Review

Anti-inflammatory Dietary Supplements in the Chemoprevention of Oral Cancer.

Shri Hari[1]*, Vijeev Vasudevan[1], Soundarya Kasibhotla[1], Deepa Reddy[1], Manjunath Venkatappa[1], Devaraju Devaiah[1].

1. Department of Oral Medicine and Radiology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore, Karnataka, India.

***Corresponding author**: Shri Hari, Assistant Professor, Department of Oral Medicine and Radiology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore-562157, Karnataka, India. Email: drshrihariomr@gmail.com. Telephone: 91-9844386188.

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ABSTRACT

Oral cancer is the most serious and growing problem now-a-days in many parts of the world. When grouped with pharyngeal cancer, it is the sixth most common cancer globally. The major risk factors being cigarette smoking that includes tobacco, alcohol abuse and viral infections such as HPV, lead to this condition by inducing inflammatory changes in the oral cavity. Inflammation, a crucial, complex host defense against biologic, chemical, physical and endogenous irritants, is considered as the seventh hallmark of cancer involved in all the stages of carcinogenesis, from initiation, progression to invasion. Inflammatory cells such as macrophages, mast cells and lymphocytes produce several tumor inducing substances such as IL1β, IL-6, TNF-α, NF-κB, MMPs, COX-2 which induce remodeling of extracellular matrix components, inhibit apoptosis resulting in immortalization of tumor cells. Although there have been significant advances in cancer therapeutic modalities, available anti-tumor drugs display limited efficacy and sometimes carry a risk of severe adverse side effects. To address this issue, a number of epidemiological examinations in human populations and experimental animal studies provide evidence that certain dietary supplements such as Curcumin, Epigallocatechin gallate, Resveratrol, etc, suppress the progression of cancer through the inhibition of inflammatory cascade and also modulation of various signaling pathways implicated in the cancer initiation, promotion and progression. In this review article, we summarize the potential role of various anti-inflammatory dietary supplements and their possible chemopreventive mechanisms against oral cancer.

Key words- Inflammation, Oral cancer, Curcumin, Chemoprevention, Reactive oxygen species, Reactive nitrogen species, Omega-3 fatty acids, Epigallocatechingallate (EGCG), Resveratrol, Beta carotene, Lycopene, Vitamin D, cytokine, Antioxidant, Anthocyanins, Genstein, COX-2, NF-kB, STAT-3, TGF-Beta.

INTRODUCTION

Oral cancer is the most serious and growing problem now-a-days in many parts of the world. By 2015, it has been reported that nearly 1,658,370 newly diagnosed cancer cases and 589,430 deaths due to these cancers are projected to occur in the USA [1]. Epidemiological studies from India reported that around1 million new cancer cases are diagnosed and

around 600,000–700,000 Indians died due to cancer by the year 2012 [2]. Oral cancer together with pharyngeal cancer attained the 7th position in the incidence in the European Union, where 70,000 new cases are reported each year [3]. The 5 years survival outcome for oral cancer patients is still at 50%, and there has been a decrease in the survival years in tongue cancer patients [4]. Of late, cancer-related inflammation, a chief component of the tumour microenvironment, has been proposed to promote tumour progression and is considered as the seventh hallmark of tumour [5]. Rudolf Virchow, in 1863 showed the functional association and had hypothesized that cancer arises in sites of inflammation [6].

Inflammation is a protective response involving host cells, blood vessels, and proteins and other mediators that is intended to eliminate the initial cause of cell injury, as well as the necrotic cells and tissues resulting from the original insult, and to initiate the process of repair [7].

Inflammatory mediators, including macrophages, lymphocytes, and neutrophils, migrate to the site of injury and release a number of reactive oxygen species (ROS)and reactive nitrogen species (RNS) and thereby protect the body from injury. Reactive Oxygen Species (ROS) is an abundant, unstable, highly reactive, mutagenic factor frequently present in inflammatory microenvironment as a result of oxidative stress induced by phagocytic cells [8] and pro-inflammatory cytokines such as IL-6, IL-1 and TNF-α, genes encoded by activation of (NF-κB) [9].

The other pro-carcinogenic products of inflammation include chemokines, prostaglandins, enzymes such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), 5-lipoxygenase, matrix metalloproteinases (MMPs), growth factors such as endothelial growth factor (EGF),vascular endothelial growth factor (VEGF),transcriptional factors such as hypoxia-inducible factor-1α(HIF-1α), nuclear factor-κB (NF-κB), nuclear factor of activated T-cells, signal transducers and activators of transcription 3 (STAT3), activator protein-1 (AP-1) and various upstream kinases, including protein kinase C (PKC), IκB kinase (IKK), phosphoinositide-3 kinase/protein kinase B (PI3K)/AKT and mitogen-activated protein kinase (MAPK) [10].Chronic inflammatory conditions of oral cavity due to tobacco use such as leukoplakia, lichen planus, oral sub-mucous fibrosis, which are collectively termed as oral potentially malignant disorders and prolonged infections such as HPV lead to tissue injury because of these highly reactive oxidative species. These, in turn, impair DNA replication in proliferating epithelium, causing deleterious effects such as point mutations, deletions or rearrangements, and subsequently the genome gets altered [11] (Figure 1).

