Review

Naturally-Occurring Bioactives in Oral Cancer: Preclinical and Clinical Studies, Bottlenecks and Future Directions

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Abstract

Oral cancer (OC) is the eighth most common cancer, particularly prevalent in developing countries. Current treatment includes a multidisciplinary approach, involving chemo, radio, and immunotherapy and surgery, which depends on cancer stage and location. As a result of the side effects of currently available drugs, there has been an increasing interest in the search for naturally-occurring bioactives for treating all types of cancer, including OC. Thus, this comprehensive review aims to give a holistic view on OC incidence and impact, while highlights the preclinical and clinical studies related to the use of medicinal plants for OC prevention and the recent developments in bioactive synthetic analogs towards OC management. Chemoprophylactic therapies connect the use of natural and/or synthetic molecules to suppress, inhibit or revert the transformation of oral epithelial dysplasia (DOK) into oral squamous cell carcinoma (OSCC). Novel searches have underlined the promising role of plant extracts and phytochemical compounds, such as curcumin, green tea extract, resveratrol, isothiocyanates, lycopene or genistein against this malignancy. However, poor bioavailability and lack of in vivo and clinical studies and complex pharmacokinetic profiles limit their huge potential of application. However, recent nanotechnological and related advances have shown to be promising in improving the bioavailability, absorption and efficacy of such compounds.

Keywords: oral cancer; head and neck squamous cell carcinoma; phytotherapy; curcumin; green tea extract; bioavailability; nanotechnology

1. Introduction

Cancer is one of the most common causes of morbidity and mortality worldwide [1], and representing nearly 10 million deaths in 2020 [2]. Furthermore, more than 50% of all cancer cases occur in developing countries [3].

Oral cancer (OC) is the 8th commonest type of cancer worldwide, being most common in developing countries [3]. OC and oropharyngeal cancer together represent the 6th most frequent neoplasm worldwide, with about 400,000 new cases diagnosed each year, despite vary greatly depending on the geographical setting. In effect, the incidence can be 20 times greater in some regions compared to others (e.g., Malaysia or Sri Lanka) [4]. Likewise, differences in both incidence and mortality have been observed by comparing the epidemiological data of industrialized countries with that of developing countries [4]. Such variations in incidence have been mainly related to environmental factors, like smoking and alcohol consumption, and diet [5]. Although both smoking and alcohol have been largely searched in relation to OC risk, data related to dietetic factors are more limited [6].

By definition, OC is a type of head and neck cancer, arising on the lip or oral cavity, with 90% being classified as squamous cell carcinoma [7]. It includes all malignant tumors, including salivary glands, metastatic tumors of other epithelial organs and nerve-related malignant tumors arising from submucosal regions (Fig. 1) [7]. Pain, sticky saliva, xerostomia (dryness of mouth), feeding and speaking difficulties are the most common physical complaints shown by OC patients [8]. The treatment depends upon the
cancer stage and location. Ideally, it comprises a multidisciplinary approach involving chemotherapy, radiotherapy, immunotherapy and surgical removal of the tumor or their combination, often accompanied by severe side effects [9].

Fig. 1. Oral cancer on the floor of the mouth in a smoking patient (There is written informed consent signed by the patient to use this image).

This type of cancer is mainly diagnosed at advanced stages, causing high morbimortality rates. However, it is known to be preventable, since most exogenous risk factors are avoidable (e.g., tobacco smoking, heavy alcohol consumption) [10,11]. Additionally, ultraviolet (UV) exposure, poor oral hygiene, herpes virus infections are other relevant risk factors [12]. Therefore, the WHO Global Oral Health Programmed recently co-sponsored international meetings with a focus on OC prevention. The main preventative approaches are the treatment of potential human papillomavirus (HPV) infections, avoidance of alcohol, and abstinence or withdrawal from tobacco use and the intake of antioxidants-rich diets [13].

On the other side, medicinal plants are characterized by a secular use for the prevention and treatment of multiple diseases [14]. Recently, plant-food-derived natural compounds have attracted researchers given their remarkable anticancer abilities [15]. The National Cancer Institute has identified about 35 plant foods that can prevent several multiple diseases [16]. Additionally, ultraviolet (UV) exposure, poor oral hygiene, herpes virus infections are other relevant risk factors [12]. Therefore, the WHO Global Oral Health Programmed recently co-sponsored international meetings with a focus on OC prevention. The main preventative approaches are the treatment of potential human papillomavirus (HPV) infections, avoidance of alcohol, and abstinence or withdrawal from tobacco use and the intake of antioxidants-rich diets [13].

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3. Incidence and Impacts of Oral Cancer

In 2018, the WHO estimated that cancer burden increased to 18.1 million new cases and 9.6 million deaths per year. Also, it has been reported that 1 in 5 men and 1 in 6 women are diagnosed with cancer over lifetime, with 1 in of 8 men and 1 in 11 women dying from this disease [25].

