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APIGENIN: A POTENT CHEMOPREVENTIVE AGENT AGAINST ORAL CARCINOGENESIS- A REVIEW

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ABSTRACT

Oral cancer is one of the most leading and challenging health problems in the entire world today. It is an important social health issue. The major risk factors of oral cancer are tobacco smoking and chewing, alcohol consumption and betel quid chewing. The estimated annual incidence of oral cancer cases is around 5,00,000 and approximately 128,000 deaths are reported to occur every year worldwide. The chemopreventive agents are to modulate and alter the molecular pathways of different stages of cancer cell progression. Apigenin, a plant derived flavone, possesses diverse pharmacological properties including antioxidant, antimutagenic and anticarcinogenic effects. This review examines the better understanding of existing research on the apigenin potent and successful development of cancer chemoprevention strategies.

Key words: chemoprevention, oral cancer, apigenin, carcinogenesis, apoptosis

INTRODUCTION

Oral cancer, the fifth most common malignancy worldwide, imposes a significant burden on public health in many parts of the world. Oral cancer is the cancer of the mouth and commonly found in lip, tongue, palate, gingiva and floor of the mouth. Oral cancer usually appears as a growth or sores in the mouth that does not go away (1). Each year, more than 5,00,000 new cases of oral cancer are diagnosed worldwide and the majority of the cases are diagnosed in the advanced stages. The World Health Organization (WHO) pointed out that oral cancer causes highest mortality ratios amongst all malignancies.

Oral cancer can be life threatening if not diagnosed and treated early (2). The estimated annual incidence of oral cancer cases is around 5,00,000 and approximately 128,000 deaths are reported to occur every year worldwide. Every year worldwide and around 50-60%

occur in South and South-East Asia (3). It has been estimated that approximately 40,000 new cases of oral cancer are diagnosed every year in USA. High rates of oral cancer incidence were also reported in Sri Lanka, Pakistan, Bangladesh, France and Brazil (4-6). India has recorded the highest incidence of oral cancer in the world and India oral cancer accounts for 40-50% of all cancers. In India's, approximately 80,000 new cases of oral cancer are diagnosed each year (7).

Risk Factors of Oral Cancer

The aetiology of oral cancer is almost certainly multifactorial and involves many alterations in the host immunity and metabolism, angiogenesis and exposure to chronic inflammation in a genetically susceptible individual. The carcinogenic changes may be influenced by oncogenes, viruses, irradiation, drugs (tobacco and alcohol), hormones, nutrients or physical irritants (8).

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Tobacco and alcohol consumption, two of the most important risk factors of oral cancer, are associated not only with the development of oral cancer, but also with the course of the disease and a poor prognosis (9).

Chemoprevention and Oral Cancer

Cancer chemoprevention an innovative area of experimental oncology, is the use of natural, synthetic, or biological, chemical agents to reverse, suppress, or prevent malignant progression to invasive cancer. (10). Advances in understanding molecular carcinogenesis in parallel have made possible for the identification of chemopreventive agents to hit key molecular targets of carcinogenesis. The most preferred chemopreventive approach lies in the intervention at the early stage of carcinogenesis to eliminate premalignant cells before they become malignant or protect normal cells from undergoing neoplastic transformation (11). Extensive studies reported the chemopreventive efficacy of natural or synthetic entities against chemical induced oral carcinogenesis (12). The list of chemopreventive agents and their possible mechanism has been reported against oral carcinogenesis (Table 1).

Table 1. Reported Chemopreventive Agents in Experimental Oral Carcinogenesis

Medicinal plants/ Bioactive constituents	Possible mechanism reported for chemopreventive potential	References
Red mold dioscorea	Reduced oxidative damage caused by DMBA and induced apoptosis against oral squamous cell carcinoma	Hsu <i>et al.</i> , 2011 (13)
Mangrove black tea extract from <i>Cerriops decandra</i>	Significantly reduced the formation of tumors in experimental oral carcinogenesis.	Boopathy <i>et al.</i> , 2011(14)