Apart from the tobacco products, there is also evidence that viruses take part in oral carcinogenesis. An association between HPV infection and chronic inflammation is biologically rational. HPV infects basal cells of the epithelium especially, and it gains access through micro abrasions. Moreover, replication of the virus is closely associated with basal cell proliferation, mucosal injury and associated breaks within the oral mucosa mediated by inflammatory cytokines may facilitate the acquisition as well as the persistence of oral HPV infection. In the presence of chronic inflammation, basal cell proliferation is also increased leading to higher viral load in the saliva, thereby leading to higher risk of transmission [12]. Moreover, there can be activation of NF-κB which interferes with p53 synthesis by attenuating p53-mediated genomic surveillance, thereby, promoting the tumour mechanism.

In HPV infected epithelium, HPV E6 and E7 proteins are expressed by the matured epithelial cells in the supra-basal layers. E6 prevents apoptosis and E7 activates the cellular DNA replication mechanism allowing matured epithelial cells to re-enter the S-phase of the cell cycle, which makes the cellular replication machinery available for viral DNA replication [13].

Studies indicated that human papilloma viruses HPV-16 and HPV-18 were the most common types detected in individuals with oral squamous cell carcinoma (OSCC). HPV-16 DNA, in particular, was detected predominantly in oro-pharyngeal SCCs located in the lingual and palatine tonsillar regions. HPV (+) OSCC are clinically found at young ages and generally in subjects without tobacco or chronic alcohol consumption. These histologically well differentiated and faster growing HPV (+) OSCC respond well to chemo-radiotherapy and have a clinical outcome in terms of overall survival better than HPV (-) OSCC patients [14].

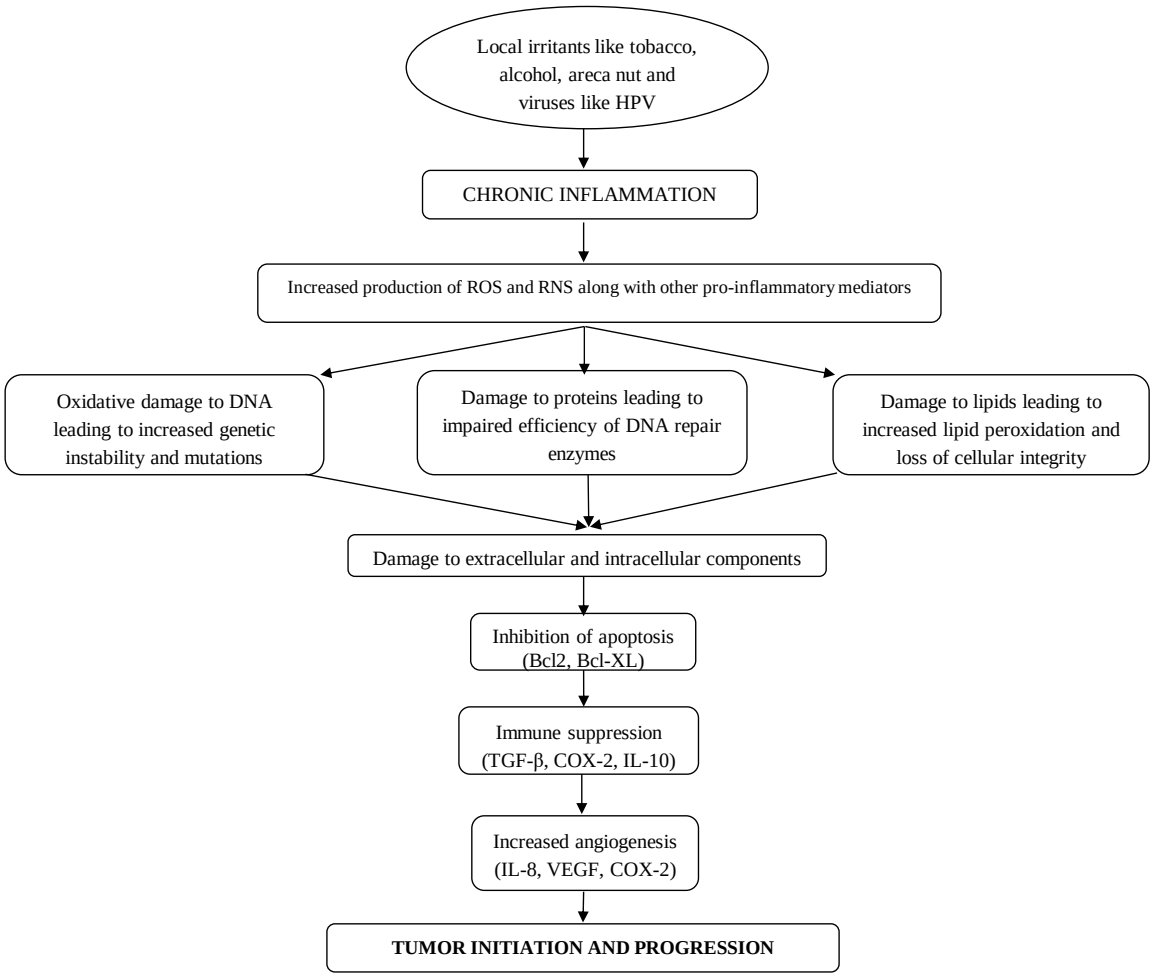

Fig1. Chronic inflammatory mediators in Cancer initiation and progression. Factors such as tobacco, alcohol and infections induce chronic inflammation and their mediators such as ROS and RNS leading to oxidative stress mediated tumor initiation, progression and promotion by the activation of cytokines and transcriptional factors (NF κB, STAT-3, HIF-1α, AP-1) which induce DNA damage, genetic mutation and breaks in the DNA strands, lipid peroxidation and loss of cellular integrity. This further leads to inhibition of apoptosis by Bcl 2, Bcl-XL, immunosuppression by TGF-β, COX 2, IL-10, Treg cells angiogenesis by IL-8, VEGF, COX-2, cell proliferation by cyclin D, PI3K/AKT, MAPK pathway and thereby, invasion and metastasis by MMPs, TGF-β and uPA.