As previously referred, OC is the 6th commonest cancer worldwide, and occurs in oral cavity environment. It can include squamous cell carcinoma (SCC), salivary gland and odontogenic neoplasms, although most cases (~90%) are SCC [26]. WHO estimates that tobacco and excessive alcohol consumption play a leading role in around 90% of OC cases [27]. In the past few decades, despite the improvement of therapeutic methods, the survival rate of OC has not changed significantly (5-year survival rate slightly >50%) [28]. Annually, more than 400,000 new cases are anticipated worldwide. In the EU, there are projected 66,650 new cases each year [29] and the American Cancer Society estimates 53,000 new cases and 10,860 deaths in 2019 [30].
Interestingly, OC incidence in higher in developing countries, namely in South-Eastern countries and parts of Eastern Asia, Central and Eastern Europe, and parts of North and South America. In South-Central Asia, OC is the 3rd commonest cancer, and in India, the age-standardized rate of incidence of OC is 12.6/100,000 individuals. Based on the WHO report, Asian countries account for 73.3% of the global deaths from the lip and oral cavity cancers and have the leading rate of mortality compared to other continents. Europe and Africa have the 2nd and 3rd highest-mortality rate, respectively. Lately, there was stated an increased rate of OC in developed countries, including Denmark, France, Germany, Scotland, and to a lesser extent, in Australia, Japan, New Zealand, and the USA, which is thought to be a result of the aging of population and of the raise in the prevalence of risk factors [27]. In most countries, OC is more frequent in men than women, with the ratio of males to females being of 1.5:1, while to oropharynx cancer is of 2.8:1 [5].

As indicated, the risk of OC increases with age, being more common in people older than 50 years [5]; however, evidence shows that the incidence of OC has clearly changed. The disease has increased in individuals younger than 45 years in the past 4 decades, and studies show a decrease in the rate of classical OC (older patients) in the last years worldwide, being related to a decrease in smoking and alcohol intake [31]. On the other hand, many young OC patients have never drunk alcohol or smoked. In fact, new evidence suggests strong connections between HPV infection, hereditary factors, immunodeficiency and OC [31, 32].

4. Phytotherapy and Ethnopharmacology as Upcoming Strategies for Oral Cancer

OC treatment is multimodal and involves radio, chemo and immunotherapy, and surgery. Despite the existing clinical interventional strategies (surgery and drug administration), the mortality rate of head and neck squamous cell carcinoma (HNSCC) remains high. In this sense, new and more effective treatments with fewer adverse effects are needed [33].

Natural plant products have been used for centuries, and their bioactive components offer promising perspectives for the development of new chemotherapies or adjuvant treatments capable of avoiding the conventional therapies-associated cytotoxic effects [34–36]. Briefly, chemoprophylactic therapies involve the use of natural and/or synthetic compounds to repress, inhibit or revert the transformation of DOK into oral squamous cell carcinoma (OSCC). For instance, it has been suggested that diet can prevent from OC, particularly through a high consumption of fruits and vegetables (rich in micronutrients, such as β-carotene, vitamin C, vitamin D, and flavonoids) [37, 38]. Recently, the beneficial role of vitamin D in preventing the OC was reviewed. The experimental evidences suggest a relationship between serum calcidiol concentration in the optimal level of ~32 ng/mL and prevention of cancer [39]. Utilization of natural products as drug in treatment of various ailments is also reviewed recently [40].

New investigations have progressively revealed the anticancer effects of multiple phytochemical compounds, being often classified according to their function and chemical structure [41–43]. However, to afford benefits following ingestion, these substances need to be absorbed and metabolized before being transported to both tissues and organs, and many challenges need to be surpassed (e.g., toxicity at the required doses, low bioavailability or triggering resistance mechanisms), and in such perspective the use of nanoparticles and other agents have been viewed as a key to resolve part of these issues [44, 45].

In the case of oral lesions, in particular, the success of intraoral administration of natural products is conditioned by the effectiveness of the administration vehicles used, i.e., the capacity to effectively deliver the agent and to maximize patient adherence to therapy. In this regard, many polymeric vehicles, such as mucosalhesive gels, patches, tablets, oral rinses and aerosols have been searched for potential use in local intraoral administration. Although many of these administration strategies have been used in OC, to date, most chemoprophylactic trials have been unable to optimize drugs’ administration based on the evaluation of local pharmacokinetic parameters (e.g., determination of oral intraepithelial concentration of the agent, metabolites formation, stability and/or release kinetics). Moreover, the inclusion criteria used (e.g., patients with benign hyperkeratotic lesions and a lack of smoking cessation) complicate the comparative chemoprophylactic efficacy analyses between trials [36, 46]. The number of clinical trials, registered and ongoing, on international databases, trial registers can be seen in Table 1. Major bioactives involved in the prevention and suppression of oral cancer are discussed in following sub-section.

4.1 Curcumin

Curcumin is a polyphenol derived from Curcuma longa L. (turmeric), a member of the Zingiberaceae family, already approved for use as a food additive. The active ingredients of curcumin are volatile, orange-yellowish colored oils, called curcuminoids [42]. Various phytochemical and plant sources targeted for the oral cancer treatment are illustrated in Fig. 2.

