0.18 gm) changes were observed in group 1 and 4 correspondingly. In Group 2, BW (23.65 ± 1.81 gm) and LW (1.10 ± 0.08) were notably (P < 0.05) diminished in the mice treated with DMBA alone. Oral supplementation of Fx and DMBA mice shows considerably increased BW (33.02 ± 2.51 gm) and LW (2.11 ± 0.16) as compared to Group 1.

Fucoxanthin suppressed in skin tumors

Table 2 depicts TI, TV, and TB in experimental mice. Group II mice showed the 100% tumor development with mean TV (583.61 mm³) and TB (1848.09 mm³) on the 25th week. Supplementation of Fx to DMBA-treated (group III) mice totally inhibited TI, TN, TV, and TB as compared with Group I. No tumor development was observed in the Group I and Group IV.

Histopathological examination of mice skin tumor tissues

The gross appearance of skin detected in a mice treated with alone squamous cell carcinoma (SCC) is depicted in Figure 1 and the histological changes were examination for any of the skin tumor tissues in the experimental mice [Figure 2]. Group 1 and IV skin tissues were found normal intact epithelial layers. Group II mice exhibited a well-transformed SC among the development of keratin pearls and

Table 1: Effect of Fxon body weight and liver weight of experimental mice

Groups/treatments	Body we	eight (g)	Liver weight (g)
	Initial	Final	
Group I	19.13±1.46	36.26±2.76	2.30±0.18
Group II	18.76 ± 1.44	23.65±1.81	$1.10{\pm}0.08$
Group III	19.02±1.45	33.02±2.51	2.11±0.16
Group IV	20.11±1.54	37.50±2.87	2.33±0.18

Values are expressed as mean \pm SD of six mice. Values not sharing a common superscript letter differ significantly at *P*<0.05. SD: Standard deviation

 Table 2: Effect of Fxon tumor incidence, tumor volume and burden of skin tumor of experimental mice

Groups/treatments	Number of mice	TI (%)	Tumor number	TV	ТВ
Group I	6	-	-	-	-
Group II	6	100	19±1.45	583.61 ± 44.69	1848.09 ± 140.75
Group III	6	-	-	-	-
Group IV	6	-	-	-	-

Values are expressed as mean \pm SD of six mice. Values not sharing a common superscript letter differ significantly at *P*<0.05. TI: Tumor incidence; TV: Tumor volume; TB: Tumor burden; SD: Standard deviation

a discernible penetration of tumor cells in the underlying skin layer. Group III treated mice skin tissues were showing a normal cellular architecture through mild-to-moderated hyperkeratosis and hyperplastic papillomatous lesion.

Status of lipid peroxidation and antioxidant activities

Status of TBARS and SOD, GPx, GSH, and CAT levels in skin tumor tissues of experimental mice [Figure 3]. The status of TBARS was notably elevated and SOD, GPx, CAT, and GSH were diminished on SC tissues while comparing it to control. Orally presupplemented of Fx to DMBA challenged mice restored the antioxidants status to normal value. Group I and IV showing no considerable variation in the skin tissue levels of antioxidant activities.

Determination of detoxification enzymes

The level of xenobiotic enzymes in the SC tissues of experimental mice is depicted in Figure 4. The status of Cyt-b5 and Cyt-p450 enzymes were higher, whereas GST, GSH, and GR agents were notably lower in the hepatic tissues of Group II mice while comparing it to control. Oral supplementation of Fx to DMBA-treated (Group III) mice has revealed the intensity of xenobiotics agents to almost normal levels. Group I and IV mice showing no notable difference in the status of biotransforming enzymes.