Chemoprevention is the use of a natural or synthetic chemical agent with the aim of reverting, retarding, or suppressing the progression of carcinogenesis to invasive cancer, or preventing the development of premalignant lesions and conditions [15]. Chemoprevention can be provided either during the initiation phase of cancer thereby blocking the damage to DNA or it can also be given during the progression phase leading to the reversal or suppressing the progression of the malignant cells, where damage to DNA has already occurred [16].

Despite significant advances in the cancer therapeutic modalities, the anti-cancer drugs that are already available, usually display limited efficacy and often carry a risk of severe adverse effects. Therefore it is essential to identify and develop cancer chemopreventive agents without toxicity.

In this review article, we list out the various anti-inflammatory dietary supplements with their mechanism of action and their role as chemopreventive agents of oral cancer (Table 1). A literature search with the search terms “anti-

inflammatory dietary supplements", "chemopreventive agents", "oral premalignant lesions", "oral cancer" was performed via Google Scholar, PubMed, Medline and Scopus databases.

CURCUMIN

Source:

Curcumin, a yellow polyphenol, is the active component of turmeric, a common Indian spice, which can be extracted from the dried rhizome of the Curcuma longa plant.

Mechanism of action:

Curcumin shows its anti-inflammatory role by down-regulating nuclear factor kappa B (NF-κB), an inducible transcription factor which is responsible for accelerating transcription of COX–2 gene and other pro-inflammatory genes such as inducible nitric oxide synthase (iNOS). The COX–2 and iNOS are key inflammatory mediators and any change in the upregulation of these, is involved in pathogenesis of many inflammatory diseases followed by cancers [17].

Curcumin also suppresses the expression levels of NF-κB-regulated gene products which include tumor necrosis factor-α (TNF-α), interleukins (IL-1, IL-6, IL-8), 5-lipoxygenase (5-LOX), chemokine receptor type 4 (CXCR-4**)**, and C-reactive protein (CRP) [18].

It is also known to inhibit the Signal transducer and activator of transcription 3 (STAT3) and signaling pathways of NF-κB, which play a pivotal role in cancer development and progression [19].

Clinical Trials on Curcumin:

In the study of Rai B et al, 25 patients with oral Leukoplakia, showed a significant symptomatic relief and also a reduction in the clinical size of the lesion by treatment with curcumin [20].

An in-vitro study was done by Zang S et al on the anti-fibrotic effect of curcumin in TGF-β1 induced myofibroblasts from human oral mucosa, and it was found that curcumin inhibits proliferation of fibroblasts and myofibroblasts and thus demonstrates the anti-fibrotic effect [21]. The two randomized controlled trials conducted by Chainani Wu et al concluded that higher dosages of curcumin (up to 6,000 mg/day) helped a significant number of OLP patients control their symptoms [22]. Whereas smaller doses of curcumin (<2,000 mg/day) have failed to provide relief [23].

In a pilot study conducted by Singh V et al, curcumin was studied as a treatment option for the treatment of oral lichen planus and positive results were found both in terms of symptomatic relief and decrease in the size of the lesion [24].

Agarwal N et al used commercially available turmeric for the treatment of 30 patients diagnosed with oral sub-mucous fibrosis and found that there is a significant decrease in burning sensation but mouth opening was not significantly improved [25].

EPIGALLOCATECHIN GALLATE(EGCG)

Source:

Tea is obtained from the leaves of the plant Camellia sinensis. Green tea is prepared from the fresh tea leaf and is widely consumed for several health beneficial effects as it is anti-inflammatory, anti-arthritic, anti-bacterial, anti-angiogenic, anti-oxidative, anti-viral, neuroprotective, and cardio-protective in nature. The major flavonoids in green tea are catechins. There are four types of catechins mainly recognized in green tea: epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). EGCG is renowned as the major catechin of green tea for its maximum health beneficial effect [26].