Some evidence has shown that curcumin modulates inflammation- and carcinogenesis-involved pathways. Preclinical studies have shown the anti-OC effects of curcumin, when administered in isolation or combined with conventional drugs [42]. Its chemoprophylaxis activity involves the regulation of a number of intracellular signaling transduction pathways at different levels, such as transcription factors. It also influences the expression of many proteins, such as p53, cyclin D1, β-catenin, epidermal growth factor receptor (EGFR), caspases, and improves apoptosis.
<table>
<thead>
<tr>
<th>Title</th>
<th>ClinicalTrials.gov Identifier</th>
<th>Objective</th>
<th>Number of patients enrolled</th>
<th>Intervention/treatment</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin biomarker trial in HNSCC</td>
<td>ClinicalTrials.gov Identifier: NCT01160302</td>
<td>To assess the short-term effects of supplementation with a turmeric extract, Curcumin C3 Complex® on HNSCC biomarkers.</td>
<td>33</td>
<td>Microgranular Curcumin C3 Complex®; 4 grams twice daily for 21–28 days</td>
<td>Early phase I</td>
</tr>
<tr>
<td>Phase I chemoprevention trial with green tea polyphenon E (PPE) and Erlotinib in patients with premalignant lesions of head and neck</td>
<td>ClinicalTrials.gov Identifier: NCT01116336</td>
<td>To examine the protective role of a combination of drugs: green tea extracts derived PPE, and Erlotinib. The safety of the combination was also evaluated alongside the effect of the combination on patient’s premalignant lesion. The highest dose of each agent without side effects was also formulated.</td>
<td>25</td>
<td>Erlotinib (50 mg, 75 mg, or 100 mg); daily continuously for 6 cycles for each cycle + Green Tea Polyphenon E (200 mg); three times daily for 6 cycles</td>
<td>Phase I</td>
</tr>
<tr>
<td>Phase II trial to evaluate the effects of green tea in oral leukoplakia</td>
<td>ClinicalTrials.gov Identifier: NCT00176566</td>
<td>To assess the effects of a green tea preparation on leukoplakia and to find out these effects in reducing the risk of cancer in or around the area of leukoplakia.</td>
<td>8</td>
<td>Green tea lozenges (6 grm), 8 times daily for 12 weeks</td>
<td>Phase II</td>
</tr>
<tr>
<td>Soy isoflavone combination with radiation therapy and Cisplatin in SCC of the head and neck</td>
<td>ClinicalTrials.gov Identifier: NCT02075112</td>
<td>To evaluate the effects of soy supplementation during chemotherapy and radiation therapy in decreasing the side effects caused by the treatments.</td>
<td>24</td>
<td>Genistein (150 mg) daily</td>
<td>Phase I</td>
</tr>
<tr>
<td>Soy Isoflavones in Preventing Head and Neck Cancer Recurrence in Patients With Stage I–IV Head and Neck Cancer Undergoing Surgery</td>
<td>ClinicalTrials.gov Identifier: NCT02007200</td>
<td>To determine how well soy isoflavones prevent head and neck cancer in patients who have undergone surgery for stage I–IV.</td>
<td>55</td>
<td>Soy Isoflavones (300 mg); daily for 14 days</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

HNSCC, head and neck squamous cell carcinoma; SCC, squamous cell carcinoma.
As a matter of fact, the curcumin activity has been investigated in a number of HNSCC cell lines, including CAL27, CCL23 (laryngeal carcinoma), UM-SCC1 and UMSSC14A (oral carcinoma), with curcumin action being fundamentally upon the nuclear factor kappa-B (NF-κB) signaling pathway. In fact, it produces a decrease in NF-κB expression while further inhibits its nuclear localization [48]. In 2017, Borges et al. [49] conducted a systematic review on the role of curcumin in HNSCC cell lines, and stated that it inhibits cell proliferation and viability, while induces apoptosis and triggers cell cycle arrest at G2/M phase, thus evidencing its potential as an adjuvant in HNSCC treatment. Preclinical studies on curcumin in rats found that it can inhibit oral carcinogenesis [50,51]. Carcinogenesis was induced by the application of 4-nitroquinoline 1-oxide (4-NQO), and curcumin was administered to rats (100 mg/kg) during 12 weeks, being found a marked reduction in proliferating cell nuclear antigen (PCNA), Bcl2, suppressor of cytokine signaling (SOCS)-1 e-3 and signal transducer and activator of transcription 3 (STAT3) expression [41]. In another study, it was reported the inhibition of oral mucosal tumors in hamsters following dietetic curcumin administration [52]. In another independent study, curcumin administered either alone or with green tea inhibited oral carcinogenesis in hamsters; the authors linked this effect to cell proliferation suppression, apoptosis induction and angiogenesis inhibition [53]. Other researchers assessed the effect of oral dosing of curcumin and piperine in cheek pouch of hamsters with carcinomas induced by 7,12-dimethyl-benzanthracine (DMBA), found that both compounds were able to avoid the tumors formation, probably as a consequence of their antioxidant properties [54]. A group of researchers also reported significant inhibitory effects of curcumin upon SAS cell line (OC cell lines) growth and proliferation, inoculated subcutaneously in mice; the cytotoxic effect of curcumin was found to be targeted at G2/M phase of cell cycle [55]. Other studies have also shown that curcumin exerts in vivo suppressive effects upon cell growth, based on nude mouse xenograft models. It was found that curcumin is highly effective in suppressing HNSCC cell xenografts growth in nude mice, suppressing carcinogenesis through inhibition of the AKT/mammalian target of rapamycin (mTOR) pathway [56].