Fucoxanthin-induced expression of inflammatory markers

Several cytokines play a key role in mediating acute inflammatory reactions that suppressed skin SCC development has been noticed, with TNF- α , IL-6, and IL-1 β . TNF- α is a cell signaling protein (cytokine) created with cells of monocytic lineage and it has been illustrated to augment anti-tumor activities.^[31] Moreover, a study showed that caspase-8 acting a pro-inflammatory function in growing the status of IL-6 and IL-1 β and is implicated in the maturation of IL-1 β .^[32] Figure 5 shows the status of serum IL-6 and IL-1 β was considerably diminished in the DMBA only (Group II). Although, Fx treatment noticeably resulted in elevated serum TNF- α , IL-6, and IL-1 β status as compared to control. Group I and IV depicted no considerable difference were observed in the TNF- α , IL-6, and IL-1 β markers.



Figure 1: Photomicrograph showing the gross appearance, skin tumor in the skin tissues of experimental mice. (Group I and IV) Control and fucoxanthin alone mice; (Group II) 7,12-dimethylbenz[a] anthracene alone depicts skin tumor cell proliferation; (Group III) 7,12-dimethylbenz[a] anthracene + fucoxanthin treated mice

Western blotting test of inflammation mediators

The expression patterns of inflammation markers in the experimental mice [Figure 6]. The Western blot analysis shows significantly (P < 0.05) increased appearance of inflammatory markers were noticed in Group II. Oral supplementation of Fx in mice treated with DMBA (Group III) notably (P < 0.05) restored the expression of the inflammatory mediators while comparing it to Group I. Group I and IV showing the normal appearance of the above markers.

DISCUSSION

SC is a major type of cancer which accounts for the highest morbidity and mortality.^[33] DMBA stimulates carcinogenesis through the accretion of reactive oxygen species (ROS) and hydroperoxides in keratinocytes that will exaggerate usual cell to malignant cells.^[34] In this study, DMBA challenged mice exhibited reduced body weight and liver mass, which may because of oxidative stress caused by inflammation in the epithelial mucosa.^[33,35-37] These attributes to the pathological process of initiation and progression of SC.^[34] This study proved that the Fx at 50 mg/kg BW, used a topical application because it was reported that the fastest way of absorption of DMBA carcinogen was found in skin tissue. In the present experiment, carcinogen-treated mice exhibited 100% TI and the highest cumulative number of tumors. However, treatment with Fx regressed of TI and tumor number as well. In addition, DMBA also causes the formation of proinflammatory cytokines (IL 1-6, IL-B, and TNF- α), inflammatory markers (NF-KB, COX-2 and iNOS), which act on the similar way to Fx mediated inflammation and oxidative stress which donate to SC progression.

Oxidative stress as a result of over accretion of ROS along with diminished free radical scavenging mechanism has promotes the pathogenesis of cancer,^[38] inducing LPO, DNA damage by transforming various biochemical mechanism and gene expression.[39] Various articles were explained the DMBA applied via topical on skin region induces ROS leads cancer.^[40] This current study proved that the Fx treatment at 50 mg/kg BW dose was exhibited a remarkable amelioration effects against the DMBA provoked SC through the inhibition of over ROS generation, reducing inflammatory mediators, and inflammatory gene markers.^[41] Fx (50 mg/kg BW) administration was brought to delay the prevalence of tumor appearance more in the promotion phase. Such diminution of tumor synthesis due to similar factors and in various plants has been reported previously.[42] Ascorbic outstanding is the most powerful antioxidant beneath physiological circumstance, which can precisely scavenge harmful radicals. Such kind of vitamin reduces H₂O₂ to water through ascorbate peroxidase reaction.^[43] Fx shows anti-hyperlipidaemic, anti-angiogenic, anti-diabetic, and anti-oxidant activities. The precise technique of action of Fx is not clearly known. In



Figure 2: Histopathological changes of the skin tissues of experimental mice. Photomicrographs of the skin tissue showing normal histology (×40) in (Group I) control and (Group IV) Fucoxanthin -alone mice; (Group II) 7,12-dimethylbenz[a] anthracene induced mice showing squamous cell carcinoma to keratin pearls generation; (Group III) 7,12-dimethylbenz[a] anthracene + fucoxanthin treated mice skin demonstrating dysplastic and papillomatous development without obviously of infiltrative tumor

the same way as a result, the induction of cell death by Fx may destroy the cancer cells. The existing work demands further study to deal with the exact mechanism and clinical applicability of Fx as a chemopreventive agent.^[19] In addition, Fx has also been revealed to anti-cancer effects in various chemical-induced different cancer through evade oxidative stress, malformed activation of biotransforming enzymes and preneoplastic lesion. In the existing study, we identified that Fx 50 mg/kg BW, dose show a chemopreventive effect against DMBA-induced skin tumor cell propagation.