Mechanism of action:

Epigallocatechin-3-gallate arrests cell cycle in the G0-G1 phase, suppresses cyclin D1 activity, increases p14ARFand/or p16 protein levels. Therefore, it stabilizes p53 and regulates apoptosis, inhibits angiogenesis by decreasing the phosphorylation of the vascular endothelial growth factor receptor (VEGFr), thereby blocking VEGF secretion by tumor cells [27]**.**

EGCG prevents invasion and metastasis of the oral cancer cell line(OC2), which is derived from buccal mucosa carcinoma, through inhibition of the matrix metalloproteinase MMP2 and MMP9. It also suppressed the ability of urokinase plasminogen activator (uPA) thereby, inducing the mobility of cancer cells in a synergistic manner with MMPs. This uPA is responsible for the degradation of the extracellular matrix [28]. EGCG reduces the transcriptional activity of NF-κB, COX-2 expression, and PGE-2 synthesis. EGCG protects from p53 mutation by activating wild-type p53. It promotes hypo-phosphorylation of pRb (tumor suppressor

Table 1: List of chemopreventive agents, their sources and mechanisms of action

Name of the chemopreventive agent	Source	Mechanism of Action	Stage of carcinogenesis affected
Curcumin	Dried rhizome of the Curcuma longa plant	Inhibition of NF-κB and STAT 3 [17-19]	Tumour promotion
EGCG	Fresh leaves of the plant Camellia sinensis [26]	Inhibition of the matrix metalloproteinase MMP 2 and MMP 9 [10, 27-29]	Tumour promotion
Resveratrol	Grape skin, red wine, berries, peanuts and many other plants	Suppression of de novo synthesis of iNOS and COX2 through inhibition of NF-κB pathway [28, 32,33]	Tumour initiation and promotion
Omega 3 fatty acids	Vegetable oils, dark leafed vegetables, cold water fishes, fish oils	Alteration in EGFR signaling; and upregulation of lipid peroxidation [38-40]	Tumour promotion
Beta carotene	Dark green, orange or yellowish fruits and vegetables	Potent antioxidant. increases the activity of tumor necrosis factor alpha (TNF α) [26, 43]	Tumour initiation
Lycopenes	Tomatoes and tomato-based products, apricot, berries, grapes, pink grapefruit, guava, papaya, peaches, and watermelon [50]	Modulates the insulin-like growth factor–binding protein 3 (IGFBP-3) system [52-54]	Tumour promotion
Anthocyanins	Berries, apples, purple cabbage and corn [60]	Inhibit nuclear translocation of NF- κB and degradation of IκBα as well as phosphorylating MAPKs [10, 61]	Tumour initiation and promotion
Genistein	Soy-derived compound	Inhibit TPA-induced c-fos expression, ERK activity and AP-1 activity; to inhibit cellular proliferation through inactivation of IGF1R/ PI3K/AKT pathway [65-68]	Tumour promotion
Vitamin D	Cheese, butter, fortified milk, healthy cereals and fatty fish	Inhibits prostaglandin synthesis by suppressing the COX2 and NF-κB signaling [74-76]	Tumour promotion

protein) and its activation, and inhibits MMPs such as MMP-9 [10]**.** Most of the studies showed that EGCG inhibits phosphorylation of EGFR tyrosine kinase in head and neck cancer. It also undermines epidermal growth factor receptor (EGFR) signaling by inducing ubiquitin mediated degradation of EGFR [29].

Clinical Trials on EGCG:

Chen et al. observed the human tongue SCC (SCC-7) culture supplemented with EGCG, and found that inhibition of cell invasion was probably through down-regulation of MMPs and u-PA (urokinase-

plasminogen activator-serine protease) expression [30].

To date, the clinical data were based on prevention studies, and no attempt has been made to treat OSSC with Green Tea Extracts (GTE)/EGCG. A phase II randomized, placebo-controlled trial was performed to examine the effects green tea extract supplementation on the outcome of high-risk oral premalignant lesions in 28 participants for 12 weeks. It was observed that high dose green tea extract (750 and 1000 mg/m2) had a significant clinical and histological outcome, though they were not associated with long-term oral cancer development. However, longer intervention trials might be able to demonstrate oral cancer prevention [26].

In rats, oral cancer was induced by 4-Nitroquinoline 1-oxide (4-NQO) and had been given green tea polyphenols extracts. The number and the volume of the tumors were smaller on an average compared with the rats that did not receive green tea [31].

RESVERATROL

Source:

Resveratrol is a component of grape skin, red wine, berries, peanuts and many other plants.

Mechanism of Action:

It is a bioactive polyphenol that activates antioxidant enzymes, prevents inflammation, directly binds to DNA and RNA, and stimulates DNA damage checkpoint kinases affecting genomics especially in malignant cells [32]. It is known to suppress de novo synthesis of iNOS and COX-2 through inhibition of NF-κB pathway [10]. Resveratrol also suppresses lipoxygenase-stimulated inflammatory responses and nitric oxide production. So, it counteracts ROS and/or RNS-associated DNA damage and inflammatory responses as a part of its potential cancer chemopreventive efficacy [33].

Clinical trials on Resveratrol:

Grape seed extract (GSE) and Resveratrol (Res) feeding for 8 weeks, moderately decreased the incidence, but remarkably prevented the multiplicity and severity of 4NQO-induced pre-neoplastic and neoplastic lesions, without any apparent toxicity. GSE and Res could effectively prevent 4NQO-induced oral tumorigenesis through modulating AMPK activation, thereby, inhibiting proliferation and inducing apoptosis and autophagy, as mechanisms of their efficacy [34].

It was evident that exposure of the OSCC cells to this polyphenol for 48 hours caused cell cycle arrest at the G2/M phase which was due to changes in the expression level of the proteins like cyclin A and cyclin B1 [35]. It can also down-regulate TGF-β production resulting in the inhibition of regulatory T (Treg) cells [CD4(+)CD25(+)FoxP3(+)] activity [36]**.** HS-1793, a synthetic resveratrol analog has been found recently in a study which modulated tumor-derived T lymphocytes, preponderantly suppressing the Treg cell population, and accordingly contributing to anti-neoplastic activity [37].