Resveratrol complexed with 2-hydroxy-propyl- β -cyclodextrin	Exhibited anti-cell proliferative effect.	Berta <i>et al.</i> , 2010 (15)
Carnosic acid	Exerted potent anti-lipid peroxidative and antioxidant effect in DMBA-induced oral carcinogenesis	Manoharan <i>et al.</i> , 2010 (12)
3-(4'-(4''-fluorophenyl)-6'-phenylpyrimidin-2'-yl)-2-phenylthiazolidin-4-one	By modulation of lipid peroxidation and enhancing the levels of GSH, GPx, and GST	Thanusu <i>et al.</i> , 2011(16)
Andrographolide	By inhibition of aberrant NF- κ B activation during experimental carcinogenesis	Wang <i>et al.</i> , 2011(17)
Geraniol	Antioxidant function and modulating effect on detoxification cascade.	Vinothkumar and Manoharan., 2011 (18)
(6)-paradol	Showed potent anti-lipid peroxidative and antioxidant potentials as well as a modulated on phase II detoxification enzyme in favour of excretion of carcinogenic metabolite in DMBA-induced hamster buccal pouch carcinogenesis.	Suresh <i>et al.</i> , 2010 (19)

Neem limonoids azadirachtin and nimbolide	Showed potent antiproliferative and apoptosis inducing effect during oral carcinogenesis.	Harish Kumar <i>et al.</i> , 2010 (20)
Neem limonoids azadirachtin and nimbolide	Showed potent antioxidant property during DMBA-induced oral carcinogenesis.	Priyadarsini <i>et al.</i> , 2009 (21)
Dietary turmeric	Augmented apoptosis of the initiated cells and decreased cell proliferation in DMBA-treated animals.	Garg <i>et al.</i> , 2008(22)

Apigenin

Apigenin (4',5,7-trihydroxyflavone), is a naturally occurring plant flavonoid (Fig.1), is found in, onions, basil, parsley, orange, tea, oregano, tarragon, cilantro, tea, chamomile and wheat sprouts (23). Apigenin possesses diverse pharmacological properties including antioxidant, antimutagenic and anticarcinogenic effects (24). Apigenin, a non-mutagenic chemopreventive agent, mediated chemopreventive activity by modulating signal transduction pathways [mitogen activated protein kinase (MAPK) cascade] in keratinocytes and colon carcinoma cell lines (25). Wei *et al.*, (26) suggested that topical application of apigenin inhibited skin carcinogenesis initiated by DMBA and promoted by 12-O-tetradecanoylphorbol-13-acetate in SENCAR mice. Caltagirone *et al.*, (27) investigated that apigenin delayed the melanoma growth and inhibited the invasive and metastatic potential of B16-BL6 melanoma cells in syngenic mice. Way *et al.*, (28) reported that apigenin exhibited potent growth-inhibitory activity and apoptosis in HER2/neu-overexpressing breast cancer cells. Gupta *et al.*, (29) reported that apigenin inhibited the growth of androgen-responsive human prostate carcinoma LNCaP cells by inducing cell cycle arrest and apoptosis. Jeyabal *et al.*, (30) reported the antioxidant and antigenotoxic

potential of apigenin in N-nitrosodiethylamine induced hepatocellular carcinogenesis in Wistar albino rats. Wu *et al.*, (31) reported that apigenin showed inhibition on the growth and clone formation of gastric carcinoma SGC-7901 cells by inducing apoptosis. Wang *et al.*, (32) suggested that the induction of apoptosis by apigenin may be attributed to its cancer chemopreventive activity. Fang *et al.*, (33) reported that apigenin significantly inhibited the expression of VEGF in human ovarian cancer cells. Liu *et al.*, (34) reported that apigenin inhibited A549 lung cancer cell proliferation and angiogenesis in a dose-dependent manner. Zheng *et al.*, (35) reported that apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells and apigenin thus has a strong potential for development as an agent for preventing cervical cancer.

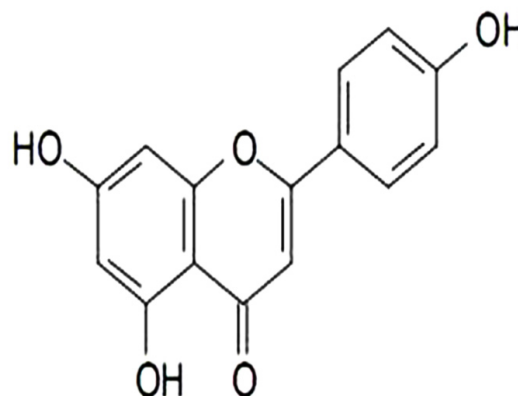


Fig.1. Molecular structure of Apigenin

DMBA-induced Oral Carcinogenesis

7,12-Dimethylbenz(a)anthracene (DMBA), one of the most potent organ and site specific procarcinogen, is emitted during the incomplete combustion of carbon-containing compounds, and predominantly found in tobacco smoke (36). DMBA is metabolized in the liver to its active metabolite, dihydrodiol epoxides, which interact with DNA and contributing to malignant transformation. DMBA, the most widely used chemical carcinogen to induce oral carcinogenesis, mediates carcinogenesis through free radicals mediated oxidative damage to cells and tissues. DMBA is commonly employed to develop oral cancer in the buccal pouch of golden Syrian hamsters since the DMBA-induced precancerous and cancerous lesions as well as biochemical and molecular marker expressions have marked similarities with human oral cancer (12).