Regarding clinical data, a number of studies have been performed with curcumin. In a study, patients with potentially malignant oral disorders, like leukoplakia, received increasing doses of curcumin extract, starting with 500 mg/day and reaching the highest tolerated dose of 8000 mg/day, over 3 months, and no toxic effects were stated at the highest dose [57,58].

Regarding limiting factors, one that sometimes limits the use of curcumin is its relatively low bioavailability. Accordingly, Boven et al., in 2019 [56], applied the substance directly to the oral cavity by means of a chewing gum formulation. Curcumin release and absorption in serum and saliva were assessed after chewing, since contact with the mucosa appears to be critical for improving its release and absorption [59]. Moreover, one of the main research criticisms related to curcumin is that most available data derives from pre-clinical studies. Consequently, the optimum therapeutic dosage remains unclear. On the other hand, there are some agents, like piperine, that can raise the curcumin bioavailability. Therefore, further studies are needed to assess the curcumin usefulness in terms of prevention and treatment [42]. Curcumin has a number of mechanisms of therapeutic action, with main disadvantages of its oral administration being its high metabolic instability and low water solubility that markedly impairs its systemic bioavailability [42]. In this way, new strategies are being investigated to overcome such difficulties, like the use of liposomal formulations containing curcumin and its encapsulation in polymeric nanoparticles.

Fig. 2. Illustration showing the plant source of phytochemical reviewed in the current study and structure of specific compound discussed in the current review against oral cancer. Where (A) Turmeric. (A1) Structure of keto form of curcumin. (A2) Structure of keto-enol form of curcumin. (B) Green tea and powder. (B1) Structure of epigallocatechin gallate. (C) Black grapes and red wine. (C1) Structure of resveratrol. (D) Strawberry. (D1) Ferulic acid. (D2) Black raspberry. (D4) Structure of cyanidin-3-rutinoside. (E) Broccoli. (E1) Structure of glucoraphanin. (F) Tomato. (F1) structure of lycopene. (G) Soybean. (G1) Fava beans. (G2) Structure of genistein.
4.2 Green Tea Extract

Green tea (Camellia sinensis L., Kuntze) is a popular beverage throughout the world, with a particular attractive aroma, flavor and health potentialities, closely linked to its content in polyphenols. Approximately 80% of commercial tea product found mainly in the western world is black tea, and the remaining corresponds to green tea [60].

Green tea contains 4 main polyphenols, known as catechins: 10–15% (-)-epigallocatechin gallate (EGCG), 6–10% (-)-epigallocatechin (EGC), 2–3% (-)-epicatechin gallate (EGC) and 2% (-)-epicatechin (EC), the first three with antineoplastic properties [43]. Briefly, EGCG is an ester of epigallocatechin and gallic acid, and is the most abundant catechin in green tea. It is also the catechin with the greatest bioactive effects [61]. The most widely used administration route for EGCG is the oral route in the form of tea or capsules, where only 0.1–1% of the administered oral dose reaches the systemic circulation [62,63]. However, the EGCG levels may be far higher in tissues that come into direct contact with the drug, such as oral cavity. Once ingested, the first transformation reactions take place in saliva, in the form of esterase-mediated hydrolysis of EGCG. Metabolization largely occurs in intestine and liver, where glucuronidation, sulphatation of the hydroxyl groups and O-methylation of the catechol groups take place mediated by UDP-glucuronyltransferase (UGT), phenol sulfotransferase (SULT) and catechol-O-methyl transferase (COMT) [43,63,64].

Laboratory and animal studies have shown that tea-derived polyphenols inhibit tumor cells proliferation while induce apoptosis. Likewise, tea-derived catechins inhibit angiogenesis and tumor cell invasion, while also protect from ultraviolet B (UV-B) radiation, and may possibly modulate the immune system function. In addition, it has been shown that green tea activates enzymes involved in detoxification processes, like glutathione S-transferase and quinone reductase, which may help to protect against tumor formation. Although most beneficial effects of tea have been linked to the remarkable antioxidant effects of polyphenols, the exact mode of action whereby tea could contribute to prevent cancer has not yet been precisely defined [62,65,66].