OMEGA-3 FATTY ACIDS

Source:

The term “omega-3 fatty acids” refers to a group of polyunsaturated fatty acids which contain a double carbon–carbon bond at the third carbon atom from the methyl end of the carbon chain. These are substrates to several enzymes obtained from the diet and are regarded as the key components of cell membrane phospholipids. The well-known omega-3 FAs which play a key role in human physiology are Alpha-linolenic acid (ALA) that is obtained from plant sources, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) both of which are obtained from the marine source. The human body has a limited ability to form EPA and DHA from ALA. This ability may even become lesser with age. Therefore, omega-3 FAs must be primarily obtained from dietary sources. These are known to reverse cancer cachexia, improve muscle mass and lean body mass, and promote weight maintenance.

Mechanism of action:

The omega-3 FAs play a crucial role in altering the membrane-associated signal transduction, such as the modification of lipid composition of membrane rafts: i.e., alteration in EGFR signaling; as well as in upregulating lipid peroxidation which causes irreversible cell damage; thus enhancing drug sensitivity and inducing apoptosis, or modulating gene expression involved in multiple signaling pathways including NF-κB and mitogen-activated protein kinases (MAPKs) [38].

A study has shown that Avocado extract, a rich source of oleic acid, upregulates tumour suppressor

genes such as p21 and p27, which are well-known to block progression through the cell cycle by inducing G2 /M cell-cycle arrest [39]. Another study has shown that aliphatic acetogenins, (2S, 4S)-2,4-dihydroxyheptadec-16-enyl acetate and (2S, 4S)-2,4-dihydroxyheptadec-16-ynyl acetate, the two components isolated from avocado, inhibit the EGRK/RAF/MEK/ERK1/2 oncogenic pathway [40].

Clinical Trials on Omega 3 fatty acids:

Gama et al conducted a study to evaluate the effects of PUFAS omega-3 or fish oil (FO) as a potential chemopreventive agent to prevent the carcinogenesis of the upper aero-digestive tract initiated by 4-NQO in Swiss mice. Chemoprevention with FO did not show any benefit in preventing the carcinogenesis process initiated by 4-NQO for oral cancer. The suggestive pro-tumour action of FO when given after tumour post-initiation seems to demonstrate that it can potentiate the action of 4-NQO in the oesophagus carcinogenesis of the Swiss mice [41].

In another study, it was found that both FA and D003 extract inhibit DMBA-induced tumorigenesis. However, neither of them markedly changed the stage or frequency of existing dysplasia induced by DMBA in the HCP. This suggests that the cancer preventive effect of the avocado extract is through the inhibition of tumor progression, rather than reversing the existing premalignant changes induced by the carcinogen [42].

BETA-CAROTENE

Source:

Beta-carotene is a vitamin A precursor commonly found in dark green, orange or yellowish fruits and vegetables, such as spinach, sweet potato, carrots, papaya, mango, and oranges.

Mechanism of action:

The main actions of beta-carotene include its potent antioxidant activity and free radical scavenging. It increases the activity of tumor necrosis factor alpha (TNF-α) and stimulates T-helper, NK cells, and cells with IL-2 receptors, thereby, displaying its immune-modulatory mechanism. In various oral premalignant lesions and conditions, serum beta-carotene levels are shown to be decreased and so its supplementation has led to the suppression of these lesions [26]**.** The expected levels of serum beta-carotene have been seen less in men who smoke cigarettes and consume alcohol. The level of beta carotene varies inversely with the risk of oral cancer [43].

Clinical Trials on beta-carotene:

Twenty-four patients with oral leukoplakia were given beta-carotene employed at an oral dose of 30 mg/day for six months. Only 2 patients (8.3%) showed a complete clinical response and 15 patients (62.5%) had shown a partial clinical response [44].

In another study, 23 patients with oral leukoplakia were treated with beta-carotene, in oral doses of 90 mg/day, for three cycles for a period of 3 months each. Among 18 patients who had completed the study, 6 patients (33.3%) showed a complete clinical response. There were no significant clinical signs of toxicity in any of the patients [45].

Results from a study done by Sankaranarayanan et al demonstrated that one-third of the patients (15 out of 46) who used 360 mg beta-carotene per week for duration of 12 months presented a complete resolution of oral leukoplakia. During the follow-up sessions one year after the treatment, 8 out of 15 patients who had a complete response presented recurrence. Moreover, 12 months after stopping the supplements, 2 out of 15 patients (5%) who had made use of beta-carotene developed malignant neoplasia adjacent to the site of oral leukoplakia. Side effects were observed in 5 patients, among which 2 patients developed muscular pain and the remaining 3 patients had headaches [46].

According to Liede et al, a diet supplemented with beta-carotene could prevent changes in the oral mucosa, especially in smoker patients, who showed low serum levels of beta-carotene and vitamin C when compared to nonsmokers. In addition, it had also been shown that beta-carotene had a better therapeutic clinical response in the prevention of oral leukoplakia lesions in smoker patients than in the non-smoker ones [47].