Oxidative Stress and Oral Carcinogenesis

Reactive oxygen species (ROS) that are harmful to human cells and tissues include superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (OH^{\bullet}) and hydrogen peroxide (H_2O_2), which are generated during normal oxidative metabolic processes as well as during pathological conditions (37). ROS can cause damage to proteins, lipids, carbohydrates and nucleic acids, if they are abnormally generated in the cells due to pathological phenomenon (38). ROS attacks membrane lipids, particularly PUFA and initiates a chain reaction known as lipid peroxidation. ROS induced lipid peroxidation has been implicated in the pathogenesis of several disorders including cancer. Over production of reactive oxygen species in the system, directly or indirectly, play a crucial role in pathogenesis of several cancers including oral cancer (39). ROS mediates the carcinogenic process by interfering with cell proliferation probably by modulating the activities of cell cycle proteins. ROS has been reported to have clastogenic and genotoxic potential (40). Under normal conditions, the production of ROS is tightly controlled with an elaborate and sophisticated enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] and non-enzymatic [vitamin E, reduced glutathione (GSH)] antioxidant defense mechanism (41). Over production of ROS or insufficient antioxidant potential in the cells or tissues thus leads to a condition known as oxidative stress, which has been implicated in the initiation and promotion steps of multi-stage carcinogenesis. Altered status of lipid peroxidation and antioxidants were well documented in both human and experimental oral carcinogenesis (42,43).

Experimental Protocol

The local institutional animal ethics committee (Register number 160/1999/CPCSEA), Annamalai University, Annamalainagar, India, approved the experimental design. The animals were maintained as per the principles and guidelines of the ethical committee for animal care of Annamalai University in accordance with Indian National Law on animal care and use.

A total of 40 hamsters were randomized into four groups of ten animals in each. Group I animals served as control and were painted with liquid paraffin alone, three times a week for 14 weeks on their left buccal pouches. Groups II and III animals were painted with

0.5% DMBA in liquid paraffin, three times a week for 14 weeks on their left buccal pouches. Group III animals were orally given apigenin at a dose of 2.5 mg/kg body weight/day, starting 1 week before exposure to the carcinogen and continued on days alternate to DMBA painting, until the end of the experiment. Group IV animals received oral administration of apigenin alone throughout the experimental period. The experiment was terminated at the end of 16 weeks and all animals were sacrificed by cervical dislocation

Apigenin and molecular markers in oral carcinogenesis

Protective effect of apigenin on glycoconjugates in oral carcinogenesis

Glycoconjugates, consist of glycoproteins, glycopeptides, peptidoglycans, glycolipids, and lipopolysaccharides; they are carbohydrates covalently linked with other chemical species. They have played a key role in biological recognition processes such as cell-cell recognition, cell growth and differentiation and cell-matrix interaction (44). Tumor associated carbohydrate changes have been utilized as diagnostic criteria for human cancers. Glycoproteins are clinically important, to be used as valuable index in establishing diagnostic and identifying patients at high risk for recurrence and evaluating therapeutic responses of oral cancer (45). Glycoconjugates have more expression of fucosylated oligosaccharides that have been noticed and reported in cancerous and inflammatory conditions. It has been reported that altered fucose metabolism may be indicative of tumor burden. In cancer conditions the fucose levels are very high; they are also reported in breast, leukemia and oral cancer (45,46).

The protective effect of apigenin may be due to its inhibitory effect on enzymes involved in glycosylation, sialylation and fucosylation process. To confirm the protective effect of apigenin on DMBA induced cell surface glycoconjugate abnormalities, studies on the activities of enzymes involved in glycosylation (glycosyl transferase) sialylation (sialyl transferase) and fucosylation (fucosyl transferase) Oral administration of apigenin at a dose of 2.5mg/kg bw significantly restored the status of glycoconjugates in DMBA treated hamsters. A result suggests that apigenin protected cell surface glycoconjugates abnormalities during DMBA-induced oral carcinogenesis (47).

Apigenin protects and increases antioxidant status against oral carcinogenesis

Epidemiological and experimental studies demonstrated that high consumption of natural products that are rich in antioxidants reduce the risk of many cancers (48,49). Procarcinogen to ultimate carcinogens was converted by the metabolic biotransformation of Phase I enzymes. Altered the activities of phase I and phase II detoxification agents in the buccal mucosa indicates the formation of active and toxic metabolite of DMBA, dihydrodiol epoxides (12). Decrease in antioxidant status (reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase and vitamin C), it leads to increase in lipid peroxidation that has been well documented in the hamsters bearing oral tumors and plasma of oral cancer patients (50,51).