Regarding clinical data, the green tea extract administration during 4 weeks in smokers led to a reduction in keratinocyte DNA damage. Furthermore, cell growth was inhibited, with a decrease in the percentage of cells at S phase, and an increase in the rate of that in phase G1. Likewise, the diploid DNA content increased, with positive regulation of the apoptosis markers [67]. In a clinical trial involving 59 patients with oral leukoplasia, a 3 g/day of a mixed tea product in the form of oral capsules plus the application of mixed tea ointment with topical glycerin, or placebo plus topical glycerin administered for 6 months, led to a reduction of oral lesions in 38% of patients treated with green tea mixture versus 10% in the placebo group [53]. Another clinical study assessed the chemoprophylactic potential of green tea extract in OC. The authors found that the two arms receiving the highest doses (0.75 and 1.0 g/m²) evidenced greater clinical response rates (58.8%) compared to those receiving the lower dose (0.5 g/m²; 36.4%) or placebo (18.2%), stating a dose-response effect. The treatment was well-accepted, being only stated the following side effects at the highest doses: insomnia, diarrhea and oral/neck pain [68]. Lastly, in patients at high risk of developing oral precancerous lesions, the EGCG application in the form of an oral rinse during 7 days triggered a reduction in level of expression of certain oral carcinogenetic biomarkers — though the results failed to reach statistical significance [69].

In 2014, Huang et al. [67] studied the association between tea consumption and head and neck cancer in Taiwan, where tea is a commonly used beverage. Surveys were made regarding tea intake (frequency, duration and types) in 396 patients with head and neck cancer and 413 controls. A marked decrease was stated in the risk of head and neck cancer linked to tea consumption, and a significant inverse correlation was recorded between head and neck cancer and tea intake — particularly green tea [70]. Similar inhibitory effects have been stated using EGCG with curcumin and resveratrol in decreasing the tumorigenicity of HPV-positive head and neck tumors [71].

4.3 Resveratrol

Resveratrol is a natural polyphenol found in a broad variety of plant species, including grape, peanut and different types of berries, being also an important constituent of red wine. Currently, resveratrol is known to be a bioactive molecule with potential beneficial health effects thanks to its numerous pharmacological properties and lack of deleterious effects [72,73]. However, the health benefits of resveratrol may be hampered by its low oral bioavailability, which has been attributed to its incomplete intestinal absorption. Nevertheless, different studies have attempted to synthesize resveratrol analogs, ultimately preserving its biological activities [74,75]. The antineoplastic properties of resveratrol have also been tested in different preclinical studies, and for instance, in HNSCC cell lines, the drug is able to inhibit its growth and proliferation [76].

In HNSCC, resveratrol given in combination with curcumin led to a higher inhibition of cancer cells growth than curcumin alone, and the effect of 5-fluorouracil (5-FU) plus resveratrol also revealed to be synergistic in HNSCC cell lines. It was also found that resveratrol (10–1000 µM) reduce cell viability in a tongue SCC cell line [77]. In another study, the effect of resveratrol on signal transducer and activator of transcription (STAT)-3 signaling cascade and its regulated functional responses in squamous cell carcinoma of the head and neck (SCCHN) cells was investigated [78]. Authors concluded that resveratrol attenuates STAT3 cascade by induction of Suppressors of cytokine signaling (SOCS)-1, thus inhibiting STAT3 phosphorylation.
and proliferation in SCCHN cells.

However, the low bioavailability and inadequate focalization of the drug is conceived as the main obstacle that blocks the use of these biomolecules in in vivo and clinical studies [79]. In this regard, there is a need for nanotechnological developments capable of administering these substances (e.g., EGCG and resveratrol), with a view to boosting their antitumor effects and overcoming the problems posed by their complex pharmacokinetic profile.

4.4 Garcinol

Garcinol (camboginol) is one of another bioactive components explored for the head and neck cancer. This compound is extracted from waste rinds of the Garcinia indica fruit. The compounds have also showed their exceptional role in prevention of other types of cancers such as colorectal, breast, gastrointestinal and leukemia [80].

Garcinol mediates its antitumor effects in squamous cell carcinoma of the HNSCC cells and mouse model through the suppression of multiple proinflammatory cascades. Garcinol reduced the growth of HNSCC mouse without exhibiting any significant toxicity, and downregulated the expression of p-signal transducer and activator of transcription (STAT)-3, p65, Ki-67, and CD31 in the treated groups as compared with the control group [80].

4.5 Freeze-Dried Strawberries and Black Raspberries

Strawberries (Fragaria × ananassa) contain a number of ingredients with potential chemoprophylactic activities, including vitamins A, C and E, folic acid, calcium, selenium, β-sitosterol, ellagic and feral acids, flavonols, like kaempferol and quercetin, and a range of anthocyanins. Strawberry extracts inhibit human cancer cell proliferation and induce apoptosis in vitro; furthermore, they inhibit activator protein 1 (AP-1) and NF-κB in cell cultures [81].

Stoner and Casto [82] reviewed the pharmacological and chemoprophylactic activities of the individual components of a number of fruits. The findings referred to strawberries and black raspberries in rodent aerodigestive tract cancer models suggest that the dietetic administration of strawberries could inhibit the development of oral malignant lesions and modify the expression of genes related to OC development [82].