Two most important stages were induced, i.e., initiation and promotion of tumorigenesis were induced by well-known LPO by the free radical chain reaction. The complex reactive Malondialdehyde compound was synthesized from LPO when levels were increased e.g., malondialdehyde. Further, the substances of LPO was informed to be mutagenic and carcinogenic with the control group.^[39] Exposure to DMBA on skin region induces LPO and ROS leading to carcinoma. Caspase was activated, when ROS initiate permeabilization of the outer mitochondrial membrane, from the inner membrane gap into the cytosol.^[44]

Taken together, in our study, oxidative stress noted on the Group I mice, enhanced the LPO (TBARS) and statuses of CAT, SOD, GSH, and GPx amount were diminished on mice were during the experimental period. The Fx orally treated is probably to stimulate free radical scavenging enzymes in cancerous cells. In skin tissues developed enzyme activity when deregulating the formation of ROS and LPO diminished the prevalence of papilloma in the region of Fx 50 mg/kg BW, treated mice. Histopathological examination revealed that DMBA produced severe skin injury. The histopathology research demonstrated that Fx (50 mg/kg b. w) restored the deteriorated dermal and epidermal layers of skin in the Group III mice. On the other hand, major changes in histological appearances identified in the hypodermis, this part mainly created as chemopreventive agents to inhibit the production of Fx was delayed the increase and



Figure 3: Levels of lipid peroxidation, enzymatic and non-enzymatic anti-oxidants in the skin tissues of experimental mice. Values are expressed as mean \pm standard deviation of six mice. Values not sharing a common superscript letter differ significantly at (a-c) P < 0.05







Figure 5: Fucoxanthin inhibits pro-inflammatory cytokines elevated in the serum of experimental mice. The status of circulating pro-inflammatory cytokines of interleukin 1-6, interleukin- β and tumor necrosis factor alpha were estimated through enzymes-linked immunosorbent test procedures. Values not sharing a common superscript differ significantly at (a-c) P < 0.05

maintain levels of LPO in DMBA-induced tumor-bearing mice, and it suggested that delay the frequency of SC.

GSH plays an essential role in normal cell metabolism, which is exposed to prevention and development, production of free radicals.^[45] We suggest that GSH actions were lowered on the DMBA-treated Group I mice, but was developed in Fx-treated mice, signifying its free radical scavenging activity. Chemopreventive properties reported as the free radical changing effect on the different cancer models.^[46] Antioxidants are believed to be secure towards ROS induced stress, which proposed that helpfulness in the conversion of the danger of oxidative problems in cancer formation.^[45] SOD, CAT, and GPx are the antioxidative catalysts that stay against ROS. We examined the effects of CAT, SOD, GPx, and GSH function on the Fx-treated mice while comparing it to control mice.

The enzymes for Phase I metabolism are accountable for the major conversion of chemical carcinogens into its active intermediate were used in the experiments. The elevated level of Cyt-P450 and b5 enzymes was acting as a biomarker for carcinoma identification along with a tendency analyzed in the carcinogen-treated mice. The modulator effect of Fx restored the status of Cyt-P450 and b5 similar to normal levels in skin tumor tissues in Fx administered mice. In staining reveals that phytochemicals improved (GR, GST, and GSH) status in biological tissues. GSTs make possible the xenobiotic of carcinogens through ligands with the carcinogen and GSH finally uridine diphosphate glucuronic acid, correspondingly, for its fast biotransformation enzymes and remove. In the current study, superior GR, GSH and GST status in the skin tissues of treated mice were evidenced after Fx treatment.