Apigenin significantly ameliorate the status of lipid peroxidation by-products and antioxidants in DMBA treated hamsters. Apigenin attenuated oxidative stress by enhancing the intracellular levels of glutathione. Nagaraja *et al.*, (52) reported that apigenin scavenged free radicals induced reactive oxygen species that is produced during chronic cyclosporine treatment. Antioxidant potential of apigenin could be attributed to the H⁺ donating potential of the phenolic hydroxyl groups (Fig.2). The results conclude that apigenin improved the antioxidant defense mechanism by scavenging excessively generated reactive oxygen species during DMBA induced oral carcinogenesis. Oral administration of apigenin at a dose of 2.5mg/kg bw to hamsters treated with DMBA not only completely prevented the formation of oral tumors, it also brought back the status of lipid peroxidation, antioxidants and phase I and phase II detoxification agents to near normal range. It concludes that apigenin might have inhibited oral carcinogenesis by improving the status of antioxidant defense mechanism and modulated the activities of phase I and phase II detoxification cascade toward increased excretion of active metabolite of DMBA, during DMBA-induced hamster buccal pouch carcinogenesis (53).

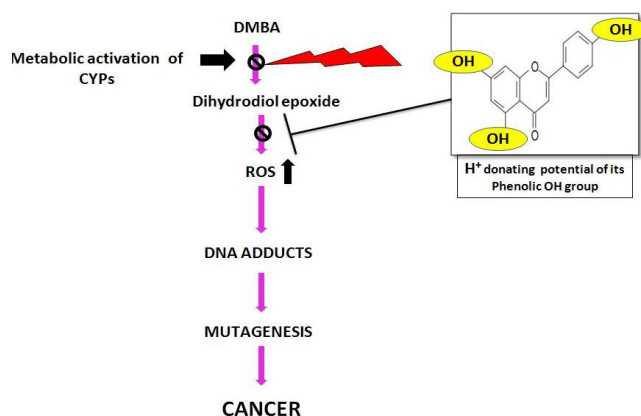


Fig.2. Chemopreventive Potential of Apigenin

Apigenin inhibits deregulation in the expression pattern of cell-proliferative, apoptotic, inflammatory and angiogenic markers during experimental oral carcinogenesis

Understanding the molecular mechanism associated with the pathogenesis of oral cancer would help for the early diagnosis and prevention. Abnormal cell proliferation (c-fos, PCNA and Cyclin D1), angiogenesis (VEGF), inflammation (NF- κ B and COX-2) and evasion of apoptosis (p53, Bax, Bcl-2, caspase-3 and -9) are the central features of a malignant tumor.

PCNA and p53, Bcl-2, and Bax received greater attention as a molecular marker of cell proliferation and apoptosis respectively not only for their known role in these processes but also for the participation in the human carcinogenesis process, especially oral carcinogenesis. Abnormal expression of PCNA has been shown in precancerous and cancerous lesions of the oral cavity (54). Choi and Kim (55) reported that apigenin induced p53 dependent apoptotic pathway might have played a role in cell cycle arrest in human breast cancer SK-BR-3 cells. It has been reported that apigenin induced a reversible G2/M and G0/G1 cell cycle arrest by increasing p53 protein stability in a wide array of malignant cells (58). Over expression of cyclin D1 can result in aggressive tumor growth and poor prognosis of oral cancer. A large number of studies have shown amplification and overexpression of cyclin D1 in oral cancer (56).

Deregulation of apoptotic machinery may contribute to the malignant transformation. p53 serves as a key element in maintaining the balance between cell growth and cell death in the living system and plays

a pivotal role in tumor growth inhibition and induction of apoptosis. (57,58). Dysfunction of apoptotic regulatory proteins, p53, bcl-2 and bax has been associated with tumor development and progression (59). Zheng *et al.*, (60) reported that apigenin induced p53 expression and decreased Bcl-2 protein, which caused cell cycle arrest and apoptosis in human cervical carcinoma cells. Shukla and Gupta (61) reported that treatment of androgen-refractory human prostate carcinoma DU145 cells with apigenin resulted in shift in Bax/Bcl-2 ratio in favour of apoptosis, associated with the release of cytochrome c and induction of apoptotic protease activating factor-1.