Black raspberries contain anthocyanins, ellagitannins and phenolic acids, that have been found to exert potent inhibitory activity upon aerodigestive tract carcinogenesis [41]. Preclinical findings in animal models indicate that black raspberries inhibit OC through mechanisms linked to cell proliferation, inflammation, angiogenesis and apoptosis [82]. Oghumu et al. [81] demonstrated that in a 4NQO OC rat model, black raspberries inhibit oral carcinogenesis through inhibition of proinflammatory and anti-apoptotic pathways. Knobloch et al. [83] studied rats exposed to the carcinogen 4NQO, that were fed with a diet supplemented with black raspberries 5% or 10%, or a control diet, during 6 weeks after exposure to the carcinogen. An RNA-seq transcriptome analysis in rat tongue was made, together with mass spectrometry and metabolomic analysis of RNA in rat urine. The authors identified 57 metabolites expressed in different ways, and over 662 modulated genes in rats fed black raspberries. The glycolysis and 5’ adenosine monophosphate-activated protein kinase (AMPK) pathways were modulated during OC chemoprophylaxis mediated by black raspberries. The glycolytic enzymes, Aldoa, Hk2, Tpi1, Pgam2, Pkl and Pkm2, as well as the genes of PKA-AMPK pathway Prkaa2, Pde4a, Pde10a, Ywhag and Crebbp were found to be negatively regulated by black raspberries during OC chemoprophylaxis [84]. Moreover, the glycolysis metabolite glucose-6-phosphate decreased in rats that fed black raspberries. These data evidence new metabolic pathways modulated by black raspberries phytochemicals, which may be directed during OC chemoprophylaxis.

However, translational clinical trials assessing the potential of phytochemicals contained in black raspberries applied to the oral mucosa remain limited [84,85]. For example, a phase 0 clinical trial was carried out in 38 patients in which biopsy confirmed the presence of OSCC. During the time before scheduled surgery (14 days on average), the patients received tablets containing freeze-dried black raspberry powder. The resected cancerous tissue of patients that received black raspberries revealed a significant decrease in the expression of genes encoding proteins of importance for cancer cell survival (AURKA, EGFR or BIRC5), as well as for proinflammatory agents’ production, like NFKB1 and PTGS2. Furthermore, the active substances in black raspberries, such as cyanidin-3-rutinoside and cyanidin-3-xylosyl rutinoside, were identified in OSCC tissues, thereby evidencing that they effectively accumulate within the target tissues [84,85]. A study conducted in 40 patients with premalignant oral intraepithelial lesions found that the topical application of a mucoadhesive freeze-dried black raspberry gel significantly reduced the lesion size and histological grade, while the lesions size was seen to increase in the control group [86]. Indeed, the success of local intraoral drug administration strategies mainly depends on the capacity of the polymeric drug vehicles to afford increased solubility and stability in physiological fluids, such as saliva, together with an adequate drug release, good penetration and local distribution of the chemoprophylactic agents, and the capacity to apply the drug at various mucosa oral loca-

tions [86,87]. In this sense, further research is necessary to improve our understanding on the ADMET of black raspberries and their respective polyphenols [88].

4.6 Isothiocyanates (ITCs)

Epidemiological studies have indicated that vegetables-rich diets, containing the genus Brassica (Cruciferae) family, e.g., broccoli, cabbage, cauliflower are linked to a lesser HNSCC risk. Indeed, cruciferous
vegetables, such as broccoli, cabbage and watercress possess a series of bioactive molecules with anticancer properties, mostly attributable to their high contents in glucosinolates, with isothiocyanates (ITCs) being the most potent ones. Briefly, ITCs are able to induce apoptosis and inhibit the NF-κB signaling pathway through a range of mechanisms [89]. In addition, by reducing EGFR signaling, they can suppress both the expression and activity of matrix metalloprotease-2 (MMP-2) and MMP-9 enzymes, resulting in metastasis inhibition [90]. In addition, the topical application of ITCs has shown to exert a potent chemoprophylactic activity against HNSCC in animal models, at same time that can improve the effects of chemotherapy. A pilot study, involving 3 broccoli extract administration regimens in 10 healthy volunteers, was performed to assess the bioavailability and effects upon NRF2 signaling in the oral epithelium. The three groups comprised the intake of glucoraphanin-rich broccoli sprout extracts; sulforaphane-rich broccoli sprout extracts; and the topical exposure to sulforaphane-rich broccoli sprout extracts. As main findings, the authors recorded an adequate bioavailability and chemoprophylactic activity, although further research is needed, since minor changes in the molecular formulas drastically modify the mechanisms of action [91,92].