Garewal et al evaluated 50 patients with oral leukoplakia, treated with beta-carotene at a dose of 60 mg/day, for six months. It was found that only 2 patients (4%) demonstrated a complete clinical response and relapses were found in 4 patients. A second biopsy was performed after 6 months of therapy in 23 patients. No change was observed in the degree of dysplasia in 14 patients, with an improvement of at least 1 grade in 9 patients (39%). In

the revised studies, a range of 4%-54% of the patients was seen with clinical resolution, with dosages ranging from 20 to 90 mg/day of beta-carotene during a time period of 3 to 12 months [48].

A multi-centre, randomized, double-blind controlled trial (RCT) was performed to evaluate the use of low-dose beta-carotene combined with vitamin C supplements for the treatment of leukoplakia as well as to prevent its malignant transformation. A total of 46 participants with oral leukoplakia were allotted randomly either to an experimental arm (10 mg/day of beta-carotene and 500 mg/day of vitamin C) or placebo arm (50 mg/day of vitamin C). Results showed that beta-carotene (10 mg/day) and vitamin C were neither efficacious for clinical remission nor for prevention of the development of cancer. Data from this RCT could not support the hypothesis that chemoprevention with beta-carotene and vitamin C is effective for oral leukoplakia [49].

LYCOPENE

Source:

Lycopene is a fat-soluble carotenoid which was discovered by Ernest et al in 1959. It is a natural constituent of red fruits and vegetables and of some algae and fungi. Tomatoes and tomato-based products are the major sources of lycopene in the human diet. Other sources of lycopene are apricot, berries, grapes, pink grapefruit, guava, papaya, peaches, and watermelon [50].

Mechanism of action:

Recent studies have shown that isomerization of lycopene to the cis-form occurs due to heat processing of tomatoes and tomato products which, in turn, increases its bioavailability [51]. The chemopreventive activities of lycopene may involve changes in pathways resulting in cell growth or cell death. It modulates the insulin-like growth factor–binding protein 3 (IGFBP-3) system, and also redox signals thereby preventing oxidative damage to DNA and potential mutations that can be associated with tumor initiation and progression [52]. It plays a major role in inhibiting 5-lipoxygenase (5-LOX), interleukin-6 (IL-6) and androgen. It causes activation of gap-junctional gene connexin 43 (Cx43) and enhances gap-junctional intercellular communication (GJC) [53]. Lycopenes stimulate the production of cellular enzymes such as glutathione-S-transferase, superoxide dismutase, and quinone reductase by upregulating the antioxidant response element (ARE) and thereby, protect the cells from electrophilic molecules and reactive oxygen species [54]. It was reported that the daily intake of lycopene showed a marked improvement of oral leukoplakia lesions in a group of patients [55].

Clinical Trials on Lycopene:

Cheng et al. analysed the chemopreventive effect of lycopene and other carotenoids in betel quid extract–induced hamster oral cancer model. They reported that no carcinoma was found in the lycopene or mixed carotenoid groups, whereas the apparent ones were observed in the control group. The expressions of PCNA by the lycopene were less in the dysplastic lesions than that of the control group [56].

The chemopreventive efficacy of lycopene with regard to oral carcinogenesis was studied using 4 -nitroquinoline-1-oxide (4-NQO)–induced squamous cell carcinoma of the tongue in rats by El-Rouby. Lycopene treatment at a dose of 2.5 mg/kg body weight by intra-gastric intubation once a day significantly reduced the incidence of 4-NQO induced tongue carcinogenesis. There was a decreased percentage of PCNA-positive nuclei with lycopene treatment. In addition, an increased immuno-expression of E-cadherin and b-catenin was recorded in the lycopene-treated group in comparison to the carcinogen group [57].

In a prospective, double blinded, placebo-controlled randomized controlled study conducted by Saawarn N et al, a significant reduction in burning sensation was noted with the lycopene supplements in the doses of 8mg/day for 8 consecutive weeks. All the patients in this study were reported to have more than 50% benefit and 73.3% patients showed 70-100% benefit [58].

Lycopene was found to be significantly efficacious in the amelioration of signs and symptoms in a study conducted in 92 patients out of which 46 patients were given 8mg Lycored TM per day in two divided doses of 4mg each for three months. Mouth opening was improved in 69.65% of patients [59].

ANTHOCYANINS

Source:

Anthocyanins confer the bright red, blue and purple colors to fruits and vegetables such as berries, apples, purple cabbage and corn [60].

Mechanism of action:

A diet rich in polyphenolic anthocyanins (ACs) has been reported as a chemo-protective agent in-vivo models by regulating inflammatory cytokines. These significantly inhibit and also down-regulate the excessive expression of induced pro-inflammatory mediators which include prostaglandin E2, nitric oxide, and also pro-inflammatory cytokines such as IL-1β and TNF-α, without any significant cytotoxicity [61]. Moreover, anthocyanins are known to inhibit nuclear translocation of NF- κB and degradation of IκBα as well as phosphorylating MAPKs [10].

Clinical studies on Anthocyanins:

Histological examination of hamster cheek pouches (DMBA-induced) revealed a significant reduction in mild and severe dysplasia following 12 weeks of treatment with 5% and 10% lypophilized strawberries (LS). Molecular analysis revealed that genes related to tumour development were modulated by LS [62].