In controlling the cellular reaction to cytokines and pathogens, the NF-KB signaling pathway acts a necessary role in regulating cellular proliferation and programmed cell death. Regular with this, improper signaling of NF-KB has been played in cancer cell development in several cancers such as lymphomas, breast, skin, and bladder tumor and excessively high NF-KB effect and decrease IKB status which indicate that uncontrolled of NF-KB may also involve to skin tumor cell growth. SC demonstrated higher status of AP-1 effector gene expression, associating by the degree of malignancy.^[47] Gene substances promoting the invasion and metastasis are also under AP-1 controlling.^[48] ROS, as causative factors in mutagenesis, tumor cell development and carcinogenesis, have been demonstrated in the etiology and pathophysiology of human infections.^[49] Further, to find the molecular action of the inhibition of tumors with Fx, the TNF-α, NF-κB, and COX-2 transcription factors were studied in tumors from all treatment groups. NF-kB has been concerned with cancer as it acting an essential part in inflammation, cell growth, cell adhesion, and differentiation.



Figure 6: Protein expression of pro-inflammatory markers in the control and experimental mice. The bar diagram is expressed as mean \pm standard deviation for six mice. ^{a-c}significantly (P < 0.05) different from control and experimental mice

Carcinoma is a hyperproliferative syndrome to grades starting tumor pre and progression stages and eventually generates metastasis into cancer. Numerous genes occupied in cellular alteration, propagation, invasion, and angiogenesis are controlled with NF-KB. Degradation of cytoplasmic NF-KB suppressors, IKB and its translocation to the nucleus results in oxidant stress.^[50] Nowadays, the mRNA expression of p65 up-regulated extensively in DMBA/Fx administered mice (50 mg/kg BW). This point of regulation of NF-κB, which is possibly payable to the prevention of IKB protein that exists in the cytoplasm and hence, developed the level of p65 expression in the skin tissue nuclear reaction. The constitutive power activation of NF-KB also come into view to contain a part in cell progression,^[51] while in control (untreated), readily available was a weak expression. Similarly, a change and lower in expression observed in DMBA + Fx and Fx-alone orally supplemented mice. This might exist remaining to control of NF-kB by these Fx treated. We believe the signaling pathway may be a goal for Fx. The numerous effects of chemopreventive drugs are suppressors of NF-KB stimulation. These inhibitors can inhibit step by step, in the mechanism of NF-KB signaling pathways, the translocation of NF-KB into the nucleus, DNA binding of the dimmers and connections through the basal transcription stimulation.[20]

In addition, Fx action to accelerate elevated serum TNF- α and IL-1b status similar to DMBA administrated. Augmented TNF- α and thus optimistic skin squamous cell death to inhibit cancer cell growth. These findings recommended that Fx-treated apoptosis may be related chiefly to the stimulation of the TNF- α receptor pathway slightly than the initiation of the mitochondrial pathway. After that, we consider caspase-8 possibly will contain encourage the development of IL-1 β and have found a pro-inflammatory function in raising the status of IL-1b in organizing to restrain the progression of skin SCC.^[52] This examined and established that Fx diminished iNOS, overexpression in SC in the DMBA-induced mice and considerably augmented IL-6, serum TNF- α , and IL-1b, representative that Fx abridged DMBA-treated oxidative damage in skin tumor growth. All mutually, these verdicts recommend that Fx is a promising drug for SC therapy.