4.7 Lycopene

Lycopene is a natural liposoluble pigment synthesized by plants and microorganisms, and responsible for the red and orange color of some fruits and vegetables, such as ripe tomatoes, water melon, pink or red grapefruit, and red chili pepper [93]. Lycopene can protect cell components against specific types of damage triggered by reactive oxygen species (ROS), with inhibit cancer cells growth and progression, blocking cell cycle progression from the G0/G1 to the S phase, at same time that can modulate immune responses by decreasing the activity of carcinogen mediators. The mechanisms underlying such effects upon carcinogenesis involve ROS elimination, positive regulation of detoxification systems, interference in cell proliferation, induction of gap-junction communication, and cell cycle progression inhibition. It has also been shown that lycopene raises the p53 protein levels in cancer cells [61]. Other authors also stated that lycopene and tomato paste given to an oral carcinogenesis hamster model, significantly reduced the incidence of oral mucosal tumors, with different mechanisms being involved in their anticancer effects [94]. In addition, Cheng et al. [92] analyzed the chemoprophylactic effects of lycopene in a hamster model. The animals in the lycopene or mixed carotenoid groups did not develop carcinomas, in contrast to the controls, and although dysplastic lesions were observed in all groups, though the expression of PCNA was less pronounced in the lycopene group than in the control group [95].

Regarding clinical evidence, the treatment of premalignant oral lesions with lycopene has been linked to significant histological and clinical changes. For example, a case-control study conducted in Japan by Nagao et al. [86] with 9536 subjects over the age of 40 stated that among males with leukoplakia, the mean serum lycopene and carotene levels were markedly lower than that of controls [96]. Indeed, lycopene appears to be effective in treating oral leukoplakia, being able to protect cells from damage, while protects from oral dysplasia progression by blocking tumor cell proliferation. Moreover, initial reports on the lycopene efficacy against human oral cancer cells have underlined marked therapeutic effects [94]. Nevertheless, further clinical trials are needed to better address the therapeutic benefits of lycopene for both OC prevention and management.

4.8 Genistein

Genistein is an abundant phytoestrogen in soya and other legumes. To date, no clinical studies have examined the genistein effects in HNSCC, despite the epidemiological data available have underlined the efficacy of genistein intake in breast, prostate and colorectal cancer. However, the routine consumption of soya products was not found to have a significant impact upon HNSCC risk in Chinese adults, though the study in question was hampered by many significant limitations [22,49]. A general mechanism of action of phytochemicals for preventing OC cells growth is illustrated in Fig. 3.

![Fig. 3. General mechanism of action of phytochemicals for preventing oral cancer.](image)

4.9 Thymoquinone

Thymoquinone is the main abundant active constituent of the seeds of Nigella sativa L.. N. sativa, or black cumin, is widely used in Middle Eastern and Far Eastern countries as a spice and food preservation. Thymoquinone has a wide range of pharmacological properties, including its ability to combat oxidative damage, and inflammation [97]. Additionally, the compounds have demonstrated exceptional anticancer properties [98]. The anticancer action of thymoquinone is mediated by a variety of mechanisms. By targeting tumor suppressor genes (p53, p73, PTEN,
Table 2. Role of important bioactive compounds in suppression of oral cancer.

<table>
<thead>
<tr>
<th>Bioactive compound</th>
<th>Type of extract</th>
<th>Model</th>
<th>Key findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>Standard curcumin</td>
<td>Human oral cancer cell lines (SCC-25)</td>
<td>Curcumin reduced SCC-25 cells proliferation and invasion through inhibiting the phosphorylation of epidermal growth factor receptor (EGFR) and EGFR downstream signaling molecules Akt, ERK1/2 and STAT3.</td>
<td>[105]</td>
</tr>
<tr>
<td>Green tea extract Epigallocatechin gallate (EGCG) extracts from green tea</td>
<td>Human oral cancer cell lines (SCC)</td>
<td>EGCG inhibited growth, with a decrease in efficacy as cells progressed from normal to cancer. A G1 cell cycle block was induced with an increase in the underphosphorylated form of retinoblastoma protein.</td>
<td></td>
<td>[106]</td>
</tr>
<tr>
<td>Resveratrol Standard resveratrol</td>
<td>Human head and neck squamous cell carcinoma (SCC4 and FaDu cells)</td>
<td>Resveratrol attenuates STAT3 cascade by induction of Suppressors of cytokine signaling (SOCS)-1, thus inhibiting STAT3 phosphorylation and proliferation in SCCHN cells.</td>
<td></td>
<td>[78]</td>
</tr>
<tr>
<td>Garcinol Garcinia indica rind extract</td>
<td>Squamous cell carcinoma of the head and neck cells</td>
<td>Garcinol reduced the growth of HNSCC mouse without exhibiting any significant toxicity, and downregulated the expression of p-signal transducer and activator of transcription (STAT)-3, p65, Ki-67, and CD31 in the treated groups.</td>
<td></td>
<td>[50]</td>
</tr>
<tr>
<td>Anthocyanins and other phenolics from berries Berries extract</td>
<td>Oral squamous cell carcinoma cell lines, CAL-27 and SCC25</td>
<td>Extracts inhibit human cancer cell proliferation and induce apoptosis in vitro; furthermore, they inhibit activator protein 1 (AP-1) and NF-κB in cell cultures.</td>
<td></td>
<td>[107]</td>
</tr>
<tr>
<td>Isothiocyanates Benzyl isothiocyanates standard</td>
<td>Human oral cancer OC2 cells</td>
<td>Isothiocyanate derivative inhibits growth, promotes G2/M phase arrest and triggers apoptosis of OC2 cells with a minimal toxicity to normal cells.</td>
<td></td>
<td>[108]</td>
</tr>
<tr>
<td>Lycopene Standard lycopene</td>
<td>OSCC cell lines that included CAL-27 and WSU-HN6 cells</td>
<td>Lycopene drastically induced cell apoptosis suppresses cell migration and tumor growth.</td>
<td></td>
<td>[109]</td>
</tr>
<tr>
<td>Genistein Standard genistein</td>
<td>HSC-3, an oral squamous cell carcinoma cell line</td>
<td>Anti-angiogenic agent, with respect to tumor growth, angiogenesis.</td>
<td></td>
<td>[94]</td>
</tr>
</tbody>
</table>
STAT3), this compound suppressed cancer through cell cycle arrest. Furthermore, thymoquinone suppressed cell proliferation and angiogenesis [99]. Treatment with thymoquinone induces apoptosis and autophagy in human OC cells. Thymoquinone increased number of autophagic vacuoles, LC3-II protein expression, autophagosome accumulation, bax expression, and caspase-9 activation in the HNSCC cell line in a concentration-dependent manner [100]. Thymoquinone/Ca-alg-PVA has the chemopreventive effects against OC. Abdelfadil et al. [101], in 2013, demonstrated that thymoquinone induces apoptosis by downregulating the p38MAPK pathway in chemically induced oral squamous cell carcinoma (T28). Pu et al. [102], in 2021, showed that thymoquinone down-regulated the inflammatory and PI3K/AKT/mTOR signaling pathway in orally treated 7,12-dimethylbenz[a]anthracene (DMBA)-stimulated hamster oral tumor. In addition to being effective as a treatment for cancer, thymoquinone is also effective as a chemopreventive agent. It also prevents premalignant lesions from progressing to cancer. However, clinical trials are needed to assess the therapeutic benefits of thymoquinone for OC prevention.