Bax expression was higher in well-differentiated carcinomas but significantly suppressed in poorly differentiated lesions (62). Decreased Bcl-2/Bax ratio was shown in oral squamous cell carcinoma (59). Decreased levels of caspase-3 and 9 were reported in oral carcinogenesis. (63). Tong *et al.*, (64) reported that apigenin suppressed UVB-induced over expression of COX-2 in mouse 308 keratinocytes. Over expression of COX-2 was frequently seen in well-differentiated squamous cell carcinoma than poorly differentiated ones (65). Liang *et al.*, (66) suggested that modulation of COX-2 and iNOS by apigenin may be important in the prevention of carcinogenesis and inflammation.

NF- κ B over expression negatively regulated the function of p53, contributing to tumorigenesis. Deregulation of NF κ B has been implicated in the pathogenesis of several disorders including oral carcinoma (67). Gupta *et al.*, (68) reported that apigenin treatment to human prostate cancer cells resulted in marked decrease in cyclin D1 protein expression and down regulation of the constitutive expression of NF κ B/p65. Elevated levels of c-fos in malignant oral lesions has been reported (69). Other studies also have reported a high c-fos expression in normal oral mucosa followed by gradual decrease at the advanced stages of oral cancer (70).

Oral administration of apigenin down regulated the expression of p53, bcl-2, PCNA, VEGF, c-fos, COX-2, NF κ B, cyclin D1 and up regulated the expression of BAX, caspase 3 and 9 in hamsters treated with DMBA. It has been reported that apigenin blocked the cell cycle progression at either the G1 \rightarrow S or the G \rightarrow M transition phases (Fig.3) (71). Apigenin significantly activated the expression of p53 and induced apoptosis

in cancer cells. Apigenin induced apoptosis was p53 dependent and caspase-3 expression dependent (72). Apigenin up regulated the expression of Bax and down regulated the expression of Bcl-2 in favour of apoptosis (73). Apigenin administration to p53-mutant cancer cell lines, HT-29 and MG63 resulted in growth inhibition and G2/M phase cell cycle-arrest (74). Apigenin inhibited apoptosis via activation of caspase 3 and 9 (75) Apigenin suppressed the expression of cyclooxygenase-2 in mouse macrophages (66). Also it reduced the expression of NF- κ B in human umbilical vein endothelial cells (55). Apigenin down regulated COX-2 expression in Human Colonic Epithelial Cells (HCEC) (76). Apigenin suppressed angiogenesis via a reduction of VEGF- mRNA expression in tumors. It has also been reported that apigenin significantly inhibited VEGF transcriptional activation in A549 lung cancer cells and ovarian cancer cells (77,78)

This review concludes that the protective effect of apigenin on the expression of molecular markers of apoptotic, cell proliferative, angiogenic, and inflammatory in DMBA induced hamster buccal pouch carcinogenesis. The antitumor effect of apigenin is probably due to its anticell-proliferative, anti-inflammatory, apoptotic and antiangiogenic potential during DMBA induced hamster buccal pouch carcinogenesis.

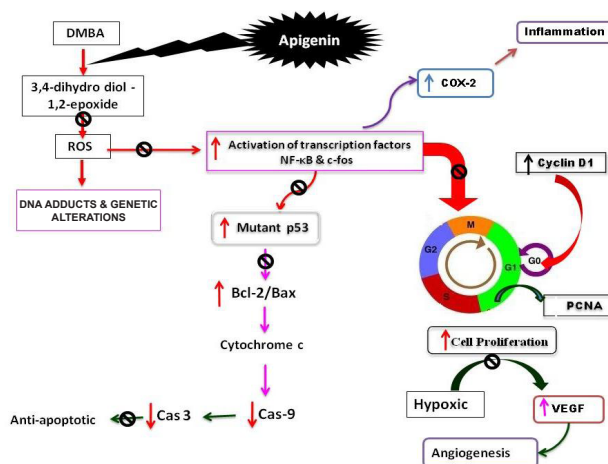


Fig.3. Mechanism Action of Apigenin Against Oral Carcinogenesis

CONCLUSION

Thus the review concludes that chemopreventive efficacy of apigenin in DMBA-induced hamster buccal pouch carcinogenesis is probably due its antioxidant

and modulating effect on detoxification cascade as well as due to its anti-cellproliferative, anti-inflammatory, apoptotic and anti-angiogenic potential during DMBA-induced hamster buccal pouch carcinogenesis. The present research works on biochemical and molecular aspects of oral carcinogenesis is giving us deeper insight into the etiology and pathogenesis of oral cancer, which will hopefully lead to more effective strategies for diagnosis and treatment of oral tumor in the future.

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