CONCLUSION

As a result, findings of this current investigation suggest that the Fx supplementation at various steps of cancer abilities to chemo-preventive possible with modulating the xenobiotic enzymes, anti-LPO, anti-oxidants and suppress inflammation in mammals. The anti-inflammatory, anti-cancerous, and antioxidative effect of Fx possibly be a result of promising chemopreventive and therapeutic target action in skin carcinogenesis. Yet, additional studies are necessary to know the particular function of Fx in the different molecular pathways, pertaining to anti-cancer potential.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Apalla Z, Nashan D, Weller RB, Castellsagué X. Skin cancer: Epidemiology, disease burden, pathophysiology, diagnosis, and therapeutic approaches. Dermatol Ther (Heidelb) 2017;7:5-19.
- Gálvez JM, Castillo D, Herrera LJ, San Román B, Valenzuela O, Ortuño FM, et al. Multiclass classification for skin cancer profiling based on the integration of heterogeneous gene expression series. PLoS One 2018;13:e0196836.
- Rangwala S, Tsai KY. Roles of the immune system in skin cancer. Br J Dermatol 2011;165:953-65.
- Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, et al. Inhibition of 7,12-dimethylbenz(a) anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. Cancer Res 2006;66:8293-6.
- Mukherjee S, Koner BC, Ray S, Ray A. Environmental contaminants in pathogenesis of breast cancer. Indian J Exp Biol 2006;44:597-617.
- Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018;9:7204-18.
- 7. Ren K, Torres R. Role of interleukin-1beta during pain and inflammation. Brain Res Rev 2009;60:57-64.
- Shklar G, Schwartz J. Tumor necrosis factor in experimental cancer regression with alphatocopherol, beta-carotene, canthaxanthin and algae extract. Eur J Cancer Clin Oncol 1988;24:839-50.
- Kushibiki S. Tumor necrosis factor-α-induced inflammatory responses in cattle. Anim Sci J 2011;82:504-11.
- Afshari JT, Ghomian N, Shameli A, Shakeri MT, Fahmidehkar MA, Mahajer E, et al. Determination of interleukin-6 and tumor necrosis factor-alpha concentrations in Iranian-Khorasanian patients with preeclampsia. BMC Pregnancy Childbirth 2005;5:14.
- Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: An integrated approach. Ayu 2012;33:10-9.
- Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med 2013;10:210-29.
- Zhang H, Tang Y, Zhang Y, Zhang S, Qu J, Wang X, et al. Fucoxanthin: A promising medicinal and nutritional ingredient. Evid Based Complement Alternat Med 2015;2015:723515.
- Wang Z, Li H, Dong M, Zhu P, Cai Y. The anticancer effects and mechanisms of fucoxanthin combined with other drugs. J Cancer Res Clin Oncol 2019;145:293-301.
- Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K. Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white

adipose tissues. Biochem Biophys Res Commun 2005;332:392-7.