4.10 Salvadora Persica L. Extract

Salvadora persica L. is a perennial shrub traditionally called miswa, which has chemopreventive and anti-oral cancer effects. S. persica has been extensively studied for its effect on oral health [103]. S. persica root extracts showed significant cytotoxic effects on DOK, oral squamous cell carcinoma (PE/CA-PJ15), and periodontal ligament fibroblast (PDL) cell lines, at concentrations of 11.25, 13.50, and 15.75 mg/mL, respectively [104]. Table 2 (Ref. [50,78,94,105–109]) shows the beneficial role of discussed bioactive compounds in prevention of oral cancer.

5. Conclusions

Although the stated advances, HNSCC requires more effective treatment strategies, with a decrease in therapy-related complications, and in this regard, natural phytochemicals are viewed as possible new chemoprophylactic agents, regarding their tolerability, safety, low toxicity and antioxidant effects. In parallel, preventive strategies have been widely investigated, though the existing data on phytochemicals are still fragmented and inconclusive; nevertheless, the findings from preclinical and clinical trials are becoming more promising in this regard. Besides, pharmacodynamic interactions should also be considered, as well as studies focusing on the pharmacokinetic interactions of different natural products. Also worth of note is that healthcare providers should also monitor herb-drug interactions routinely in cancer patients. In addition, many natural products are limited in their anticancer efficacy for relatively low bioavailability, lack of in vivo and clinical evidence, standardization of the optimum dose for treating disease, high metabolic instability and poor aqueous solubility during oral administration, inadequate focalization, complex pharmacokinetic profile, side effects and poor patient adherence. Thus, and in view of the aforementioned, further studies should be done to overcome these constraints and to enhance the absorption, metabolism and bioactivity herbal products through nanotechnological and related developments, besides attempting for minor changes in molecular formulas to modify their mechanisms of action.

Author Contributions

MB, CQ, JS-R, EP-F, PL-J, WZ, TD, AD, MK, MP, AHE, AU, J-TC, made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas. That is, revising or critically reviewing the article; giving final approval of the version to be published; agreeing on the journal to which the article has been submitted; and, confirming to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest. MB is serving as one of the Editorial Board members and Guest Editors, J-TC is serving as one of the Guest Editors of this journal. We declare that MB and J-TC had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to LT.

References


Abstract

In this study, we investigated the effects of benzyl isothiocyanate (BITC) on oral squamous cell carcinoma (OSCC) cells. BITC was found to induce apoptosis in OSCC cells in a concentration-dependent manner. The mechanism of apoptosis was further explored, and the involvement of the p38 signaling pathway was observed. BITC also inhibited cell proliferation and migration, and induced cell cycle arrest in the G1 phase. These findings suggest that BITC may serve as a potential therapeutic agent for the treatment of OSCC.

Keywords: benzyl isothiocyanate, apoptosis, p38 signaling pathway, oral squamous cell carcinoma.