Hamster cheek pouches (HCPs) were treated with a carcinogen for 6 weeks to initiate a high at-risk mucosa (HARM) microenvironment. Subsequently, the hamster cheek pouches were topically administered a black raspberry (BRB) suspension in short-term or long-term studies. It was observed after 12 weeks that SCC multiplicity (-41.3%), tumour incidence (-37.1%), and proliferation rate (-6.9%) were reduced in HCPs receiving BRBs. The application of topical BRBs correlated with an increased expression in Rb1 in developing oral lesions [63].

In another study, patients with biopsy-confirmed oral squamous cell carcinomas (OSCC) were administered oral troches containing freeze-dried BRB powder and the transcriptional biomarkers were evaluated. Following BRB troche administration, the expression of pro-survival genes (EGFR, AURKA, BIRC5) and pro-inflammatory genes (PTGS2, NFKB1) were reduced significantly. No BRB-associated grade 3-4 toxicities or adverse events were observed, and 30 patients (79.2%) had successfully completed the study with high levels of compliance (97.2%) [64].

GENISTEIN

Source:

Genistein is a soy-derived compound isoflavone and is known as a phytoestrogen that has a similar structure of estrogen.

Mechanism of action:

This phytochemical has multiple biological functions including cancer chemopreventive function. Genistein is known to inhibit TPA-induced c-fos expression, ERK activity and AP-1 activity in human breast cancer cells [65]. In the transgenic mouse model for prostate cancer, supplementation of genistein significantly down-regulated the activation of EGFR and IGF-1R and their downstream signaling [66]**.** Genistein also down-regulates IκB phosphorylation and thereby inhibits nuclear translocation of NF-κB resulting in decreased DNA binding and NF-κB activation in prostate cancer cells [67]. It is known to inhibit cellular proliferation through inactivation of IGF1R/ PI3K/AKT pathway [68]. Especially, genistein-treated HN4 SCC (Head and Neck Squamous cell carcinoma) cells underwent morphological changes suggesting growth arrest, cell differentiation, and eventual cell death. After a flow cytometric analysis it was confirmed that genistein induced S/G2M phase cycle arrest. Genistein induced apoptosis in HN4SCC cells. No toxicity could be observed on genistein-treated normal keratinocytes [69].

Clinical Trials on Genistein:

In an animal study, genistein (0.5 mg/kg) was injected into the tumor (HSC-3)-bearing mice to compare the tumor growth rate and metastasis to lymph node or lung and the micro vessel density (CD31) was subsequently examined by immunohistochemistry. It was noticed that the genistein-treated group showed a down-regulation in VEGF mRNA expression, but not in bFGF and MMP-2 mRNA expression. In the genistein-treated mice, a significantly lower CD31 immuno-reactivity was found. However, no significant differences were found in the tumor growth and metastatic behavior in the experimental group and the control group. These results demonstrate the possible use of genistein as a potential chemopreventive agent in oral squamous cell carcinoma [70].

In another study, the left cheek pouches of male Syrian golden hamsters were topically applied with DMBA solution (0.5% in mineral oil), three times a week for 6 weeks. Two days after the last treatment

of DMBA, genistein suspended in distilled water (10 mg/kg body wt/day) or the same volume of distilled water was administered to the animals by gavage daily for 12 weeks. The treatment with genistein decreased the visible oral tumor incidence from 53.6% (15/28) of the positive control to 40.7% (11/27), but the difference was not statistically significant. In addition, there was no statistically significant difference observed between the control group and the genistein-treated group in the average number of tumors or tumor-bearing hamsters, the average tumor volume, or latency. The vascular density in OSCC of the study group and that of the control group was similar. The results showed no inhibitory effect of genistein on the chemically-induced post-initiation stage of oral carcinogenesis. Conversely, genistein appeared to promote oral sub-mucosal stromal tumorigenesis in combination with DMBA. So caution should be warranted for people with a predisposition to oral cancer [71].

Another study demonstrated that the treatment of cells with the dual anti-EGFR agents such as genistein and cetuximab decreased the expressions of p-EGFR, and p-Akt in HSC3 cell line with no significant difference in down-regulation between cetuximab alone and in concert with genistein in KB cells. Both HSC3 and KB cells showed a dose-dependent reduction in cell proliferation significantly with single agent treatment and in combination [72].

VITAMIN D

Source:

Vitamin D3 is the precursor to the most potent steroid hormone calcitriol (1, 25 dihydroxyvitamin D3 (1,25(OH)2D3)) that regulates the expression of many genes which are present in all body tissues. In the skin, Vitamin D can be synthesized in adequate amounts by using the energy of ultraviolet (UV) radiation which comes from the sunlight. Therefore, this is not considered as an essential element. Most of the foods have a little amount of vitamin D unless they are fortified. This implies that humans are dependent on sunlight to maintain adequate levels of vitamin D. The foods that are rich in vitamin D are cheese, butter, fortified milk, healthy cereals and fatty fish [73].

Mechanism of action:

Calcitriol inhibits prostaglandin synthesis by suppressing the cyclooxygenase 2 (COX2) and prostaglandin signaling by increasing the expression of 15-hydroxyprostaglandin dehydrogenase (a catabolic enzyme) and decreasing the expression of prostaglandin receptors [74]. It plays a major role in the inhibition of NF-κB signaling. It regulates the expression of the components of the plasminogen activator system and suppresses MMP-9 activity and increases the expression of TIMP-1 (tissue inhibitor of metalloproteinase 1) [75]. It also down-regulates the vascular endothelial growth factor (VEGF) via transcriptional repression of HIF-1α (hypoxia inducible factor 1 alpha) and interleukin-8 (IL-8) in an NF-κB-dependent manner [76].