- Nishikawa S, Hosokawa M, Miyashita K. Fucoxanthin promotes translocation and induction of glucose transporter 4 in skeletal muscles of diabetic/obese KK-A(y) mice. Phytomedicine 2012;19:389-94.
- Kim KN, Heo SJ, Yoon WJ, Kang SM, Ahn G, Yi TH, *et al.* Fucoxanthin inhibits the inflammatory response by suppressing the activation of NFκB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. Eur J Pharmacol 2010;649:369-75.
- Zhang L, Wang H, Fan Y, Gao Y, Li X, Hu Z, *et al.* Fucoxanthin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE and Nrf2-autophagy pathways. Sci Rep 2017;7:46763.
- Martin LJ. Fucoxanthin and its metabolite fucoxanthinol in cancer prevention and treatment. Mar Drugs 2015;13:4784-98.
- Liu Y, Zheng J, Zhang Y, Wang Z, Yang Y, Bai M, et al. Fucoxanthin activates apoptosis via inhibition of PI3K/Akt/mTOR pathway and suppresses invasion and migration by restriction of p38-MMP-2/9 pathway in human glioblastoma cells. Neurochem Res 2016;41:2728-51.
- Hou LL, Gao C, Chen L, Hu GQ, Xie SQ. Essential role of autophagy in fucoxanthin-induced cytotoxicity to human epithelial cervical cancer HeLa cells. Acta Pharmacol Sin 2013;34:1403-10.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
- 24. Beutler E, Kelly BM. The effect of sodium nitrate on RBC glutathione. Experientia 1963;19:96-7.
- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
- Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
- 27. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
- Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. evidence for its hemoprotein nature. J Biol Chem 1964;239:2370-8.
- Habig WM, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1994;249:7130-9.
- 30. Mannervik CB. Glutathione reductase. Methods Enzyme 1985;113:484-90.
- Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014;2014:149185.
- Gurung P, Kanneganti TD. Novel roles for caspase-8 in IL-1β and inflammasome regulation. Am J Pathol 2015;185:17-25.
- Singh M, Suman S, Shukla Y. New enlightenment of skin cancer chemoprevention through phytochemicals: *In vitro* and *in vivo* studies and the underlying mechanisms. Biomed Res Int 2014;2014:243452.
- 34. Kwon YJ, Ye DJ, Baek HS, Chun YJ. 7,12-Dimethylbenz[α]anthracene increases cell proliferation and invasion through induction of Wnt/ β -catenin signaling and EMT process. Environ Toxicol 2018;33:729-42.
- Gopalakrishnan T, Ganapathy S, Veeran V, Namasivayam N. Preventive effect of D-carvone during DMBA induced mouse skin tumorigenesis by modulating xenobiotic metabolism and induction of apoptotic events. Biomed Pharmacother 2019;111:178-87.
- Bhandari PR. Crocus sativus L. (saffron) for cancer chemoprevention: A mini review. J Tradit Complement Med 2015;5:81-7.
- Kotecha R, Takami A, Espinoza JL. Dietary phytochemicals and cancer chemoprevention: A review of the clinical evidence. Oncotarget 2016;7:52517-29.
- 38. Liou GY, Storz P. Reactive oxygen species in cancer. Free Radic Res 2010;44:479-96.
- Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014;2014:360438.
- Sati J, Mohanty BP, Garg ML, Koul A. Pro-oxidant role of silibinin in DMBA/TPA induced skin cancer: 1H NMR metabolomic and biochemical study. PLoS One 2016;11:e0158955.
- Shimoda H, Tanaka J, Shan SJ, Maoka T. Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules. J Pharm Pharmacol 2010;62:1137-45.
- Deghan Manshadi S, Ishiguro L, Sohn KJ, Medline A, Renlund R, Croxford R, et al. Folic acid supplementation promotes mammary tumor progression in a rat model. PLoS One 2014;9:e84635.

- Noctor G, Foyer CH. Ascorbate and sglutathione: Keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 1998;49:249-79.
- 44. Ali H, Dixit S, Ali D, Alqahtani SM, Alkahtani S, Alarifi S. Isolation and evaluation of anticancer efficacy of stigmasterol in a mouse model of DMBA-induced skin carcinoma. Drug Des Devel Ther 2015;9:2793-800.
- Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4:118-26.
- Klaunig JE, Dekant W, Plotzke K, Scialli AR. Biological relevance of decamethylcyclopentasiloxane (D5) induced rat uterine endometrial adenocarcinoma tumorigenesis: Mode of action and relevance to humans. Regul Toxicol Pharmacol 2016;74 Suppl: S44-56.
- Sever R, Brugge JS. Signal transduction in cancer. Cold Spring Harb Perspect Med 2015;5:a006098.

- Baranwal S, Alahari SK. miRNA control of tumor cell invasion and metastasis. Int J Cancer 2010;126:1283-90.
- Waris G, Ahsan H. Reactive oxygen species: Role in the development of cancer and various chronic conditions. J Carcinog 2006;5:14.
- Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol 2009;1:a000034.
- Gille A, Stojnic B, Derwenskus F, Trautmann A, Schmid-Staiger U, Posten C, *et al*. A Lipophilic Fucoxanthin-Rich *Phaeodactylum tricornutum* Extract Ameliorates Effects of Diet-Induced Obesity in C57BL/6J Mice. Nutrients 2019;11. pii: E796.
- Maelfait J, Vercammen E, Janssens S, Schotte P, Haegman M, Magez S, *et al.* Stimulation of toll-like receptor 3 and 4 induces interleukin-1beta maturation by caspase-8. J Exp Med 2008;205:1967-73.