Some calcitriol analogs might have been more effective in restraining tumor growth than calcitriol at equivalent doses, while exerting reduced calcaemic effects in animal models. Combinations of calcitriol with other anticancer drugs show more efficacy than the individual drugs in inhibiting the tumor growth. For instance when calcitriol is combined with CYP24A1 inhibitors, anti-cancer actions are increased along with the hyper-calcaemic effects [73].

Clinical Trials on vitamin D:

A clinical trial comprised of 16 patients with newly diagnosed HNSCC being untreated and 16 patients being treated with 1,25$(OH)_2$D3 during a 3-week interval between cancer diagnosis and surgical treatment. The HNSCC tissues of patients who received treatment with 1, 25$(OH)_2$D3 had had increased levels of CD4(+) cells and, more prominently, CD8(+) T cells. There was also a significant increase in cells expressing the lymphoid activation marker CD69. The results of this study demonstrate that the patients treated with 1, 25$(OH)_2$D3 had a lengthier time to tumor recurrence compared with patients who were not treated before the surgery [77].

Katelyn et al investigated the efficacy of short-term 1, 25$(OH)_2$D3 treatment in combination with the EGFR inhibitor called erlotinib against HNSCC. The results of the studies conducted in PDX-bearing mice showed a significant inhibition of tumour growth with combination treatment. This combination treatment was well tolerated without any significant change in the body weight. The pathologic assessment revealed a significant decrease in the severity of dysplasia with

combination treatment compared to either monotherapy [78].

CONCLUSION

Oral cancer, one of the most usual causes of mortality all over the world, displays a five-year survival rate of only 50%. More often, oral cancer patients get treated primarily by surgery with adjuvant therapies such as radiotherapy and/ or chemotherapy due to which there is an immediate chance of many body cells getting affected along with cancer cells and the patients end up with severe adverse effects. Moreover, there can also be post treatment morbidity secondary to recurrences. In this context, the dietary phytochemicals which have been discussed above not only have a safe toxicological profile and are inexpensive but can also be administered at any stage of carcinogenesis as potent chemopreventive agents of cancer with better patient acceptance. So, the future perspective should, therefore, be focused on the dosage of administration of these anti-inflammatory dietary supplements as an adjuvant to the conventional therapies to have a better synergistic effect in combating cancer.

ABBREVIATIONS:

1, 25 $(OH)_2D3$ – 1,25 dihydroxy vitamin D3
AC –Anthocyanins
ALA – Alpha-linolenic acid
AMPK - adenosine monophosphate-activated protein kinase
AP-1 – Activator Protein – 1
ARE – Antioxidant Response Element
AURKA - Aurora kinase A
BIRC5 - baculoviral inhibitor of apoptosis repeat-containing 5
BRB – Black Raspberry
CRP – C-reactive protein
COX-2 – Cyclooxygenase 2
Cx43 – connexin 43
CXCR-4 – Chemokine receptor type 4
DHA – Docosahexanoic acid
DMBA - Dimethylbenz[a]anthracene
DNA – Deoxyribonucleic acid
EC – Epicatechin
ECG – Epicatechin-3-gallate
EGC – Epigallocatechin
EGCG – Epigallocatechin-3-gallate
EGF – Endothelial Growth Factor
EGFR - Endothelial Growth Factor Receptor
EPA – Eicosapentanoic acid
ERK - extracellular signal–regulated kinase
FA – Fatty acid
FGF – Fibroblast Growth Factor
FO – Fish oil
GJC – Gap junctional intercellular communication
GSE – Grape seed extract
GTE – Green tea extract
HARM – High at-risk mucosa
HCP – Hamster cheek pouch
HIF – Hypoxia inducible factor
HNSCC – Head and neck squamous cell carcinoma
HPV – Human papilloma virus
IκK – IκB kinase
IGFR - insulin like growth factor receptor
IGFBP-3 – insulin like growth factor-binding protein - 3
IL – interleukin
iNOS – inducible nitric oxide synthase
LOX – lipoxygenase
LS – Lypophilized Strawberries
MAPK – mitogen activated protein kinase
MMP – matrix metalloproteinases
NF κB – nuclear factor κB
NQO – nitroquinoline oxide
OL – oral leukoplakia
OLP – oral lichen planus
OSMF – oral sub mucous fibrosis
OSCC – Oral squamous cell carcinoma
P14 ARF – p14 alternate reading frame
PCNA – proliferating cell nuclear antigen
PGE2 – prostaglandin E2
PI3K – phosphoinositide-3 kinase
PKC – protein kinase C
PTGS - Prostaglandin-endoperoxide synthase
RNA – ribonucleic acid
RNS – reactive nitrogen species
ROS – reactive oxygen species
STAT 3 – signal transducers and activators of transcription 3
TGF – transforming growth factor
TIMP – tissue inhibitor of metalloproteinase
TNF – tumour necrosis factor
uPA – urokinase plasminogen activator
VEGFr – vascular endothelial growth factor receptor

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